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Vanilla insignis Ames. (Orchidaceae): morphological variation of the labellum in Quintana Roo, Mexico

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Abstract

The shape of the flower can vary based on the type of pollinator or the environment in which the plant develops. In *Vanilla insignis*, there are no studies that analyse the shape of the labellum of the flower as has been done in *V. planifolia*. Therefore, the study aimed to determine the variation in the shape of the labellum of *V. insignis* through a morphometric analysis in different environmental conditions in the state of Quintana Roo, Mexico. The results showed that there were significant differences in the variables analysed. Principal component analysis and dendrogram analysis reveal that four *V. insignis* morphotypes were possibly associated with soil water availability conditions because there were significant differences between the variables that define the apical region. In addition, the distribution of the morphotypes corresponded with the presence of humidity regardless of geographical distances such as in the populations of Tenampulco, Puebla and Caobas, Quintana Roo. The presence of these morphotypes allows the development of conservation programmes and genetic improvement of the species of *V. insignis* and related commercial species.

Introduction

The range of geographic and environmental distribution of plants is closely related to their capacity to maintain morphological and physiological variation at the infraspecific level as a response to their interaction with differential biotic and abiotic conditions (Paiaro *et al*., 2012). Particularly in lineages with broad geographic distribution, it has been reported that diverse environmental factors, both biotic and abiotic, can habilitate new niches by which they increase phenotypic differentiation among populations or individuals throughout the entire distribution area (Nattero *et al*., 2011; Paiaro *et al*., 2012). When there is a high correlation between the plant phenotype and the geographic distribution of a particular environmental factor, this relationship is known as '*a geographic pattern of phenotypic variation*' (Paiaro *et al*., 2012).

In general, within the expression of geographic patterns of phenotypic variation, it has been observed that abiotic factors mainly affect vegetative structures such as leaves. In reproductive or floral structures, the phenotypic variation is associated with qualitative (shape) or quantitative (size) modifications promoted by biotic factors, such as interaction with pollinizers (Bateman and Rudall, 2011; Essenberg, 2021).

In some angiosperms, however, the abiotic environment can also have a major effect on the morphology of floral traits (Carroll *et al*., 2001; Strauss and Whittall, 2006; Toji *et al*., 2021). Specifically, in environments that are extremely limited in terms of resources, resource allotment for floral display can involve a very high energy cost that may compromise the growth and survival of the individuals (Galen, 1999). Thus, when the shape or size of the flower that optimizes pollinator attraction differs from the shape and size that optimize the consumption of plant resources, the selection pressures in conflict favour the divergence of flower shapes adequate to the available abiotic resources (soil, light and moisture) among microhabitats or over environmental gradients (Galen, 1999). For example, a reduction of approximately 33% in flower size and flowering in *Epilobium angustifolium* L. (Onagraceae) has been described in populations subjected to soil drought conditions (Carroll *et al*., 2001; Essenberg, 2021; Westerband *et al*., 2021). In flowers of *Tolumnia variegata* (Sw.) Braem. (Orchidaceae) modifications in total flower length, labellum length and petal length: width ratio were identified, determining variation in morphotypes associated with availability of water between sites (spatial) and years (temporal) (Morales *et al*., 2010). In the case of *Vanilla planifolia*

Andrews, variations in the shape of the labellum have been reported in Oaxaca where the morphotypes were associated with different environmental conditions (Hernández-Ruiz *et al.*, 2020). In the Huasteca of Hidalgo (Maceda *et al.*, 2023) and San Luis Potosí (Lima-Morales *et al.*, 2021), the morphotypes were distributed heterogeneously in the different habitats, for which reason the variation could be associated with pollinators.

Given that some models describe a clinal effect of environmental and geographic factors, such as water availability or elevation, on the structure of floral morphological variation among populations; the objective of this work was to identify the variation in the morphology of the labellum of *V. insignis* in different environmental conditions in Quintana Roo, Mexico.

Materials and methods

Study species

Vanilla insignis Ames. (Orchidaceae, Vanilloideae, Xanata) is distributed in the Caribbean basin of Central America (Soto-Arenas and Dressler, 2010). In Mexico is distributed in the states of Campeche, Chiapas, Quintana Roo, Oaxaca, Puebla, Tabasco, Veracruz and Yucatán, in elevations ranging from sea level to 900 m asl, with diverse climate and moisture conditions (1000 to 4000 mm mean annual precipitation). *Vanilla insignis* is hemi-epiphyte and vegetatively abundant, flowering in April and May and with a system of pollination by deceit (Lubinsky *et al.*, 2006; Soto-Arenas and Dressler, 2010; Pansarin, 2021).In the Yucatan peninsula, *V. insignis* is common to find associated with seasonally dry environments, for this reason, it has finely wrinkled stems (Rodríguez-López and Martínez-Castillo, 2019).

Biological material

During 2017, flowers were collected from different *V. insignis* plants, three flowers were taken from each plant, giving a total of 141 flowers from 47 plants. The collections were made in the localities of Santa Elena (fifteen plants), Laguna Guerrero (nineteen plants), Caobas (four plants) and Huatusco (three plants) in the state of Quintana Roo. In addition, samples from Tenampulco (six plants), in the state of Puebla, Mexico were collected to have an external group to verify that the shape of the labellum corresponded to environmental factors. The collection sites had a mean annual temperature of 22°C, similar climate conditions and mean solar radiation. The differences between localities were the elevation and the soil moisture regime, which were considered environmental gradient criteria (Table 1). The species were identified during the plant collection with the key of Soto-Arenas and Dressler (2010).

The flowers collected in the field were vigorous and free of visible damage. The cut was made at the base of the peduncle and the flowers were stored in 125 ml glass recipients with a preservative solution (ethanol 27%, lactic acid 4%, benzoic acid 3%, glycerine 3%, and distilled water 63%) until dissection and measurement (Salazar-Rojas *et al.*, 2010; Lima-Morales *et al.*, 2021).

Evaluated traits

To characterize the shape of the labellum, morphometric analysis was performed based on the methods proposed by Catling (1990) and modified by Salazar-Rojas *et al.* (2010) with some modifications. The labellum was used to identify the infraspecific variation due to it was useful to identify variation in different populations of *V. planifolia* (Hernández-Ruiz *et al.*, 2020; Lima-Morales *et al.*, 2021; Maceda *et al.*, 2023). First, the flower column, sepals, petals and labellum were dissected, then the labellum was expanded on an anti-reflective glass plate, and the labellum was stained with methylene blue (0.08%) to contrast the contour. Finally, the image was digitized with a Sony Alfa 65v reflex camera equipped with a Sony DT 30 mm F/2.8 SAM macro lens. The digital image of the labellum was vectorized in Corel Draw Graphics Suite X4 with a grid composed of 69 lines, seven angles and one index obtained from the variables A, B, C, D and E to evaluate the proportion of the labellum length using the formula index = (ΣB,C, D)/A. The 76 traits were grouped into three regions (Fig. 1): basal region (7 traits), middle region (49 traits) and apical region (21 traits).

Statistical analysis

Two sources of variation were considered in the analysis of the labellum morphology of *V. insignis*: The first one was analysing

Table 1. Main characteristics of the five environmental conditions where the *V. insignis* populations analysed are located

Environment	Locality	Collection	Climate	Mean solar radiation (μmols/m²/s)	Altitude (m)	Soil moisture regime (SMR)
1	Santa Elena, Quintana Roo	*Vi*1 *Vi*2 *Vi*3	Aw1(x´)	82 to 87	10	Aquic (365 days)
2	Laguna Guerrero, Quintana Roo	*Vi*5 *Vi*8 *Vi*9 *Vi*15 *Vi*16	Aw1(x´)	82 to 87	10	Ustic (180–270 days)
3	Caobas, Quintana Roo	*Vi*11	Aw1(x´)	78 to 82	150	Udic (270–330 days)
4	Huatusco, Quintana Roo	*Vi*13	Aw1(x´)	78 to 82	100	Xeric (90–180 days)
5	Tenampulco, Puebla	*Vi*18	Am	69 to 73	210	Udic (270–330 days)

Constructed with data from CONABIO (2021).

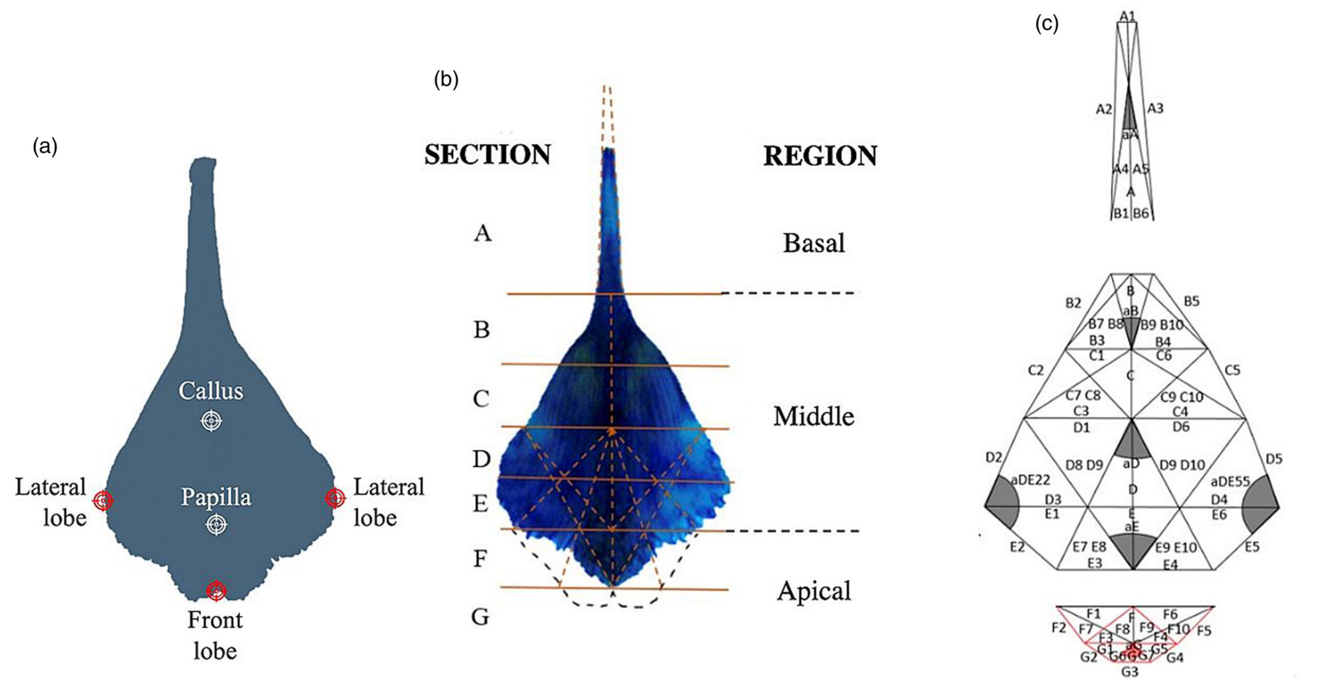

Figure 1. (a) Characterization of the *V. insignis* labellum. (b) Grid used to mark seven sections (A, B, C, D, E, F and G) and three regions (Basal, Middle and Apical). (c) The diagram illustrates the position of 69 lines and seven angles used as morphological traits.

the environmental condition in which the populations were distributed, so there were five treatments (environments) with different numbers of repetitions. The second was with the original population, so 11 collects with different numbers of repetitions were considered. The data were analysed under a completely randomized design model (PROC GLM, SAS 2011). Means of environments and collections were compared with ANOVA with unequal sample size and Tukey tests (SAS, 2011).

Numerical analysis

Two multivariate analyses were performed to characterize the variation of the labellum of *V. insignis*: principal components analysis (PC) and conglomerate clustering with Euclidean distance and average linkage as the measure of distance and method of grouping (Sneath and Sokal, 1973). The numerical analysis considered the means of each of the 77 traits analysed for all the collects using the program SAS v 9.3 (SAS, 2011).

Results

The effect of the environment and the total population on the morphology of *V. insignis* labellum was analysed and statistically significant differences were obtained between the accessions ($P \leqslant 0.01$–0.001) and the environmental conditions ($P \leqslant 0.01$–0.001) for 72 of 77 variables analysed (Table S1). The five variables that were not significantly different (B5, B9, D, E7, E10) corresponded to variables of the middle region of the labellum.

In the case of the coefficient of variation (CV), three groups of variables were distinguished based on their CVs (Table S1). The first group with CV values less than 10% was made up of 56% of the total variables. The variables of this group form 90% of the basal region of the labellum, 59% of the middle region of the labellum and 38% of the apical region (Table S1). The second group with a CV of 10 to 17% was compounded by 34% of the total variables. These variables make up 41% of the middle region of the labellum, 10% of the basal region and 24% of the apical region. The third group with CVs greater than 17% were obtained only in the variables of the apical region, mainly those that form the apical lobes.

Distribution and grouping of V. insignis populations

Dispersion of *V. insignis* populations represented in space was determined by the first three principal components, which together explained 85% of the accumulated variation for the 77 analysed variables (Table S2).

PC1 explained 46% of the total variation, the main variables that determine the variation of PC1 are related to the size and shape of the middle region of the labellum and callus (sections B and C). PC2 explained 28% of the variation and the variables that mainly influenced the separations of the groups were the sections D and E that form the regions of the median labellum adjacent to the papilla and the size of the lateral lobes. PC3 explained 11% of the variation and the variables that separate the groups were sections A, E and G, which delimit the size of the basal region and the shape and size of the apical region and the apical lobes of the labellum.

According to the spatial distribution of the first three principal components, four groups of studied populations of *V. insignis* were obtained. Figure 2 shows that populations located on the positive side of the PC1 axis presented a labellum with a middle region that gives a larger tubular shape (GII) (Fig. 2), while on the negative side were populations with smaller tubular spaces in the labellum. For PC2, the populations with the largest papilla in the middle region (GI and GII) were located on the positive side (Fig. 2). In the centre of the axis, populations with the middle region of the papilla of rectangular shape and medium size were

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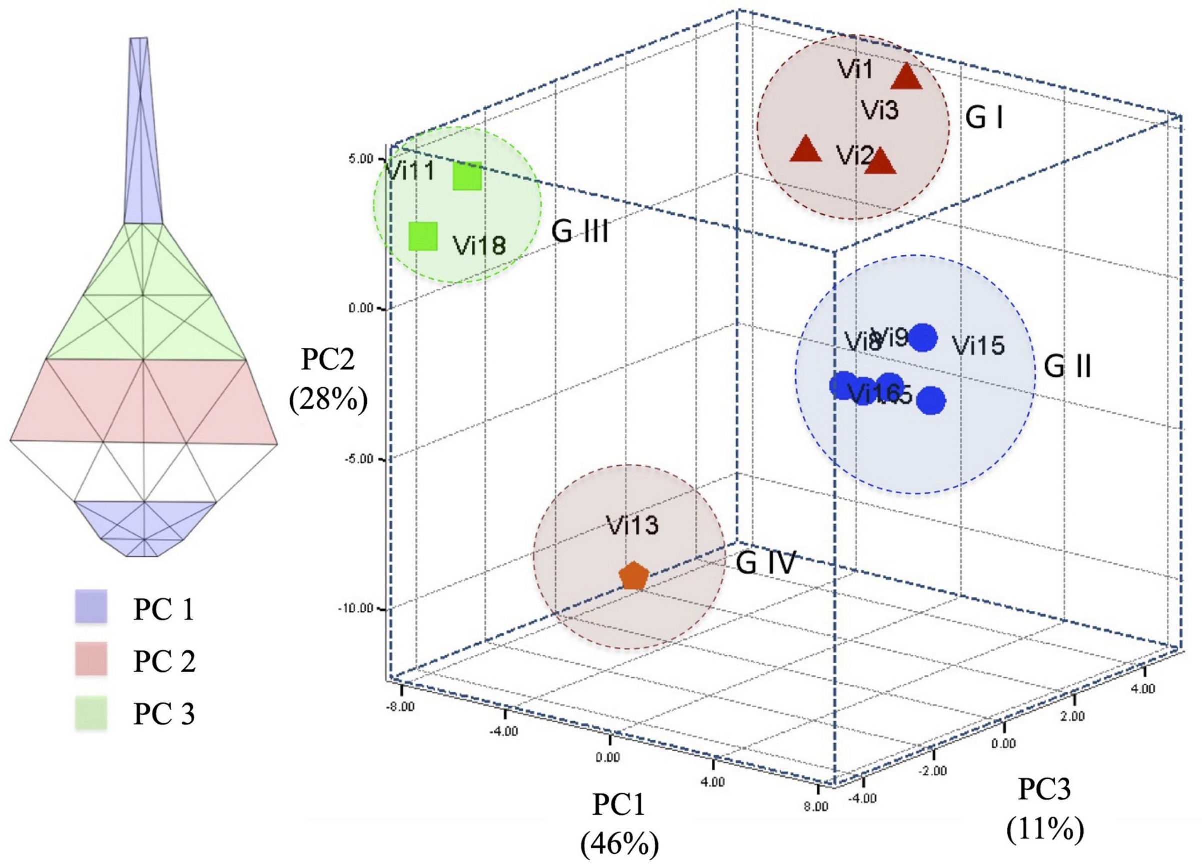

Figure 2. Dispersion of 11 *V. insignis* collections in the space determined by the first three principal components.

observed, while populations with a trapezoidal shape and smaller size were grouped on the negative side of PC2 (GIV) (Fig. 2).

In PC3, on the positive side near the centre were the populations that presented a pronounced basal region and ornamentation of the apical lobes (GI) (Fig. 2), while on the negative side were the populations without apical lobes (GIII) (Fig. 2). Therefore, the following groups of *V. insignis* populations were identified: Group I (*Vi*1, *Vi*2 and *Vi*3), Group II (*Vi*5, *Vi*8, *Vi*9, *Vi*15, *Vi*16), Group III (*Vi*11, *Vi*18) and Group IV (*Vi*13) (Fig. 2).

Using the cluster analysis, a grouping pattern similar to that obtained with the PC analysis was observed. Figure 3 shows the identification of four morphological groups based on the labellum of *V. insignis* populations at a Euclidean distance of 0.8. The grouping of the populations coincides with the SMR in which they are located. Table 2 shows that in most of the variables, there are significant differences between the different SMRs, therefore the separations of the groups in the PCs and cluster analysis are due to the SMR conditions as confirmed in Table 2.

Discussion

The shape of the labellum of *V. insignis* had significant differences between the populations in general and between the populations that were located in different environmental conditions. The five variables that did not have significant differences and that represent the middle region of the labellum are possibly stable (conserved traits) between populations and different environments and would serve as morphological markers for the definition of morphospecies in the *V. planifolia* complex of the section Xanata, within the subgenus Xanata (*sensu*) (Soto-Arenas and Cribb, 2010).

An important point to understand the behaviour of the labellum variables is the values of the coefficient of variation (CV). In studies with *Phlox cuspidata* (Polemoniaceae), Schlichting and Levin (1984) defined low CVs as those that were below 10%, intermediate those that were between 10 to 20% and that associated with floral structures, and high CVs (greater than 20%) were associated with vegetative structures. In *V. insignis*, three groups of CVs were identified, most of the variables had CV values less than 10%, while only 10% of the collected individuals has CV values above 17%. The Cvs less than 10% have less dispersion in the data, while the CVs above 10% have a greater dispersion of the values and therefore a greater standard deviation that reflects the variation between the collects (Bedeian and Mossholder, 2000). CV values above 17% are reported for orchid floral structures (Bateman and Rudall, 2006; 2011; Morales *et al*., 2010; Ackerman *et al*., 2011). The results of this work are consistent with previous work in *L. anceps* subsp. *dawsonii* f. *chilapensis* L. (Orchidaceae) that recorded very similar values (16–20%) in the CV of the variables that define the morphology of the apical lobes between morphotypes (Salazar-Rojas *et al*., 2010).

In the Orchidaceae family, it has been reported that the CV is higher in vegetative structures (15.3–38%) than in reproductive structures (8.7–12.4%); most of the CVs identified in floral structures between populations are in an average range of 12–17% (Pellissier *et al*., 2010; Bunpha *et al*., 2014). The biological interpretation of high CVs in the floral structures of orchids is associated with the expression of plasticity of plants (Morales *et al*.,

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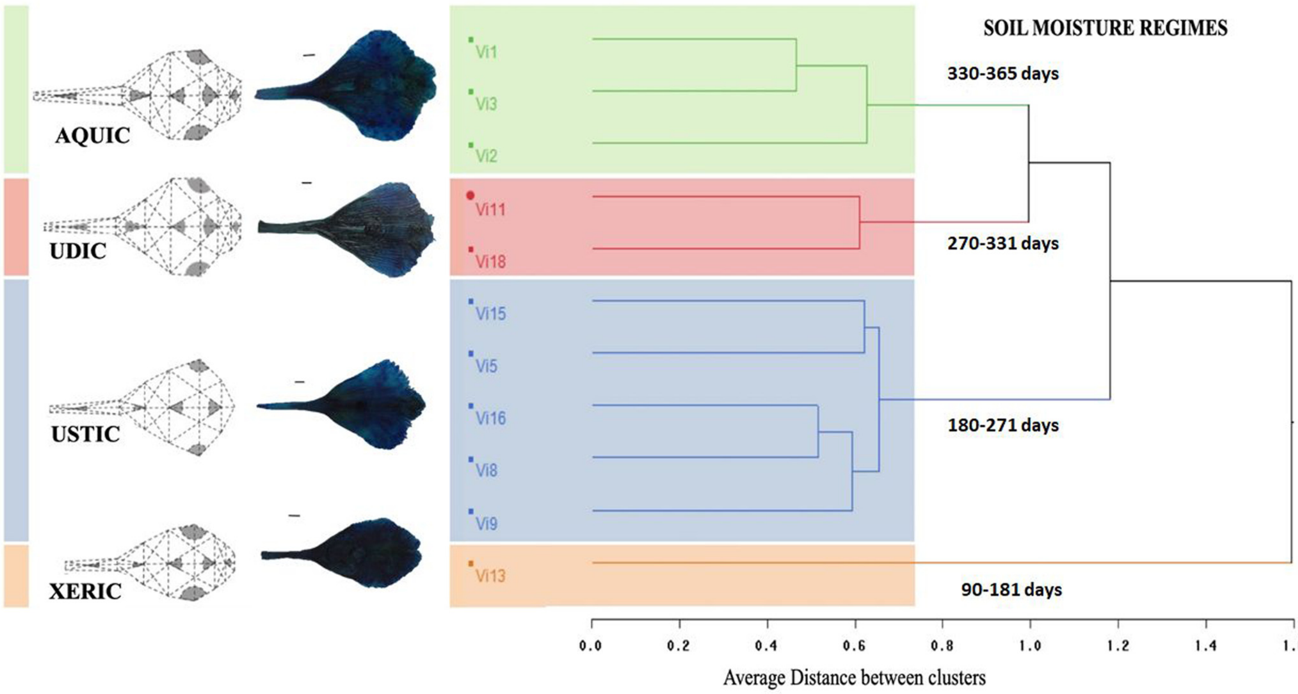

Figure 3. Cluster analysis by hierarchical grouping, constructed with the average of 77 variables analysed in 11 collections *V. insignis*. The scale bar of all the photographs of the labellum is 1 cm.

2010). Variables with CV values >20% correspond to traits that are less heritable and more susceptible to the effects of environmental or geographic heterogeneity (Borba *et al.*, 2007; Morales *et al.*, 2010; Bateman and Rudall, 2011; Bunpha *et al.*, 2014). Therefore, the results obtained in *V. insignis* showed that most of the traits with CV values >20% were concentrated in the apical region of the labellum, and presented highly significant differences ($P \leqslant 0.001$) between environments (Table S1).

Distribution and grouping of V. insignis *populations*

With the information obtained in the principal components and cluster analyses, a grouping pattern was identified in labellum morphology that is probably associated with the soil moisture regime (SMR) in which *V. insignis* populations are located. Climate, solar radiation and altitude did not affect the structure of labellum variation. A reduction in size and modification in the shape of the middle region of the labellum (mainly in the

Table 2. Synthesis of the nine principal traits of the four morphological groups used to describe the infraspecific variation of *V. insignis* in different environmental conditions

Morphotype		I	II	III	IV
Environment		Aquic	Udic	Ustic	Xeric
Traits	Units	Basal Region			
A	(mm)	31.8 ± 2.9a	28.5 ± 2.3ab	27.4 ± 2.2bc	25.0 ± 1.9c
A1		4.8 ± 0.7a	4.1 ± 0.7ab	3.5 ± 0.7bc	3.8 ± 0.2c
		Middle Region			
Σ B + C		26.0 ± 0.7a	25.9 ± 0.7ab	24.9 ± 0.7bc	20.2 ± 0.7c
Σ D + E		22.3 ± 1.6a	21.6 ± 1.5a	22.5 ± 2.3a	26.3 ± 6.3a
ΣD3 + D4		42.9 ± 1.8b	46.4 ± 1.2a	41.7 ± 1.0b	32.0 ± 0.2c
aDE55	Angle (°)	119.4 ± 6.6c	132.0 ± 5.4b	123.0 ± 4.8bc	149.0 ± 1.5a
		Apical Region			
Σ F3 + F4	(mm)	13.3 ± 0.8c	20.4 ± 0.9a	0d	17.7 ± 0.5b
G		2.0 ± 0.7b	5.0 ± 0.7a	0c	4.2 ± 1.0a
aG	Angle (°)	116.4 ± 14.1a	74.5 ± 14.5b	0c	66.3 ± 6.8b

Different letters in each column indicate significant differences ($P < 0.05$). Mean ± standard deviation (SD).

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papilla area) as an effect of decreased availability of soil moisture was observed (Fig. 3). This agrees with what has been reported for other plant species that decrease the size of the petals due to the dry conditions where they grow (Glenny *et al.*, 2018). Halpern *et al.* (2010) mentioned that the decrease in the size of the flower in dry conditions could improve the water status of the plants, improve their photosynthetic capacity and increase the capacity to produce seeds.

At the population level, plants can decrease their adaptation and affect both their reproduction and mortality rates (Edwards *et al.*, 2012), so water availability works as a major selection factor that affects the evolution of morphological traits (Edwards *et al.*, 2012). In the case of *V. insignis*, there are no data on the reproductive success of each morphotype. However, morphological variation among populations reveals gradual adaptation observed in very specific traits of the labellum. Such adaptations were from conditions with high water availability, as is the case of the Aquic SMR, to a dry environment with fewer than 181 days of moisture in the Xeric SMR. In this way, it was possible to identify floral traits of *V. insignis* that are affected by the reduction of soil moisture and that represent possible adaptations to these environmental conditions.

Some authors mention that the morphological variation may be due to the great geographical distances between populations (Hodgins and Barret, 2008). However, it is not the only factor involved in the morphological variation and sometimes there are no differences between distant populations (Rech *et al.*, 2018; Rodríguez-Peña and Wolfe, 2023). This was similar to what we obtained in this work between the populations of Tenampulco, Puebla and Caobas, Quintana Roo, which were grouped in the analysis of PCs and the dendrogram because they share similar Udic conditions. On the other hand, the populations may have cross-pollinations and genetic recombinations, which promotes the flower variation (Rodrigues *et al.*, 2018), however, the separations of the groups in the PCs and the dendrogram is associated with the SMR conditions, so possibly this is the factor that delimits the distribution of the morphotypes.

Soil moisture availability (SMR) has a determining effect on the morphology of floral traits of *V. insignis*. This has been reported for other orchids, especially those with a pollination system by deceit, such as *T. variegata* (Sw.) Braem. In addition, it has been recorded that species present high rates of floral morphological variation associated with abiotic factors such as light and water availability, which suggests floral plasticity phenomena that have been little recognized in orchids (Ackerman and Galarza-Pérez, 1991; Harder and Johnson, 2005; Morales *et al.*, 2010; Hemborg and Després, 2011; Blinova, 2012; Gratani, 2014).

The floral structures of *V. insignis* imply a high expenditure of resources (carbon, nutrients and water) extracted from the vegetative portion of the plants during the life of the flower. The colonization of environments with limited resources, such as the xeric SMR, and the high ecophysiological cost of displaying the flower have generated in *V. insignis* adjustments in the basic structure of the flower. These changes optimize the consumption of plant resources and favour the divergence of four floral morphotypes related to the availability of water availability in the different habitats. However, the floral variation of *V. insignis* can also be related to the populations of pollinators that inhabit these conditions (Glenny *et al.*, 2018), since it has been proposed that environmental conditions can influence the presence and shape of pollinators (Weber *et al.*, 2020), so they are also a key factor in understanding the interrelationships between environment, flower and pollinator, so it is important in the future to carry out studies on the morphology of possible pollinators and their relationship with *V. insignis* morphotypes.

The labellum variables are expressions of genetic variation that can be used for the development of productive models through the genetic improvement of both *V. insignis* and *V. planifolia*. This last species presents problems of genetic erosion, which is why genetic improvement projects have been developed (Hasing *et al.*, 2020; Ramos-Castellá and Iglesias-Andreu, 2022) and possible compatible hybridizations (Ellestad *et al.*, 2022a, 2022b). Currently, few improvement works have been carried out on vanilla due to a lack of understanding of the genetic and phenotypic diversity that exists in the species and compatible species (Hammer and Khoshbakht, 2015; Chambers, 2019). Therefore, by identifying the variation of the floral structure of *V. planifolia* in different environments of Quintana Roo, it will serve for the development of strategies for the conservation of this species, as well as for the improvement of commercial species such as *V. planifolia* that allow the capitalization and preservation of the genetic resource of vanilla.

Conclusions

There is variation in the shape of the labellum due to environmental differences in which the analysed populations of *V. insignis* live, therefore they were grouped into four morphotypes, which suggests that there is floral plasticity. Soil moisture regimes (SMR) are one of the possible factors determining the morphology of the labellum of *V. insignis*. The identification of the morphological variation of the labellum of *V. insignis* contributes to the knowledge of the germplasm of the species to develop strategies for the conservation and use of vanilla.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S147926212300062X.

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