Morphological variation of the labellum of *Vanilla planifolia* Andrews (Orchidaceae) in Oaxaca, Mexico

Variación morfológica del labelo de *Vanilla planifolia* Andrews (Orchidaceae) en Oaxaca, México

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Originales: *Recepción:* 21/05/2019 - *Aceptación:* 04/04/2020

Abstract

Infraspecific variation has transcendental ecological consequences for species adaptation to new niches. Estimating levels of variation is key for understanding the life history of a species, as well as for designing strategies for use and conservation. For this reason, the objective of this study was to characterize infra-specific morphological variation of the labellum of *V. planifolia*, of 122 flowers from 28 specimens from the state of Oaxaca and two specimens from Veracruz, Mexico. The labellum of each flower was dissected and analyzed by morphometry. Sixty variables were obtained and grouped into basal, middle and apical regions. An analysis of variance was performed considering the collections and the origin of each individual as sources of variation. Principal components and cluster analyses were also conducted. Differences in the 60 variables analyzed were highly significant among the collections. Among environments, 18 variables showed significant differences, which were situated in the lateral and middle lobes of the labellum. Thus, these structures were considered susceptible to environmental changes. The remaining 42 variables situated in the basal and middle regions of the labellum, which were fused to the edges of the floral column were not significantly different among environments. With the first three principal components, the model explained 73% of the total variation studied. Morphological variation of the flower labellum was represented by four morphotypes distributed in three environments.

Keywords
environment • primary pool • morphometry • vanilla

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Resumen

La variación infraespecífica tiene consecuencias ecológicas trascendentales en la adaptación de las especies a nuevos nichos. De manera que estimar los niveles de variación es un componente clave para comprender la historia de vida de una especie, así como para el diseño de estrategias de aprovechamiento y conservación. En este sentido, el objetivo de este estudio fue caracterizar la variación morfológica infraespecífica del labelo de *V. planifolia,* de 122 flores de 28 especímenes del estado de Oaxaca y dos especímenes de Veracruz, México. El labelo de cada flor fue diseccionado y analizado por morfometría. Se obtuvieron 60 variables y se agruparon en región basal, media y apical. Se efectuó un análisis de varianza considerando las colectas y el origen de cada individuo como fuentes de variación. También se realizó un análisis de componentes principales y conglomerados. Las 60 variables analizadas fueron altamente significativas entre colectas. Entre los ambientes, 18 variables mostraron diferencias significativas, que se situaron en los lóbulos laterales y medios del labelo. Así, estas estructuras fueron consideradas susceptibles a los cambios ambientales. Las 42 variables restantes situadas en la región basal y media del labelo, que están fusionadas con los bordes de la columna floral no fueron significativamente diferentes entre los ambientes. Con los tres primeros componentes principales, el modelo explicó 73% de la variación total estudiada. La variación morfológica del labelo de la flor estuvo representada por cuatro morfotipos distribuidos en tres ambientes.

Palabras clave

medio ambiente • fondo primario • morfometría • vainilla

Introduction

Vanilla planifolia Andrews, is native to the tropical forests of southeastern Mexico (31). It is the source of vanilla, the most popular aromatic substance in the world (4, 11). This orchid is listed in the category of "high degree of genetic erosion" (9). According to NOM-059-SEMARNAT-2010, it is at risk and subject to special protection in Mexico. Commercial plantations of vanilla were established with the clone called 'mansa' (5), and given this clonal multiplication, it has been subjected to genetic erosion factors (19). For this reason, identifying natural variation is of utmost importance in conservation actions, as they are potential sources of variation and will serve in the rescue and improvement of this irreplaceable genetic resource.

Isoenzyme analysis, nucleotide sequences and RAPDs (27, 29) show major differentiation among vanilla populations in two regions of Mexico: Veracruz, mostly homozygotes related to crops, and northeastern Oaxaca, broader genotypic diversity with some heterozygous individuals, which are different potentially wild specimens.

Information on variation among Oaxaca wild *V. planifolia* populations does not exist, making it difficult to conserve and potentiate use, reproductive and quality characteristics of this wild genetic resources (16, 20). Because the Oaxaca populations are considered to have greater heterozygosity, the hypothesis was that, at the phenotypic level, there is morphological variation of reproductive structures among the different populations. Of the most used methodological strategies for estimating the level of intraspecific phenotypic variation in orchids, morphometric analysis of the labellum has permitted precise identification of variation, even in clone populations (3, 22, 24). It is assumed that because the labellum has a reproductive function, it is less susceptible to the effects of the environment, and thus, it is a valuable diagnostic trait in orchids (6, 13, 22). For this reason, the objective of this study was to identify infra-specific morphological variation of the labellum of *V. planifolia* in 28 collections from the state of Oaxaca and to compare them with two reference specimens collected previously in the state of Veracruz, Mexico.

Materials and methods

Study site

The study was conducted in the state of Oaxaca, Mexico, located between 15°39' and 18°42' N and between 93°52' and 98°32' W, with an area of 95,364 km^2 (18). In each of the 14 sites where *Vanilla planifolia* was collected, the factors precipitation, moisture regime and ecological zone were taken into account since they determine the distribution of the species (14). Environmental variables (table 1) were processed with JMP v. 10 software (25) to generate hierarchical clusters with the 30 collections. Afterwards, environmental grouping was contrasted with the species labellum morphology.

Table 1. Abiotic factors of the collection sites *Vanilla planifolia* in Oaxaca, Mexico.
Tabla 1. Factores abióticos de los sitios de colecta de *Vanilla planifolia* en Oaxaca, México.

Site	Collection	Mean anual precipitation	Soil moisture regime	Ecological zone
Oaxaca				
Cerro Caída	1, 2	> 4000	Type I Udic	Humid temperate
Cerro Hoja	3 - 8	> 4000	Type I Udic	Humid temperate
Usila	9 - 11	2500 a 4000	Type I Udic I	Humid temperate
Stg. Tlatepusco	12, 13	2500 a 4000	Type I Udic	Humid temperate
Cutzoo Loo	14, 15	1500 a 2000	Type II Udic	Humid temperate
Eluruua	16, 17	1500 a 2000	Type II Udic	Humid temperate
Yacuini	18, 19	1500 a 2000	Type II Udic	Humid temperate
Arroyo Chorro	20, 21	1500 a 2000	Type II Udic	Humid temperate
Agua Mohecida	22 - 24	1500 a 2000	Type II Udic	Humid temperate
Agua Concuabe	25	1500 a 2000	Type II Udic	Humid temperate
Cerro Azul	26	1500 a 2000	Ustic	Humid tropical
Pluma Hidalgo	27, 28	1500 a 2000	Ustic	Pacific humid temperate
Veracruz				
Rancho 20 Soles	29	2500 a 4000	Type II Udic	Humid temperate
1ro. de Mayo	30	2500 a 4000	Type II Udic	Humid temperate

Biological material

In April 2013, 122 flowers from 30 specimens (collections) of *V*. *planifolia* were collected in the sites Cerro Caída, Cerro Hoja, Usila, Stg. Tlatepusco, Cutzoo Loo, Eluruua, Yacuini, Arroyo Chorro, Agua Mohecida, Agua Concuabe, Cerro Azul, Zaragoza, Rancho 20 Soles and 1ro. de Mayo (table 2).

Collection and preservation of *V. planifolia* flowers in the field consisted of the following steps. (i) Selection, flowers (vigorous with no visible damage) were cut at the base of the peduncle. (ii) Preservation, the flower was submerged in an ethanol-based preservative solution (50%), lactic acid (4%), benzoic acid (0.5%), glycerin (2.5%) and distilled water (43%) in a 150 mL glass recipient. (iii) Labeling, each recipient was identified inside and outside by date, place UTM coordinates and collection number (table 2). (iv) Conservation, the recipients were placed at room temperature in a dark room.

Evaluated traits

Morphological characterization of the *V. planifolia* labellum was based on the technique and scheme of Herrera *et al.* (2006), which was modified through the following steps. i) Dissection of the flower and extending the labellum on a glass surface.

Table 2. Geographic location of the collection sites and number of replications *V. planifolia* in Oaxaca, Mexico.

Tabla 2. Ubicación geográfica de los sitios de colecta y número de repeticiones de *V. planifolia* en Oaxaca, México.

Municipality	Locality	Site	Longitude[†]	Latitude[†]	Collection (N°)	Num. flowers per collection
San Felipe Usila	Arroyo Aguacate	Cerro Caída	1981808	765380	1, 2	7
	Arroyo Iguana					
	San Felipe Usila	Cerro Hoja	198514	766867	3 – 8	8
		Usila	1978103	761904	9 – 11	5
	Stg. Tlatepusco	Stg. Tlatepusco	1972242	763881	12, 13	6
Santiago Lalopa	Quera Quiets	Cutzoo Loo	1928187	791220	14, 15	6
Yae	Lachichina	Eluruua	1931801	791964	16, 17	8
		Yacuini	1931507	792295	18, 19	6
Sochiapam	Sochiapam	Arroyo Chorro	1972072	747411	20, 21	7
Chiquihuitlan de B. Juárez	Loma Ojiteca	Agua Mohecida	1992850	736520	22 – 24	6
		Concuabe	1993074	736191	25	7
Santa Ma. Chimalapa	Zacatal	Cerro Azul	1865068	315789	26	3
Pluma Hidalgo	Pluma Hidalgo	Zaragoza	1761267	775017	27, 28	6

* UTM Coordinates. / * UTM Coordenadas.

ii) Impregnation of the labellum with methylene blue (0.08%). iii) Photographic capture of the labellum with a Sony alpha Reflex camera 65v, equipped with a Sony DT 30mm F/2.8 SAM macro lens. iv) Image processing with the software CorelDraw X7 for generation and evaluation of 53 lines and 7 angles.

The 60 variables analyzed were grouped in three regions of the labellum (figure 1): basal region formed by section A (A, A1, A2, A3, A4, A5, aA, B1x), middle region made up of four sections, section B (B, B2, B5, B7, B8, B9, B10, aB), section C (C, C1x, C2, C5, C7, C8, C9, C10), section D (D, D1x, D2, D5, D7, D8, D9, D10, aD, aDE22, aDE55), section E (E, E1x, E2, E5, E7, E8, E9, E10, aE), and the apical region comprising two sections, section F (F, F1x, F2, F5, F7, F8, F9, F10) and section G (G, G1x, G2, G3, G4, G6, G7, aG).

Statistical analysis

Two analyses of variance were performed. In the first, the collections were considered sources of variation, so that 30 treatments were evaluated with different numbers of replications (number of flowers) (table 2, page 163). The traits per treatment were analyzed using a model equivalent to the completely randomized unbalanced design.

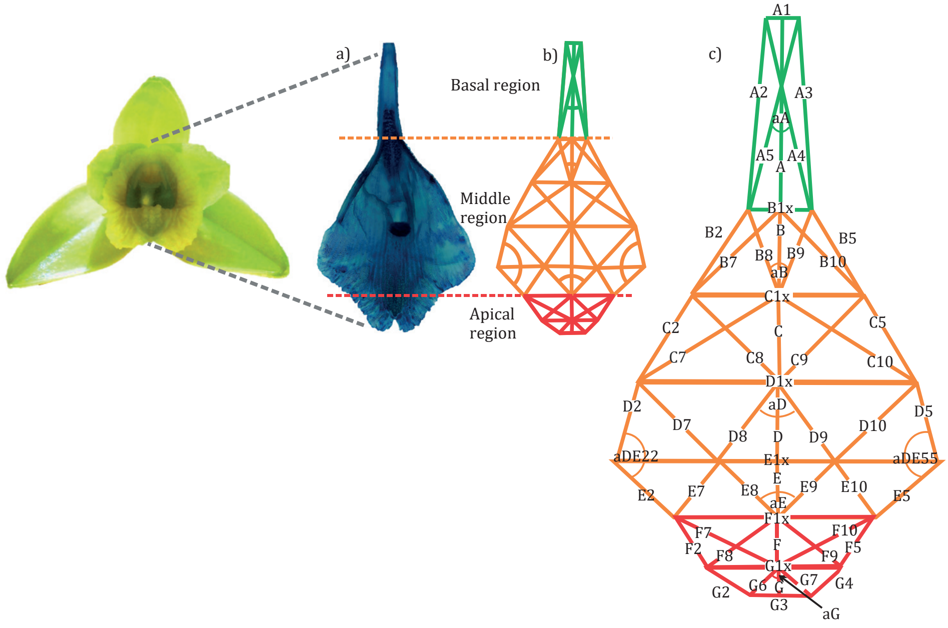


Figure 1. Flower and scheme of the *V. planifolia* labellum. a) Extended labellum in which the basal, middle and apical regions are shown. b) Lines and angles used for the analysis. c) Name and location of the variables used.

Figura 1. Flor y esquema del labelo de *V. planifolia*. a) Labelo extendido donde se muestra la región basal, media y apical. b) Líneas y ángulos utilizados para el análisis. c) Nombre y ubicación de las variables utilizadas.

Comparison of collection means was calculated based on the harmonic mean (n) with the Tukey test (26). In the second, averages of the data from 30 collections were used; the ecological zone of origin of each collection was considered (table 1, page 162) using an analysis of variance with a nested factor (collection nested within the interaction site-environment) in the software SAS V.9.0. (26) to estimate how labellum shape is structured relative to the environment. A multivariate analysis of the *V. planifolia* collections was conducted with two numerical analysis methods: principal components (PCA) based on the matrix of correlations between selected variables, and cluster analysis with mean distance between clusters, in the software SAS v 9.0 (26). For the numerical analysis, the means of each of the 60 evaluated traits of the specimens from each site of origin were used.

Results and discussion

Environments of the study sites

The cluster analysis of the environmental characteristics of the *V. planifolia* collections at a euclidian distance of 1.8 defined three groups: Teh (Temperate humid), PTe (Pacific Temperate humid) and Trp (Tropical humid) (figure 2).

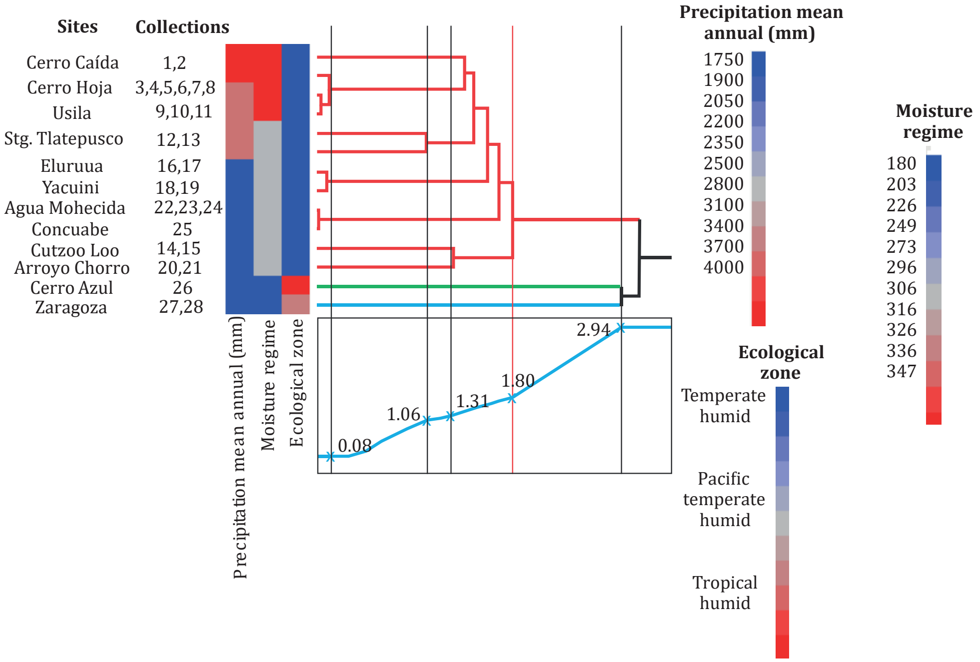


Figure 2. Clustering of sites and *V. planifolia*, collections, based on environmental characteristics in Oaxaca, Mexico.

Figura 2. Agrupación de sitios y colectas de *V. planifolia*, basadas en características ambientales en Oaxaca, México.

The Teh group relates 27 collections with characteristics of the temperate humid ecological zone, which has a type I udic soil moisture regime (330 to 365 days of moisture), warm climate and precipitation of 2500 to 2000 mm. The PTe group, comprising collections 27 and 28, has characteristics of the Pacific temperate humid ecological zone, which has a ustic soil moisture regime (180 to 270 days of moisture), warm subhumid climate (Aw1), and precipitation of 1500 to 2000 mm. The Trp group is made up of collection 26, having characteristics of the tropical humid ecological zone, with a type II udic soil moisture regime (270 to 330 days of moisture), hot humid climate and precipitation of 1500 to 2000 mm.

The *V. planifolia* collections that are distributed in the state of Oaxaca exhibit highly significant statistical differences in the 60 evaluated traits of the labellum ($p \leq 0.01$), suggesting the existence of morphological variation among the collections (table 3, page 167-168). According to the coefficients of variation, some variables had slightly higher values (14.01 to 15.33) associated to the shape and size of the middle lobe.

For comparison of the collections of vanilla from different environments, statistical variation was in function of the labellum trait evaluated. Nine variables (D5, E2, E5, E7, aE, F2, F7, F10, G7) had significant differences ($p \leq 0.05$), nine (D2, F1x, F8, F9, G1x, G2, G4) had highly significant differences ($p \leq 0.01$), and the 42 remaining variables, located in the basal region and in sections B and C of the middle region of the labellum, were not statistically different (table 3, page 167-168).

For the specimens collected in Oaxaca, the 60 traits evaluated in the *V. planifolia* labellum contribute information for determining morphological variation within the species. This confirms that in the Orchidaceae family and specifically in *V. planifolia*, the use of labellum traits is useful for identification of infra-specific variation (24).

Regarding environment, the statistically significant variables were situated mainly in two sites (figure 3, page 169). The first is on the edges of the lateral lobes and where the penicillate callus, formed by eight scales, flabellate and premorsas, is found and is characteristic of the species *V. planifolia* (29). The second site was on the edges of the middle lobe, where two conspicuous rows and 2 to 4 less conspicuous rows of papillae are found (31). The differences found in the variables of the middle and apical region of the labellum (figure 3, page 169) should be interpreted as a result of the process of adaptation (7), in which survival in the wild state requires adaptation to biotic and abiotic surroundings (8, 21). Moreover, it can be inferred that the variables situated in the basal region of the labellum (figure 3, page 169) and in section B and C of the middle region (figure 1, page 164), areas that are fused to the edges of the flower column (30), seem less susceptible to environmental changes than the upper middle region (section D, E and F) and the apical region of the labellum. It has been stated that floral organs require an elevated expenditure of resources (carbon, nutrients and water) that are extracted from the vegetative portion of the plants during the live of the flower. For this reason, in environments with limited resources allotment of resources for floral development can be very costly in terms of growth and survival. The shape or size of the flowers when they are optimized to attract pollinators are different from the shape and size when they optimize plant consumption of resources (23). The selection pressures in conflict favor divergence of flower shapes related to availability of abiotic resources in different microhabitats or over environmental gradients (1, 10).

Table 3. Average values, coefficients of variation, square mean error, collection and environment related to 60 labellum traits in the evaluation of 30 collections of *V. planifolia*, Oaxaca, México.

Tabla 3. Valores promedio, coeficientes de variación, cuadrado medio del error de colección y ambiente relacionados con 60 rasgos del labelo en la evaluación de 30 colecciones de *V. planifolia*, Oaxaca, México.

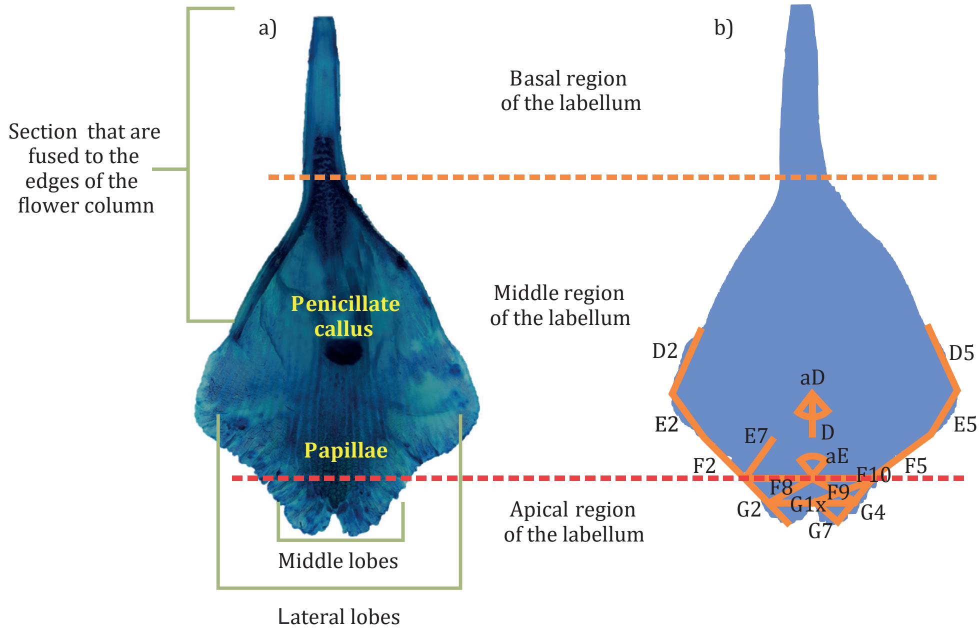
Variable	Mean (mm)	Coefficient of variation	Mean squares		
			Error	Collection	Ecological Zone
I. Basal labellum region					
A	16.32	4.57	0.55	5.13**	13.65^{ns}
A1	1.97	13.68	0.07	0.26**	0.25^{ns}
A2	16.42	4.45	0.53	4.60**	14.55^{ns}
A3	16.40	4.51	0.54	4.46**	14.18^{ns}
A4	16.56	4.45	0.54	4.63**	13.56^{ns}
A5	16.59	4.48	0.55	4.65**	13.54^{ns}
aA	21.99	8.79	3.73	16.44**	45.59^{ns}
B1x	4.45	9.42	16.20	0.56**	1.26^{ns}
II. Middle labellum region					
B	8.17	6.52	0.28	0.84**	2.22^{ns}
B2	9.26	7.19	40.92	1.13**	2.23^{ns}
B5	9.34	5.92	0.30	0.91**	0.24^{ns}
B7	10.48	6.41	0.45	1.17**	2.75^{ns}
B8	8.49	6.22	0.27	0.86**	2.42^{ns}
B9	8.50	5.82	0.24	0.85**	1.49^{ns}
B10	10.50	5.87	0.38	1.05**	1.90^{ns}
aB	30.11	10.1	9.26	42.32**	99.24^{ns}
C	8.21	4.92	0.16	0.69**	0.59^{ns}
C1x	13.29	7.83	1.08	2.70**	3.30^{ns}
C2	9.28	5.40	0.25	0.84**	0.21^{ns}
C5	9.35	5.48	0.26	1.11**	0.41^{ns}
C7	13.39	6.23	0.69	2.74**	0.67^{ns}
C8	10.60	5.34	0.32	1.16**	0.20^{ns}
C9	10.51	5.57	0.34	0.76**	0.41^{ns}
C10	13.54	5.75	0.6	2.53**	0.62^{ns}
D	7.37	7.3	0.29	1.05**	6.71**
D1x	21.85	5.68	1.54	7.48**	0.92^{ns}
D2	7.48	8.52	0.40	1.46**	8.62**
D5	7.38	7.47	0.30	1.19**	5.28*
D7	10.06	6.61	0.44	1.94**	7.56^{ns}
D8	8.40	6.62	0.30	0.84**	2.61^{ns}
D9	8.41	6.49	0.29	0.87**	2.05^{ns}
D10	10.00	6.70	0.44	2.17**	5.45^{ns}

Table 3 (cont). Average values, coefficients of variation, square mean error, collection and environment related to 60 labellum traits in the evaluation of 30 collections of *V. planifolia*, Oaxaca, México.

Tabla 3 (cont). Valores promedio, coeficientes de variación, cuadrado medio del error de colección y ambiente relacionados con 60 rasgos del labelo en la evaluación de 30 colecciones de *V. planifolia*, Oaxaca, México.

Variable	Mean (mm)	Coefficient of variation	Mean squares		
			Error	Collection	Ecological Zone
aD	57.74	7.56	19.07	130.72**	22.96**
aDE22	124.88	5.48	46.84	210.89**	884.33^{ns}
aDE55	124.82	5.35	44.62	171.61**	667.88^{ns}
E	4.36	8.40	0.13	0.28**	0.45^{ns}
E1x	23.32	5.29	1.52	8.31**	2.89^{ns}
E2	6.73	7.67	0.26	1.33**	4.74*
E5	6.7	9.76	0.42	1.22**	5.82*
E7	4.99	8.79	0.19	0.54**	1.97*
E8	5.92	6.69	0.15	0.68**	1.68^{ns}
E9	5.94	7.52	0.20	0.58**	0.57^{ns}
E10	5.01	8.96	0.20	0.58**	1.18^{ns}
aE	86.12	4.71	16.47	39.99**	161.86*
II. Apical labellum region					
F	2.63	8.84	0.05	0.12**	0.20^{ns}
F1x	13.04	8.05	1.10	5.58**	36.38**
F2	3.12	10.25	0.10	0.35**	4.12*
F5	3.17	12.08	0.14	0.28**	0.33^{ns}
F7	5.62	9.97	0.31	0.96**	4.11*
F8	6.91	10.14	0.49	1.81**	14.80**
F9	6.88	9.68	0.44	1.58**	8.80**
F10	5.60	12.13	0.46	1.05**	4.35*
G	2.42	15.33	0.13	0.54**	1.50^{ns}
G1x	9.67	8.54	0.68	3.27**	9.11**
G2	3.62	14.01	0.25	0.87**	11.97**
G3	4.15	14.48	0.36	1.11**	1.08^{ns}
G4	3.71	14.85	0.30	1.17**	7.94**
G6	3.24	12.36	0.16	0.67**	1.69^{ns}
G7	3.23	12.00	0.15	0.47**	1.47*
aG	81.81	9.20	56.76	418.37**	522.45^{ns}

** = $p \leq 0.01$; * = $p \leq 0.05$; ns = not significant. / ** = $p \leq 0,01$; * = $p \leq 0,05$; ns = no significativo.



a) regions and main parts of the labellum, b) variables with statistically significant differences among environments.
a) regiones y partes principales del labelo, b) variables con diferencias estadísticamente significativas entre los ambientes.

Figure 3. The extended labellum of *V. planifolia*.

Figura 3. Labelo extendido de *V. planifolia*.

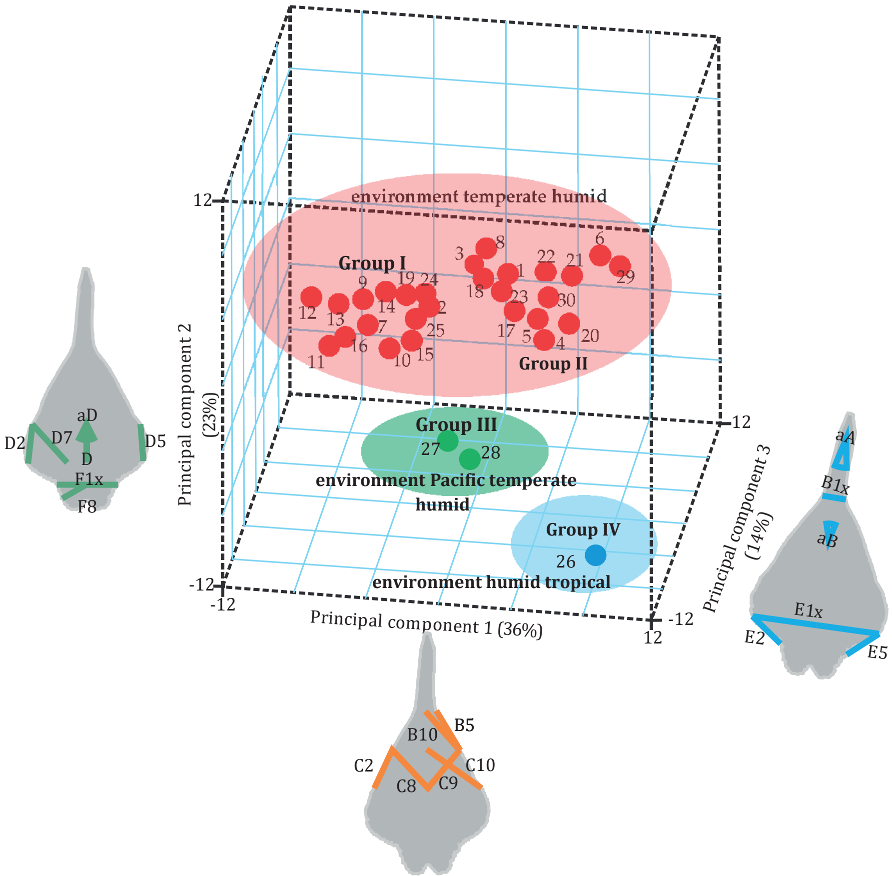
In the case of vanilla, apparently, resource limitations do not affect the entire flower. In the specific case of the labellum, the region related to the location of the pollinators for transfer of pollen is the most conserved organ in different environments, and it is only the apical portion, which corresponds to the labellum ornaments that has the function of attracting pollinators, that is modified, possibly in response to changes in resource availability.

Distribution of morphological variation

With the average value of the 60 evaluated variables, the 122 flowers grouped in 30 collections of *Vanilla planifolia* were subjected to an analysis of principal components (PCA). The dispersion of the 30 collections represented in the space determined by the first three principal components explained 36%, 23% and 14% of the total variation, respectively.

The first principal component (PC1) was more associated with variables that determine shape and size of sections B and C of the middle region of the labellum (B5=0.17, B10=0.18, C2=0.17, C8=0.18, C9=17.9, C10=0.18). The second, PC2, was determined by dimensions and shape of section D (D=0.22, D2=0.20, D5=0.19, D7=0.19, aD=-0.26) and size of the middle lobe (F1x=-0.19, F8=-198). PC3 expressed the size and opening of the lateral lobes

(E1x=0.24, E2=0.22, E3=0.23) and the horizontal size of the basal region that is fused to the floral column (aA=0.29, aB=0.25, B1x=0.26). Spatial distribution of the collections, according to principal components 1, 2, and 3 (figure 4), shows that the material collected in the temperate humid (Teh) environment form two groups, that are close to each other. In contrast, collections 27 and 28 from the Pacific temperate humid (PTh) environment and collection 26 from the humid tropical (Trh) environment, constitute independent groups, completely separated from each other.



The scheme of each labellum in one of the PC indicates the variables with the greatest weight in explaining total variation of the model.

El esquema de cada labelo en cada uno de los PC indica las variables con el mayor peso para explicar la variación total del modelo.

Figure 4. Dispersion of 122 flowers grouped in the mean of 30 collections of *V. planifolia*, based on the first three principal components (PC).

Figura 4. Dispersión de 122 flores agrupadas en la media de 30 colectas de *Vanilla planifolia*, basado en los tres primeros componentes principales (PC).

Morphological distribution, based on PC1, shows that the collections with greater vertical dimension and opening in the middle regions (B5, B10, C2, C8, C9, C10) of the labellum are distributed toward the right side of the graph and constitute collection 26 of group IV. Meanwhile, the smaller proportions of the same variables are placed on the left side and make up collections 11, 12, 13, 16 9, 7, 14 10, 19, 15, 24, 25 and 2 of group I. PC2 places groups I and II on the positive side. These groups have larger horizontal dimensions of the lateral lobes (D, D2, D5, D7, aD) and larger diameter and length of the middle lobe of the labellum (F1x, F8). Groups III and IV are placed in the negative quadrant. These groups have flowers with smaller dimensions in these sections. PC3 places the collections of groups I and II in the positive quadrant because the basal region of the labellum is larger (aA, B1x). They were also larger in section E (E1x, E2, E5), unlike group IV, which is found in the negative quadrant of PC3 and has smaller dimensions in these two regions.

The 122 flowers grouped in the mean of 30 *Vanilla planifolia* collections were subjected to a cluster analysis. Using the software Past. V. 3.0 (12), the optimum number of groups based on a bootstrap of 70% similarity among groups was calculated using Euclidian distance and average distance. At a distance of 0.9 in the dendrogram of figure 5, grouping of *Vanilla planifolia* complex is observed.

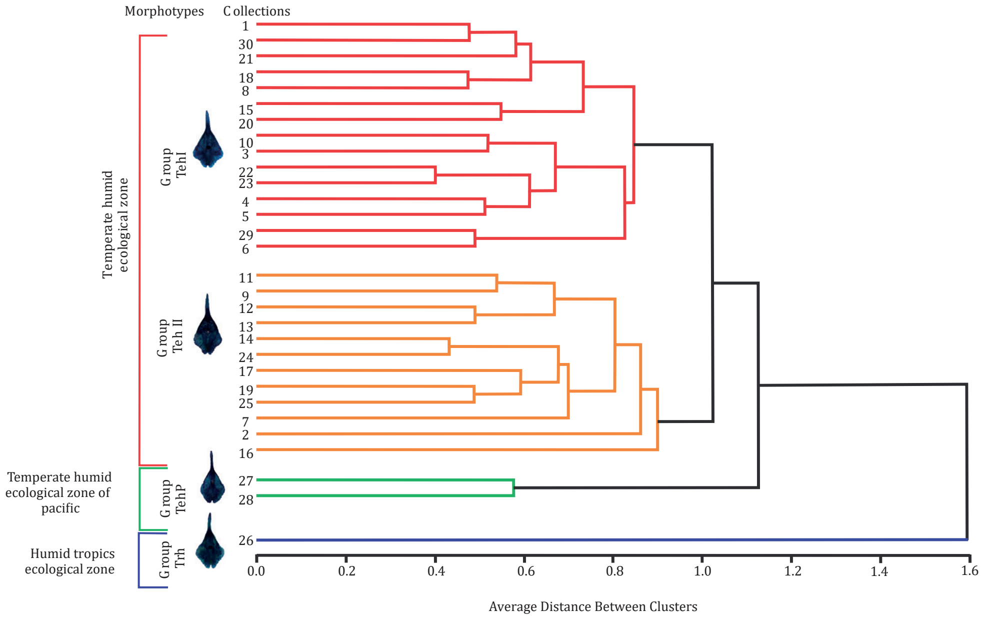


Figure 5. Dendrogram of the means of 30 grouped *V. planifolia* collections (122 flowers) from the state of Oaxaca, Mexico, based on the averages of 60 traits at a mean distance between clusters.

Figura 5. Dendrograma de las medias agrupadas de 30 colectas de *V. planifolia* (122 flores) del estado de Oaxaca, México, sobre la base de los promedios de 60 caracteres a una distancia media entre grupos.

The description of infra-specific morphological variation exhibited in the labellum of *V. planifolia* Andrews, within the environmental complex of the state of Oaxaca, was the following.

a) Group TehI comprises 15 collections. It is characterized by a labellum that has the largest dimensions of width, with a mean of 22.84 mm and inverted trapezoid shaped lateral lobes narrow at the base (9.89 mm) and wider toward the apex (13.40 mm) (figure 5, page 171). This group contains two specimens from Veracruz, suggesting that of the plantations of this site have plants from the state of Oaxaca. The collections of this group are distributed in the temperate humid ecological zone.

b) Group TehII comprises 12 collections. The middle region (20.62 mm long) and the apical region (9.07 mm long) of the labellum are the smallest among the analyzed material. The trapezoid-shaped division between the middle and apical regions exists, but has smaller dimensions than the TehI group (figure 5, page 171). The collections are distributed in the temperate humid ecological zone.

The populations of *V. planifolia* located in the northeastern part of the state that have TehI and TehII morphotypes are in an area delimited by the Sierra Norte mountains of Oaxaca. This mountain system, which reaches altitudes above 3000 m, is a geographic barrier. The populations found outside this barrier and subjected to humid tropical and Pacific temperate humid environmental conditions express Trh and Tehp morphotypes, respectively (figure 5, page 171). This is interpreted as an adaptation of a wild species to the surroundings where it develops (2, 28).

c) Group TehP comprises two collections that are longer at the base of the apical lobes (10.68 mm) than groups ThpI and ThpII, but they have similar diameter in the middle region of the labellum (22.3 mm). For this reason, the division between the middle and the apical regions blurs and takes on a trapezoidal shape, elongated with a base of 10.68 mm and wider toward the apex (14.55 mm), with a diameter of 4.37 mm (figure 5, page 171). This group is distributed in the temperate humid ecological zone, influenced by the Pacific coasts, so that the soil moisture regime is drier (180 to 279 days) than that where the TehI and TehII groups are distributed.

d) Group Trh has only one collection. It is defined by the presence of a labellum that is narrower in the basal region (3.70 mm) than that of other groups. Width of the middle region of the labellum is medium (21.83 mm), relative to the other groups. The apical lobes are longer (13.05 mm) and the aperture is smaller (63°) than those of the rest of the analyzed specimens. In addition, the division in the transition from the middle to the apical region create a rhomboid shape (figure 5, page 171). This collection is found in an isolated complex, east of the state of Oaxaca where environmental characteristics are those of the humid tropics: altitude is not above 480 m, mean annual temperature is 25° C and the moisture regime is 180 days. This is the most tropical site from where the species was collected.

The populations that express morphotypes Tehp and Trh are closer to the Pacific coast and separated by the mountain complex belonging to the Sierra Madre del Sur, where the highest altitude in the state of Oaxaca is found. These characteristics have facilitated isolation of the biotic communities in this region (31, 32). The Sierra Madre del Sur is an effective geographic barrier that impedes genetic drift or exchange of pollen (27) between the *V. planifolia* populations of the southeast and those of the north-eastern part of the state of Oaxaca. Thus, the differences found among groups should be interpreted as a result of the process of adaptation of the populations to environments (1, 6, 15, 17) where mountainous complexes have served as geographic barriers.

Conclusions

The use of morphometric traits of the labellum of *V. planifolia* is useful to identify the variation at the infraspecific level in this species. Particularly, traits related to the shape of basal and middle regions of labellum, were the most informative for distinguishing variation among specimens. While the traits related to the shape of the upper and apical region of the labellum, explained variation related to environmental factors. Morphometric analysis of the labellum revealed infra-specific variation in the germplasm of *V. planifolia* from Oaxaca, represented by four floral morphotypes.

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