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1 **Phenotypic plasticity rather than local adaptation explains the broad realized**
2 **niche of a Neotropical orchid species**

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18 **Running head:** Local adaptation in a Neotropical orchid

19 **Keywords:** Common garden, Isolation by environment, Niche overlap, Orchidaceae.

20 **One-sentence summary:** Integrating genetic, climatic and phenotypic data we show
21 that local adaptation between coastal and inland populations of a long-lived, Neotropical
22 orchid is unlikely and that phenotypic plasticity is a better explanation for the broadness
23 of this species' niche.

Abstract

- Local adaptation is common in plant species, and knowing whether a population is locally adapted has fundamental and applied relevance. However, local adaptation in tropical plants remains largely understudied, and covering this gap is not simple since reciprocal transplantation – the gold standard for detecting local adaptation – is unfeasible for most species. Here, we combined genetic, climatic and phenotypic data to investigate local adaptation in coastal and inland populations of the orchid *Epidendrum fulgens* Brongn., a long-lived tropical plant for which reciprocal transplantation would be unfeasible.
- We used nine microsatellite markers to estimate genetic divergence between inland and coastal populations. Moreover, occurrence data and climatic data were used to test for differences in the realized niche of those populations. Finally, we assessed saturated water content, leaf specific area, height and stomatal density in common garden and *in situ* to investigate the effects of local adaptation and plasticity on the phenotype.
- Northern and southern groups' niches do not overlap, the former occupying a wetter and warmer area. However, this differentiation does not seem to be driven by local adaptation since there was no positive correlation between genetic structure and climatic dissimilarity. Moreover, specific leaf area and leaf saturated water content, which are important phenotypic traits related to soil fertility and drought stress, were rather plastic.
- We conclude that local adaptation between northern and southern groups of *E. fulgens* is unlikely and that phenotypic plasticity is an important mechanism explaining the niche broadness of this species.

49 **Introduction**

50 When natural selection overcomes migration and drift, local adaptation emerges
51 (Gonzalo-Turpin & Hazard, 2009; Jakobsson & Dinnetz, 2005; Santamaría et al., 2003).
52 This can be defined as a positive genotype by environment interaction on fitness which
53 appears in the sympatric combination of deme and environment (Kawecki & Ebert, 2004).
54 One of the first studies of local adaptation was carried out by the Swedish botanist Göte
55 Turesson (Turesson, 1922). The synthesis of the knowledge generated since Turesson
56 reveals that local adaptation in plants is common but not ubiquitous (Leimu & Fischer,
57 2008; Hereford, 2009). However, there are still important knowledge gaps. For instance,
58 Chen & Schemske (2015) note that of the 71 studies on plant local adaptation surveyed
59 by either Leimu & Fischer (2008) or Hereford (2009), only 3 involved tropical species.
60 While there are some more recent studies on long-lived tropical plants, they remain a very
61 small portion of the local adaptation literature (e.g., Chen & Schemske, 2015; Brancalion
62 et al., 2018; Barton et al., 2020).

63 Understanding how pervasive is local adaptation in wild populations has become
64 even more important recently because its presence can considerably affect the outcome
65 of climate niche models used for predicting the impact of climate change on species
66 ranges (Valladares et al., 2014; Ikeda et al., 2017; DeMarche et al., 2019). Unfortunately,
67 the gold standard method for detecting local adaptation – that is, reciprocal transplants
68 (Blanquart et al., 2013) – is not practical and its application is virtually unfeasible for
69 most species. When reciprocal transplants are unfeasible, one can look for other
70 phenomena that are associated with local adaptation. One option is to investigate niche
71 differentiation between populations, which is necessary for local adaptation to happen
72 (Kawecki & Ebert, 2004). Most of the time researchers estimate the niche of a species
73 using widely available occurrence record data and grided environmental (usually

climatic) data (reviewed by Elith & Leathwick, 2009; Peterson & Soberón, 2012). This approach usually cannot capture the fundamental niche, one prominent example of this being the problems that arise when trying to predict the area invaded by an alien species using such an approach (see Jiménez-Valverde et al., 2011 for a discussion). Therefore, these niche estimations cannot tell us if the differences in the niches of two biological entities are due to differences in their fundamental niches (i.e., local adaptation) or if they are just reflecting differences in the realized niches (Sobel et al., 2010). Nevertheless, this analysis can still inform us about how different are the habitats of each group, which is an important factor in the evolution of local adaptation.

Another interesting phenomenon associated with local adaptation is isolation by environment (IBE). This is defined as the positive correlation between genetic dissimilarity – usually represented by F_{st} – and environmental dissimilarity (Wang & Bradburd, 2014). It can be thought of as a counterpart of the well-known pattern of isolation by distance (IBD) (Wright, 1943). Examples of processes that can generate this pattern are natural selection against immigrants and reduced hybrid fitness (Wang & Bradburd, 2014). These processes in turn are some of the side effects of local adaptation (e.g., Lowry et al., 2008; Montgomery et al., 2021). Therefore, IBE is suggestive evidence of local adaptation. Lastly, a third pattern that is associated with local adaptation is genetically fixed phenotypic differentiation. To detect this differentiation and exclude the possibility of plasticity-induced differences, common garden experiments are generally performed (e.g., Platenkamp, 1991; Ramírez-Valiente et al., 2010; Vidaller et al., 2020). When local adaptation is present, we expect that most of the phenotypic difference will be genetically determined and maintained between the ecotypes in common garden (e.g., Ramírez-Valiente et al., 2010; López et al., 2020; Vidaller et al., 2020).

98 The genus *Epidendrum* L. is one of the emerging models for plant evolution in the
99 Neotropics (Pinheiro & Cozzolino, 2013; Karremans, 2021). Here, we studied
100 *Epidendrum fulgens* Brongn., which is a long-lived terrestrial orchid. It is endemic to
101 southern and southeastern Brazil (Figure 1) (Pinheiro et al., 2011), and its distribution has
102 a discontinuity located around the Portal de Torres, an important biogeographical barrier
103 that marks the transition between contrasting biomes, the Mata Atlântica – a tropical
104 rainforest – and the Pampas – a subtropical grassland (Rambo, 1950). To the north of
105 Portal de Torres, the species is within the Mata Atlântica, where it occurs in a sandy
106 coastal plain vegetation type (i.e., restinga, Figure 1). Its distribution extends further south
107 of Portal de Torres, beyond the Mata Atlântica, occurring in the interior of the continent,
108 in granitic rock outcrops (Figure 1). Therefore, its distribution spans contrasting degrees
109 of continentality and latitude, which should reflect the type of climate experienced by
110 each population. Interestingly, previous studies have documented a genetic differentiation
111 between northern and southern populations, but whether this could represent IBE remains
112 to be tested (Pinheiro et al., 2011).

113 Our goal here was to combine environmental, genetic and phenotypic evidence to
114 test whether *E. fulgens* northern and southern populations are locally adapted. More
115 specifically, we tested 1) if northern and southern populations have different climatic
116 realized niches, 2) if there is a positive correlation between environmental distance and
117 genetic distance (i.e., IBE) and 3) if the phenotype of northern and southern populations
118 differ in the field, and if those differences are preserved in the common garden.

119

120 **Methods**

121 *Ecological niche analysis*

122 The occurrence data were compiled from GBIF (GBIF, 2023), speciesLink
123 (<http://www.splink.org.br/>) and the occurrence data set of our research group. Points were
124 manually cleaned, removing incongruous points (i.e., outside the species distribution,
125 within urban centers, falling into the ocean, near the centroids of administrative areas).
126 Finally, we used spThin R package (Aiello-Lammens et al., 2015) to thin the occurrence
127 data set so that no two points were less than 9km apart. The distance used was the one
128 determined by a Mantel correlogram between the environmental and geographic
129 distances. The environmental distance was calculated as the Euclidian distance in the
130 environmental space delimited by the axes of a principal components analysis (PCA)
131 generated using all 19 bioclimatic variables (see ahead). In the end, our cleaned data set
132 contained 41 occurrence points, 31 in the northern group and 10 in the southern group.

133 As environmental data, we imported the 19 bioclimatic variables commonly used
134 in niche analyses from the CHELSA database (<https://chelsa-climate.org/>). The variables
135 we arranged into sets of high correlation (i.e., Spearman's $\rho \geq 0.7$), and for each set a PCA
136 was performed. Only the covariate of each set with the highest contribution to the PCA
137 was kept. More details about the method and the R scripts used can be found in van
138 Proosdij et al. 2016. The selected covariates were average annual temperature (BIO 1),
139 average diurnal temperature variation (BIO 2), isothermality (BIO 3), average
140 temperature of the wettest quarter (BIO 8), average temperature of the driest quarter (BIO
141 9), annual precipitation (BIO 12) and precipitation seasonality (BIO 15).

142 This niche analysis requires the definition of a study area for each of the two
143 groups, hereafter named background environment. Here, the background environment
144 was delimited as a circular buffer around the occurrences of each group, using an estimate
145 of the maximum seed dispersal distance as the buffer's radius. Pinheiro et al. 2011 used
146 nuclear and plastid DNA data to assess the relevance of pollen and seed dispersal in *E.*

147 *fulgens*, concluding that the latter is not frequent. Based on that study we used the distance
148 of 120km as the buffer's radius. This is the distance between Morro São Pedro (SP) and
149 Torres (TO) populations, which are the closest pair of northern-southern populations
150 showing different chloroplast haplotypes and, thus, no evidence of seed dispersal (see
151 Figure 1 and Table S1, Pinheiro et al. 2011).

152 Niche overlap analysis was performed using the ecospat v.3.1 R package (Di Cola
153 et al., 2017). The methodology behind this package is described in Broennimann et al.
154 2012. Briefly, it combines an environmental PCA and a kernel density distribution
155 generated from the occurrences to estimate the niche overlap using the D index (Schoener,
156 1968) which ranges from 0, no overlap, to 1, full overlap. The final step is testing the
157 hypothesis of niche equivalence and niche similarity (Warren et al., 2008). Niche
158 equivalence is a one-tailed test that asks whether the observed overlap is higher or lower
159 than expected by chance. Niche similarity is a one-tailed test that asks whether the
160 observed overlap is higher or lower than expected given the background environment.
161 Since we are interested in niche divergence, we performed the left-tailed version of both
162 tests. We simulated random overlap values one hundred times to test the significance.

163 *Isolation by environment and isolation by distance*

164 To test whether IBD or IBE explain better the genetic differentiation already
165 documented between northern and southern populations, regression analyses were
166 performed between matrices quantifying genetic structure, environmental dissimilarity,
167 and geographic distance. We included 17 populations in the analysis, five from the
168 southern group and twelve from the northern group (see Figure 1 and Table S1 for more
169 information about sampled populations). The environmental dissimilarity was calculated
170 using the same climatic variables used in the niche overlap analyses, which were
171 standardized and then used to generate a PCA. The first four axes of this PCA, containing

172 98% of the variation, were used to generate an environmental distance matrix calculated
173 as the Euclidean distance between each pair of points. Finally, as an estimate of the
174 genetic structure of populations, we used F_{st} calculated from nine microsatellite markers
175 (for more details on sampling and genotyping procedures, see Pinheiro et al. 2011; Sujii
176 et al. 2019.). This F_{st} matrix was linearized ($F_{st} - 1/F_{st}$) before being used in the analyses.

177 Using these three matrices (i.e., geographic, environmental and genetic), we
178 performed three Mantel tests with 10,000 permutations estimating the effect of IBD and
179 of IBE, as well as the correlation between geographic distance and environmental
180 dissimilarity. Given that environmental dissimilarity and geographical distance are
181 expected to be correlated, partial Mantel tests were performed to estimate separately the
182 contribution of IBD and IBE in the genetic structure. To deal with the high Type I error
183 rate associated with this test, the significance level was adjusted to 0.001 (Oden & Sokal,
184 1992). Using the same data, we also performed multiple matrix regression with
185 randomization (MMRR) (Wang, 2013). By estimating a correlation coefficient for each
186 matrix, this analysis allows estimating and comparing the effect of IBD and IBE on
187 genetic structure. The three analyses were performed using the vegan R package
188 (Oksanen et al., 2013) for the Mantel tests and the scripts provided by Wang (2013) for
189 the MMRR analysis.

190 *Phenotypic differentiation between in situ and common garden plants*

191 To assess possible differences between the two groups in phenotypic traits and
192 their reaction norms, four populations of *E. fulgens* growing in two conditions – growing
193 naturally *in situ* and cultivated in common garden – were analyzed. The four populations
194 were UB and TO, belonging to the northern group, and IT and PE, belonging to the
195 southern group (Table S1, Figure 1). *In situ* populations were visited between November
196 and December 2021. These same four populations were represented in a common garden

197 maintained at an experimental garden which is outside the species' natural range.
198 Common garden individuals had been in cultivation for several years before the onset of
199 this project, and all phenotypic measurements were performed on new stems generated
200 when plants were already growing in common garden, thus mitigating the maternal effect.
201 The common garden cultivation took place in the open, with partial shading (net of 30%)
202 and watering performed every three days. Plants were cultivated on plastic pots using a
203 substrate medium equally composed by pine bark and charcoal (10-15mm, Mogifertil,
204 São Paulo, Brazil).

205 Four phenotypic traits were evaluated: saturated water content (SWC, a measure
206 of succulence), specific leaf area (SLA), stomatal density and stem height. In the field,
207 we sampled ten individuals per population, measuring each individual's highest stem's
208 height in cm and collecting this same stem to measure other traits in the laboratory. The
209 third or fourth younger leaf was immediately stored in 70% alcohol for stomatal density
210 determination and the stems were then stored in plastic bags for SWC and SLA estimation
211 in the lab. We used an optical microscope to visualize and count the stomata. SWC was
212 estimated as $[\text{turgid mass} - \text{dry mass} / \text{dry mass}]$ (Ogburn & Edwards, 2012), and these
213 masses were obtained by clipping 3-4 one cm^2 discs from each leaf, hydrating then for
214 24h till maximum turgor (turgid mass) and then drying them for three days (dry mass).
215 Formally, this succulence index is dimensionless (Ogburn & Edwards, 2010).
216 Nevertheless, some authors prefer to present it using the unit gram of H_2O per gram of
217 dry mass ($\text{gH}_2\text{O g}^{-1}$). Finally, the SLA was estimated using the same dataset by dividing,
218 for each leaf, the discs' total leaf area by the discs' total dry mass, with units of $\text{m}^2 \text{kg}^{-1}$.
219 These same protocols were repeated for populations in common garden, however, in that
220 case sample effort was limited by the number of individuals of each population in
221 cultivation, which ranged from 8-12 (see Table S1 for detailed sample size).

222 To test for differences in reaction norms between groups, we fitted a two-way
 223 REML mixed-effect ANOVA of the following nature:

$$224 \quad Y_{ijk} = \mu + E_i + C_k + P(E)_{ij} + EC_{ik} + \varepsilon_{ijk}, \quad (1)$$

225 where Y_{ijk} is the trait's value measured in the n individual, of the j population and i
 226 group, growing in the condition k . μ is the trait's grand mean. E_i is the fixed factor that
 227 estimates the effect of the group (northern or southern). C_k is the fixed factor that
 228 estimates the effect of the growing condition (*in situ* or common garden) and allows us
 229 to infer the magnitude of the plasticity. The random factor $P(E)_{ij}$ estimates the effect of
 230 the population nested within the group. EC_{ik} estimates the interaction between group and
 231 growing condition and allows us to infer differences between groups in the reaction norm.
 232 Finally, ε_{ijk} is the residual error term for each observation. Succulence values needed to
 233 be log-transformed prior to analysis to alleviate heteroscedasticity, but it should be
 234 noticed that linear mixed models themselves are already robust to violations of
 235 distributional assumptions (Schielzeth et al., 2020).

236 Next, to test for differences between groups in common garden and *in situ*
 237 conditions we ran separately for each growing condition a one-way REML nested
 238 ANOVA of the following nature:

$$239 \quad Y_{nij} = \mu + E_i + P(E)_{ij} + \varepsilon_{nij}. \quad (2)$$

240

241 **Results**

242 *Niche differentiation*

243 The PCA for climatic data generated two principal components that together
 244 explained 67.70% of the variation (Figure 2). Thermal variables such as isothermality

(BIO 3), rainfall variables such as annual precipitation (BIO 12), and mixed variables such as mean temperature of the driest quarter (BIO 9) contributed considerably to PC1. On the other hand, PC2 was mainly influenced by thermal variables such as mean annual temperature (BIO 1) and mean diurnal thermal amplitude (BIO 2). The niches of the two groups were differentiated in both axes, but especially in PC1 (Figure 2). Considering both axes, the climatic niches of the two groups did not overlap ($D=0$), with the northern group occupying warmer and rainier regions with less seasonal variation in temperature and rainfall. According to the equivalence test, the niches of the two groups were significantly different ($p<0.01$). On the other hand, the similarity test was non-significant ($p>0.05$).

Isolation by environment

The Mantel test revealed a significant effect of IBD ($R^2 = 0.34$, $p<0.001$, Figure S1) and IBE ($R^2 = 0.18$, $p<0.001$, Figure S1) on genetic structure, with the effect of IBD being the largest. However, as expected, environmental dissimilarity and geographic distance are also correlated with each other ($R^2 = 0.60$, $p<0.001$, Figure S1). According to the Mantel partial tests, IBD persists even when ambient dissimilarity is accounted for (Table 1). However, when geographic distance is controlled for, IBE disappears (Table 1). This same result appears in the MMRR, according to which IBD is significant and 6 times greater than IBE, which is non-significant (Table 1). Therefore, geographic distance was the main factor responsible for the genetic structure of populations, the effect of environmental dissimilarity being negligible.

Plasticity and genetic determination of the phenotype

The two-way ANOVA testing for phenotypic plasticity showed that the condition factor effect was significant, except for stomatal density, the only non-plastic trait (Figure

269 3). Moreover, the *in situ* growing condition had a positive effect (i.e., numeric increase)
270 on all plastic traits. The reaction norms for SLA and height are more complex due to
271 group-condition interactions: the southern population's mean phenotype was more
272 sensitive to the growing condition than the northern one (Figure 3).

273 Moving forward to the comparisons between groups in one or another growing
274 condition, we found out northern and southern individuals were more different from one
275 another when growing *in situ*. Succulence and SLA were different between groups
276 growing under *in situ* condition, these two traits being larger for the southern group
277 (Figure 3ab). Under common garden conditions, these same two traits did not differ
278 between groups. On the other hand, the pattern for height was the opposite: northern
279 plants were significantly higher in common garden, but this difference is non-significant
280 *in situ* (Figure 3d). Finally, stomatal density did not differ significantly between groups
281 in any of the conditions (Figure 3c).

282

283 Discussion

284 Species occurring in coastal and inland environments have been studied by
285 different authors and signs of local adaptation have been detected for most of them,
286 probably due to the strong abiotic contrast between these two habitats (e.g., Köhl 1997;
287 Lowry et al. 2008; White et al. 2014; Liu et al. 2016). Our results demonstrate that
288 northern and southern groups of *E. fulgens* have different and non-overlapping realized
289 climatic niches, therefore inhabiting climatically different areas. Nevertheless, the neutral
290 genetic differentiation between these two groups was better explained by geographic
291 distance rather than environmental dissimilarity. Finally, the phenotypic traits analyzed
292 presented variable and contrasting patterns of phenotypic plasticity. In the next

293 paragraphs, we integrate these three lines of evidence and discuss whether they weigh in
294 favor or against the hypothesis of local adaptation.

295 *Northern and southern populations occupy climatically different environments*

296 The realized niche of *E. fulgens* is composed of two very different and non-
297 overlapping niches at the subspecific level (Figure 2). This result can be interpreted in
298 two ways. On one hand, the niche differentiation could have been caused by local
299 adaptation, in which case we would expect also phenotypic differentiation in common
300 garden (e.g., van Boheemen et al. 2019) and isolation by environment. On the other hand,
301 the same pattern can be generated by phenotypic plasticity, in which case we would
302 expect no phenotypic differentiation in common garden and no isolation by environment
303 (e.g., Williams et al. 1995; Riis et al. 2010). We use evidence from the other two analyses
304 – the genetic and the phenotypic ones – to try to distinguish which of these cases better
305 describes the pattern observed for *E. fulgens* populations.

306 *IBD rather than IBE better describes the pattern of genetic differentiation*

307 Analogous to geographical distance, the environmental dissimilarity between sites
308 can lead to non-random mating, generating what is called isolation by environment
309 (Wang & Bradburd, 2014). Here, when controlling for confounding variables, we
310 detected IBD but not IBE (Table 1, Figure S1). The previous analysis of this data also
311 reported IBD (Pinheiro et al. 2011), however, that study did not account for the
312 confounding factor of environmental differentiation, which was shown to be present by
313 our niche analysis. Therefore, our results offer an important contribution by comparing
314 both IBD and IBE hypotheses within the same statistical framework and demonstrating
315 the IBD rather than IBE better describes the observed pattern. Therefore, the genetic
316 structure between southern and northern groups does not support local adaptation.

317 *Trait-environment relationships and reaction norms*

318 *E. fulgens* seems to cope with environmental heterogeneity inside its distribution
319 by possessing considerable phenotypic plasticity, which argues against the hypothesis of
320 local adaptation. One of the traits that responded plastically was specific leaf area (SLA).
321 There was no difference in this trait between northern and southern populations in
322 common garden, but SLA was higher for the southern population *in situ* (Figure 3ab).
323 Higher values of SLA are related to a more acquisitive syndrome of resource use in
324 general (Wright et al., 2004). SLA is expected to increase with soil fertility because of
325 the decrease in leaf density (LD) with soil fertility (e.g., Witkowski & Lamont, 1991;
326 Hodgson et al., 2011). That pattern has been verified by other empirical studies (Ordoñez
327 et al., 2009, De Frenne et al., 2011) and it is also present here, since the populations with
328 the lower SLA (i.e., northern populations) grow on coastal soils, which are known to be
329 consistently poorer than inland ones, on which southern populations grow (Du & Hesp,
330 2020).

331 Similar to SLA, SWC was similar in common garden but higher for southern
332 plants *in situ*. The northern populations inhabit coastal sites, which are commonly
333 exposed to salt spray (Du & Hesp, 2020). Interestingly, a parallel study conducted by our
334 research group found that northern populations are more exposed to salt spray and that
335 succulence seems to be an important factor related to their resistance to it (de Lima in
336 press.). Nevertheless, the highest values of SWC *in situ* are found in the southern/inland
337 populations. One possible explanation would be that inland populations are more
338 susceptible to another kind of stress to which higher SWC might be beneficial, such as
339 drought stress. Indeed, our niche analysis shows that southern populations seem to occupy
340 habitats with lower annual precipitation (BIO 12), but further studies in the field are

341 needed to confirm that these populations are really exposed to some kind of drought
342 stress.

343 *Evidence for ecotypic differentiation between southern and northern populations?*

344 The process by which lineages split and evolve separately – which might
345 eventually lead to speciation – is a long continuous and telling where a given lineage is
346 in this process is by no means simple because different lines of evidence might lead to
347 contrasting conclusions (De Queiroz, 2007). Here the data on phenotype argues against
348 the existence of two ecotypes. We expect that the high plasticity detected here would
349 allow northern and southern plants to adjust their phenotypes to each other's habitats.
350 Moreover, the data on nuclear neutral markers confirms that the north-south genetic
351 structure found previously (Pinheiro et al., 2011) is better explained by IBD rather than
352 IBE, which weakens even further the hypothesis of local adaptation. Therefore, the
353 congruency between phenotypic and genetic data allows us to infer that local adaptation
354 is most probably absent in this species and that its niche broadness is likely the result of
355 its plastic phenotype.

356 That said, a future study venue that might be interesting to pursue is to study the
357 effect of temperature in each of the groups. While succulent plants are known for
358 inhabiting very hot areas, their lower thermal resistance threshold is considerably high,
359 especially due to their high tissue water potentials, which results in a higher freezing point
360 (Ogburn and Edwards 2010). Indeed, temperature variables were the dominant ones in
361 both axis of the environmental PCA that differentiated the coastal and inland niches,
362 showing that inland areas are colder and have a more thermal amplitude (Figure 2). Most
363 of *E. fulgens* distribution occurs within the tropical region, where temperatures virtually
364 do not reach negative values. However, the extreme south of Brazil, where the inland
365 populations are located, is within the subtropical region and is known to occasionally

366 experience frost and below-zero temperatures during the winter (Wrege et al. 2018).
367 Interestingly, a recent study identified that niche modelling using only climate data
368 accurately predicts the southern range of this species (de Mattos et al. 2023), suggesting
369 that the climate is indeed an important factor limiting the southern edge of *E. fulgens*
370 distribution. Therefore, future studies could assess the resistance of *E. fulgens* to cooling
371 and freezing, which would enable a deeper understanding of the evolution of this species'
372 populations and how this species copes with the contrasting environments it inhabits.

373

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387

388 **Supporting information**

389 Additional supporting information may be found online in the Supporting Information
390 section at the end of the article.

391 **Figure S1.** Linear regression plots associating geographic distance and genetic structure
392 (a), environmental dissimilarity and genetic structure (b), and geographic distance and
393 environmental dissimilarity (c) considering all 17 *Epidendrum fulgens* populations
394 sampled. The coefficients of determination (R^2) and their associated p-values are
395 presented. Significant values are in bold. The significant codes are * $p < 0.05$, ** $p < 0.01$,
396 *** $p < 0.001$.

397 **Table S1.** Name, ID, geographic coordinates, and geographic group of all *Epidendrum*
398 *fulgens* populations included in this study. For each population the sample sizes (N) for
399 nuclear F_{st} estimation using microsatellites (F_{st} sample), phenotypic analysis *in situ*
400 (Phenotype IS) and in common garden (Phenotype GC) are provided. For more
401 information on sampling and F_{st} estimation methods, please see Pinheiro et al. 2011; Sujii
402 et al. 2019.

403

404 **Data accessibility**

405 The datasets used is available in a Zenodo repository (DOI: 10.5281/zenodo.10462803).

406

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586 **Figure 1.** Distribution of *Epidendrum fulgens* (black silhouette), and its southern (blue
587 dots) and northern (yellow dots) populations included in this project. Notice that the
588 distribution is disjunct, and the gap is located at the transition between the Mata Atlântica
589 (green area) and the Pampas (brown area), known as the Portal de Torres biogeographical
590 barrier. The inset photos show the two characteristic habitats of each group of
591 populations: coastal sand plains (northern populations, top photo) and inland granitic
592 outcrops (southern populations, bottom photo). Brazil Federal States: Rio Grande do Sul
593 (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP) and Rio de Janeiro (RJ). Biomes
594 borders are those defined by the Brazilian Institute of Geography and Statistics (IBGE).
595 See **Error! Reference source not found.** for the full name and additional information on
596 each population.

597

598 **Figure 2.** Estimated realized climatic niches of *Epidendrum fulgens* northern (yellow
599 shade) and southern (blue shade) groups. The niches are projected onto an environmental
600 space defined by the first two axes of a principal components analysis of climate data.
601 The percentage of variance explained by each axis is in parentheses. The solid line and
602 the dotted line represent, respectively, 100% and 50% of the available background
603 environment for each group of corresponding color. A correlation circle shows the
604 correlation of each individual variable with the axes.

605

606 **Figure 3.** Reaction norm of saturated water content (SWC) (a), specific leaf area (SLA)
607 (b), stomatal density (c), and stem height (d) measured for 82 individual plants originated
608 from northern (N = 2, squares) and southern (N = 2, circles) populations, all growing
609 under common garden (N = 42) and in situ (N = 40) conditions. The error bars represent

one standard error. The asterisks inside a rectangle indicate significant differences between the two groups on a given condition, either in common garden (left) or *in situ* (right). The asterisks outside a rectangle indicate significant interaction between group and growing condition (G-C interaction) or significant effect of growing condition on the traits (Condition effect, i.e., phenotypic plasticity). Significant codes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

† Originally, this succulence index was described as dimensionless (Ogburn and Edwards 2012). However, some studies present it as having units of gram of H₂O per gram of dry mass (gH₂O g⁻¹).

1 **Table 1.** Effect of isolation by environment (IBE) and isolation by distance (IBD) on the
 2 genetic structure of 17 *Epidendrum fulgens* populations. The association was tested using
 3 partial Mantel tests and multiple matrix regression with permutation (MMRR). For both
 4 tests, the r (Pearson's correlation coefficient) and its associated p-value are presented. For
 5 the MMRR, the geographic (β_G) and environmental (β_E) coefficients are also presented.
 6 For MMRR the significant codes are $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. For partial
 7 Mantel test the significant codes are $*p < 0.001$, $**p < 0.0001$, $***p < 0.00001$.

	MMRR	Partial Mantel	
	IBE + IBD	<u>IBD</u>	<u>IBE</u>
r	0.348***	0,452*	-0,069
β_G	0.658***	-	-
β_E	-0.090	-	-

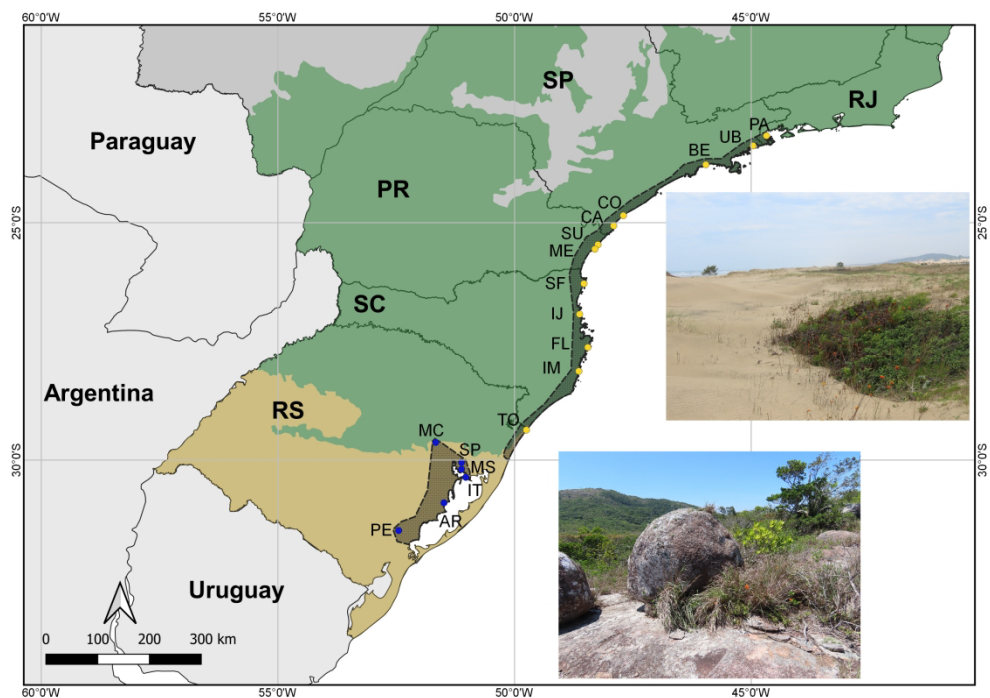


Figure 1. Distribution of *Epidendrum fulgens* (black silhouette), and its southern (blue dots) and northern (yellow dots) populations included in this project. Notice that the distribution is disjunct, and the gap is located at the transition between the Brazilian Atlantic Forest (green area) and the Pampas (brown area), known as the Portal de Torres biogeographical barrier. The inset photos show the two characteristic habitats of each group of populations: coastal sand plains (northern populations, top photo) and inland granitic outcrops (southern populations, bottom photo). Brazil Federal States: Rio Grande do Sul (RS), Santa Catarina (SC), Paraná (PR), São Paulo (SP) and Rio de Janeiro (RJ). Biomes borders are those defined by the Brazilian Institute of Geography and Statistics (IBGE). See Table S1 for the full name and additional information on each population.

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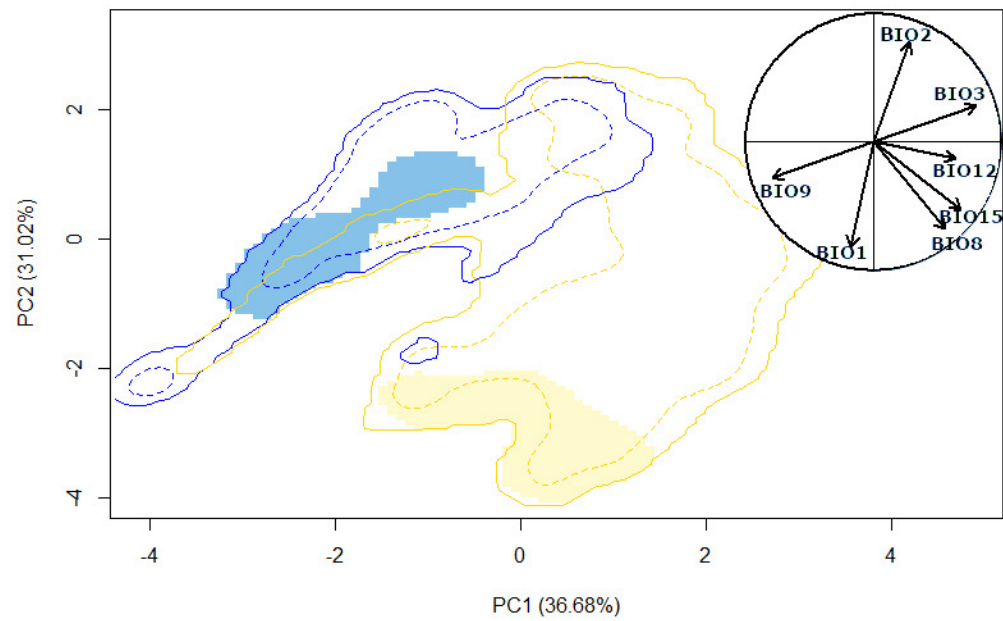


Figure 2. Estimated realized climatic niches of *Epidendrum fulgens* northern (yellow shade) and southern (blue shade) groups. The niches are projected onto an environmental space defined by the first two axes of a principal components analysis of climate data. The percentage of variance explained by each axis is in parentheses. The solid lines envelop all the available background environment of each group. The dotted lines represent the 50% most common conditions in each background environment. A correlation circle shows the correlation of each individual variable with the axes.

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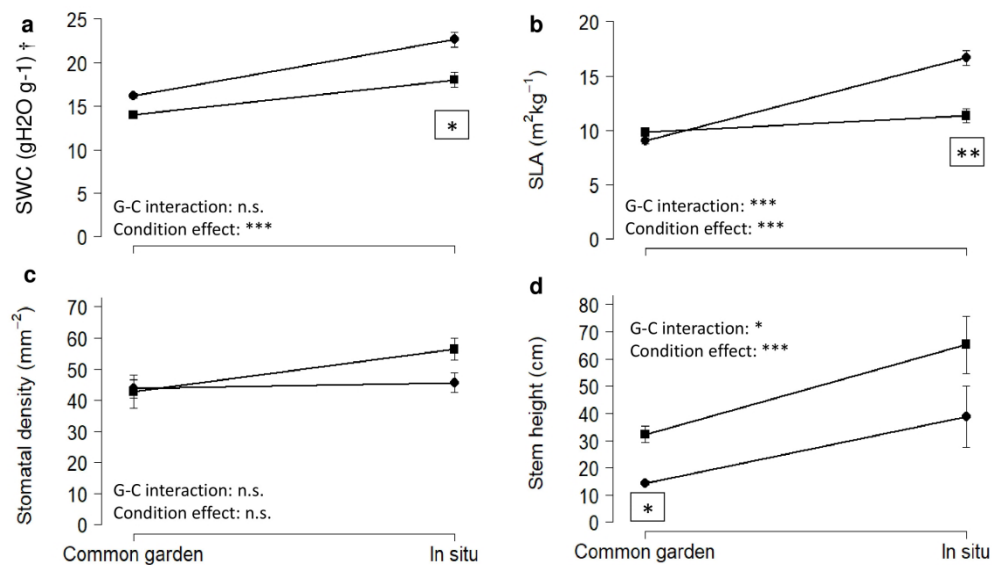
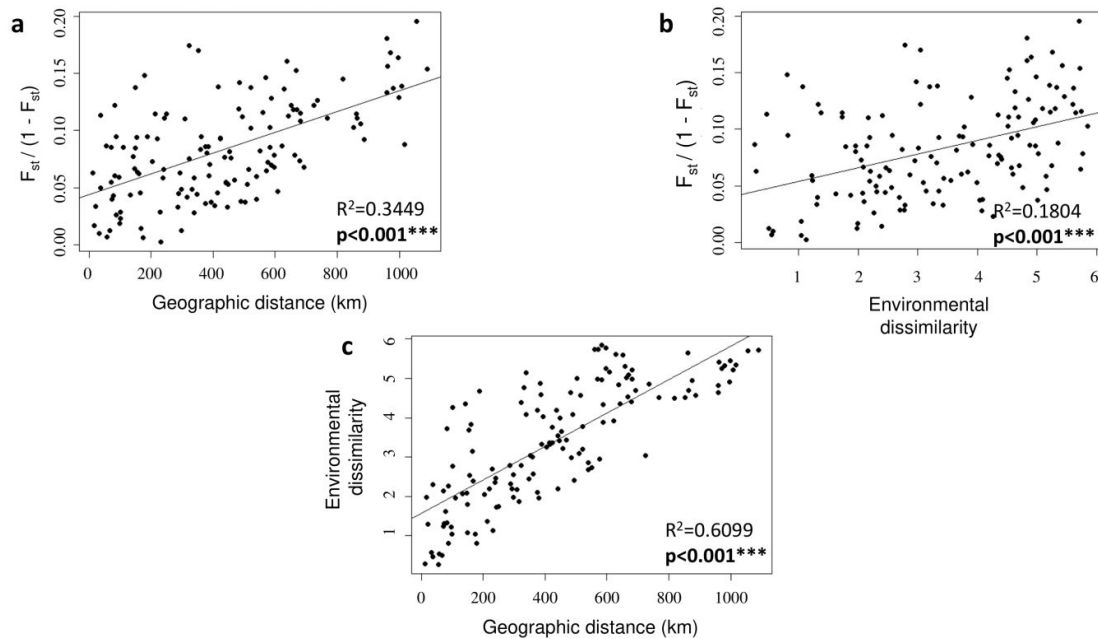


Figure 3. Reaction norm of saturated water content (SWC) (a), specific leaf area (SLA) (b), stomatal density (c), and stem height (d) measured for 82 individual plants originated from northern (N = 2, squares) and southern (N = 2, circles) populations, all growing under common garden (N = 42) and in situ (N = 40) conditions. The error bars represent one standard error. The asterisks inside a rectangle indicate significant differences between the two groups on a given condition, either in common garden (left) or in situ (right). The asterisks outside a rectangle indicate significant interaction between group and growing condition (G-C interaction) or significant effect of growing condition on the traits (Condition effect, i.e., phenotypic plasticity). Significant codes: *p < 0.05, **p < 0.01, ***p < 0.001.

† Originally, this succulence index was described as dimensionless (Ogburn and Edwards 2012). However, some studies present it as having units of gram of H₂O per gram of dry mass (gH₂O g⁻¹).



1

2 **Figure S1.** Linear regression plots associating geographic distance and genetic structure (a),
 3 environmental dissimilarity and genetic structure (b), and geographic distance and
 4 environmental dissimilarity (c) considering all 17 *Epidendrum fulgens* populations sampled.
 5 The coefficients of determination (R^2) and their associated p-values are presented. Significant
 6 values are in bold. The significant codes are * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table S1. Name, ID, geographic coordinates, and geographic group of all *Epidendrum fulgens* populations included in this study. For each population the sample sizes (N) for nuclear F_{st} estimation using microsatellites (F_{st} sample), phenotypic analysis *in situ* (Phenotype IS) and in common garden (Phenotype GC) are provided. For more information on sampling and F_{st} estimation methods, please see Pinheiro et al. 2011; Sujii et al. 2019.

Population	ID	Latitude	Longitude	Group	F_{st} (N)	Phenotype IS (N)	Phenotype CG (N)
Paraty	PA	23S 10'	44W 40'	North	34	-	-
Ubatuba	UB	23S 22'	44W 57'	North	18	10	11
Bertioga	BE	23S 46'	45W 57'	North	20	-	-
Ilha Comprida	CO	24S 51'	47W 42'	North	20	-	-
Ilha do Cardoso	CA	25S 04'	47W 54'	North	29	-	-
Ilha de Superagui	SU	25S 27'	48W 13'	North	18	-	-
Ilha do Mel	ME	25S 31'	48W 17'	North	24	-	-
Ilha de São Francisco	SF	26S 16'	48W 31'	North	12	-	-
Itajaí	IJ	26S 55'	48W 37'	North	20	-	-
Florainópolis	FL	27S 37'	48W 27'	North	52	-	-
Imbituba	IM	28S 10'	48W 41'	North	50	-	-
Torres	TO	29S 22'	49W 45'	North	25	10	8
Morro Santana	MS	30S 03'	51W 07'	South	20	10	12
Morro São Pedro	SP	30S 11'	51W 06'	South	21	-	-
Morro do Cabrito	MC	29S 37'	51W 39'	South	22	-	-
Itapua	IT	30S 21'	51W 02'	South	37	-	-
Arambaré	AR	30S 11'	51W 07'	South	22	-	-
Pelotas	PE	31S 29'	52W 26'	South	-	10	11

For Peer Review