Morphological variation of leaf traits in the *Ternstroemia lineata* species complex (Ericales: Penthaphylacaceae) in response to geographic and climatic variation

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Variation in leaf morphology is correlated with environmental variables, such as precipitation, temperature and soil composition. Several studies have pointed out that individual plasticity can largely explain the foliar phenotypic differences observed in populations due to climatic change and have suggested that the environment plays an important role in the evolution of plant species by selecting for phenotypic variation. Thus, the study of foliar morphology in plant populations can help us identify the environmental factors that have potentially influenced the process of species diversification. In this study, we analyzed morphological variation in the leaf traits of the *Ternstroemia lineata* species complex (Penthaphylacaceae) and its relation to climatic variables across the species distribution area to identify the patterns of morphological differentiation within this species complex. Based on the collected leaves of 270 individuals from 32 populations, we analyzed nine foliar traits using spatial interpolation models and multivariate statistics. A principal component analysis identified three main morphological traits (leaf length and two leaf shape variables) that were used to generate interpolated surface maps to detect discrete areas delimited by zones of rapid change in the values of the morphological traits. We identified a mosaic coarse-grain pattern of geographical distribution in the variation of foliar traits. According to the interpolation maps, we could define nine morphological groups and their geographic distributions. Longer leaves, spatulate leaves and the largest foliar area were located in sites with lower precipitation and higher seasonality of precipitation following a northwest – southeast direction and following significant

latitudinal and longitudinal gradients. According to the phenogram of the relationships of the nine morphological groups based on morphological similarity, the putative species and subspecies of the *T. lineata* species complex did not show a clear pattern of differentiation. In this study, we found a complex pattern of differentiation with some isolated populations and some other contiguous populations differentiated by different traits. Further genetic and systematic studies are needed to clarify the evolutionary relationships in this species complex.

2 MORPHOLOGICAL VARIATION OF LEAF TRAITS IN THE *TERNSTROEMIA LINEATA*
3 SPECIES COMPLEX (ERICALES: PENTHAPHYLACACEAE) IN RESPONSE TO
4 GEOGRAPHIC AND CLIMATIC VARIATION

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Abstract

22 Variation in leaf morphology is correlated with environmental variables, such as precipitation,
23 temperature and soil composition. Several studies have pointed out that individual plasticity can
24 largely explain the foliar phenotypic differences observed in populations due to climatic change
25 and have suggested that the environment plays an important role in the evolution of plant species
26 by selecting for phenotypic variation. Thus, the study of foliar morphology in plant populations
27 can help us identify the environmental factors that have potentially influenced the process of
28 species diversification. In this study, we analyzed morphological variation in the leaf traits of the
29 *Ternstroemia lineata* species complex (Penthaphylacaceae) and its relation to climatic variables
30 across the species distribution area to identify the patterns of morphological differentiation
31 within this species complex. Based on the collected leaves of 270 individuals from 32
32 populations, we analyzed nine foliar traits using spatial interpolation models and multivariate
33 statistics. A principal component analysis identified three main morphological traits (leaf length
34 and two leaf shape variables) that were used to generate interpolated surface maps to detect
35 discrete areas delimited by zones of rapid change in the values of the morphological traits. We
36 identified a mosaic coarse-grain pattern of geographical distribution in the variation of foliar
37 traits. According to the interpolation maps, we could define nine morphological groups and their
38 geographic distributions. Longer leaves, spatulate leaves and the largest foliar area were located
39 in sites with lower precipitation and higher seasonality of precipitation following a northwest –
40 southeast direction and following significant latitudinal and longitudinal gradients. According to
41 the phenogram of the relationships of the nine morphological groups based on morphological
42 similarity, the putative species and subspecies of the *T. lineata* species complex did not show a
43 clear pattern of differentiation. In this study, we found a complex pattern of differentiation with
44 some isolated populations and some other contiguous populations differentiated by different
45 traits. Further genetic and systematic studies are needed to clarify the evolutionary relationships
46 in this species complex.

47

Introduction

48 Intraspecific variation is one of the main sources of information for recognizing evolutionary
49 patterns. Identifying the causes of intraspecific variation is essential to understanding the
50 evolutionary processes that maintain diversity and promote speciation (*Futuyma, 1998*). In
51 plants, variation occurs in populations of species that are separated in space, encompassing both
52 the genotype and phenotype (*Thorpe, 2002*). The causes of phenotypic variation among
53 individuals across the geographical distribution range of a species can be broadly divided into
54 current environmental conditions within particular habitats and historical processes and
55 phylogenesis (*Thorpe, 1987; Peppe et al., 2011*). Plant populations of the same species growing
56 under different environmental conditions respond to different selection pressures, producing
57 genetic and phenotypic divergence between populations (*Ramsey, Cairns & Vaughton, 1994;*
58 *Fenster & Stenøien, 2001; Albarrán-Lara et al., 2019*).

61 Plants have developed particular adaptations to the surrounding local climate that allow them to
62 be better fitted to their environment (*Chevin et al., 2010; Valladares et al., 2014*). The leaf is the
63 structure in which changes in morphology in response to their environment are more readily
64 acquired by plants (*Malhado, 2009a*; *Yang et al., 2015*) because leaves are the organs that
65 perform essential functions, such as photosynthesis and regulation of water content (*Givnish,*
66 *1979; Wright et al., 2004; Adam & Ichiro, 2018; Tsukaya, 2018*).
67 Latitudinal and altitudinal variations in leaf size and shape are the result of plastic and adaptive
68 responses of plants to varying environmental conditions, with differences in plant physiological
69 responses also present among populations (*Rico-Gray & Palacios-Ríos, 1996; Niinemets 2001;*
70 *Uribe-Salas et al., 2008; Frenne et al., 2013; Moles et al., 2014*). The correlation between the
71 shape and size of leaves and the surrounding habitat has been interpreted as the result of an
72 evolutionary response of plants to varying environmental conditions (*Givnish, 1987; Westoby et*
73 *al., 2002; Peppe et al., 2011*). For instance, leaf size has been shown to decrease with increasing
74 altitude because of the differences in precipitation and nutrient content in the soil decrease
75 (*McDonald et al., 2003; Nicotra, 2011*). Additionally, smaller leaves appear to be better adapted
76 to dry environments because the smaller size reduces the hydraulic vulnerability of leaves and
77 makes the plants more tolerant to drought (*Scoffoni et al., 2011*). Light intensity also plays a key
78 role in the adjustment of leaf size and shape in later stages of development, promoting the
79 expansion of the leaf petiole and inhibiting the growth of the leaf blade (*Tsukaya, 2005*).
80 Several environmental factors have been shown to play key roles in the evolution of plant
81 species by selecting for phenotypic variation (*Pfennig et al. 2010*), especially variation related to
82 leaf traits. Thus, the study of foliar morphology in plant populations can help us to identify the
83 environmental factors that have potentially influenced the process of species diversification and
84 phenotypic variability (*Ashcroft et al., 2011*). The relationships of leaf variation with
85 environmental variables have been focused on the global (*Ordoñez, 2009; Yang et al., 2015;*
86 *Wright et al., 2017*), regional (e.g., North America) (*Royer et al., 2008*) and local scales (e.g.,
87 South Australia, Amazonia and Bolivia) (*Sokal, Crovello & Unnasch, 1986; Wright & Ladiges,*
88 *1997; Gregory-Wodzicki, 2000; Malhado, 2009a,2009b; Guerin, Wen & Lowe, 2012*). However,
89 similar patterns are not always identifiable across spatial scales, and in some cases, contrasting
90 trends can be found (*Malhado, 2009b; Wright et al., 2017*). Studies on how plants respond to
91 environmental variables within regions with a high degree of spatial heterogeneity could be
92 useful for understanding the differential response of plants to climatic gradients (*Moeller &*
93 *Merilä, 2004*). In turn, this knowledge would allow us to know the degree and direction of
94 evolutionary divergence between populations.
95 The genus *Ternstroemia* (Penthaphylacaceae, order Ericales) is a group of plants that is poorly
96 understood taxonomically, with the total number of species accepted in the genus varying from
97 90-110 species (*Stevens, 2001; Weitzman, Dressler, Stevens, 2004; Xiang, 2007*) to over 160
98 species (*Weitzman, 1995*). The genus is distributed from Sri Lanka to SE and E Asia and exists
99 in tropical and subtropical America and in Africa (two species) (*Weitzman, Dressler, Stevens,*
100 *2004*). In Mexico and Central America, *Ternstroemia* can be found in regions with a great

101 variety of climates and habitats, ranging from 50 m to more than 3000 m (*Luna-Vega, Alcántara-*
102 *Ayala & Contreras-Medina, 2004*). One of the most widely distributed and common species of
103 *Ternstroemia* in Mexico is *Ternstroemia lineata*. In particular, the taxonomic circumscription of
104 *T. lineata* has been difficult and thus is considered a species complex composed of several taxa:
105 *T. lineata* subsp. *chalicophila* (Loes.) B.M. Barthol., *T. dentisepala* B.M. Barthol., *T. lineata*
106 subsp. *lineata* and *T. impressa* Lundell. The *T. lineata* species complex is distributed exclusively
107 in Mesoamerica and is restricted to mountain ecosystems at elevations ranging from 1400 to
108 3140 m asl in habitats characterized by high precipitation (>1000 mm annually), such as cloud
109 forests and mixed humid forests (*Alcántara-Ayala, Luna-Vega, Velázquez, 2002; Luna-Vega,*
110 *Alcántara-Ayala, Contreras-Medina, 2004*). In Mexico and Guatemala, these types of forests
111 occur within a relatively narrow altitudinal zone under humid, temperate climates with
112 continuously foggy conditions. However, different forest patches show local climatic variations
113 that generate heterogeneity in floristic composition. Accordingly, individuals in the *T. lineata*
114 species complex show great variation in leaf shape and size among populations (*Kobuski, 1942;*
115 *Bartholomew & McVaugh, 1997; González-Villarreal, 2001*).
116 In this study, we used the *Ternstroemia lineata* complex as a model to test how climates
117 influence foliar morphology in forest species under environments with high humidity but great
118 geographic heterogeneity. We analyzed the leaf morphological variation of the *Ternstroemia*
119 *lineata* species complex and its relation to climate variables across its geographic distribution to
120 detect the environmental variables that have influenced the leaf morphological differentiation in
121 this species complex. We also tested the degree of leaf morphological differentiation among the
122 putative species and subspecies complex of *T. lineata*.
123

Materials & Methods

125 *Taxon sampling and morphological trait analysis*
126 We collected leaves of 270 individuals (8-10 mature leaves per individual) from 32 populations
127 located throughout the distribution range of the species (Fig. 1). A field permit of scientific
128 collection (Official number SGPA / DGVS / 12770/16) was issued by the Secretaria de Medio
129 Ambiente y Recursos Naturales, of Mexico. Leaf samples were pressed and dried for further
130 measurements in the lab and for herbarium specimens. For each specimen, we measured nine
131 foliar traits: 1. total length including lamina and petiole (TL); 2. lamina length (LL); 3. maximum
132 width of the lamina (MW); 4. petiole length (PL); 5. distance from the base to the maximum
133 width of the lamina (DW); 6. petiole diameter (PD); 7. angle of the lamina apex (ALA); 8. ratio
134 between MW and LL (WLR); and 9. ratio between DW and LL (DWLR) (Fig. 2A). All variables
135 (except ALA) were measured using a Mitutoyo® Vernier caliper (0.05 mm resolution) and are
136 recorded in mm. For ALA, we used a Jeppesen PJ-1 Rotating Azimuth Plotter.
137

138 *Variation of morphological traits*
139 We performed a principal component analysis (PCA) of the nine variables using the software
140 Statistica (*Statsoft, 2009*) to group the variables according to their variability and to select those

141 with the higher values per component to account for most of the morphological variation among
142 individuals (Table 1). With the selected morphological traits (TL, WLR and DWLR),
143 interpolated surface maps were generated in a geographic information system (*GIS, ArcMap 10.1*
144 *ESRI, 2011*) using the Geostatistical Analyst extension (*Johnston et al., 2001*). The variables
145 DWLR and WLR are descriptors of leaf shape. DWLR refers to the degree to which a leaf is
146 spatulate or elliptical, and WLR is indirect evidence of the foliar area (*Nautival et al., 1990*;
147 *Cittadini & Peri, 2006; Singh, 2007*), where high values signify small foliar areas and low values
148 signify large areas.
149 Finally, the interpolated maps were clipped with a map of the selected hydrographic basins of
150 Mexico (*INEGI, INE, CONAGUA, 2007*) based on the presence of *T. lineata* complex records
151 and the altitudinal range obtained from the herbarium specimens and field collections to
152 eliminate the interpolation areas outside the known distribution of the *T. lineata* complex.
153 We used an empirical Bayesian kriging method, which is based on a semivariogram estimated
154 from the spatial structure of the data (*Oliver & Webster, 1990; Kidd & Ritchie, 2006; Brito et al.,*
155 *2008*). This kriging method is a geostatistical tool that generates an estimated surface from a set
156 of dispersed points with Z values. The method is based on statistical models that consider the
157 spatial autocorrelation among data points and provide a measure of precision of the predicted
158 values. We considered the mean error, the square root of the quadratic mean error, the mean
159 standard error, and the square root of the quadratic mean error to evaluate the efficiency of the
160 interpolation (*Johnston et al., 2001*).
161
162 *Geographical areas of morphological homogeneity*
163 The three interpolated maps were used to detect discrete areas delimited by zones of rapid
164 change in the values of morphological traits because the variation inside the area is lower than
165 that among the areas. For this method, we used a boundary delineation method with the
166 BoundarySeer software (settings used: crisp boundary type, top 20% in boundary in threshold(s),
167 thresholds 90 deg. vector-to-vector and 30 deg. vector-to-line gradient angle). Different
168 boundaries are zones of rapid change, and for detection, wombling methods are used, which
169 estimate the average amount of change in the variable across the space. The locations that have
170 high values of change are referred to as boundaries (*BioMedware, 2013*).
171 Each map generated by the boundary analysis was vectorized, and all were intersected in a single
172 map using ArcGIS (*ESRI, 2011*). The resulting polygons represented areas of geographical
173 coincidence of the distribution of the three morphological traits: those polygons where the
174 presence of *T. lineata* complex was not corroborated were eliminated based on the records of
175 biodiversity information systems (REMIB, GBIF, specimens of herbaria). To reduce the number
176 of polygons and group them into homogeneous areas, a cluster analysis was carried out based on
177 the mean values of each of the morphological traits present in each polygon. This analysis was
178 performed using NTSYSpc ver 2.11 (*Rohlf, 2000*) with a taxonomic distance coefficient and the
179 UPGMA algorithm, and group formation was taken at a value of 0.88. Finally, the resulting
180 groups were evaluated using discriminant analysis to identify their differences (Table 2). Finally,

181   the distributions of the values of each of the three variables within each group were represented
182   using boxplots. Discriminant analysis and boxplot were carried out using SPSS Statistics ver. 19
183   software (*IBM Corp., 2010*).
184

185   *Relationship of morphological traits with climate and geography*
186   To evaluate the relationship of the morphological traits with the climate across the range of the
187   distribution of this species complex, we used the average values of each polygon, considering 19
188   bioclimatic variables (*Hijmans et al., 2005*) (Table 1) and three geographical variables. Values
189   were obtained through a zonal analysis in the GIS. We used the redundancy analysis (RDA)
190   implemented in the package 'vegan' (*Oksanen et al., 2018*) in R 3.5.1 software (*R Core Team,*
191   *2018*) to determine the combination of environmental and geographical variables that better
192   explain the morphological variation. This analysis was carried out as follows: first, all the
193   bioclimatic variables (BIO01 to BIO19) were included, and an iterative analysis was carried out
194   so that the variables showing high collinearity were eliminated according to their values of
195   variance inflation factor (VIF). Then, to evaluate the effects of climate and geography on leaf
196   trait variation, we performed RDA analysis in three phases: a) an analysis that includes both
197   climatic and geographical variables (RDAfull); b) a partial RDA analysis including only
198   bioclimatic variables without collinearity (pRDA1); and finally, c) a partial RDA analysis
199   including only geographical variables (pRDA2).
200   We also conducted linear correlation analyses between the three morphological traits selected
201   after the PCA and the bioclimatic variables without collinearity after an iterative analysis. We
202   included geographic variables such as latitude, longitude and altitude in these linear correlation
203   analyses to detect patterns of leaf morphological variation through latitudinal, longitudinal or
204   altitudinal gradients.
205

Results

207   *Leaf morphological variation and interpolation maps*
208   The principal component analysis of the morphological traits showed that the first three
209   components explained 79.71% of the accumulated variance (41.83%, 24.32% and 13.56%). For
210   the first component, the higher loading values were associated with leaf length (TL and LL) and
211   width (DW and MW), and the second and third components were associated with leaf shape
212   (WLR and DWLR, respectively) (Table 2). The relation among the components showed two sets
213   of variables with maximum correlation associated with the leaf length and width (LL, TL and
214   DW) and the lamina angle and leaf shape (ALA and WLR) (Figs. 2B and 2C).
215   For the three variables identified as important for each component (TL, WLR and DWLR), we
216   obtained the interpolated surface maps (Figs. 3A-3C). We identified a coarse-grain geographical
217   mosaic distribution in the variation of these foliar traits. The longest leaves occurred in southern
218   Mexico (Sierra Madre del Sur), and the plants with the shortest leaves were mainly located in the
219   southernmost region of the distribution (Sierra Norte y Los Altos de Chiapas and Sierra Madre
220   de Chiapas and Cuchumatanes) and a small area in the western part of the Eje Neovolcánico

221 (Fig. 3A). Plants with spatulate leaves (higher values of DWLR) were mainly located in the
222 western part of the Eje Neovolcánico and in the southernmost part of the Sierra Madre de
223 Chiapas and Cuchumatanes. Plants with elliptical leaves (lower values of DWLR) were located
224 mainly in the Sierra Madre del Sur and Sierra del Norte y Altos de Chiapas (Fig. 3B). The
225 regions with plants with the largest foliar area (low values of WLR) were mainly located in the
226 Sierra Madre del Sur, Eje Neovolcánico and Sierra Madre Occidental. The highest WLR values
227 (smaller foliar area) are located in Sierra Madre de Chiapas and Cuchumatanes (Fig. 3C).
228
229 *Geographical areas of morphological homogeneity and the morphological delimitation of the T.*
230 *lineata species complex*
231 The boundary analyses and the vectorization of the boundary maps produced 228 polygons for
232 the TL map, 395 polygons for the DWLR, and 270 for the WLR (Figs. 3D-3F). The intersection
233 of these three maps and the subsequent elimination of polygons, using only the records of
234 presence of the *T. lineata* complex, produced a single map with 108 polygons (Fig. 4A). The
235 cluster analysis with the average values of the three morphological traits in these 108 polygons
236 generated nine clusters (morphological groups) (Fig. 4B). The discriminant analysis showed
237 significant differences among these nine clusters (Table 3) with the eigenvalues of the first two
238 functions discriminated by 92.7% with small Wilks' lambda values, which confirms the high
239 variability among these groups. Finally, the percentage error among the groups was minimal
240 (0.9%) (Fig. 5A). Figure 5B shows the differences of the three morphological traits among the
241 nine morphological groups.
242 The nine morphological groups have some correspondence with the species and subspecies of
243 the *T. lineata* species complex. *T. lineata* subsp. *lineata* were formed by the populations of group
244 1 but also by populations of groups 3, 7 and 9 and some populations of group 4 (in allopatry) and
245 other populations of groups 4 and 5 (in parapatry). Populations of group 2 belong to *T.*
246 *dentisepala*, which is distributed in Nayarit state (northwest Mexico), populations of group 6
247 belong to *T. lineata* subsp. *chalicophila* (Chiapas state in southern Mexico) and populations of
248 group 8 belong to *T. impressa*, which is distributed in Guatemala (Fig. 5C). However, according
249 to the phenogram of the relationships of the nine morphological groups based on morphological
250 similarity, the putative species and subspecies did not show clear patterns of differentiation. For
251 example, the morphological similarity within morphological group 2 (*T. dentisepala*) was higher
252 and was also higher within the morphological groups of *T. lineata* subsp. *lineata* (groups 1, 3 and
253 4). Group 5 was an interesting case because the populations of this group were distributed
254 parapatrically and differentiated from the main cluster of *T. lineata* subsp. *lineata*. Populations of
255 the morphological groups 6, 7, 8 and 9 were geographically isolated and differentiated in
256 different clusters, and plants of group 8 that were distributed in Guatemala were described as *T.*
257 *impressa* (Fig. 5C).
258
259 *Relationship of geography and climate to morphological traits*

260   We selected six bioclimatic variables (BIO01, BIO04, BIO13, BIO14, BIO15 and BIO19) based
261   on an iterative analysis. The results of the full redundancy analysis (F = 10.037, p < 0.001, N =
262   999 permutations), including geographic and bioclimatic variables, explained 47.79% of leaf
263   variation (p < 0.001) with two significant axes RD1 and RD2; the first axis explained 83.72% of
264   the variance, and the second axis explained 13.61%. The most significant variables for the first
265   axis were precipitation of the driest month (BIO14, p < 0.001), precipitation seasonality (BIO15,
266   p < 0.043), precipitation of the coldest quarter (BIO19, p < 0.007) and longitude (p < 0.001); for
267   the second axis, the most significant variables were latitude (p < 0.001), precipitation of the
268   wettest month (BIO13, p < 0.024), temperature seasonality (BIO04, p < 0.014) and annual mean
269   temperature (BIO01, p < 0.001). The most important morphological variables were total leaf
270   length (TL) and width-length ratio (WLR). The first axis of the full RDA separated those
271   populations mainly located in the south in Sierra del Norte y Los Altos de Chiapas and Sierra
272   Madre de Chiapas and the populations of the Sierra Madre del Sur (in the state of Oaxaca) and
273   the most western area of the Eje Neovólcanico (state of Nayarit) (Fig. 6).
274   The analysis of leaf trait variation indicated that climate had a higher impact than geography.
275   The percentage of the variance explained only by climate was 31.08%, the percentage explained
276   by geography was 13.38% and the join effect, geography – climate, explained 2.38%.
277   The partial redundancy analysis using climate as control of geography (pRDA1) was significant
278   only in its first axis (p < 0.001), explaining 87.26% of the variation, with precipitation of the
279   coldest quarter (BIO19, p < 0.009) as the most important variable. The partial redundancy
280   analysis using geography as a control climate (pRDA2) was only significant in its first axis (p <
281   0.001), which explains 99.45% of the variation, with latitude being the variable of greater
282   importance (p < 0.001) (Fig. 6).
283
284   *Relationship between morphological traits and environmental and geographic variables*
285   After the principal component analysis defined TL, WRL and DWLR as the morphological traits
286   that explain most of the variance in the *T. lineata* species complex and the iterative analysis
287   selected the climatic variables without collinearity, we conducted linear correlations between
288   both groups of variables. Total leaf length (TL) was positively correlated with annual mean
289   temperature (BIO01) (r = 0.30; p < 0.01) and precipitation seasonality (BIO15) (r = 0.48; p <
290   0.01) and negatively correlated with the precipitation of the driest month (BIO14) (r = -0.53; p <
291   0.01), precipitation of the wettest month (BIO13) (r = -0.13; p < 0.01) and precipitation of the
292   coldest quarter (BIO19) (r = 0.45; p < 0.01) (Fig. 7).
293   The ratio between the maximum width of the lamina (MW) and leaf length (LL) (WRL) as a
294   descriptor of leaf shape was positively correlated with the precipitation of the wettest month
295   (BIO13) (r = 0.39; p < 0.01) and precipitation of the driest month (BIO14) (r = 0.31; p < 0.01)
296   and was negatively correlated with temperature seasonality (BIO04) (r = -0.27; p < 0.01) and
297   precipitation seasonality (BIO15) (r = -0.37; p < 0.01) (Fig. 8).
298   The ratio between the distance from the base to the maximum width of the lamina (DW) and leaf
299   length (LL) (DWLR) was positively correlated with temperature seasonality (BIO04) (r = 0.40; p

300   < 0.01) and precipitation seasonality (BIO15) (r = 0.25; p < 0.05) and negatively correlated with
301   the precipitation of the wettest month (BIO13) (r = -0.21; p < 0.05), precipitation of the driest
302   month (BIO14) (r = 0.32; p < 0.01) and precipitation of the driest quarter (r = -0.41; p < 0.01)
303   (Fig. 9).
304   Additionally, TL and DWLR were positively correlated with latitude (r = 0.20; p < 0.05; r =
305   0.22; p < 0.05, respectively) and negatively correlated with longitude (r = -0.30; p < 0.05; r = -
306   0.24; p < 0.05, respectively), but WLR was negatively correlated with latitude (r = -0.53; P <
307   0.01) and positively correlated with longitude (r = 0.49; p < 0.01). The three morphological traits
308   were not correlated with altitude (TL: r = -0.12; p > 0.05; WLR: r = -0.08; p > 0.05; DWLR: r = -
309   0.14; p > 0.05) (Fig. 10).
310

311   **Discussion**
312   Here, we analyzed leaf morphology in the *T. lineata* species complex, a group of plants
313   characteristic of the humid forests of Mesoamerica. To detect the role of environmental factors in
314   morphological population differentiation, we studied the general geographic trends in leaf
315   morphology and their relationships with climatic variables such as temperature and precipitation.
316   The populations of the *T. lineata* species complex show great levels of variation in leaf
317   morphology throughout their distribution range. Accordingly, our analyses allowed us to define
318   nine morphologically homogenous groups within the complex and to define their geographic
319   distributions. In general, the pattern we uncovered is a patchy mosaic geographic distribution
320   with some groups of populations having a continuous distribution along the central and southern
321   mountains of Mexico (Faja Volcánica Transmexicana, Sierra Madre del Sur). However, we also
322   detected some morphological groups that are distributed parapatrically and others that are
323   isolated at the northern and southern edges of the distribution range of the species complex.
324   Although some groups are consistent with the present-day accepted taxonomic status (*Kobuski,*
325   *1942; Bartholomew & McVaugh, 1997*), such as the populations identified as *T. dentisepala* in
326   the north and *T. impressa* in the south, other groups do not have a clear association with the
327   currently accepted taxonomic circumscription.
328   Since the 19th century, biogeographers have found that wet tropics harbor plants with larger
329   leaves than those observed in temperate regions, suggesting that small leaves are more frequently
330   found at high latitudes and elevations. Recently, some authors (e.g*., Jones, 2014; Michaletz, et*
331   *al. 2016; Wright, et al. 2017*) have attempted to explain the global climatic drivers of leaf size
332   and shape. In general, morphological variation in plants follows changes in environmental
333   variables along latitudinal, longitudinal and altitudinal gradients at different geographic scales
334   (*Rico-Gray & Palacios-Ríos, 1996; Niinemets 2001; Chalcoff, Ezcurra & Aizen, 2008; Uribe-*
335   *Salas et al., 2008; Frenne et al., 2013; Moles et al., 2014*). In particular, complex leaf
336   morphology in the *T. lineata* species showed clear patterns of latitudinal and longitudinal
337   variation, but no patterns in morphology were detected across altitude. However, we uncovered
338   strong correlations between leaf morphology and environmental variables. More specifically,
339   leaf area showed a close association with environmental variables, where leaves with less surface

340   area were found in regions with higher precipitation throughout the year (*i.e.*, high precipitation
341   during the wettest and driest months), whereas leaves with greater surface area were found in
342   regions with pronounced temperature and precipitation seasonality. Interestingly, this pattern
343   does not follow the general trend observed in plants at a global scale, in which smaller leaves
344   tend to occur in drier sites (*Wright et al., 2017*).
345   Variation in leaf shape along environmental gradients has been tied to the response of plant
346   populations to environmental factors (*Givinsh, 1987; Malhado et al. 2009a; Malhado et al.*
347   *2009b; Werger & Ellenbroek, 1978*). Accordingly, longer leaves in *T. lineata* were found in
348   regions with higher temperatures and with strong precipitation seasonality, whereas the shortest
349   leaves were found in regions with higher levels of rainfall. In general, longer, narrower and more
350   spatulate leaves were located in regions with lower precipitation and higher precipitation
351   seasonality, following a northwest – southeast direction. However, populations of the *T. lineata*
352   species complex also show differentiation in leaf shape (*i.e.,* narrow spatulate vs elliptical and
353   obovate leaves) within similar mixed cloud forest habitats but still followed significant
354   latitudinal and longitudinal gradients.
355   The relationship we observed between the length and the surface area of leaves with precipitation
356   seasonality is difficult to explain. Traditionally, a reduction in leaf size has been reported for
357   many species as aridity increases, which represents an adaptive advantage because small leaves
358   have low evapotranspiration (*Chalcoff, Ezcurra & Aizen, 2008*). In contrast, our results show that
359   individuals of the *T. lineata* complex with the longest leaves and with the largest leaf areas are
360   found in regions with the lowest extreme rainfall. We believe that the pattern we are observing is
361   due to the habitats in which these plants live. These habitats are characterized by high levels of
362   humidity in the form of fog, which is more persistent precisely in the driest and coldest periods
363   of the year. In this case, the constant presence of fog tends to form a thin layer of water on the
364   surface of the leaf, which reduces perspiration and therefore growth (*Leigh, 1975, Lightbody,*
365   *1985*). In addition, this water film reflects sunlight, causing a reduction in photosynthetic activity
366   and therefore affecting the growth rate of the leaves (*Lightbody, 1985*).
367   The adaptive value of having more spatulate or elliptical leaves with respect to varying
368   environments is also difficult to explain. Recently, *Kidner & Umbreen (2010)* argued that leaf
369   shape is extremely variable between and within species, with great levels of variation in leaf
370   shape among populations and individual plants. The shape of the leaf has been related to the
371   capacity to capture light within distinct habitats and to the regulation of water balance and
372   temperature. In this case, a detailed study of microclimatic variation among populations and
373   analyses of leaf vascular patterns are needed to determine the factors that drive the variation of
374   leaf shape in the *T. lineata* complex.
375   Variation in leaf traits is due to phenotypic plasticity in response to environmental gradients, and
376   the observed morphological variation is frequently strongly associated with climate (*Givnish,*
377   *1987; Westoby et al., 2002; Gratani 2014*). In this context, the recognition of geographical and
378   morphological groups along the distribution range of the *T. lineata* species complex helped us
379   identify regions where environmental variation mirrored the observed morphological variation

380   across populations. There is a clear mosaic-type geographical pattern of variation in some foliar
381   traits, but there is also evidence of correspondence between environmental gradients and leaf size
382   and shape. These results indicate that environmental factors play a relevant role in the observed
383   variation in foliar traits in the *T. lineata* species complex. However, this trait variation is
384   clustered within discrete groups of populations, which are well differentiated geographically.
385   Interestingly, the observed patterns suggest that several groups of populations are
386   morphologically differentiated as a result of geographic isolation. However, there is evidence of
387   significant differentiation in leaf morphology among geographically contiguous populations,
388   suggesting that factors other than climate might influence variation in leaf morphology in
389   *Ternstroemia.*
390

Conclusions

392   In this study, we analyzed the relationship between environmental variables and variation in leaf
393   morphology among populations throughout the distribution range of the *T. lineata* species
394   complex in Mexico and Guatemala. Our analysis showed that the effect of climate varies among
395   populations, which showed different morphological trends leading to varying patterns of
396   geographic differentiation. Our results indicate that converging leaf morphologies can be
397   observed among individuals from different populations, which appears to be a parallel response
398   to similar environmental factors rather than to geographical proximity. Although the climate-
399   morphology association may eventually lead to adaptive evolution in this species complex, the
400   observed patterns should be corroborated with analyses of genetic structure among populations.
401   The combination of multivariate statistical and geographic analyses of leaf morphology allowed
402   us to establish variability patterns that are a fundamental first step to understanding the process
403   of population differentiation in this group of closely related species. In turn, the integration of
404   genetic data with morphological variation in vegetative and reproductive structures can lead to a
405   better understanding of the reproductive barriers and the processes of species formation in these
406   species. Our research highlights the key role of the environment in molding morphological
407   variation among plant populations at the local and regional scales. Last, a formal taxonomic
408   treatment that includes morphological traits of the leaf, flowers and fruits, together with
409   molecular markers, is needed to evaluate the degree of differentiation among species and
410   subspecies of the *T. lineata* complex.
411

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421

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List of climatic variables using of the redundancy analysis.

Climatic variables used in the redundancy analysis. (** Climatic variables with VIF>10)

1   Table 1. Climatic variables used in the redundancy analysis. (** Climatic variables with VIF>10)

Abbreviations	Climatic variable names
BIO1**	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp)
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4**	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of the Warmest Month
BIO6	Min Temperature of the Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of the Wettest Quarter
BIO9	Mean Temperature of the Driest Quarter
BIO10	Mean Temperature of the Warmest Quarter
BIO11	Mean Temperature of the Coldest Quarter
BIO12	Annual Precipitation
BIO13**	Precipitation of the Wettest Month
BIO14**	Precipitation of the Driest Month
BIO15**	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of the Wettest Quarter
BIO17	Precipitation of the Driest Quarter
BIO18	Precipitation of the Warmest Quarter
BIO19**	Precipitation of the Coldest Quarter

Principal Component Analysis of nine morphological variables of the *T. lineata* species complex.

Principal Component Analysis of nine morphological variables of the *T. lineata* species complex. Variables: 1) total length of leaf (includes lamina and petiole, TL); 2) lamina length (LL); 3) maximum width of the lamina (MW); 4) petiole length (PL); 5) distance from the base to the maximum width of the lamina (DW); 6) petiole diameter (PD); 7) angle of the lamina apex (ALA); 8) ratio between MW and LL (WLR); and 9) ratio between DW and LL (DWLR).

1 Table 2. Principal Component Analysis of nine morphological variables of the *T. lineata* species
2 complex. Variables: 1) total length of leaf (includes lamina and petiole, TL); 2) lamina length
3 (LL); 3) maximum width of the lamina (MW); 4) petiole length (PL); 5) distance from the base
4 to the maximum width of the lamina (DW); 6) petiole diameter (PD); 7) angle of the lamina apex
5 (ALA); 8) ratio between MW and LL (WLR); and 9) ratio between DW and LL (DWLR).
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Contributions	C_1	C_2	C_3	C_4	C_5
PL	0.041737	0.000777	0.161636	0.726383	0.016466
PD	0.054816	0.186583	0.009767	0.068551	0.258501
MW	0.189874	0.083223	0.015169	0.012927	0.000201
TL	0.244426	0.029179	0.002038	0.001309	0.011538
LL	0.239342	0.032236	0.000070	0.018755	0.016813
DW	0.228145	0.028064	0.052958	0.000150	0.001161
WLR	0.000166	0.379410	0.026536	0.000723	0.038457
DWLR	0.000956	0.000000	0.663112	0.148503	0.081381
ALA	0.000538	0.260529	0.068714	0.022698	0.575481
% ACUMULATED VARIANCE	41.83	66.15	79.71	94.04	99.59

The discriminant analysis showing significant differences among the nine clusters

(A) Eigenvalues of the functions show that the first two functions discriminate by 92.7%. (B) Wilks Lambda values and Chi-square, p = 0.05.

1 Table 3. A) Eigenvalues of the functions show that the first two functions discriminate by 92.7%.
2 B) Wilks Lambda values and Chi-square, p = 0.05.

A) Eigenvalue

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	10.251[a]	60.3	60.3	.955
2	5.506[a]	32.4	92.7	.920
3	1.249[a]	7.3	100.0	.745

[a] We used the three first canonical discriminant functions in the analysis.

B) Wilks' Lambda

Test of functions	Wilks' Lambda	Chi-square	df	Sig
1 to 3	0.006	515.478	24	.000
2 to 3	0.068	271.010	14	.000
3	0.445	81.858	6	.000

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Figure 1

Location of the sampled populations throughout the *T. lineata* complex distribution range

Distribution of the *Ternstroemia lineata* species complex in the main mountain systems of Mexico (*sensu* INEGI, 2001) and Guatemala (*sensu* Marshall, 2007).
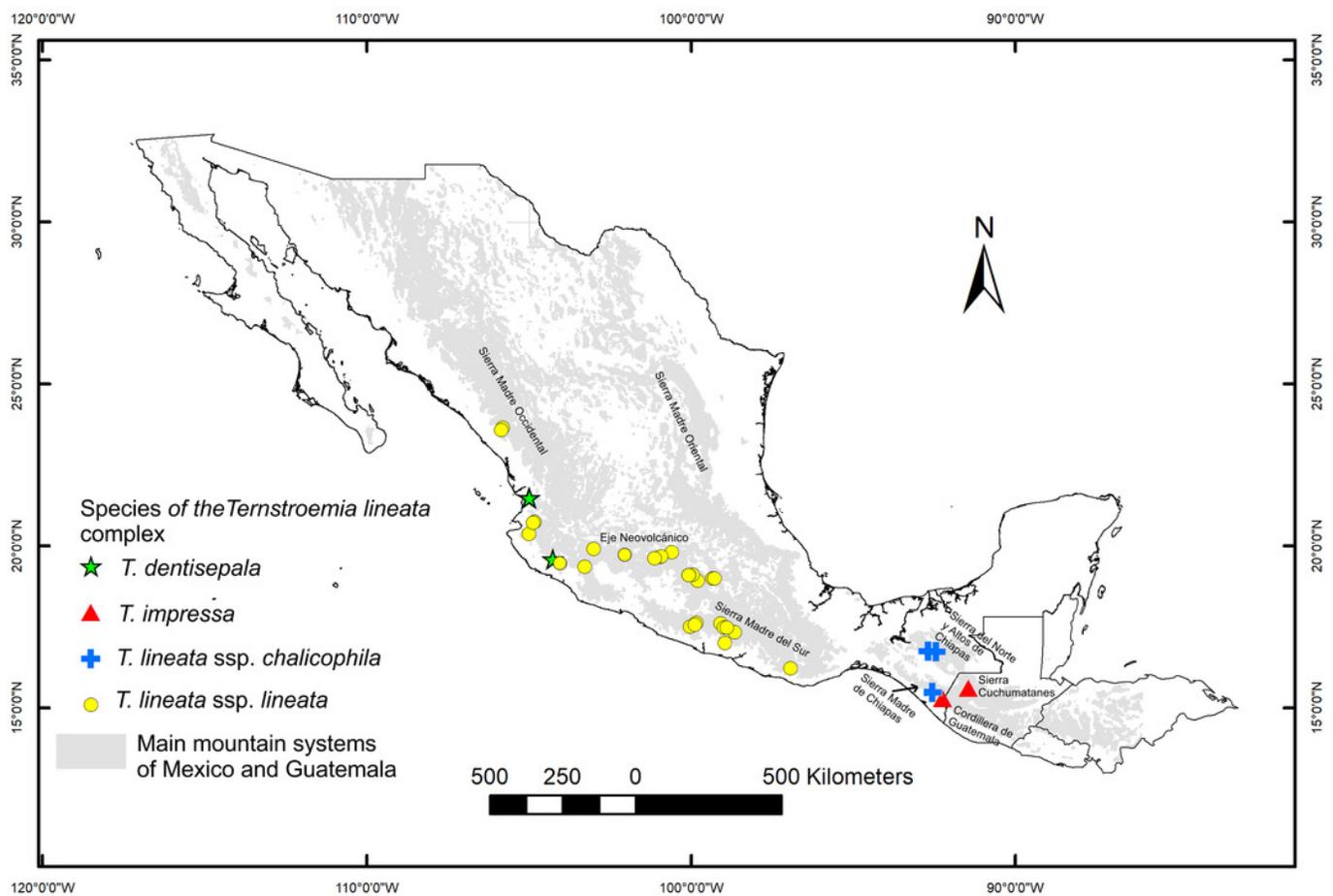
Figure 2

Nine foliar traits and Principal Component Analysis (PCA) of this variables

Foliar measurements and PCA analysis of the morphological variables. A) The nine foliar traits measured in each individual: 1) total leaf length (including lamina and petiole, TL); 2) lamina length (LL); 3) maximum width of the lamina (MW); 4) petiole length (PL); 5) distance from the base to the maximum width of the lamina (DW); 6) petiole diameter (PD); 7) angle of the lamina apex (ALA); 8) ratio between MW and LL (WLR); 9) ratio between DW and LL (DWLR). B-C) We plotted the PCA values of the morphological variables: first component vs. second component (B) and second component vs. third component (C) we show two sets of variables with maximum correlation: LL-TL-DW and ALA-WLR. The number (B and C) corresponds to species of *T. lineata* complex, 1. *T. lineta* ssp. *chalicopila*, 2. *T. dentisepala*, 3. *T. lineata* ssp. *lineata* and 4. *T. impressa*.
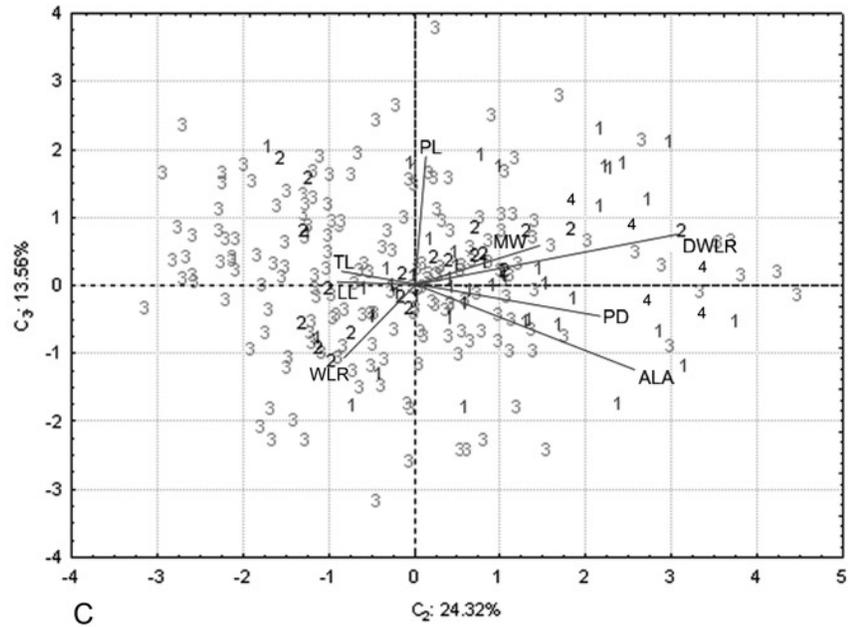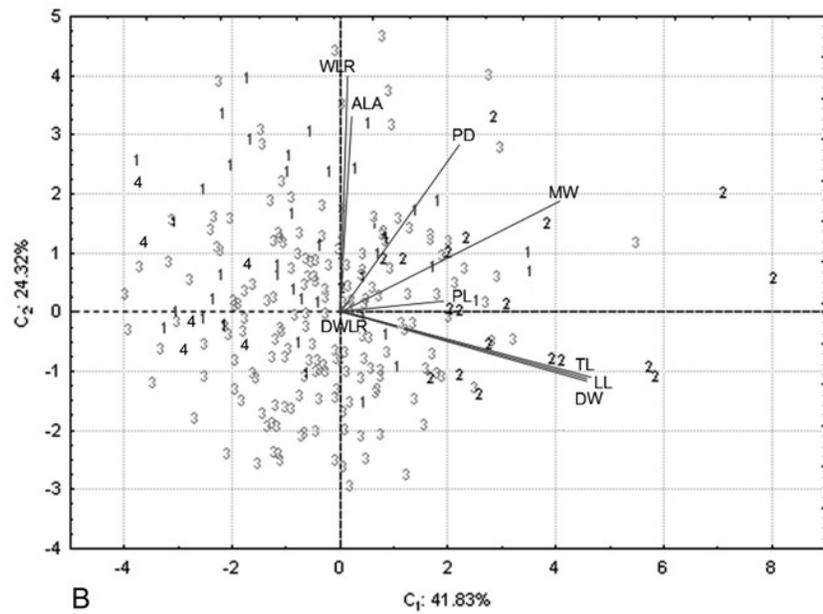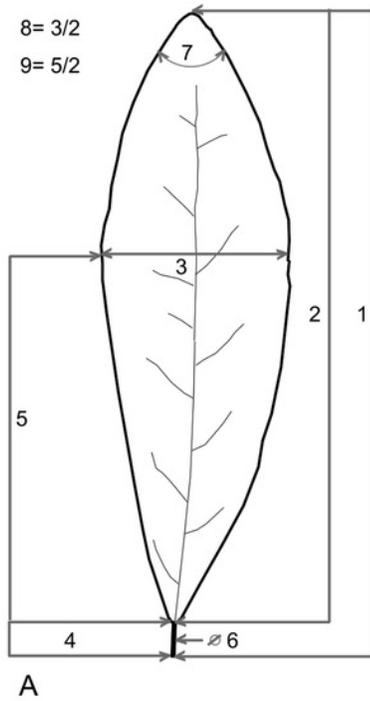
Figure 3

The interpolated surface maps

Interpolation maps for (A) total leaf length (TL), (B) ratio between the distance from the base to the maximum width of the lamina (DW) and leaf length (LL) (DWLR) and (C) ratio between maximum width of the lamina (MW) and leaf length (LL) (WLR). (D-E) Boundary detection analyses based on the interpolated surface maps of the three leaf traits (TL, DWLR, WLR, respectively).
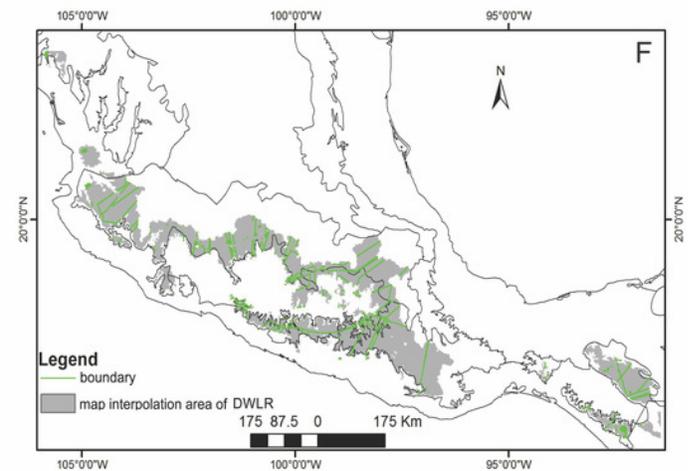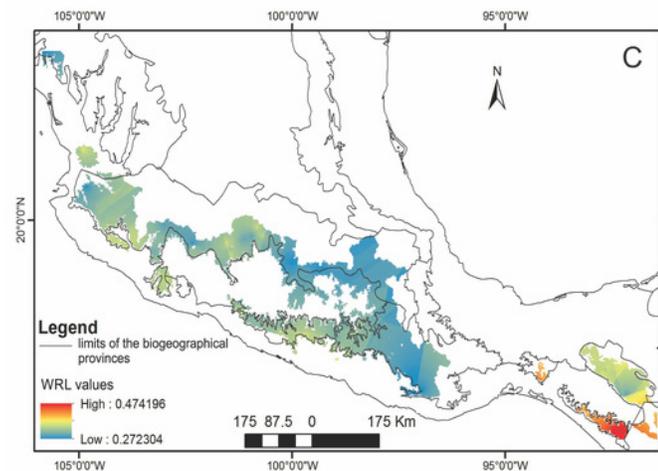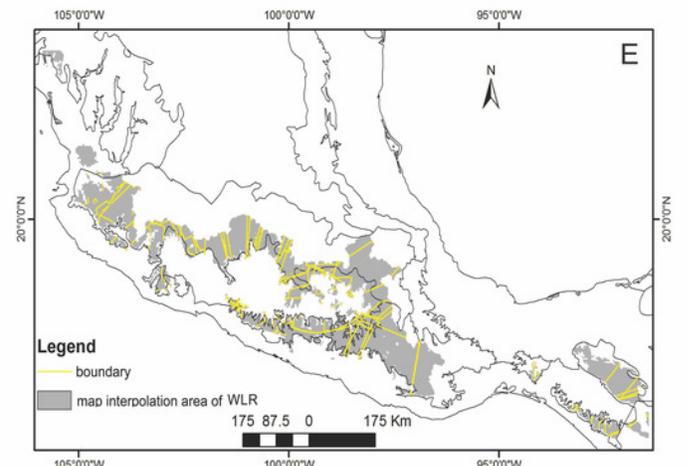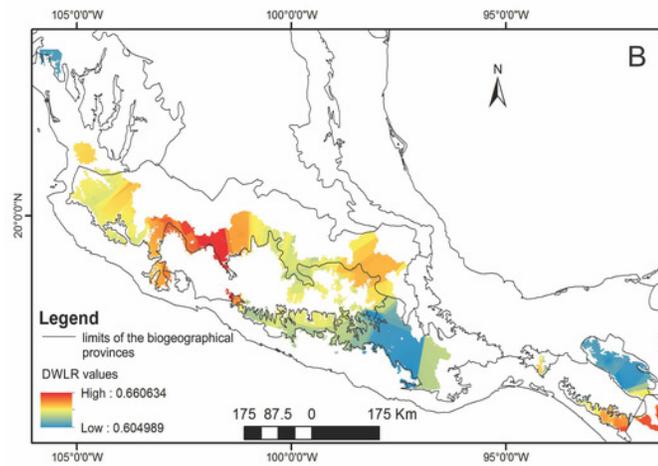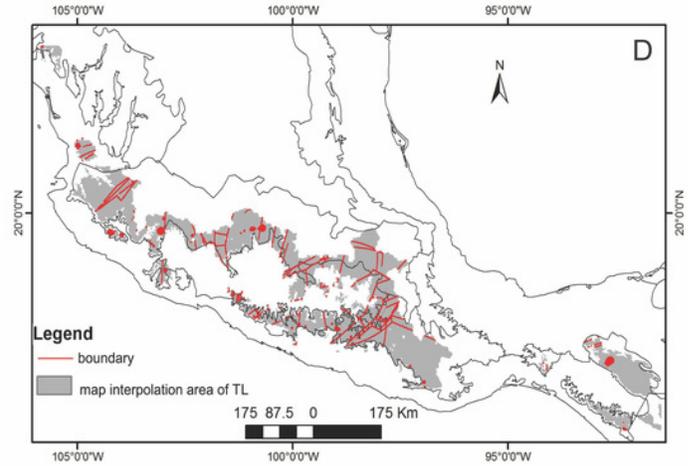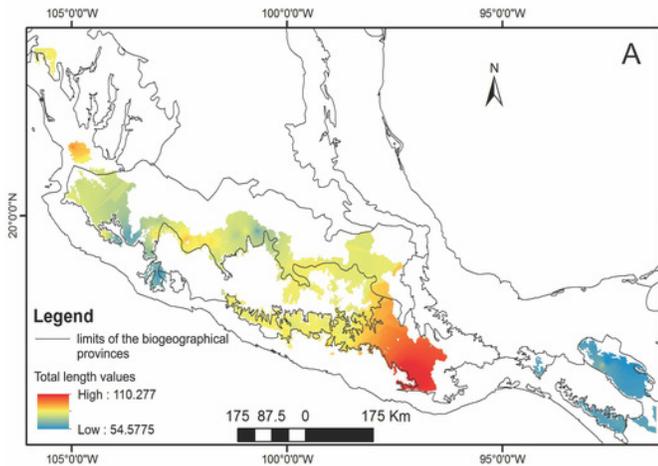
Figure 4

The intersection of three maps and phenogram resulted from a cluster analysis of the 108 polygons

(A) Map with polygons generated by the intersection of the three maps of morphological variables (see Figure 3D-F) after the boundary analysis and the vectorization of the boundary maps using only the records of presence of the *T. lineata* complex (green dots). (B) Phenogram resulted from a cluster analysis of the 108 polygons.
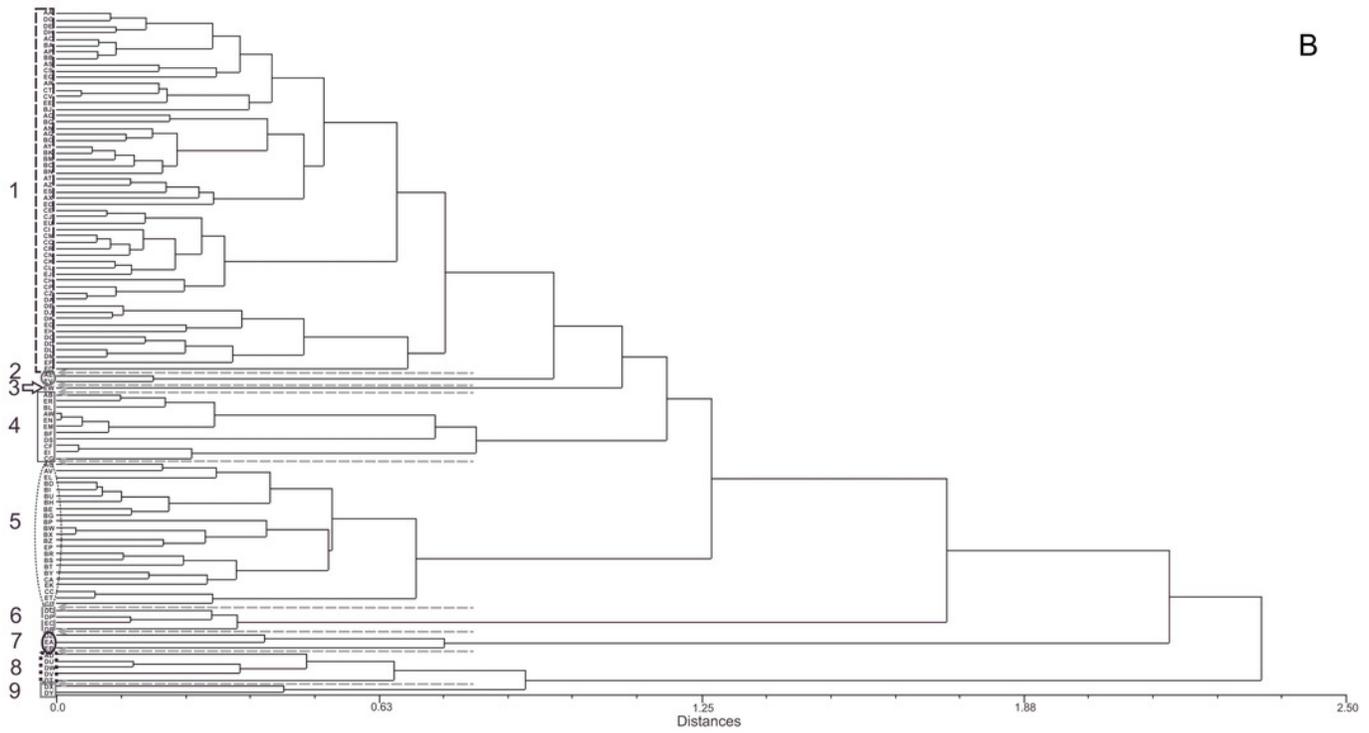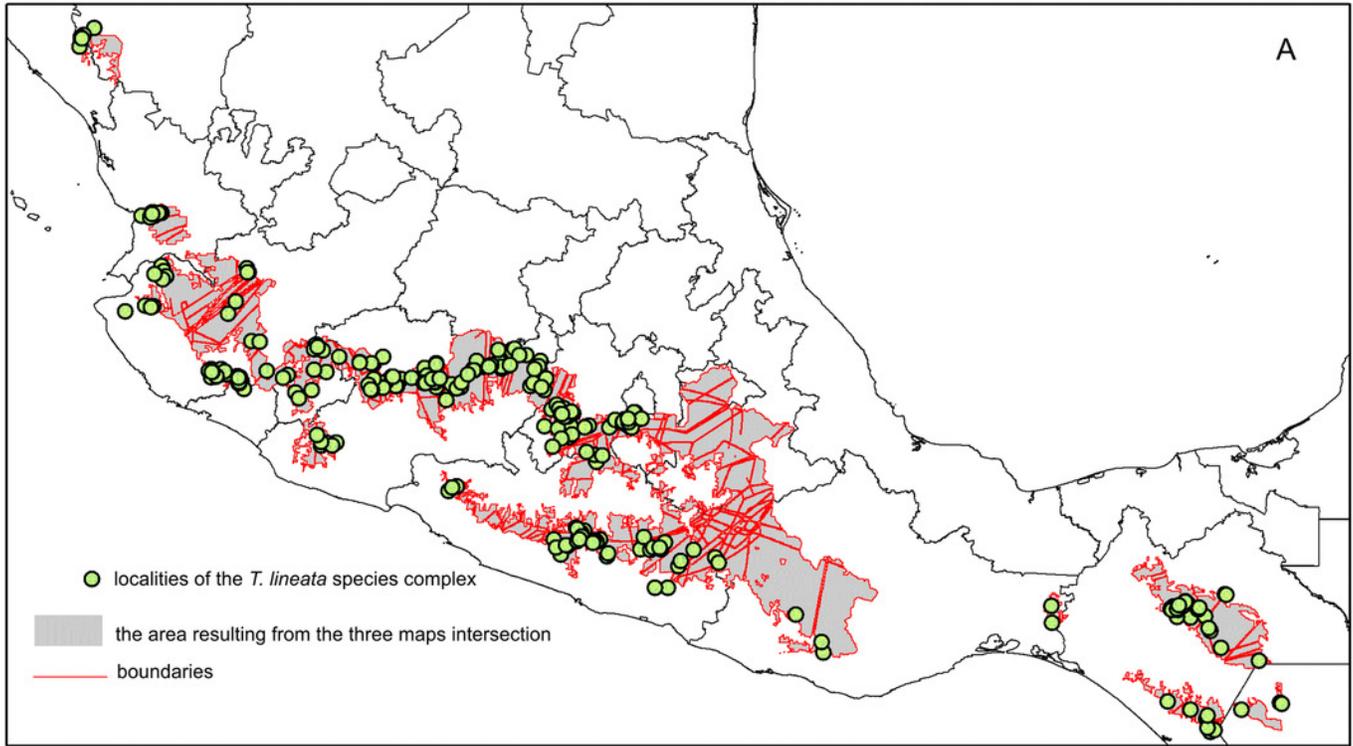
Figure 5

Biplot discriminant analysis, boxplot of the differences of the three morphological traits among the nine morphological groups and distribution map of this nine morphological groups.

(A) Canonical discriminant charts where the integrity of the nine groups was tested; (B) boxplot of the nine morphological groups; (C) Distribution map of the nine morphological groups (with a typical leaf sample).
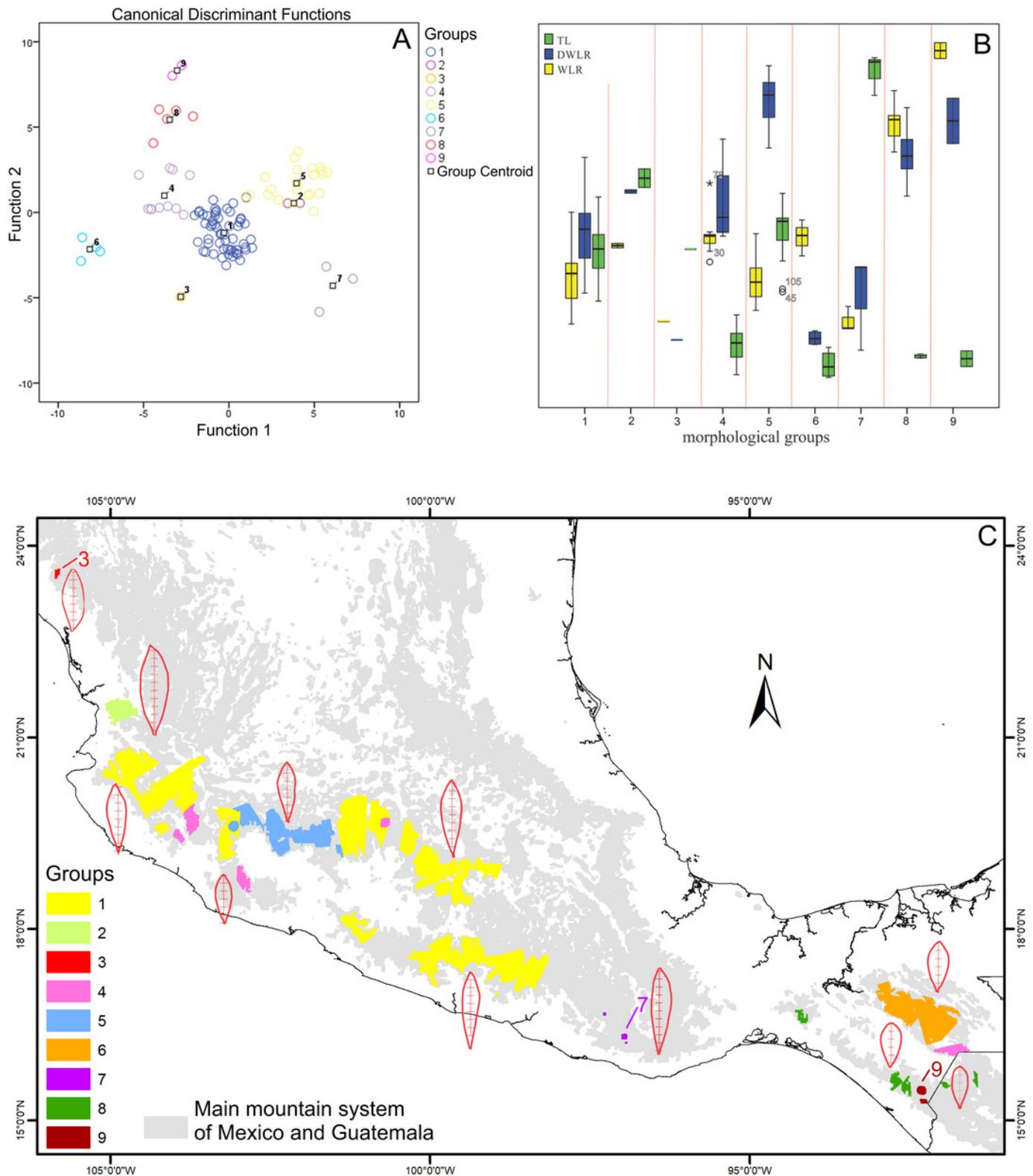
Figure 6

Graph of the redundancy analysis that includes both climatic and geographical variables (RDAfull).

Graph of full redundancy analysis (Full RDA) showing the relationship between response variables (morphological characters) and explanatory variables (bioclimatic and geographic variables).
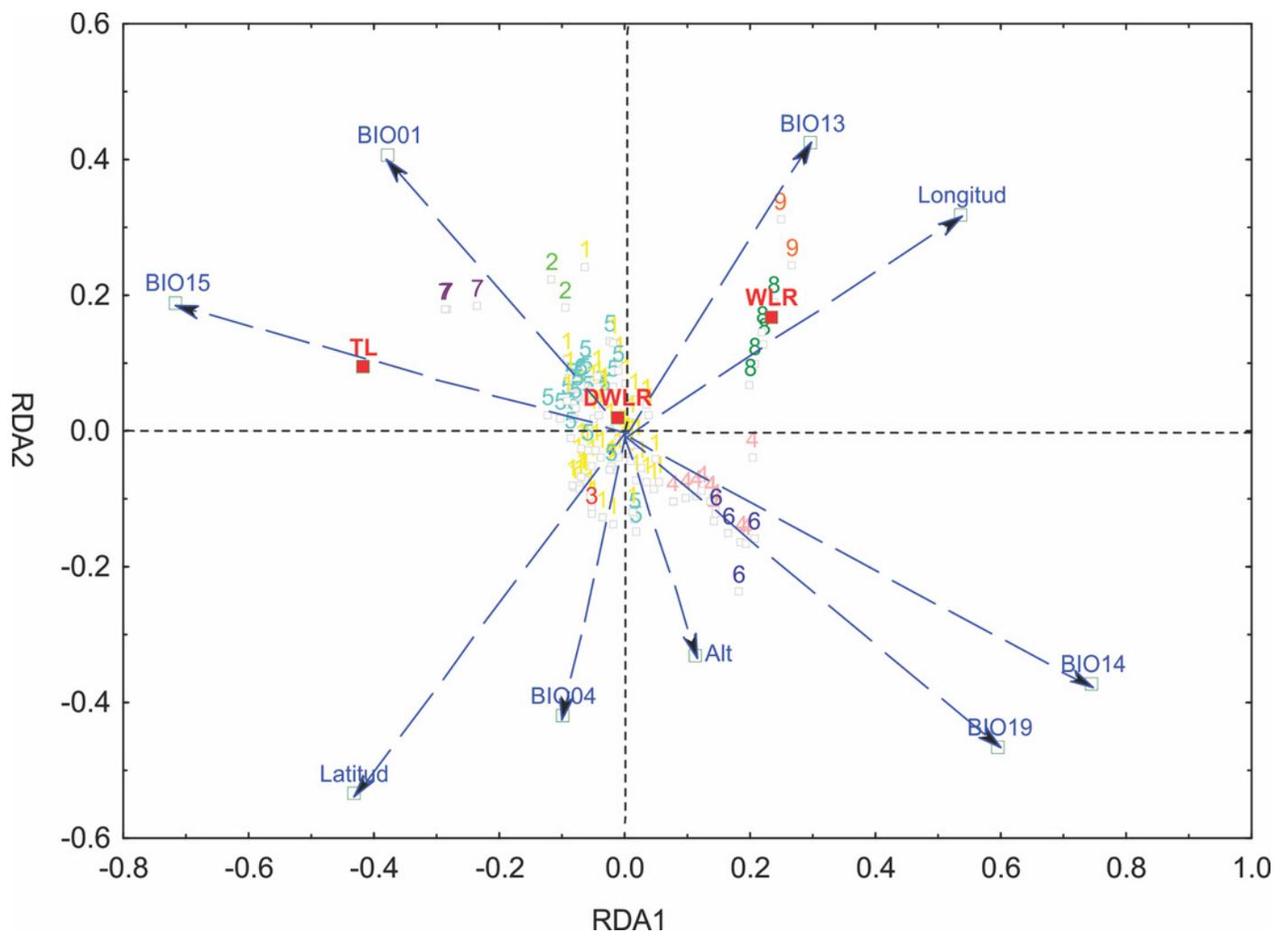
Figure 7

Correlations between total leaf length and bioclimatic variables

(A) Correlations between total leaf length (TL) and bioclimatic variables were positively correlated with annual mean temperature (BIO01) and (B) precipitation seasonality (BIO15) and (C) negatively correlated with precipitation of the driest month (BIO14), (D) precipitation of the wettest month (BIO13) and (E) precipitation of the coldest quarter (BIO19).
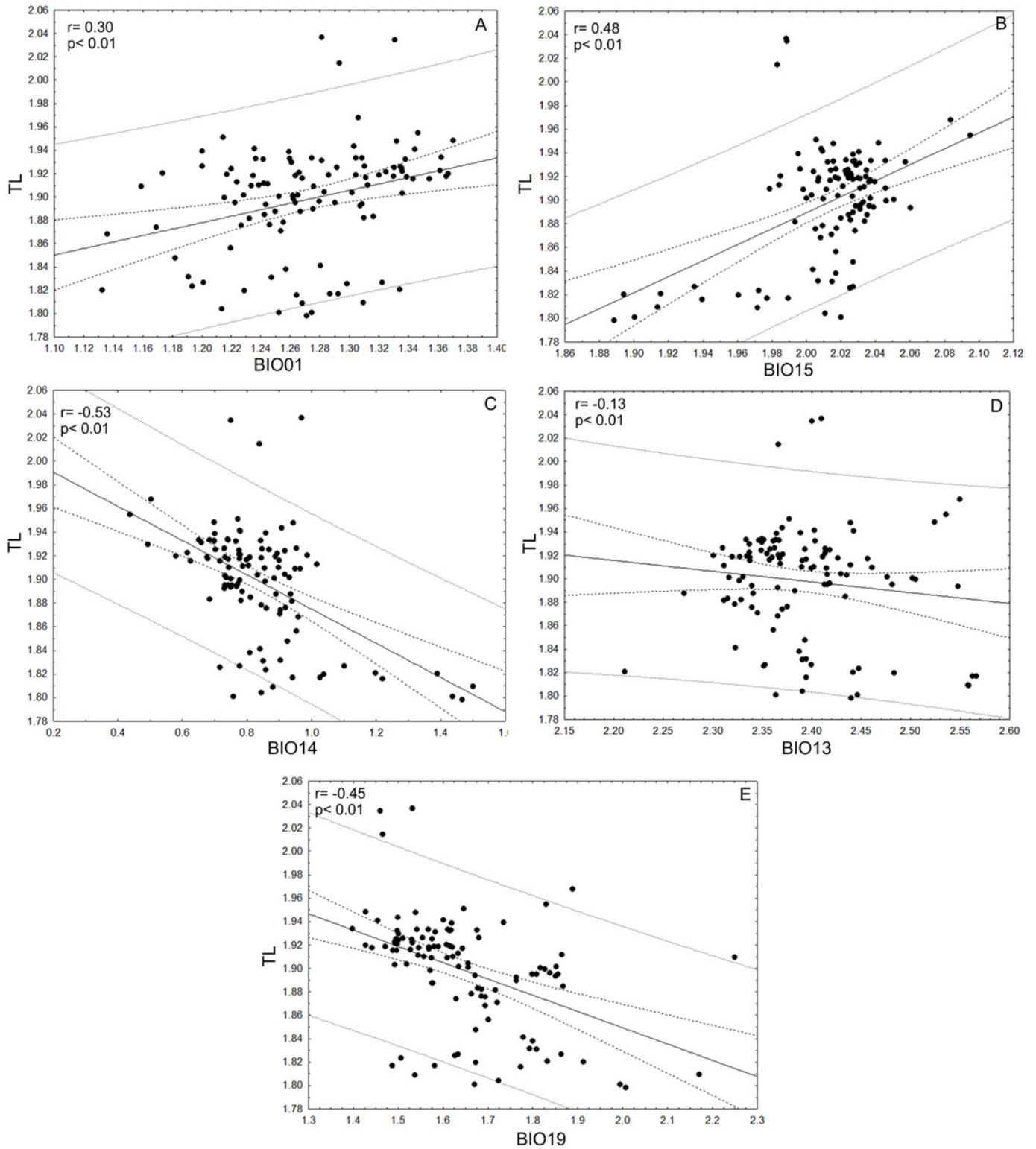
Figure 8

Correlations of the ratio between maximum width of the lamina/leaf length (WRL) and bioclimatic variables

The ratio between maximum width of the lamina (MW) and leaf length (LL) (WRL) as descriptor of leaf shape was (A) positively correlated with precipitation of the wettest month (BIO13) and (B) precipitation of the driest month (BIO14) and (C) negatively correlated with temperature seasonality (BIO04) and (D) precipitation seasonality (BIO15).
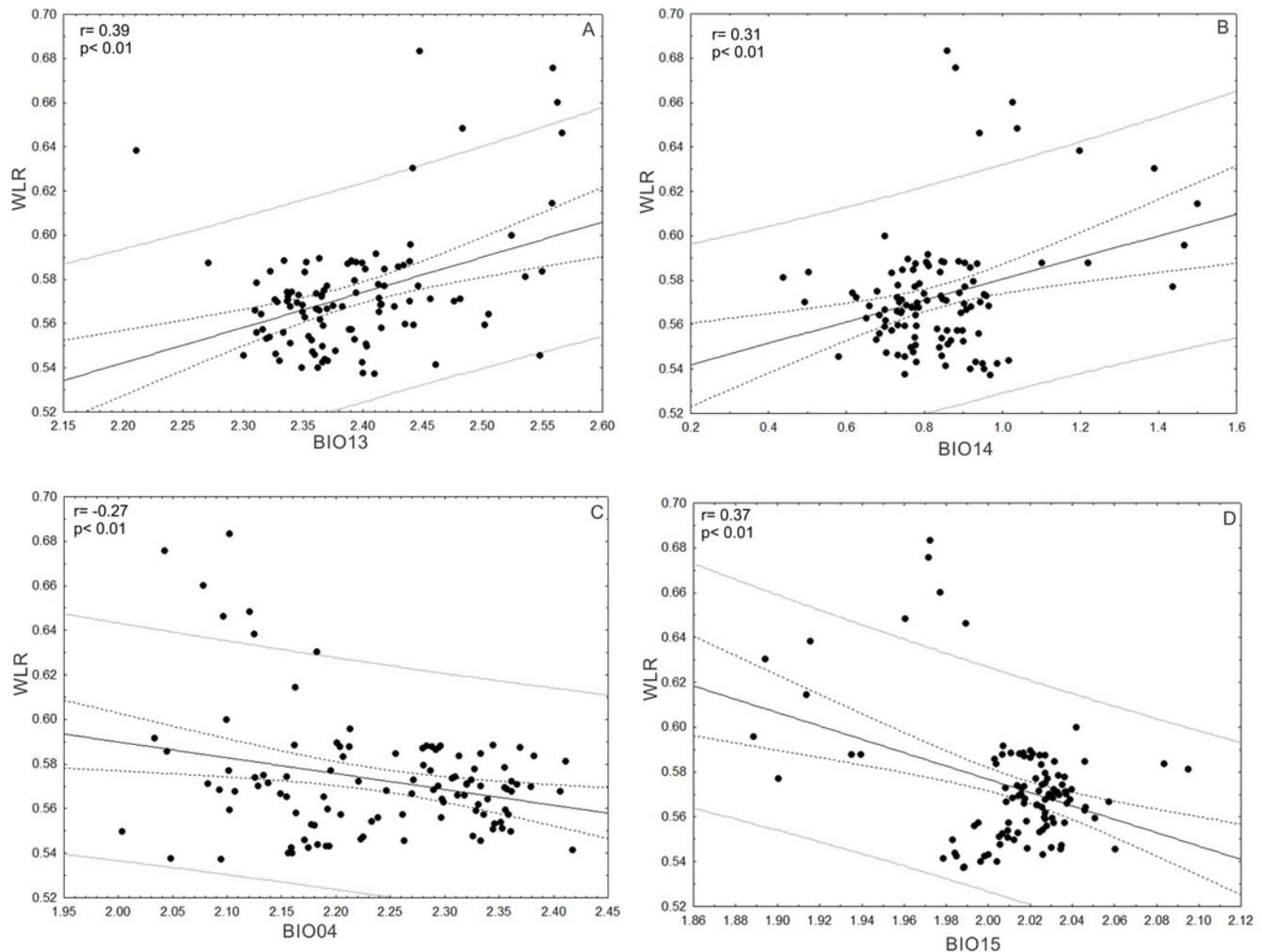
Figure 9

Correlations between the ratio between the distance from the base to the maximum width of the lamina/ leaf length (DWLR) and bioclimatic variables

The ratio between the distance from the base to the maximum width of the lamina (DW) and leaf length (LL) (DWLR) was (A) positively correlated with temperature seasonality (BIO04) and (B) precipitation seasonality (BIO15) and (C) negatively correlated with precipitation of the wettest month (BIO13), (D) precipitation of the driest month (BIO14) and (E) precipitation of the driest quarter.
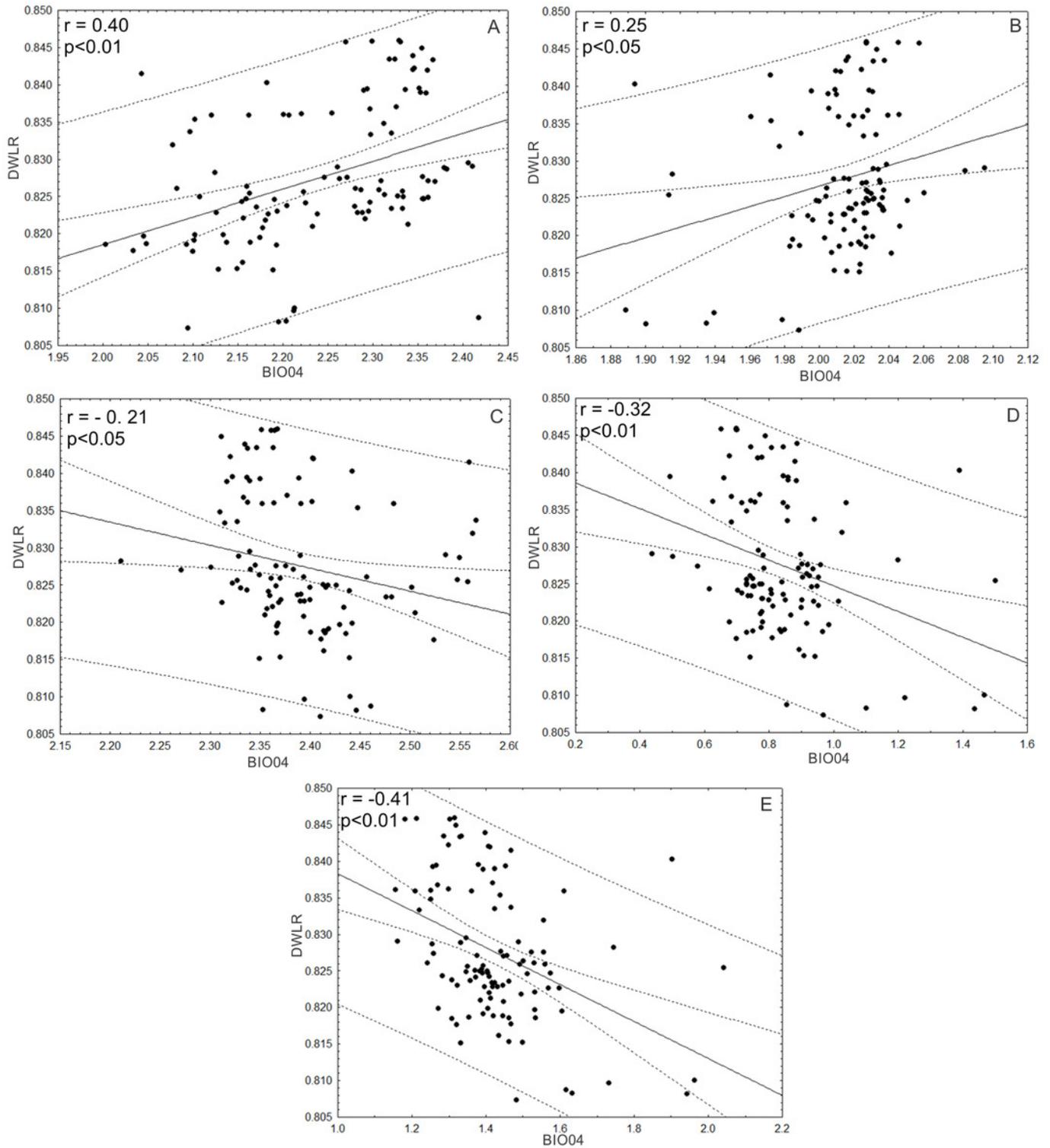
Figure 10

Correlations between morphological variables and latitude and/or longitude.

(A and B) TL and DWLR were positively correlated with latitude and (C and D) negatively correlated with longitude, (E) WLR was negatively correlated with latitude and (F) positively correlated with longitude.