Morphological characterization of Colombian *Passiflora* species

Caracterización morfológica de especies del género *Passiflora* de Colombia

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Abstract

21 Colombian *Passiflora* species, including three subgenera of the genus, were morphologically characterized using 66 quantitative and 100 qualitative descriptors. Principal component analysis of quantitative data identified two components with own value superior to one, associated with leaves and flowers, which explained 80% of total variance of the studied accessions. Factorial analysis of multiple correspondence identified three dimensions, which explained 82% of total variance. The second dimension was associated with grown habit, number of flowers per node and color of the last filaments series in the apex. The third dimension was associated with bract morphology and margin, and the first dimension with the remaining qualitative variables. Cluster analysis (city-block-Manhattan distances) revealed two major groups, according to flower tube size (short or long flower tube). Classification analysis also showed that flower morphology was very important for infrageneric discrimination in *Passiflora*.

Key words: *Passifloraceae*, plant anatomy, flower morphology.

Resumen

Se caracterizaron 21 especies de *Passiflora* las que incluyeron tres subgéneros, mediante 66 descriptores cuantitativos y 100 cualitativos. El análisis de componentes principales (ACP) de los descriptores cuantitativos identificó dos componentes con valor propio superior a 1, asociados con hoja y flor, los cuales explicaron 80% de la varianza total de las accesiones estudiadas. Con el análisis factorial de correspondencia múltiple (AFCM) de los descriptores cualitativos se identificaron tres dimensiones, que explicaron 82% de la varianza total; a la segunda se asociaron tipo de crecimiento, número de flores por nudo y color de la última serie de los filamentos en el ápice; a la tercera, forma y margen de la bráctea, margen de la bráctea; y a la primera las demás variables cualitativas. El análisis de agrupamiento (distancias de 'city-block-Manhattan') evidenció dos grandes grupos, según la longitud del hipantio —especies con flor de hipantio corto y largo—. El análisis de clasificación también mostró que la morfología floral fue determinante en la discriminación infragenérica en *Passiflora*.

Palabras-clave: *Passifloraceae*, anatomía de la planta, morfología floral.

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Introduction

The genus *Passiflora* L., the most important of the *Passifloraceae* family, is distributed in tropical and subtropical regions from sea level to altitutdes greater than 3000 m.a.s.l., however the greatest diversity of species is found in moderately warm and temperate regions, between 400 and 2000 m.a.s.l.. The most recent taxonomic revision of the genus (Feuillet & McDougal, 2003; Ulmer & McDougal, 2004) reduced the number of subgenera from 22 (Killip, 1938) to four: *Astrophea* (57 species), *Deidamioides* (13 species), *Decaloba* (214 species) and *Passiflora* (236 species). In the present study the taxonomy proposed by Feuillet & McDougal (2003) is considered, with the subgenus *Tacsonia* (following Killip, 1938) proposed as a supersection of the sungenus *Passiflora*. Similarly, *P. mollissima* (Killip, 1938) is re-admitted and not *P. tripartita* var. *mollissima* (Holm-Nielsen et al., 1988).

Nearly 90% of the 520 species of *Passiflora* are native to America (Escobar, 1988). In Colombia 164 species have been inventoried (Ocampo et al., 2007), and 58 of these are considered endemic. However, some Andean species are threatened with extinction, and some are already considered extinct. The high diversity of the Colombian Coffee Zone, home to 59% of the species of *Passifloraceae*, has been affected by the intense fragmentation of the Andean forests (Kattan, 1997).

The studies of morphological diversity of passifloras are relatively new. Previously, studies were limited to taxonomic descriptions (Killip, 1938; 1960, Escobar, 1988; Holm-Nielsen et al., 1988). In the genus *Passiflora* morphological characterization studies have been initiated, mainly for species of the subgenus *Passiflora* and the supersection *Tacsonia* (taxonomy according to Feuillet and McDougal, 2003).

The morphological study of Ecuadorian material (Villacis et al., 1998) showed little variation in the cultivated species of 'curuba' *P. tripartita* var. *mollissima* and *P. tarminiana*, and a strong affinity between the former and the wild 'curuba' species *P. mixta*. This study also tested for the first time a list of descriptors and revealed the necessity to give greater importance to the floral morphology. A new version of the list was used in the study of Colombian material, giving results concordant with the criteria that maintained the classification of the subgenus *Tacsonia* by Killip (1938) and, partially, with that of Escobar (1988). In particular, similarity was seen amongst the four most common species of 'curuba' (*P. tripartita* var. *tripartita*, *P. tarminiana*, *P. mixta* and *P. cumbalensis*) and, at the intra-specific level, a geogrpaphic differentiation in these species, and in *P. manicata*, with a strong morphological diversity in *P. mixta*, and much less diversity in the cultivated species (Rioux, 1999; Coppens d'Eeckenbrugge et al., 2002). A study focused on accessions from the

Department of Valle del Cauca, Colombia, suggested the existence of introgression between var. *mollissima*, the 'curuba de Castilla', and *P. mixta* (Primot et al., 2001). In other studies, the morphological data separated the species, and recognisied strong associations between *P. ligularis* and *P. tiliifolia*, and between *P. alata* Dryand. and *P. quadrangularis* L. (Ocampo et al., 2001; Olaya, 2002).

Given the minimal information for certain species, and the total lack of information for others, the present study had the objective to investigate the diversity of 21 species of *Passiflora* in Colombia through morphological characterization, generating new knowledge on these important plant genetic resources.

Materials and Methods

Using as a base, the morphological descriptors for *Passiflora* developed by CIRAD, IPGRI (now Bioversity International) and the Colombian Corporation for Agricultural Research (Corpoica), a new list was developed that included 66 quantitative and 100 qualitative descriptors, encompassing the subgenera and supersections of *Passiflora*. In particular, foliage nectaries were added, to discriminate species of the subgenus *Decaloba*, and the peduncles, pedicels and nerves, for the subgenus *Astrophea*. The material was collected during 2004 in different locations in the Departments of Caldas, Chocó, Cundinamarca, Nariño, Quindío, Risaralda, Santander, Tolima and Valle del Cauca, under the framework of the project " Study of Diversity of the Families Passifloraceae and Caricaceae of the Colombian Coffee Zone", by John Ocampo Pérez, Creucí Maria Caetano and Mónica María Marín Tangarife. 35 accessions were characterized, distributed across three subgenera (Box 1). In the subgenus *Passiflora* most accessions (19) and species (9) were evaluated, followed by the subgenus *Decaloba* (11 and 8), and then by the subgenus *Astrophea* (5 accessions from 3 species). In Box 2 a list of the morphological descriptors is provided, together with their respective abbreviations.

The quantitative traits were submitted to Principal Component Analysis (PCA), and the qualitative traits to a Factorial Analysis of Multiple Correspondence (FAMC). With the traits retained in the PCA and the dimensions obtained in the FAMC, a cluster analysis was performed using the City-Block-Manhattan distances, and the genetic distances of Ward. From the morphological descriptors a dendrogram was generated using the metric distances of Chebychev. Analyses were performed in the programs SAS 8.1 and Statistica 98.

Box 1. Accessions of *Passiflora* characterized morphologically (taxonomy according to Feuillet & McDougal, 2003).

Subgenus	Species	Number of accessions	Providence	Abrev.
Astrophea	*P. arborea*	1	Caldas	ARCA
	P. emarginata	1	Caldas	EMCA
		1	Valle del Cauca	EMVA
	P. sphaerocarpa	1	Valle del Cauca	SPVA
		1	Caldas	SPCA
Decaloba	*P. alnifolia*	2	Valle del Cauca	ALVA 1,2
	P. andreana	1	Nariño	ANNA
	P. bauhinifolia	2	Nariño	BANA
	P. capsularis	1	Santander	CASA
	P. coriacea	1	Valle del Cauca	COVA
	P. cuspidifolia	1	Cundinamarca	CUCU
	P. filipes	1	Risaralda	FIRI
	P. rubra	1	Caldas	RUCA
		1	Valle del Cauca	RUVA
Passiflora	*P. edulis* f. *edulis*	1	Nariño	EDNA
	P. edulis f. *flavicarpa*	2	Risaralda	EDRI 1,2
	P. ligularis	1	Nariño	LINA
	P. maliformis	1	Quindío	MAQU
		1	Valle del Cauca	MAVA
	P. mixta	2	Nariño	MINA 1,2
	P. mollissima	3	Nariño	MONA 1,2,3
	P. nitida	1	Chocó	NICH
	P. tarminiana	4	Nariño	TANA 1,2,3,4
	P. tenerifensis	1	Valle del Cauca	TEVA
	P. vitifolia	1	Chocó	VICH
		1	Tolima	VITO

Box 2. The main morphological descriptors for *Passiflora* used in this study.

Descriptor	Abbreviation
Angle between lateral veins	Anl
Length of right lateral lobe	Llld
Distance from the insertion of the petiole to the right lateral lobe	Dipslld
Peduncle length	Lped
Flower length	Lf
Stamen filament length	Lfe
Length of androgynophore	Lan
Type of stem growth	Tcrt
External form of stem	Fet
Pubescence in the tendrils	Pz
Anthocyanin in the tendrils	Anz
Anthocyanin in the stipules	Ae
Leaf polymorphism	Pf
Form of the leaf base	Fbh
Pubescence on leaf underside	Peh
Predominant leaf	Hp
Presence of nectaries in limbo	Pnl
Anthocyanin in the petiole	Ap
Anthocyanin in the underside	Aeb
Bract form	Fb
Bract margin	Mb
Number of inflorescences per node	Nfn
Dominant color of the petals	Cdp
Sepal union	Us
Color of the final series of filaments in the base	Csfb
Color of the final series of filaments in the apex	Csfa
Pubescence in the ovary	Pov
Ovary color	Cov
Androgynophore color	Ca
Internal color of the hypanthium	Cih

Results and Discussion

Principal Component Analysis (PCA)

The PCA identified the components leaf form and flower variables with a value greater than 1, explaining 80% of the total variation of the studied accessions (Box 3). Leak form comprised the traits angle between lateral veins (**Anl**), length of right lateral lobe (**Llld**) , and distance from thr insertion of the petiole to the right lateral lobe (**Dipslld**). The last two traits were those that most supported the coformation of principal component 1. The variables related to the flower were the lengths: of the peduncle (**Lped**), the flower (**Lf**), the stamen filament (**Lfe**), and the androgynophore (**Lan**). Lf and Lan particularly supported the Principal Component 2 (Box 4).

As can be seen in Figure 1, species with similarity in the variables corresponding to flower size, such as those belonging to the supersection *Tacsonia,* are situated on axis 1 of the principal plane. The species with similarity in leaf variables are situated on axes 3 and 4, amongst which are found *P. maliformis*, *P. ligularis*, *P. sphaerocarpa*, *P. nitida*, *P. rubra*, *P. capsularis*, *P. emarginata* and *P. edulis.*

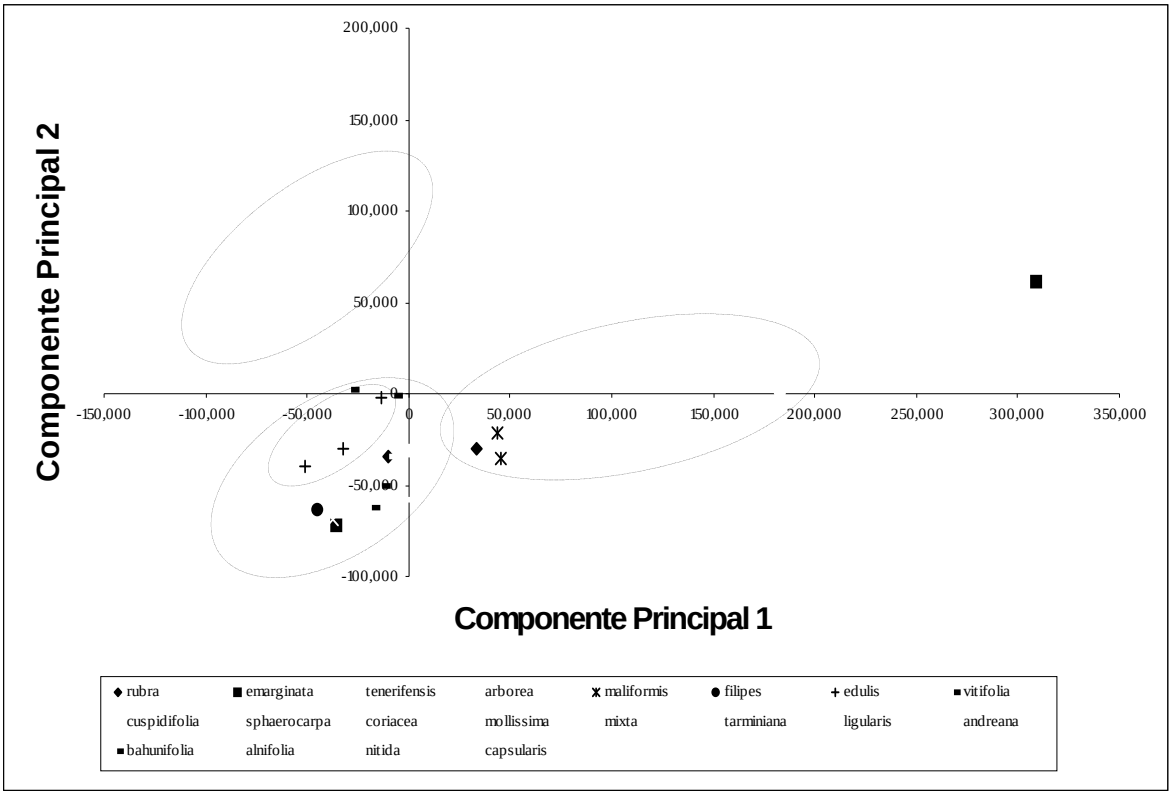

Figure 1. Distribution of the evaluated *Passiflora* accessions of in the main plane**.** Leaf form supprots CP1 and flower variables CP2.

Box 3. Values derived from the PCA, for the morphological traits considered in *Passiflora*.

Variables	Values	Difference	Proportion	Accumulated
1	6084.82	2557.63	0.50	0.50
2	3527.20	1851.44	0.29	0.80
3	1675.76	1040.75	0.14	0.93
4	635.01	487.35	0.05	1.00
5	147.67	126.19	0.01	1.00
6	21.48	15.78	0.00	1.00
7	5.70	—	0.00	1.00

Box 4. Principal Components of the quantitative morphological traits retained.

Variables	CP1	CP2
Anl	-0.37	0.25
Llld	0.56	0.27
Dipslld	0.69	0.22
Lped	0.01	0.42
Lf	-0.18	0.58
Lfe	-0.03	0.10
Lan	-0.19	0.54

Factorial Analysis of Multiple Correspondence (FAMC)

The FAMC identified three dimensions, which explained 82% of the total variance in the studied accessions (Boxes 5 and 6). In the first dimension the majority of all the qualitative variables are associated, except growth habit (2), bract form (3), bract margin (3), number of flowers per node (2) and the color of the final filament series in the apex (2). In Figure 2 the accessions appear in the principal plane.

Box 5. Values of the inertia and X^2 for the qualitative morphological traits found in the accessions of *Passiflora* evaluated.

			Percentage	Percentage accumulated	10 20 30 40 50
					----+----+----+----+----+---
0.47	0.23	623.23	50.83	50.83	**********
0.27	0.07	205.36	16.75	67.57	********
0.23	0.05	139.91	11.41	78.98	******
0.15	0.02	61.33	5	83.99	***
0.14	0.02	53.4	4.35	88.34	**
0.12	0.01	36.81	3	91.34	**
0.09	0.01	24.57	2	93.35	*
0.09	0.01	20.23	1.65	95.00	*
0.07	0.01	12.46	1.02	96.01	*
Total	0.44	1226.2	100		

Cluster Analysis

Five groups were identified with a distance of 1.5 units (Box 6; Figure 3). Group 1 comprised 23 accessions (65% of the total), pertaining to the three subgenera *Decaloba* (*P. rubra*, *P. cuspidifolia*, *P. andreana*, *P. alnifolia*, *P. bauhinifolia*, *P. coriacea*, *P. filipes* and *P. capsularis*), *Passiflora* (*P. edulis*, *P. maliformis*, *P. ligularis, P. nítida* and *P. vitifolia*) and *Astrophea* (P.

sphaerocarpa, P. *emarginata*, P. *arborea*). This group was characterized by the length of the androgynophore (9.83mm), the absence of foliar polymorphism, and free sepals.

Box 6. Factorial analysis of multiple correspondence for 23 qualitative variables evaluated in accessions of *Passiflora*.

Variables	Dim1	Dim2	Dim3
Tcrt	0.04	0.59	0.00
Fet	0.68	0.09	0.08
Pz	0.59	0.13	0.05
Anz	0.67	0.00	0.01
Ae	0.72	0.11	0.00
Pf	0.69	0.15	0.00
Fbh	0.69	0.00	0.01
Peh	0.66	0.01	0.03
Hp	0.85	0.01	0.04
Pnl	0.70	0.15	0.00
Ap	0.67	0.017	0.00
Aeb	0.65	0.00	0.00
Fb	0.15	0.13	0.52
Mb	0.09	0.01	0.65
Nfn	0.14	0.55	0.00
Cdp	0.59	0.16	0.01
Us	0.71	0.11	0.03
Csfb	0.68	0.21	0.03
Csfa	0.04	0.83	0.07
Pov	0.66	0.00	0.15
Cov	0.63	0.05	0
Ca	0.51	0.25	0.03
Cih	0.77	0.01	0.00

Box 7. Cluster analysis (Manhattan distance) for *Passiflora* accessions (subgenera *Astrophea*, *Decaloba* & *Passiflora*), as a function of the retained variables, related to all floral morphology.

Groups	Variables						
	Anl	Llld	Dipslld	Lepd	Lf	Lfe	Lan
1	44.19	78.26	58.67	31.92	21.81	6.01	9.83
2	36.73	102	67.47	44.94	41.48	19.13	24.07
3	0.0	198.6	198.6	12.8	13.5	2.00	6.40
4	0.0	301.6	301.6	16.62	25.60	7.08	11.98
5	0.0	121.2	121.2	255	123	93.2	98.4

Group 2, with nine accessions (25.7% of the total), comprised species from the supersection *Tacsonia* of the subgenus *Passiflora* (Feuillet and McDougal, 2003; Ulmer and MacDougal, 2004). This group was characterized by flower length (100 mm), a trait that

differentiates between subgenera and, in some cases, within subgenera. The two accessions of *P. mixta* must be highlighted for the intraspecific variation in the flower length.

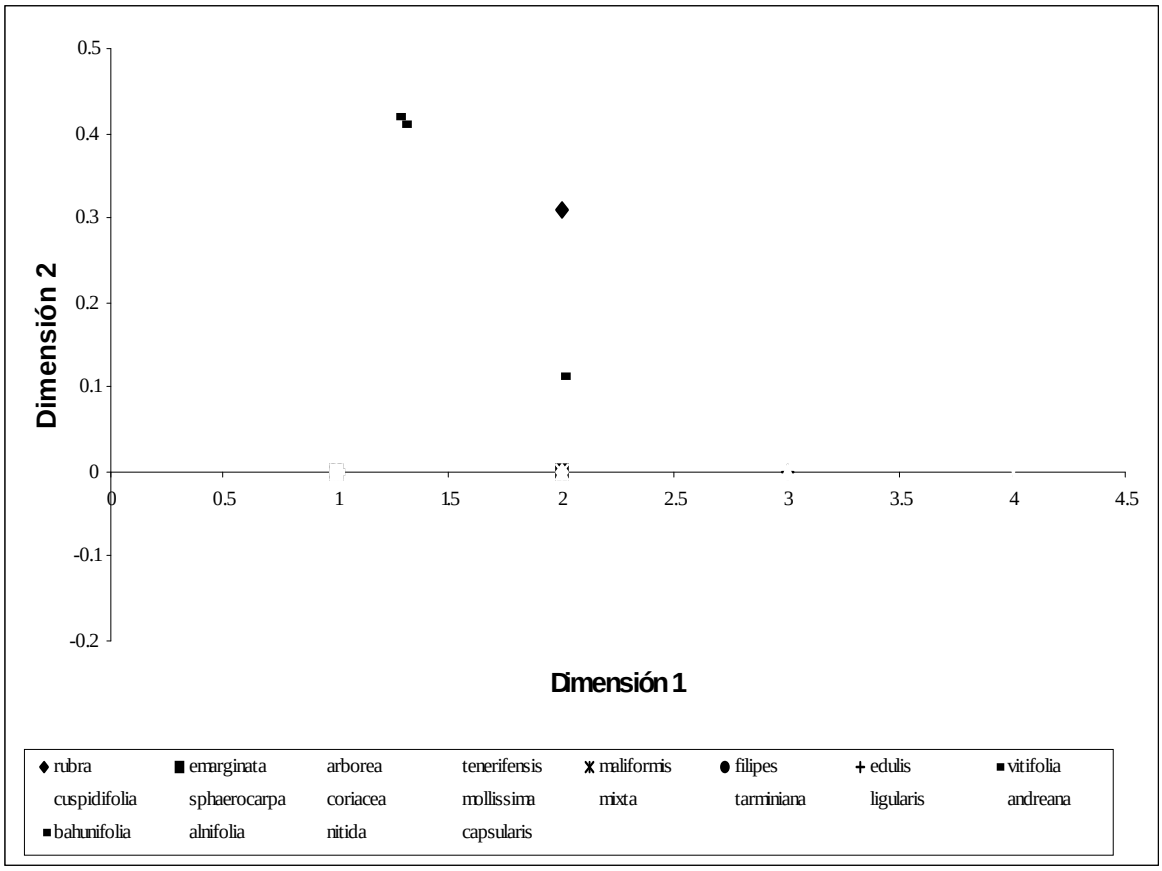

Figure 2. Graphical distribution of the accessions in the main plane, FAMC..

Groups 3 and 4 comprised 5.7% of the accessions, with the species from the subgenus *Astrophea* *P. sphaerocarpa* and *P. emarginata* respectively, separated by the large leaf size.

Group five comprised *P. tenerifensis*, (2.8% of the total), displaced from the subgenus *Tacsonia* (Killip, 1938) to *Passiflora* (Feuillet and McDougal, 2004; Ulmer and MacDougal, 2004). This group was distinguished from the species in group 2 by the lengths of the peduncle (255 mm), the petals (61.3 mm), and the flower (123 mm). The leaf is unilobed, in contrast to the other accessions in group 3. The dendrogram was generated with the Chebychev distance metric (Figure 3).

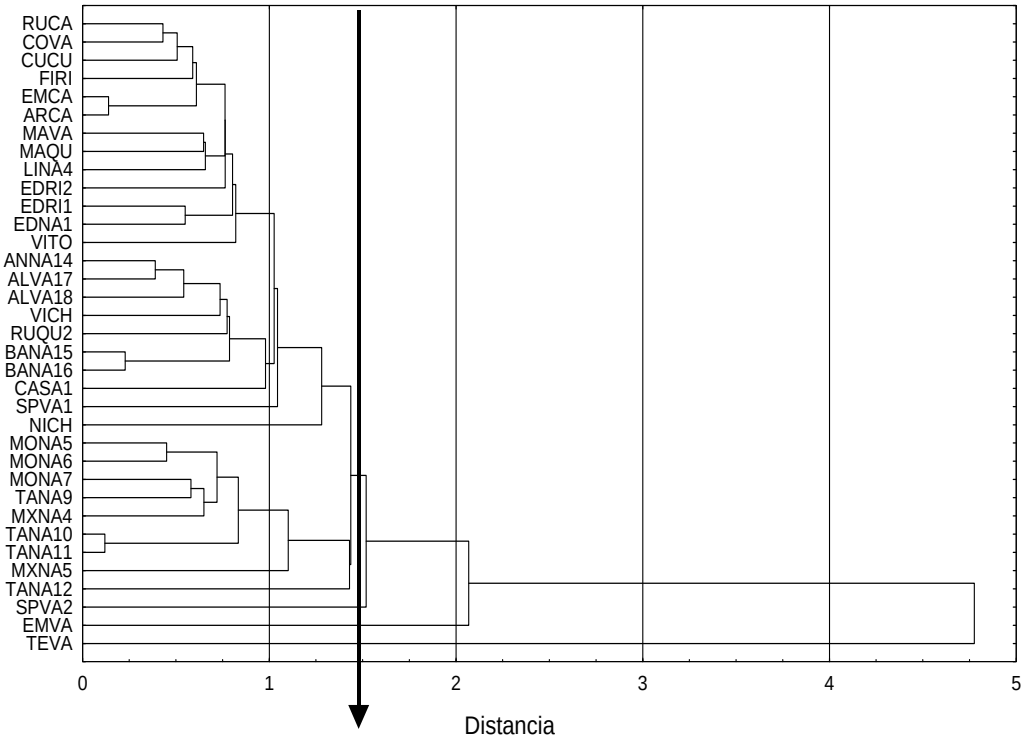

Figure 3. Dendrogram (Chebychev metric distance) of *Passiflora* accessions, generated from the morphological descriptors.

Although a characteristic pattern is seen, the floral morphology in *Passiflora* is highly variable in flower size, form, color, anthesis time, longevity and aroma presence, to such a degree that Killip (1938) based his classification of the 22 different subgenera on this. The different forms and functions of the floral organs may be related to pollination biology (Büchert Christensen, 1998).

The PCA showed that in the second component, the variables related to flowers were associated, amongst them the length of the peduncle, the flower itself, the stamen filament, and the androgynophore. In a similar manner, the three dimensions identified in the FAMC were associated with floral variables, including the color of the final series of filaments in the apex, and the number of flowers per node. The cluster analysis separated the major groups, according to length of hypanthium. Thus, the flower is one of the determining structures in the infrageneric discrimination in *Passiflora*.

Another important aspect is the coevolution of the floral morphology in *Passiflora* in relation to its pollinators. In species with a short hypanthium and filamentous crown (visually appealing and orientated horizontally like a platform) insect pollination occurs. In contrast, in those with a large hypanthium pollination by bird (hummingbird) occurs. In these latter

flowers, the crown is reduced, - and composed of tubers, the perianth is colored, the hypanthium is long, and the simple operculum in the base, for protection against nectar sucking insects. Finally, the orientation of the flower is pendulum. Bat and moth pollination have been reported for this genus (Büchert Christensen, 1998).

The classification analysis showed concordance with the criteria that supported the taxonomic classification of the subgenera, in agreement with the floral morphology (Killip, 1938), with three exceptions. These were *P. sphaerocarpa* and *P. emarginata* (subgenus *Astrophea*), separated by the greater leaf length. In *P. tenerifensis* (supersection *Tacsonia*, previously homonymous subgenus), the trait that distinguished this species from the same supersection is the great difference in the lengths of the peduncle, petals, sepals and the flower. Also, the leaf is unilobed. In general, the quantitative variables are complementary in differentiating the supersections. For both, the variables related to the flower are those that most support the separation of the species evaluated into two groups.

Conclusion

This study complemented previous classifications (Killip, 1938, 1960; Escobar, 1988; Hernández, 2003) and the recent revision (Feuillet and McDougal, 2003; Ulmer and MacDougal, 2004), and contributed new information regarding the differences in traits such as form and size. The species in which the description has become more precise are *P. andreana*, *P. vitifolia* and, particularly, *P. rubra*, clearly distinguished from *P. capsularis* by the fruit form.

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