Vanilla pompona Schiede: Morphological Variation of the Labellum in Localities of Veracruz, Puebla, Jalisco and Oaxaca, Mexico

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Abstract: *Vanilla pompona* is the third most important commercial species of the genus *Vanilla*, but its morphometric variation is little known. In orchids, infraspecific variation is expressed in the labellum. This study had the objective of analyzing the floral variation of *V. pompona* collections from localities in Veracruz, Puebla, Jalisco and Oaxaca, Mexico. During the flowering period, we obtained 55 collections of *V. pompona* flowers, and in the dissected labellum of each flower 54 lines and 7 angles were measured and portrayed with the ImageJ program. With the data obtained, we performed an analysis of variance, a principal components analysis, and a cluster analysis. The results showed significant differences among the collections and localities in the means of the lines of the basal, middle, and apical regions of the labellum. Also, six morphotypes were obtained, and we found that variation was associated with factors such as the environment, geographic barriers, and pollinator pressure. Moreover, we observed variation in flowering dates in one locality of Veracruz. We concluded that there is infraspecific variation within and between the collection localities of *V pompona* of the Atlantic region (Puebla and Veracruz) and the Pacific region (Jalisco and Oaxaca), Mexico.

Keywords: orchids; morphometry; vanilla; infraspecific variation

1. Introduction

Vanilla pompona Schiede and *Vanilla* p*lanifolia* Andrews are two relevant species of the genetic reservoir of the genus *Vanilla* because of their economic importance [1–4]. *V. pompona* pertains to the family Orchidaceae, subfamily Vanilloideae, tribe Vanilleae, genus *Vanilla*, species *V. Pompona* [5,6], subspecies *pompona*, *grandiflora* and *pittieri* [4]. *V. pompona* is a robust plant of broad leaves, resistant to diseases, such as anthracnosis (*Collectotrichum* sp.) and root rot (*Fusarium oxysporum* Schltdl) [7], and tolerant to xerophyte climates [4,5], with a flowering period from April to June [4]. This species is distributed in several humid subtropical forests (deciduous, evergreen, gallery forests) and in pine and oak forests in warm climates [8,9]. Currently, *V. pompona* is an endangered species because its fragmented populations are decreasing [9]. However, there are reports that the processed fruits are used in the cosmetic and perfume industries [1] and in the preparation extracts for flavoring [10].

In Mexico, some morphological differences in the flower have been registered in two biological populations of *V. pompona*. The first population is characterized by flowers with extended segments of large dimensions, located in northern Oaxaca and in Veracruz, on the slope facing the Gulf of Mexico. The second population is characterized by semi-closed flowers of smaller dimensions and is located on the Mexican Pacific coast [5].

Morphological traits of plants are used to analyze variation within and between species, particularly reproductive morphological traits, such as flowers, which are more stable than

morphological traits of vegetative organs [11,12]. The *V. pompona* flower is made up of sepals, petals, labellum, penicillate callus, column, stigma, and one ovary [4]. In orchids, the labellum is a modified petal [13], which owes its existence and aspect to the presence of specific genes involved in the evolutionary floral development of this plant family [14,15]. It functions as a platform for insects to land on and to attract pollinators [16]. The labellum, as a reproductive structure of orchids, is less susceptible to the environment [11], and for this reason, it is used to identify species within this family [4,17] and to analyze infraspecific variation [18,19]. For the genus *Vanilla*, flowers are used to differentiate leafless species in the Southwestern Indian Ocean region, considering differences in the shape of the labellum [20]. Infraspecific variation has also been evaluated by characterizing the labellum shape of species such as *V. planifolia* [21] and *V. pompona* [22]. In the Mesoamerican region, *V. planifolia* variation is identified by four morphotypes in Oaxaca [23], four morphotypes in the San Luis Potosí Huastec region, Mexico [21], and five morphotypes in the Hidalgo Huastec region, Mexico [24]. For *V. pompona*, floral morphological variation of the labellum is still being studied; to date, there is only one study that reports four morphotypes in collections from localities in Oaxaca. This variation is explained by the constant selection pressure exerted by pollinators together with the environment [22].

Genetic diversity or genetic variation within species, referring to populations and individuals that comprise them [25], is one of the forms of biodiversity that should be conserved [26]. Knowledge and understanding of the variation within species are vital for the conservation of ecosystems and for the social and cultural wellbeing of man [27,28].

It is not known whether clonal reproduction, disappearance of the habitat where the species is distributed, such as deciduous tropical forests, gallery forests, and tropical pine and oak forests [9], and problems of genetic erosion [29] have caused low genetic variation in *V. pompona*. Recently, studies have been conducted to determine genetic variation [30,31] and floral morphological variation [21–23,26] of the most used *Vanilla* species, in a search for different stable populations that would increase genetic variation [3,31]. However, for *V. pompona*, detailed information on genotypes and phenotypes that make up the primary genetic pool and its interaction with the environment is limited. Its cultivation is recent, and thus, both traditional and technological knowledge of its cultivation and its infraspecific variation is also limited.

Plantations with incipient traditional management located close to natural forests maintain structural diversity, endemic species, and regional native species [32,33], contributing to conservation of adjacent ecosystems [27]. Perennial plants, such as *V. pompona*, can grow in agroforestry systems where the trees that provide support and shade for this species maintain biogeochemical cycles and help to preserve the local landscape and biodiversity [34]. For example, agroforestry systems where *V. planifolia* is cultivated in northeastern Madagascar, are recognized as important areas for the conservation of birds [35]. For this reason, traditional *Vanilla* systems could be considered a determining factor in the preservation of this genetic resource [3,12,31].

In the study of orchid variation, it is important to use an integrated approach, with classic taxonomy [20] as well as morphometric analysis of the labellum [19,21,23], relating it with biotic factors (pollinators) and abiotic factors (environment). Because variation of *V. pompona* on the Mexican Altantic slope or the Pacific Coast is unknown, this study aims to demonstrate that variation of the flower labellum of *V. pompona* exists. Also, it will contribute knowledge on the floral labellum variation related to *V. pompona* flowering dates. In orchids, biotic and abiotic factors, such as temperature and light, have been reported to influence the flowering period [36.37]. For this reason, the objective of this study was to determine morphological variation of the *V. pompona* labellum in collections from localities of Veracruz, Puebla, Oaxaca, and Jalisco. We believe that this knowledge is useful for conservation, management, and use of this species.

2. Materials and methods

2.1. Geographic location

In this study, *V. pompona* flowers were collected in the states of Jalisco, Oaxaca, Veracruz and Puebla, Mexico (Figure 1, Table 1).
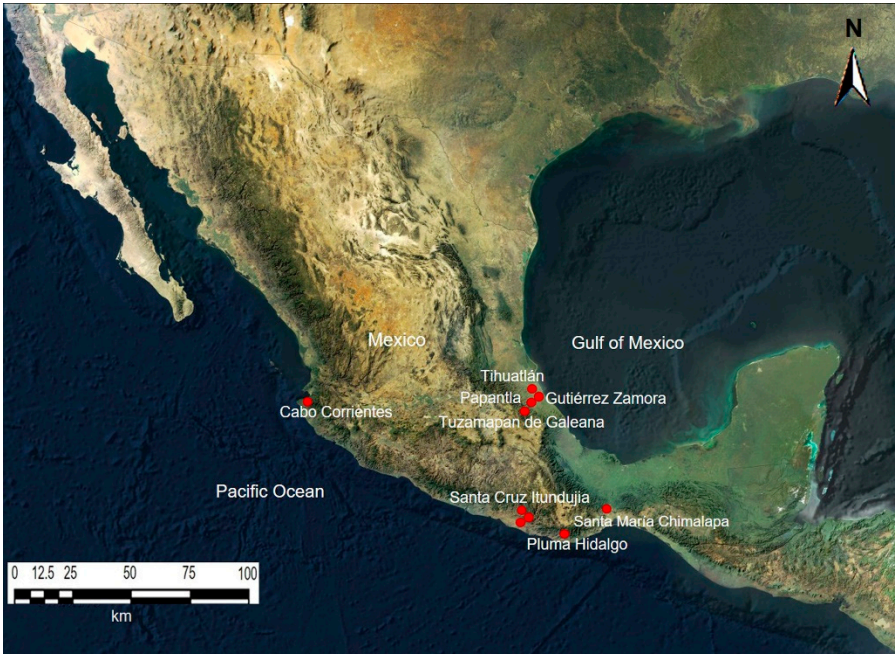
Figure 1. Geographical location of the collection sites of *Vanilla pompona* flowers in Mexico. The red dots show the collection sites: Cabo Corrientes (Jalisco), Santa Cruz Itundujia, Pluma Hidalgo and Santa María Chimalapa, (Oaxaca), Tuzamapan de Galeana (Puebla), Papantla, Tihuatlán and Gutiérrez Zamora (Veracruz). .

Table 1. Localities where *Vanilla pompona* were sampled in several states of Mexico.

State	Municipality	Locality	Altitude (m)
	Papantla	Cazuelas	69
Veracruz	Gutiérrez Zamora	Paso de Barriles	29
	Tihuatlán	La Pasadita	159
Puebla	Tuzamapan de Galeana	Reyes de Vallarta	365
Jalisco	Cabo Corrientes	Cabo Corrientes	379
		Hidalgo	1075
	Sta. Cruz Itundujia	Morelos	1668
Oaxaca		Primavera	651
	Sta. Ma. Chimalapa	Sta. Ma. Chimalapa	362
	Pluma Hidalgo	Pluma Hidalgo	1010

At each of the sampling sites, we obtained climate type, mean annual precipitation (mm), mean annual temperature (°C), soil moisture regime, and ecological zone [38] (Table 2).

Table 2. Environmental variables of the *Vanilla pompona* flower collection localities. **.**

Locality	Climate	Mean annual precipitation (mm)	Mean annual temperature (°C)	Soil moisture regime	Ecological zone
Cazuelas	Warm subhumid	800-1200	>22	Ustic	Humid tropical
Paso de Barriles	Warm subhumid	1500-2000	>22	Udic type II	Humid tropical
La Pasadita	Warm subhumid	1200-1500	>22	Ustic	Humid tropical
Reyes de Vallarta	Warm humid	2500-4000	>22	Udic type I	Humid tropical
Cabo Corrientes	Warm subhumid	1500-2000	>22	Xeric	Subhumid tropical
Hidalgo	Semi-warm subhumid	2000-2500	>22	Xeric	Subhumid temperate
Morelos	Semi-warm subhumid	2000-2500	>18	Xeric	Humid temperate
Primavera	Semi-warm subhumid	2000-2500	>22	Xeric	Subhumid temperate
Sta. Ma. Chimalapa	Warm humid	1500-2000	>22	Ustic	Humid tropical
Pluma Hidalgo	Warm subhumid	1500-2000	>22	Ustic	Humid temperate

Udic type I = 330 to 365 days of rain; Udic type II = 270 to 330 days of rain; Ustic = 180 t0 270 days of rain; Xeric = 90 to 180 days of rain [38].

Geographic localities of Mexico where *Vanilla pompona* flowers were collected.

2.2. Study area

Vanilla pompona Schiede flowers were sampled during the flowering period (March-May) in the localities of Cazuelas, Paso de Barriles, La Pasadita, Cabo Corrientes, Hidalgo, Morelos, Primavera, Santa María Chimalapa, Pluma Hidalgo and Reyes de Vallarta. These are the places where we had the opportunity to collect flowers since there is not much information on where specimens of this species are found. Also, the collections we obtained could represent part of the *V. pompona* variation that exists in Mexico (Table 1)

2.3. Material vegetal

We obtained 55 collections of *V. pompona* flowers during the flowering period (March-May) in the years 2019 to 2022. Each collection had three to five replications, and we obtained a total of 192 flowers with pollinia and no observable damage (Figure 2). In the locality of Cazuelas, Veracruz, at the suggestion and empirical knowledge of the owner of the lot, the flowers were collected on different dates of the year. The clones were named according to the flowering period: early flowering clone (March-May), continuous flowering clone (March-October), and late flowering clone (June-August).
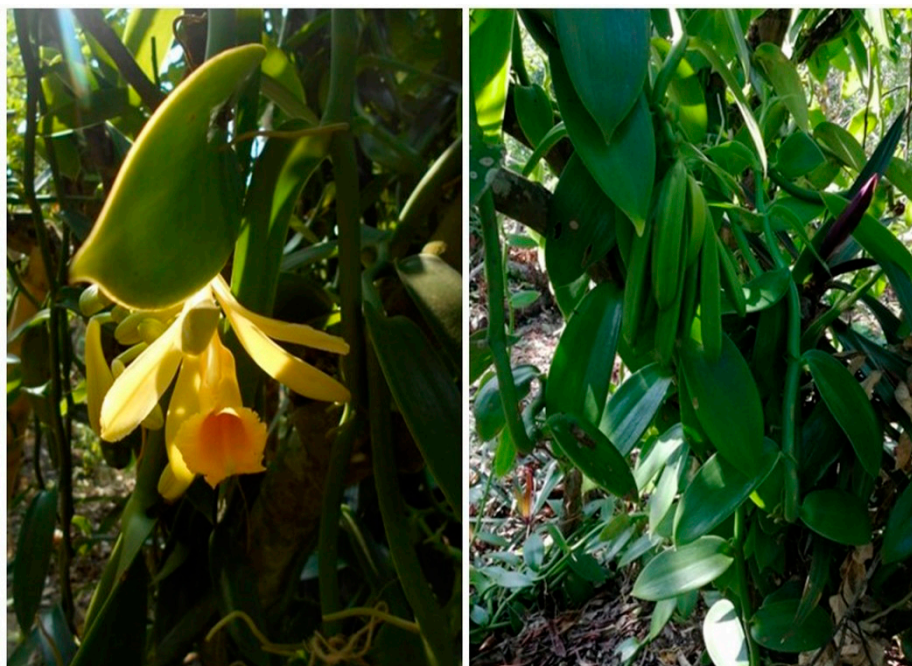
Figure 2. Phenological stages of *Vanilla pompona*; flowering in April and mature fruits in December.

The collected flowers were stored in a preservative solution. Each 150 mL recipient contained a preservative solution of ethanol (50%), lactic acid (4%), benzoic acid (0.5%, glycerin (2.5%), and distilled water (43%). The recipients were stored at room temperature (23-24 °C) in a dark room [18,22].

2.4. Morphological characterization of the labellum

Evaluation of the variation in the flower labellum was based on the technique of Catling [18] and Hernández-Ruíz et al. [22]. First, the flower was dissected, and the labellum was extended on a glass surface. The labellum was then impregnated with methylene blue (0.08%) and photographed with a Sony reflex alpha 65v camera, fixed on a tripod and equipped with a Sony DT macro lens 30 mm F/2.8 SAM. The images were processed with the ImageJ program to generate 54 lines and 7 angles and to measure the lines and angles.

To generate the lines and angles, the labellum was divided into three regions, following the protocol of Hernández-Ruíz et al. [22]. The basal region was formed by lines A, A1, A2, A3, A4, A5, B1x and the angle aA (Figure 3a).
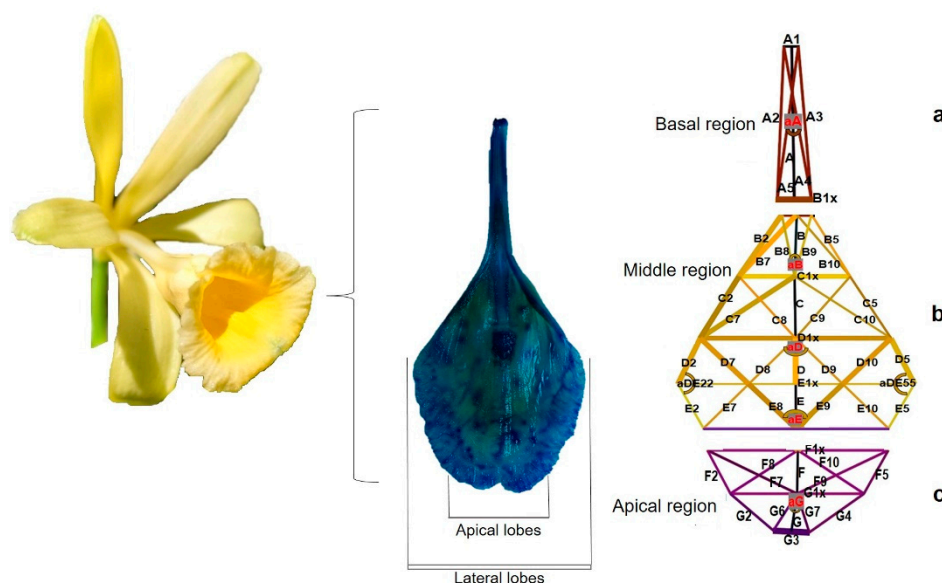
Figure 3. *Vanilla pompona* flower, labellum separated and dyed, fragmentation of the labellum into a. basal region, b. middle region, c. apical region.

The middle region contained lines B, B2, B5, B7, B8, B9, B10, C, C1x, C2, C5, C7, C8, C9, C10, D, D1x, D2, D5, D7, D8, D9, D10, E, E1x, E2, E5, E7, E8, E9, and E10, while the angles were aB, aD, aDE22, aDE55 and aE (Figure 3b). The apical region was made up of lines F, F1x, F2, F5, F7, F8, F9, F10, G, G1x, G2, G3, G4, G6, G7, and angle aG, and to obtain the length of the labellum, lines A, B, C, D, E, F and G were added (Figure 3c).

2.5. Statistical analyses

With the measurements of the 54 lines and seven angles of the labellum of the 55 collections of *V. pompona* obtained from different localities, we performed an analysis of variance (ANOVA). The collections were the source of variation. Fifty-five collections (treatments) were analyzed, each with different numbers of replications (labella) under a model equivalent to the completely randomized unbalanced design (PROC GLM; SAS, 2002). With the 61 traits (lines and angles) defined previously for *V. pompona* [22], a multivariate principal components analysis (PCA) and a cluster analysis were performed [39]. The principal components analysis was conducted based on the matrix of correlations between the selected variables and the cluster analysis with a mean distance between clusters, using the statistical software SAS version 9.0 (2002) [40].

3. Results

3.1. Characterization of the labellum

The analysis of variation showed significant differences in the 61 variables evaluated on the *V. pompona* labellum. For example, with line A (3.65 ***), which is at the base of the labellum and line C1x (4.88 ***), which is on the apical part of the labellum, we found differences in the average measurements of the labellum between collections. The coefficients of variation of the lines were, in general, low; exceptions were lines A1 (10.63) and G3 (12.84%) and angle aG (10.80%). Even so, we detected variation in the measurements of the labella of the collected flowers (Table 3). Therefore, the results indicated that there are differences in the means of the lines of the basal, middle and apical regions of the labellum among collections and localities.

Table 3. Coefficients of variation and mean squares of the 61 variables of the *Vanilla pompona* labella analyzed.

Variable	Mean (mm)	Coefficient of variation	Mean squares			
			Error	Locality		Collection
	I. Labellum basal region					
A	23.33	3.80	0.79	34.08 ***	3.64	***
A1	2.18	10.63	0.05	1.53 ***	0.30	***
A2	23.37	3.85	0.81	33.58 ***	3.64	***
A3	23.38	3.82	0.80	33.17 ***	3.57	***
A4	23.59	4.15	0.96	34.14 ***	3.61	***
A5	23.57	4.13	0.95	34.86 ***	3.73	***
aA	17.17	8.19	1.98	50.91 ***	5.15	***
B1x	4.87	8.03	0.15	3.09 ***	0.51	***
	II. Labellum middle region					
B	11.63	4.75	0.31	8.64 ***	0.93	***
B2	13.25	4.72	0.39	9.58 ***	1.14	***
B5	13.40	4.72	0.40	8.06 ***	1.12	***
B7	14.57	4.58	0.45	8.69 ***	1.15	***
B8	11.92	4.54	0.29	7.36 ***	0.83	***
B9	11.87	4.57	0.29	8.09 ***	0.97	***
B10	14.76	4.56	0.45	6.77 ***	1.16	***
aB	23.67	8.16	3.73	153.82 ***	16.61	***
C	11.62	4.78	0.31	8.65 ***	0.93	***
C1x	17.78	7.07	1.58	18.58 ***	4.88	***
C2	13.86	5.38	0.56	13.41 ***	1.57	***
C5	13.95	5.42	0.57	9.71 ***	1.96	***
C7	20.08	5.26	1.11	20.64 ***	2.26	**
C8	14.69	4.65	0.47	5.90 ***	1.02	**
C9	14.62	4.51	0.44	9.45 ***	1.17	***
C10	20.23	5.00	1.02	14.39 ***	2.97	***
D	8.35	7.37	0.38	5.29 ***	1.57	***
D1x	32.90	5.95	3.84	57.94 ***	9.48	***
D2	8.74	6.87	0.36	4.43 ***	1.25	***
D5	8.77	6.74	0.35	4.31 ***	1.28	***
D7	11.84	7.37	0.76	11.70 ***	2.37	***
D8	11.61	5.66	0.43	6.49 ***	0.93	**
D9	11.64	5.25	0.37	6.61 ***	0.84	**
D10	11.84	7.27	0.74	11.37 ***	2.53	***
aD	88.25	4.53	16.00	230.14 ***	102.01	***
aDE22	146.00	4.32	39.71	321.71 ***	118.57	***
aDE55	145.98	4.47	42.55	348.61 ***	120.61	***
E	8.12	6.39	0.27	4.75 ***	0.78	***
E1x	37.45	4.67	3.05	78.02 ***	6.58	**
E2	8.65	6.33	0.30	3.59 ***	0.92	***
E5	8.63	6.37	0.30	4.25 ***	1.03	***
E7	11.37	5.98	0.46	14.08 ***	1.49	***
E8	11.47	4.53	0.27	7.35 ***	0.51	**
E9	11.43	4.65	0.28	7.81 ***	0.54	**
E10	11.41	6.32	0.52	13.18 ***	1.48	***
aE	89.69	4.25	14.55	76.97 ***	62.19	***
	III. Labellum apical region					
F	6.22	7.45	0.22	5.62 ***	0.70	***

F1x	32.02	5.06	2.62	96.25	***	11.02	***
F2	7.25	7.82	0.32	5.76	***	0.94	***
F5	7.20	8.11	0.34	6.49	***	0.88	***
F7	17.20	5.64	0.94	28.32	***	3.39	***
F8	13.82	6.07	0.70	21.96	***	2.64	***
F9	13.92	6.27	0.76	19.98	***	3.15	***
F10	17.13	5.33	0.83	28.96	***	2.92	***
G	6.25	9.31	0.34	5.55	***	1.23	***
G1x	24.77	6.14	2.31	65.83	***	10.69	***
G2	11.30	9.46	1.14	17.49	***	4.06	***
G3	6.16	12.84	0.63	22.79	***	3.90	***
G4	11.20	9.40	1.11	26.84	***	4.78	***
G6	6.92	9.75	0.46	7.23	***	1.53	***
G7	6.98	9.60	0.45	5.43	***	1.19	***
aG	51.99	10.80	31.55	1168.06	***	221.56	***
Labellum length	75.47	3.29	6.17	286.36	***	21.27	***

***: $p \leq 0.0001$ highly significant, **: $p \leq 0.005$ moderately significant.

3.2. Distribution of the variation

To identify the variation of the measurements of the 54 lines and 7 angles in the *V. pompona* flower labellum, we used a principal components analysis. The dispersion of the 55 collections represented by the first three principal components together explained 75% of the accumulated variation. The first principal component (PC1) explained 43% of the general variation and was associated with labellum length (0.187) (Table 4). For this reason, this variable determined part of the morphological variation of the labellum of *V. pompona* specimens found at the collection sites.

Table 4. Eigenvalues, eigenvectors, and accumulated proportion of the variation explained by each variable in the first three dimensions of the characterization of 55 collections of *Vanilla pompona*.

Variable	Principal component (PC)			Variable	principal Component (PC)		
	PC1	PC2	PC3		PC1	PC2	PC3
I. Labellum basal region				D9	0.150	-0.047	0.205
A	0.156	-0.123	-0.133	D10	0.097	-0.135	0.217
A1	0.101	-0.055	-0.026	aD	0.011	0.205	-0.232
A2	0.156	-0.123	-0.134	aDE22	0.047	-0.055	-0.052
A3	0.156	-0.122	-0.134	aDE55	0.034	-0.092	-0.075
A4	0.159	-0.121	-0.130	E	0.129	0.092	0.180
A5	0.158	-0.122	-0.130	E1x	0.139	0.137	0.084
aA	-0.062	0.083	0.107	E2	0.117	0.025	0.251
B1x	-0.025	0.073	0.069	E5	0.117	0.038	0.249
II. Labellum middle region				E7	0.125	0.196	0.029
B	0.157	-0.118	-0.133	E8	0.161	0.124	0.115
B2	0.168	-0.116	-0.065	E9	0.159	0.125	0.123
B5	0.158	-0.113	-0.066	E10	0.126	0.196	0.021
B7	0.165	-0.101	-0.033	aE	-0.006	0.017	-0.188
B8	0.156	-0.119	-0.132	III. Labellum apical region			
B9	0.159	-0.115	-0.128	F	0.121	0.182	0.047
B10	0.151	-0.099	-0.026	F1x	0.125	0.209	-0.058
aB	-0.093	0.104	0.110	F2	0.110	0.191	0.065
C	0.157	-0.118	-0.133	F5	0.109	0.175	0.046
C1x	0.064	-0.013	0.146	F7	0.125	0.211	-0.036
C2	0.166	-0.098	-0.088	F8	0.132	0.186	-0.067
C5	0.153	-0.080	-0.091	F9	0.125	0.195	-0.050

C7	0.170	-0.077	0.020	F10	0.129	0.205	-0.050
C8	0.160	-0.096	-0.033	G	0.113	0.176	-0.087
C9	0.161	-0.105	-0.029	G1x	0.126	0.185	-0.084
C10	0.149	-0.054	0.020	G2	0.092	0.148	-0.066
D	0.095	-0.137	0.264	G3	0.034	0.111	0.040
D1x	0.132	-0.025	0.092	G4	0.122	0.119	-0.097
D2	0.110	-0.084	0.274	G6	0.104	0.181	-0.064
D5	0.109	-0.077	0.275	G7	0.105	0.187	-0.056
D7	0.101	-0.143	0.208	aG	-0.027	0.027	0.072
D8	0.148	-0.042	0.214	Long_lab	0.187	-0.044	-0.029
				Eigenvalues	26.126	12.174	7.401
				Proportion	0.43	0.20	0.12
				Accumulated	0.43	0.63	0.75

Values in bold type indicate variables that have the most influence in each principal component.

The second principal component (PC2) expressed 20% of the total variation and was associated with the variables aD (0.205), E7 (0.196), E10 (0.196), F1x (0.209), F2 (0.191), F7 (0.211), F9 (0.195), F10 (0.205) and G7 (0.187). The third principal component (PC3) defined 12% of the variation, explained by the variables D (0.264), D2 (0.274), D5 (0.275), D7 (0.208), D8 (0.214), D9 (0.205), D10 (0.217), E2 (0.251), E5 (0.249), and aE (-0.188) (Table 4). Thus, the variables that explain PC2 and PC3 contribute to the shape, width and amplitude of the *V. pompona* labellum.

In accord with the first three principal components, the spatial distribution of the evaluated collections revealed six groups (six morphotypes) (Figure 4). The distribution of the collections based on principal component 1 (PC1) placed the collections with the longest labella on the positive axis from the center upward (Groups I, II, III). The rest of the groups (Groups IV, V, VI) are on the negative axis, from -5 to -20.
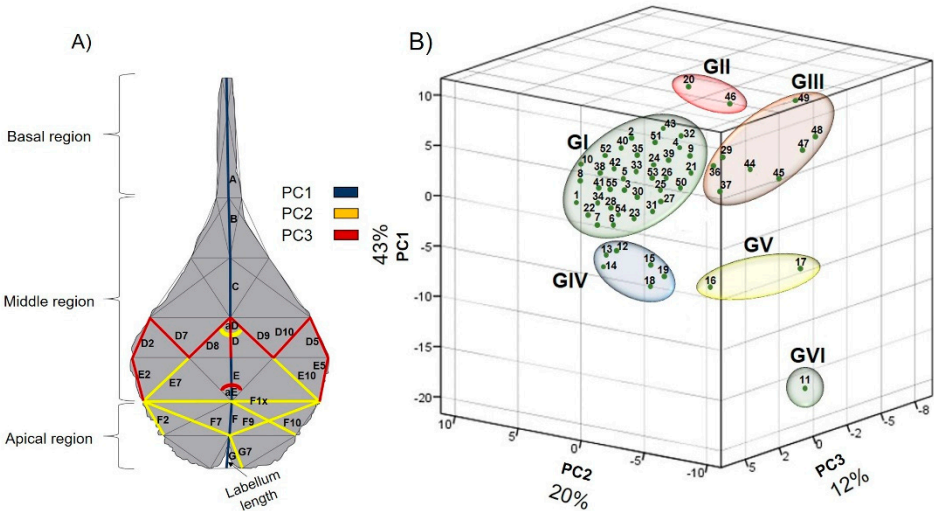
Figure 4. (**A**) Variables that define each of the first three principal components. (B) Dispersion of 55 collections of the flower labellum of *Vanilla pompona* based on the first three principal components, from localities of Veracruz, Jalisco, Puebla, and Oaxaca, Mexico.

Principal component 2 (PC2) concentrated the collections that have the shortest lines in the apical region of the labellum (E7, E10, F1x, F2, F7, F9, F10 and G7) on the negative axis (Groups IV, V and VI), while collections with longer and wider edges on the lateral lobes and wider labellum were placed in the callus region (aD) on the positive axis (Groups I, II, and III).

Principal component 3 (PC3) was represented by the collections with smaller amplitude (aE) of the middle region of the labellum and short lines in the middle region of the labellum (D, D2, D5, D7,

D8, D9, D10, E2 and E5). These collections were placed on the negative axis (Groups IV, V and VI). Likewise, the collections with acute angles of 88-89° in the middle region of the labellum (aE) and some lines of the middle region of the labellum (D, D2, D5, D7, D8, D9, D10, E2 and E5), which define the shape and size of the labellum, were placed on the positive axis (Groups I, II and III).

We identified six morphological groups (morphotypes) in function of the labellum.

Group I included collections 1, 8, 10, 22, 34, 40, 51, 52, 53, 50, 2, 7, 35, 5, 38, 39, 55, 41, 42, 24, 30, 28, 32, 33, 25, 26, 3, 4, 6, 31, 54, 23, 27, 9, 21 and 43. These collections are from the localities of Cazuelas, Papantla; Barriles, Gutiérrez Zamora, state of Veracruz, and from Cabo Corrientes, state of Jalisco. Among these collections are early, continuous, and late flowering clones.

Group II was made up of collections 20 and 46 of the locality Cazuelas and are continuous flowering clones, which begin flowering in March and continue into October.

Group III included collections 29, 36, 37, 44, 45, 47, 48, 49 from La Pasadita, Tihuatlan, Veracruz; Reyes de Vallarta, Tuzamapan de Galeana, Puebla, and Cabo Corrientes, Jalisco. This group also includes collections from Cazuelas land Papantla, Veracruz, that are early-flowering (March-May).

Group IV was represented by collections 12, 13, 14, 15, 18 and 19 from Hidalgo, Morelos, and Primavera, municipality of Santa Cruz Itundujia and Pluma Hidalgo, state of Oaxaca.

Group V included collections 16 and 17 from the municipality of Santa María Chimalapa, Oaxaca.

Group VI was made up of only collection 11 from the municipality of Santa María Chimalapa, Oaxaca.

3.3. Grouping diversity

The cluster analysis, with a distance between clusters of 0.9, confirmed the identification of the six groups defined by the principal components analysis. These groups were denominated morphotypes (Figure 5).
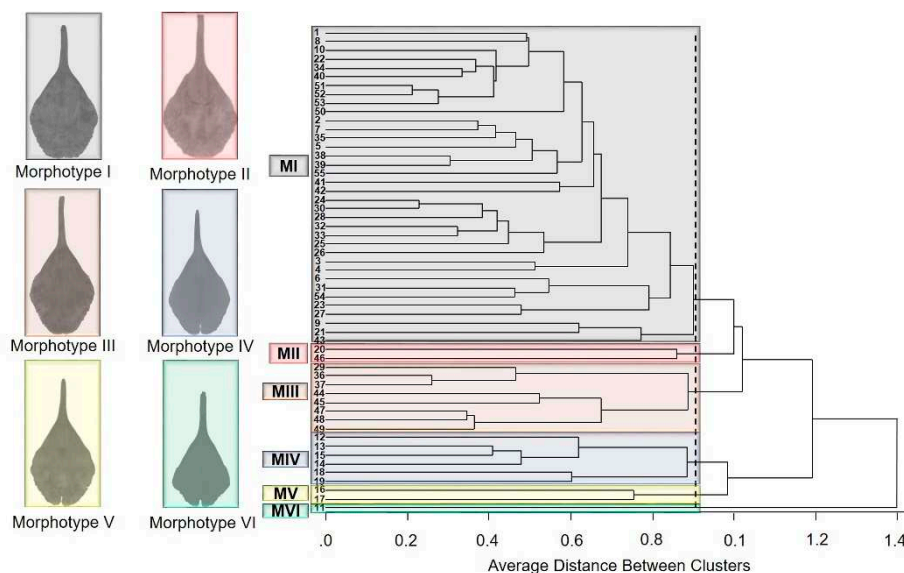
Figure 5. Dendrogram of labellum morphotypes of *Vanilla pompona* from localities of Veracruz, Jalisco, Puebla and Oaxaca, Mexico. Based on the average of 61 variables and on grouping by similarity distance.

Morphotype I (MI) included 36 collections that were distinguished by an irregular pentagon shape and a rounded shape of the lateral lobes. Total length of the labellum was 76.00 mm and its angles in the middle region were 146° and 146°. The lengths of each region were the following: basal region (B1x) 4.88 mm, middle region (D1x) 33.22 mm, and apical region (F1x) 32.90 mm.

Morphotype II (MII) was represented by collections 20 and 46 from the locality of Cazuelas. This morphotype was characterized by a longer labellum (81.44 mm) and by rounded lateral lobes that

significantly differentiate them from the apical lobe of the labellum. The labellum lengths, by region, were basal region (B1x) 4.94 mm, middle region (D1x) 34.50 mm, and apical region (F1x) 36.33 mm. Amplitude of the middle labellum region was 147° and 149°.

Morphotype III (MIII) included the collections 29, 36, 37, 44, 45, 47, 48 and 49. These collections were distinguished by the rhomboid shape of their labellum; length was 80.74 mm and amplitude of the middle region was 149° and 149°. The length of each region of the labellum were the following: basal region (B1x) 4.49 mm, middle region (D1x) 33.72 mm, and apical region (F1x) 29.99 mm.

Morphotype IV (MIV) was represented by collections 12, 13, 14, 15, 18 and 19, which had oval-shaped lateral lobes. This shape was defined by a labellum 68.28 mm long and angles of the middle region of 146° and 142°. They had the following lengths of each region of the labellum: basal region (B1x) 5.31 mm, middle region (D1x) 31.45, and apical region (F1x) 30.85 mm.

Morphotype V (MV) was made up of collections 16 and 17, which had a shape with marked lateral lobes, compared with the lobes of the apical region. Labellum length was 71.34 mm, and the angles of the lateral lobes were 140° and 149°. The lengths of each region were the following: basal region (B1x) 4.44 mm, middle region (D1x) 29.85 mm, and the apical region (F1x) 26.28 mm.

Morphotype VI (MVI) was represented by collection 11, which was differentiated from the other morphotypes by a shorter labellum (60.65 mm) and smaller amplitude in the angles of the lateral lobes (129° and 137°). The different regions of the labellum were defined by the following lengths: basal region (B1x) 4.69 mm, middle region (D1x) 26.47 mm, and apical region (F1x) 22.34 mm.

4. Discussion

4.1. Characterization of the V. pompona labellum

The analysis of variance indicated that there were differences in the means of the lines in the basal, middle, and apical regions of the labellum and of the lengths of the labellum among collections and localities. This agrees with Hernández-Ruiz et al. [22], who report that for *V. pompona* from Oaxaca, Mexico, the differences were highly significant in 58 of the variables evaluated on the labellum.

The coefficients of variation of the lines and angles evaluated oscillated between 3.29 and 12.84%, percentages that are lower than those found in other studies on orchid variables [21,23]. These results show that the labellum is an important structure of the vanilla flower in the analysis of variation and has been used to analyze infraspecific morphological variation in orchids [19,41]. Also, the labellum has been used to differentiate between subspecies, as reported for *Vanilla pompona* Schiede subsp. *pompona*, for which one of the main characteristics that distinguishes this subspecies was the length of the labellum (50 to 80 mm), while the length of *Vanilla pompona* subsp. grandiflora Lindl. was more than 80 mm [4].

4.2. Distribution of the variation

Dispersion of the labella of the *V. pompona* collections from the Gulf of Mexico slop and from the Pacific coast was represented by the first three principal components, which together explained 75% of the accumulated variation, a percentage that is lower than the study conducted by Hernández Ruíz et al. [22], who detected 81% of the variation in *V. pompona* collected only in Oaxaca, suggesting that the variation in floral traits of the first three components of this study is due to the use of collections from localities in different states of Mexico.

The horizontal length of the labellum, the variable that determines the size of the flowers, separated the PC1 groups and explained most of the variation (43%). This contrasts with Hernández-Ruíz et al. [22], who state that, for the populations of *V. pompona* from Oaxaca, the PC1 is associated with the variation in shape and size of some sections of the middle region of the labellum and with a variable of the apical region of the labellum.

The variables of the apical region of the labellum and one angle of the middle region differentiated the PC2 groups. This was similar to the variables reported that defined the PC2 groups from the *V. pompona* populations of Oaxaca, the apical region together with the dimension of the basal

region [22]. For PC3, the morphological variables that separated the groups were those of the middle region of the labellum, which was likewise one of the variables that influenced the definition of groups of the third component in *V. pompona* collections from Oaxaca [22].

In our study, the variables that explain PC2 and PC3 determined the shape, size and amplitude of lateral and apical lobes of the *V. pompona* labellum from localities of Veracruz, Jalisco, Puebla, and Oaxaca. In studies on *V. planifolia*, with the PCA, it was found that PC2 and PC3 were explained by variables that form part of the labellum shape and size of the middle lobes, aperture, and size of the lateral lobes labellum [23], also in the shape of the labellum in the apical region and amplitude of the lobes [21]. Thus, in the *V. pompona* collections evaluated in our study, the middle and apical regions and the length the labellum explain the variation. According to studies conducted on variation of the labellum of the genus *Vanilla* [21–24], the variables used are suitable since, despite differences in the measures, shapes, and size of the labellum in *V. pompona* and *V. planifolia*, these traits allow analysis of the infraspecific variation and the variation between species.

4.3. Morphotype grouping

Variation of the *V. pompona* labellum grouped in six morphotypes by PCA and cluster analysis indicates that these morphotypes represent the variation that exists in the localities of Veracruz, Jalisco, Puebla, and Oaxaca.

Morphotype I is represented by collections from localities of Veracruz and Jalisco. The collections from Veracruz are influenced by the slope of the Gulf of Mexico on the Atlantic side, while Cabo Corrientes is found on the Pacific coast on the other side of Mexico. However, despite their geographic separation by the Sierra Madre Oriental and the Sierra Madre Occidental [42] both have warm subhumid climate (Table 2) and are located on the same latitude. This could indicate that environmental and geographic characteristics have an influence in the fact that the collections from Veracruz and Jalisco are the same labellum morphotype (Figure 5).

The collections from Cazuelas, Papantla, Veracruz, are morphotype II, which stands out because the labellum (81.44 mm) is longer than that of the other morphotypes. This coincides with Soto-Arenas [5], who reported that *V. pompona* is made up of two population sets in Mexico, one in Veracruz on the Gulf of Mexico slope, characterized by its flower with extended segments of large dimensions.

Morphotype III is the widest and comprises the collections from Cabo Corrientes, Jalisco, and Papantla, Tihuatlán, Veracruz, and Tuzamapan de Galeana, Puebla. The localities of the collections from Veracruz and Puebla belong to the humid tropical ecological zone, while Cabo Corrientes belongs to the sub-humid tropical ecological zone, but the climate is warm subhumid, like that of Veracruz (Table 2), and allows them to share the same labellum morphotype.

Morphotypes IV, V, and VI of the Oaxaca localities have smaller labella. This coincides with Soto-Arenas [5], who mention that the second *V. pompona* population is found on the Mexican Pacific coast and is characterized by smaller semi-closed flowers, while Hernández-Ruíz et al. [22] report similar findings in the study of floral morphological variation in the Oaxaca populations, where the morphotype CAZ is found. This morphotype was the smallest labellum.

Morphotypes IV, V, and VI are found on the Mexican Pacific coast, and localities where the flowers were collected are in the Sierra Madre del Sur [42], which facilitates isolation of biological communities and separates ecological zones [43]. Moreover, the collection sites are found in the humid temperate, subhumid, and humid tropical zone (Table 2), which explains the variation found in Oaxaca. Also, a study of the *V. pompona* labellum from of Oaxaca reports four morphotypes and the variation found was due to the mountain complex belonging to the Sierra Madre del Sur, which separates the ecological zones where the species is found and functions as a geographic barrier [22], while for *V. planifolia* the study reports that some characteristics of the upper and apical region of the labellum are related to environmental factors [23].

Specifically, morphotype VI from Oaxaca has a horizontally narrower labellum (60.65 mm) and is different from morphotype II from Veracruz, with a horizontal length of 81.44 mm. Morphotype VI is found on the Pacific coast and is in the Sierra Madre del Sur [42], while morphotype II is found

on the Gulf of Mexico slope and belongs to the Sierra Madre Oriental [44]. These mountain chains function as geographic barriers and could partly explain the genetic diversity and speciation in orchids [45]. Moreover, it has been documented that the Gulf of Mexico has greater influence in the warm subhumid climate [43] where morphotype II is found, while morphotype VI is found in a warm humid climate (Table 2). It should be considered that the longer labellum of morphotype II could be influenced by the slope of the Gulf of Mexico since it is more humid than the Pacific coast 46]. *V. pompona* is considered a wild plant because it has not undergone the process of domestication that leads to changes in plant morphology [47]. Another cause of variation in the *V. pompona* labellum could be the environment, caused by the geographic location different from the collection sites. In *Vanilla*, the environment where the plants are found plays a highly important role in terms of availability of resources such as water, carbon, and nutrients, and only the apical region of the labellum can be modified by the availability of these resources [23].

Variation into the six labellum morphotypes of *V. pompona* identified in this study may also have been in part caused by pressure from pollinators or by genetic drift, which has been reported for other orchids [48]. An example of pressure from pollinators is reported for *Vanilla palmarum* Salzm. ex Lindl., whose flowers have no aroma, and the labellum underwent a modification to guide the beak of the hummingbird *Amazilia fimbriata* Gmelin to the nectar chamber [49]. *V. pompona* has a reward strategy for attracting pollinators offering both nectar and fragrance [50]. One of the specific pollinators of this plant is the male of the species *Eulaema cingulata* Fabricius [51].

The collections that form morphotypes I, II, and III are early-flowering (March-May), continuous-flowering (March-October), and late-flowering (June-August) floral labella from the locality of Cazuelas, Papantla, Veracruz. Studies have reported that *V. pompona* flowering in Mexico is April to June [4], but in *V. pompona* populations in Costa Rica and Peru they found two flowering periods, one January to February and another in September [51]. In *V. pompona* populations in the state of Maranhão, Brazil, flowering occurs in July and August [52], and in the Amazonian wetlands of Madre de Dios, Peru, the *V. pompona subsp. grandiflora* populations have two flowering periods that can vary depending on their geographic location [53], responding to specific environmental conditions, such as temperature and light during the flowering period [36,37]. It is interesting in Mexico that in a collection locality (established approximately 10 years ago) in Veracruz, specimens of *V. pompona* can be found with different flowering dates.

This study contributes knowledge on the variation of the *V. pompona* labellum in the states of Veracruz, Puebla, Jalisco and Oaxaca. The knowledge generated will help producers to better use the plant by selecting which plants to cultivate with different flowering dates. Growers will also be able to decide which specimens to plant according to the morphotype that is found near the area where they live. Moreover, the information contributes to conservation of genetic diversity. However, more studies are needed on genetic variation and with floral ecology of the *V. pompona* germplasm in Mexico.

5. Conclusions

In localities of the states of Veracruz, Puebla, Oaxaca and Jalisco, Mexico, we obtained six morphotypes of the *V. pompona* labellum. The variation existing in the labella of this species is possibly associated with pollinator pressure and the presence of geographic barriers, which can generate genetic drift. Because *V. pompona* is a wild plant, environment plays a highly important role in the variation of the species, and plant diversity is related to phenological-environmental factors since in the locality of Cazuelas, Papantla, Veracruz, plants were found with different flowering dates. Therefore, infraspecific variation exists in the *V. pompona* germplasm.

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