

## Phenotypic plasticity of *Vaccinium meridionale* (Ericaceae) in wild populations of mountain forests in Colombia

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**Abstract:** *Vaccinium meridionale* is a promising crop for the Andean region of South America and is currently available only in the wild. Spontaneous populations of this plant are found across the Colombian mountains, but very few published records on this plant morphology are available. A zonification study of *V. meridionale* was conducted in four principal areas of a low mountain forest of Colombia (Provinces of Boyacá, Cundinamarca, Santander and Nariño) in 2007. A total of 20 populations and 100 plants of *V. meridionale* were individually characterized and surveyed, using a list of 26 characters of morphological variables (9 quantitative and 17 qualitative characters). Our results indicated that natural populations of *V. meridionale* might be found in the tropical forest under a highly heterogeneous climate and microclimate conditions, at different mountain regions between 2 357 and 3 168masl. The shrubs of *V. meridionale* exhibited a high level of intra-population variation in several quantitative (plant height, stem diameter) and qualitative (growth habit, ramification density, presence of anthocyanins in stems) morphological characters, suggesting an environmentally induced phenotypic plasticity. Plant height, stem diameter and foliar density were the most variable morphological traits, with coefficients of variation higher than 50%. However, several quantitative characters of its reproductive potential, such as berry dimensions, rachis length and number of flowers per inflorescence, resulted with low plasticity with coefficients of variation lower than 30.2%, indicating that these characters were genetically determined. The highest correlation coefficients ( $p < 0.05$ ) resulted to be between fruit length and fruit width (0.90), leaf length and leaf width (0.78), plant height and stem diameter (0.60), and inflorescence length and flowers number per inflorescence (0.57). The results suggest that an important genetic resource exists for this species in the wild. Low variation in fruit size, which constitutes a target trait for plant breeders, could be useful for selection of cultivars of *V. meridionale*. The results of this study could also be applied in conservation programs aimed to protect these diverse populations in the mountain forests of Colombia. Rev. Biol. Trop. 59 (2): 569-583. Epub 2011 June 01.

**Key words:** plant morphological characters, *Vaccinium meridionale*, tropical forest, *in situ* characterization, Andean region, Colombia.

The genus *Vaccinium* of the family Ericaceae includes about 450 species of trees, shrubs, subshrubs, and hemiepiphytes that are mainly distributed in the tropics of the Old World, Malaysia, and Southeast Asia (Luteyn 2002, Vander Kloet & Dickinson 2009, Vander Kloet & Avery, 2010). According to Luteyn (2002), the neotropics are part of the center of diversity for the family Ericaceae, with most species in Colombia and Ecuador. From the approximately 40 species of *Vaccinium*

present in the neotropics (Luteyn 2002), five native species are reported in Colombia: *V. meridionale* Swartz, *V. floribundum* Kunth., *V. corymbodendron* Dunal., *V. euryanthum* A C Smith and *V. singularis* Salinas (Salinas & Betancur 2007). These species occur in Andean cloud forests and the regions of páramo (Gentry 1994, Luteyn 2002), with an exception of *V. euryanthum*, which may be encountered in the lowlands of Guiana Shield (Salinas & Betancur 2007). Among these plants, *V. meridionale* and

*V. floribundum* are the foremost species that have edible fruits, but these are underutilized and are not domesticated species (Ávila Diaz-Granados *et al.* 2009 and references therein, Vasco *et al.* 2009). *V. meridionale*, known by its local names as agraz, mortiño, Colombian blueberry, Andean blueberry, or Jamaican bilberry, is a wild evergreen shrub in the low mountain forests of Colombia, Ecuador, Peru, Venezuela and Jamaica (Tanner 1982, Gentry 1994, Izco *et al.* 2007). In mountain areas of Colombia, *V. meridionale* and *V. floribundum* may be frequently found growing in associations, in which the latter species is generally of smaller size, higher ramification and smaller fruits when compared to *V. meridionale*. The species of genus *Vaccinium* compose the group of clonal plants (Albert *et al.* 2005) that could be propagated both sexually and vegetatively, with vegetative progenitors well established rapidly through rhizomes or stolons (Wilbur & Luteyn 2008). *V. meridionale* is known to be partially self-fertile (Tanner 1982), and, in Colombia, the European honey-bee (*Apis mellifera*) may be important for its cross-pollination (Arjona 2001, pers. comm.), however vegetative growth is considered to be a principal strategy of the species propagation in natural habitats (Tanner 1982, McDonald *et al.* 2003, Ávila Diaz-Granados *et al.* 2009).

The fruits of *V. meridionale* have significant economic potential for the international markets due to the high contents of polyphenolic compounds that are known for their beneficial effects for human health including anticarcinogenic properties. The average contents of total anthocyanins and total phenols in fruits of *V. meridionale* were measured 201 cya-3-glu mg/100 g FW and 609 gallic ac. mg/100 g FW, respectively; whereas total antioxidant activity (FRAP) reached 58.1 TE mkM/g (Gaviria *et al.* 2009). The values comparable to or exceeded those reported for the wild berries of *V. myrtillus* L. (Giovannelli & Buratti 2009) or *V. ovalifolium* Smith (Lee *et al.* 2004). In the Andean region, consumption of fruits of *V. meridionale* over the centuries maintained importance due to their nutritional and organoleptic qualities.

However, all activities related to plant usage are limited to recollection of berries in the wild and selling these on the market places or roads nearby to recollection sites as well as artisanal methods of fruit processing for juices, vines and marmalades. Tree damage made through inadequate practices of fruit harvest, tree lodging and branches recollection for charcoal in a low mountain forest (which constitutes a natural habitat of *V. meridionale*), turns the species currently under risk of genetic erosion (Hernández *et al.* 2009).

In South America, *V. meridionale* is currently accessible only in the wild and, in Colombia, a wide variation in the plant morphology is anticipated across the country. According to Ligarreto (2009), the quantitative characteristics of the species in a single Colombian metapopulation, exhibited a broad phenotypic diversity among the studied plants, with coefficients of variation ranging between 18 and 34%. This range implies that phenotypic features are affected by the environment and may be considered as an indicator of genetic variability, especially in variables of broad dispersion of values. Additionally, a wide qualitative variation, with detection of polymorphism in 16 qualitative variables in plants of *V. meridionale*, was reported (Ligarreto 2009). This type of variation, in general, refers to a high heritability  $h^2$  (Hill *et al.* 1998) that corresponds, according to Van Hintum (1995), to an alleles expression of specific genes, due to which these qualitative variables could be employed as genetic markers.

Colombia is one of the main natural centers where *V. meridionale* might be encountered in the wild, nevertheless, the distribution of its natural populations in the country is poorly characterized. To our knowledge, the populations of *V. meridionale* in the North East (Province of Santander) and South Pacific (Province of Nariño) mountain regions of the country were neither assessed nor characterized, and the knowledge on their possible existence in these areas was based essentially on local reports. Additionally, almost no published records on plant morphology in locations

where the spontaneous populations were earlier found (Provinces Cundinamarca and Boyacá) are available (Medina & Lobo 2004, Ligarreto 2009). Thus, the goal of this study was to characterize *in situ* the basic aspects of phenotypic plasticity of *V. meridionale* within its natural populations in four principal Provinces of Colombia: Cundinamarca, Boyacá, Santander and Nariño; occupied with a tropical mountain forest. The morphological variation degree among wild populations of *V. meridionale*, constitutes a fundamental question that should be addressed in order to: a) conservation efforts to the diverse populations of *V. meridionale in situ* and *ex situ* and b) to obtain plant material for breeding purposes, and promote its cultivation in world agriculture.

## MATERIALS AND METHODS

**Plant material:** Information from previous field studies and reported cases of *V. meridionale* Swartz from the Colombian mountain areas were studied using herbaria of Instituto de Ciencias Naturales, Universidad Nacional de Colombia as well as publications (Luteyn 2002) and personal communications. These data further was used to systematize the reports and to elaborate maps of possible location of the species in the wild.

**Field survey:** According to the preliminary field studies conducted in a pilot zone in Cundinamarca, the presence of *V. meridionale* populations was tested in the similar habitats through the Provinces, making this study one of the first ones to make a geographic

distribution of this species in Colombia. Spontaneous populations of *V. meridionale* were surveyed in the Provinces of Boyacá, Cundinamarca, Santander and Nariño located in a low mountain area (between 2 100 and 3 200m elevation) in Colombia (Table 1). Each plant was labeled with an identification code, where the first and the second letters represent province and municipality, respectively, the subsequent letters designate the exact location of a population, and a number indicates the plant organism assessed in the population (Table 2). The field studies were conducted during May-June 2007, that corresponded to the dry season, when berries of *V. meridionale* are usually collected in the mountain areas across the country. The populations were assessed randomly and the sampling scheme was intended to cover the range of precipitation and temperature regimes anticipated for the species (Luteyn 2002), and also taking into account that, in Colombia, *V. meridionale* is scarcely reported growing at elevations above 3 200m and below 2 300m. The population was considered a group of plants in a reproductive state growing under free solar exposure or in understory. Data were taken on populations located at 5km minimal distance one from another. In total 100 plants of *V. meridionale* were characterized individually in 20 spontaneous populations located in the mountain forests of four Provinces. For a given population, each morphological character was accessed in 5 randomly selected plants to evaluate intra-population variability and raw data were then averaged for the statistical analyses.

Soil samples that included the whole topsoil were collected for chemical analysis in all

TABLE 1  
Environmental characteristics of the mountain forest locations of Colombia in which 20 populations of *V. meridionale* were surveyed

Province	Annual precipitation (mm)	Annual temperature (°C)	Solar radiation (MJ/m <sup>2</sup> )
Cundinamarca	1117-1237	16.5-17.0	18.3-18.8
Boyacá	1074-1370	10.2-15.3	20.4-20.9
Nariño	948-958	17.5-18.5	18.0-18.4
Santander	1000-1205	12.0-17.2	19.4-21.3

TABLE 2  
Sampling sites of 20 wild populations of *V. meridionale* in the mountain forest of Colombia

Province	Municipality	Location	Population code	Elevation (m)	Latitude (N)	Longitude (W)
Cundinamarca	Machetá	Boquerón	CMB	2 726	05° 05' 38"	73° 40' 23"
		Vía Boquerón	CMVB	2 447	05° 05' 50"	73° 39' 06"
Boyacá	Chiquinquirá	Arboleda ha	BCHA	2 625	05° 34' 45"	73° 44' 50"
		Arboleda ar	BCAR	2 645	05° 35' 25"	73° 42' 58"
		Quiches	BCHQ	2 682	05° 34' 45"	73° 44' 18"
		Sasa	BCHS	2 613	05° 36' 56"	73° 44' 08"
		Ráquira	Pueblo Viejo	BRPV	2 811	05° 29' 47"
	Tinjacá	Nirque	BRN	2 699	05° 29' 36"	73° 38' 53"
		Firita	BRF	2 927	05° 28' 41"	73° 37' 40"
		Peñas Alto	BRPA	2 382	05° 34' 31"	73° 41' 10"
		La Chorrera	BRCH	2 357	05° 31' 58"	73° 39' 10"
		Arrayanes	BTA	2 544	05° 35' 30"	73° 42' 37"
		Moyavita	BTM	2 620	05° 36' 24"	73° 43' 16"
		Vía Ráquira	BTVR	2 537	05° 35' 23"	73° 41' 38"
Nariño	Pasto	Santa Bárbara	BTSB	2 388	05° 32' 43"	73° 41' 22"
		Alto de Daza	NPAD	2 889	01° 16' 23"	77° 16' 45"
	Relleno Sanitario	NPRS	2 763	01° 16' 42"	77° 15' 57"	
	Buesaco	Meneses	NBM	2 809	01° 17' 55"	77° 13' 54"
Santander	California	La alta	SCLA	3 022	07° 23' 06"	72° 53' 36"
		Veta'e Barro	SCVB	3 168	07° 23' 23"	72° 53' 25"

Five individual plants were randomly sampled within each population. The first and the second letters of a population code represent province and municipality, respectively, and the consequent letters designate the exact location of a population.

surveyed sites and soil pH (soil:water 1:1) was measured in the Laboratory for Soil Analyses of the Faculty of Agronomy, National University of Colombia, Bogotá campus. The climate data were obtained from the meteorological stations of IDEAM, Colombia.

**Morphological measurements:** Description of plant morphology *in situ* was performed using a list of variables consisting of 9 quantitative and 17 qualitative characters according to Lopera (2005). Quantitative variables, excluding plant height, were measured with a digital caliper in mm to two decimal places. Plant height (PH) was measured as a distance between the soil level and the highest point of a principal orthotropic shoot. For measurements of stem diameter (SD), the mid-section of a principal stem was evaluated in each plant. In

order to measure leaf density (LD), number of leaves was counted on 10cm segments of a single branch taken from the mid-part of the plant. Equally, leaf length (LL) and leaf width (LW) were measured in the mid-section of fully expanded leaves. For quantitative fruit variables, diameter of mature fruits was measured in the longest (fruit length, FL) and the shortest (fruit width, FW) sections of the fruits. Number of flowers per inflorescence (FI) and inflorescence length (IL) were counted on a terminal inflorescence located at the same branch, where the fruit characters were evaluated. The stem, leaf, and floral qualitative characters were evaluated subjectively as following: growth habit (1=prostrate, 2=intermediate, 3=erect), ramification density (1=low, no branches; 2=intermediate, 1 or 2 orders of ramification; 3=high, 3 or 4 orders

of ramification), stem pubescence (1=absent, 2=low, 3=abundant), form of leaf margin on fully expanded leaves (1=serrate, number of points: >12/cm; 2=serrulate, number of points: 12-6/cm; 3=crenate, number of points: <6/cm), form of leaf lamina (1=elliptic, 2=ovate, 3=lanceolate, 4=ovulate, 5=oblong-lanceolate), form of leaf base (1=cuneate, 2=rounded), form of leaf apex (1=acuminate, 2=acute, 3=aristate, 4=obtuse), anthocyanins in stem (1=absent, 2=low, 3=medium, 4=high), anthocyanins in leaf margin (1=absent, green margins; 2=present, maroon margins), anthocyanins in terminal inflorescence (1=absent, 2=present), colour of calyx (1=green, 2=maroon, 3=light green or dark green, 4=combination of 1 and 2), colour of pedicels and bracteoles (1=green, 2=maroon, 3=light green or dark green, 4= combination of 1 and 2), flower colour (1=white, 2=pink-white), fruit wax (1=absent, 2=present), fruit colour (1=blue, 2=purple, 3=black violet), persistence of sepals on mature fruit (1=absent, 2=present), petiole attachment to lamina (1=leveled, 2=slightly sunken).

Summary statistics were used to describe the variation in morphology of *V. meridionale*, and a correlation analysis was conducted on the full set of quantitative morphological traits. Differences were considered significant at  $p < 0.05$  level. A Principal Component Analysis (PCA) was undertaken on quantitative characters using SPAD 4.5 program (Pagès 2004). Since distributions of all quantitative characters were approximately normal, no data transformation for PCA was required.

## RESULTS

**Natural environments of the populations:** Wild populations of *V. meridionale* were found in four mountain forest regions of Colombia, within the elevations 2357 and 3168m (Table 2). Based on the preliminary zonification studies conducted for this species in the Province of Cundinamarca, the wild populations of *V. meridionale* in Boyacá, Santander and Nariño were identified and characterized. In general, the populations were

found in locations with slopes of 3 to 50%, and vegetation typical of life zones of lower dry montane and lower wet montane forests, that were rich in arrayán (*Myrcianthes leucoxylla* (Ortega) McVaugh), laurel (*Myrica parvifolia* Benth.), Ericaceae of genera *Pernettya* and *Cavendishia*, epiphytes (Bromeleaceae), and hemiepiphytes (Clusiaceae).

Most of the plants of *V. meridionale* in each population were found growing under free solar exposure; also, the plants were observed as a co-dominant species growing over the litter coverage and associated with oak (*Quercus humboldtii* Bonpl.) and pine patula (*Pinus patula* Schiede ex Schltdl. & Cham.), making this last association very common in all studied Provinces. Other frequent species in forest locations of *V. meridionale* were *Dendropanax macrophyllum* Cuatrec. and *Bombacopsis patinoi* Dugand & Robyns (Nariño) and *Alnus acuminata* Kunth. (Cundinamarca, Boyacá and Santander). For Nariño locations, the third typical species were *Ossaea macrophylla* (Bentham) Cogniaux as well as various Rubiaceae species, especially *Elaeagia utilis* (Goudot) Wedd. and *Rubus* spp. Ground vegetation in all studied sites consisted mainly of mosses (*Sphagnum* spp.) and ferns, having total percentage coverage of more than 80%.

The climate factors that apparently affected the distribution of populations *V. meridionale* in the wild were temperature, precipitation, and radiation. Climate conditions, where the populations were found across the studied zones, highly differed in mean daily temperatures (8.4-23.6°C) and annual precipitation rates that ranged from 948mm (very dry areas) up to 1237mm (very humid areas); the variation in solar radiation was relatively low, between 18.0 and 21.3MJ/m<sup>2</sup> (Table 1).

A constant condition was the soil acidity, where pH ranging from 3.8 to 5.3, with pH 4.6 on average. All soils of the study zones presented good drainage and udic moisture regime. Plants were found growing in soils ranging from large A horizons that exceeded 50cm to very superficial soils, e.g. directly on the parental rock material, as in a particular

case of populations BRPV and BRF located in Ráquira, Boyacá.

**Quantitative characterization of species morphology in populations:** Plant height, stem diameter and foliar density were the most variable quantitative morphological traits, with coefficients of variation higher than 50% (Table 3). The mean plant height of 159.63cm was characterized with a standard deviation of 90.77cm indicating high degree of data dispersion (Table 3). The stem diameter presented a mean value of 3.51cm with a standard deviation of 2.39cm, apparently affected by the presence of very dissimilar data, as may be seen in Table 3. High data variation was equally observed for foliar density, with a standard deviation of 108.68cm with respect to a mean value of 212.21cm (Table 3).

Leaf and fruit size characters, namely length and width, had narrow amplitude of variation, with coefficients of variation lower

than 21%, indicating that these were relatively homogeneous characters of low phenotypic plasticity in all populations (Table 3). The leaves were on average 2.24cm long and 0.96cm wide with standard deviations of 0.39 and 0.20cm, respectively, while fruit length and fruit width presented mean values of 0.80 and 0.83cm with standard deviations of 0.18 and 0.17cm, respectively. Although the behavior of fruit size characters was of low plasticity in the studied metapopulation, in some habitats of the highest elevations, plants had a tendency to produce leaves and fruits of the smallest sizes. These patterns were also observed with increasing number of fruits per inflorescence, suggesting a competition among the fruits; however, on rare occasions fruit diameters were less than 6mm. Inflorescence length and number of flowers per inflorescence were characterized as more heterogeneous, with coefficients of variation of 26.68% and 30.12%, respectively (Table 3).

TABLE 3  
Descriptive simple statistics and associated abbreviations for 9 quantitative variables evaluated in 20 wild populations of *V. meridionale*

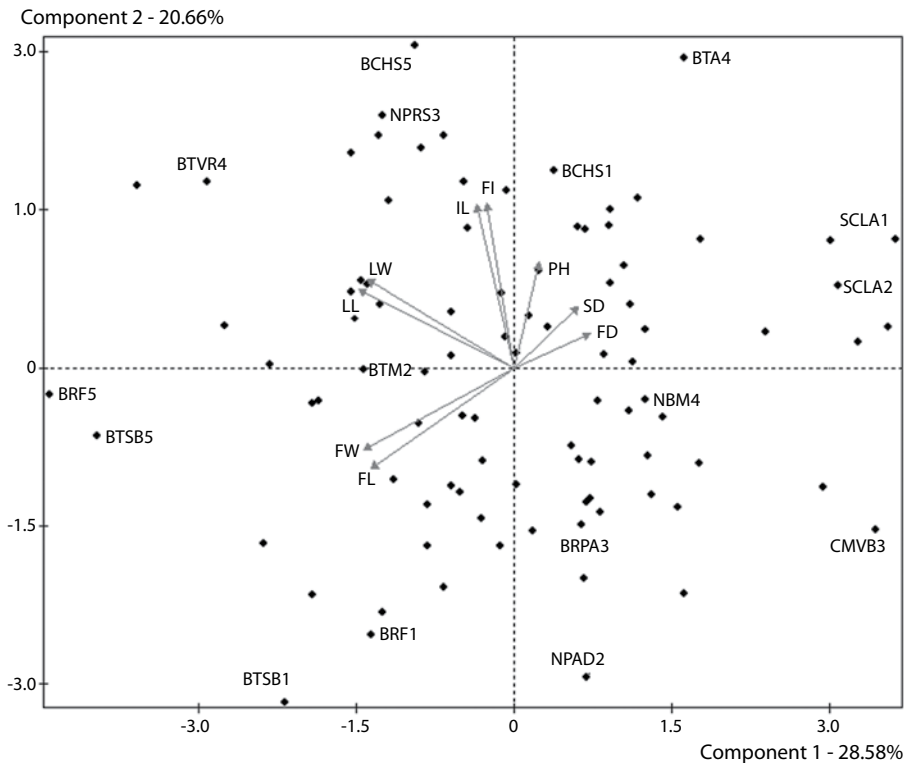
Variable	Mean	Standard deviation	Coefficient of variation (%)	Maximum value and correspondent plant	Minimum value and correspondent plant
Plant height PH, cm	159.63	90.77	56.86	500.0 NPRS3	16.0 NPAD2
Stem diameter SD, cm	3.51	2.39	68.13	15.00 SCLA1	0.65 NBM4
Foliar density FD	212.21	108.68	51.21	583.00 SCLA2	45.00 BTM2
Leaf length LL, cm	2.24	0.39	17.37	3.34 BTSB5	1.00 CMVB3
Leaf width LW, cm	0.96	0.20	20.58	1.52 BTVR4	0.50 CMVB3
Inflorescence length IL, cm	3.03	0.81	26.68	5.00 BCHS5	1.20 NPAD2
Number of flowers per inflorescence FI	12.14	3.66	30.12	22.00 BCHS1	3.00 BRPA3
Fruit length FL, cm	0.80	0.14	18.02	1.26 BRF5, BTSB1	0.50 BTA4
Fruit width FW, cm	0.83	0.14	17.19	1.20 BRF1	0.60 BTA4

The identification of each plant corresponds to a population code plus number of plant (from 1 to 5) surveyed.

Among the characters observed, the highest correlation coefficients ( $p < 0.05$ ) were between fruit length and fruit width (0.90), leaf length and leaf width (0.78), plant height and stem diameter (0.60), and inflorescence length and number of flowers per inflorescence (0.57). No relationships between inflorescence length and fruit length or fruit width were found. The weak negative correlations were observed between foliar density, on one side, and leaf length (-0.20) or leaf width (-0.21), on the other side, indicating that, in a ramet with smaller number of leaves, these tended to be larger. Stem diameter had a positive correlation with foliar density (0.36). The leaf size characteristics were correlated with fruit size and inflorescence size, such as the positive correlations were

established between leaf length, on one side, and fruit length (0.29), fruit width (0.38), or inflorescence length (0.20) on the other side. Leaf width had the positive correlations with fruit length (0.20), fruit width (0.28) or inflorescence length (0.21).

The PCA revealed that fruit size variables, namely fruit length and fruit width, contributed to the first component (F1) that accounted for 28.58% total variance (Fig. 1). Number of flowers per inflorescence and inflorescence length were the major loading characters to the second component (F2) that explained 20.66% total variance. It may be noticed that the plants with extreme (maximum or minimum) states of quantitative characters were observed within the same area as well as in different



**Fig. 1.** Biplot on the first and second principal components of 9 quantitative characters of 20 spontaneous populations of *V. meridionale* sampled in the mountain forest of Colombia. Abbreviations: PH: plant height; SD: stem diameter; FD: foliar density; LL: leaf length; LW: leaf width; IL: inflorescence length; FI: number of flowers per inflorescence; FL: fruit length; FW: fruit width. Codes designate individual plants characterized with the highest or the lowest value of each quantitative character, as presented in Table 3.

geographical locations (Fig. 1, Table 3). For example, the plants located in Pasto, Nariño presented both the highest (5.0m, NPRS3 that exceeded the height ranges typical for the shrubs of Vaccinioideae (Gentry 1994, Lens *et al.* 2004) and the lowest height (0.16m, NPAD2) within all populations evaluated. The latest individual also had the smallest inflorescence length (1.2cm), while the longest inflorescence (5.0cm) was observed on a plant in Chiquinquirá, Boyacá (BCHS5), located in the positive quadrant of F2, for which the variable of inflorescence length has the highest contribution (Fig. 1). The highest leaf length (3.34cm) was measured in a plant from Santa Bárbara, Boyacá (BTSB5) located in the negative quadrant of F1, whereas the smallest leaf length (1.0cm) was recorded for a plant CMVB3 from Vía Boquerón, Boyacá (Table 3), located in the opposite quadrant of the plot

(Fig. 1). In general, the PCA revealed a high dispersion of characters without correlation with their location, which indicates a genetic diversity of plants within the metapopulation. Equally, the PCA demonstrated a high dispersion in quantitative characters within some populations, showing a high intrapopulation morphological variation in plants of *V. meridionale*.

**Qualitative characterization of species morphology in populations:** A qualitative description of *V. meridionale* in wild populations illustrated that plant growth habit varied among intermediate and erect, with similar frequencies of 41.9% and 45.74%, respectively. Ramification was predominantly dense with 47.87% individual plants located within this category; however, there were 32.98% plants of intermediate density (Table 4).

TABLE 4  
Qualitative characters evaluated in 20 wild populations of *V. meridionale*

Variable	Type	Frequency	Frequency %
Growth habit	1=prostrate	12	12.77
	2=intermediate	39	41.49
	3=erect	43	45.74
Ramification density	1=low	18	19.15
	2=intermediate	31	32.98
	3=high	45	47.87
Stem pubescence	1=absent	0	0.00
	2=low	35	37.23
	3=abundant	59	62.77
Leaf margin form	1=serrate	18	19.15
	2=serrulate	68	72.34
	3=crenate	8	8.51
Leaf lamina form	1=elliptic	7	7.45
	2=ovate	1	1.07
	3=lanceolate	48	51.06
	4=ovulate	3	3.19
	5=oblong-lanceolate	35	37.23
Leaf base form	1=cuneate	90	95.74
	2=rounded	4	4.26
Leaf apex form	1=acuminate	8	8.51
	2=acute	66	70.21
	3=aristate	5	5.32
	4=obtuse	15	15.96



TABLE 4 (continued)  
Qualitative characters evaluated in 20 wild populations of *V. meridionale*

Variable	Type	Frequency	Frequency %
Anthocyanins in stem	1=absent	10	10.64
	2=low	14	14.89
	3=medium	31	32.98
	4=high	39	41.49
Anthocyanins in leaf margin	1=absent	26	27.66
	2=present	68	72.34
Anthocyanins in terminal inflorescence	1=absent	25	26.60
	2=present	69	73.40
Colour of calyx	1=green	32	34.04
	2=maroon	3	3.19
	3=light green or dark green	0	0.00
	4=combination of 1 and 2	59	62.77
Colour of pedicels and bracteoles	1=green	54	57.45
	2=maroon	39	41.49
	3=light green or dark green	0	0.00
	4= combination of 1 and 2	1	1.06
Flower colour	1=white	23	24.47
	2=pink-white	71	75.53
Fruit wax	1=absent	9	9.57
	2=present	85	90.43
Fruit colour	1=blue	0	0.00
	2=purple	41	43.62
	3=black violet	53	56.38
Persistence of sepals on mature fruit	1=absent	0	0.00
	2=present	94	100.00
Petiole attachment to lamina	1=leveled	13	13.83
	2=slightly sunken	80	85.17

The variables of leaf form, such as forms of margin, apex, lamina and base had lower ranges of dispersion than the above-mentioned characters, so that the predominant leaf forms may be distinguished. In the 51.06% of studied plants, leaf lamina form was lanceolate followed by 37.23% oblong-lanceolate. Serrulate leaf margins constituted a distinctive feature of *V. meridionale* plants having a frequency of 72.34% in all populations. The majority of plants presented leaves with cuneate foliar base (95.74%) and acute apex (70.21%) (Table 4).

Anthocyanin contents in the mid-section of plant stems were mostly high (32.98%), medium (41.49%), low (14.89%) and only

10.64% of plants completely lack this product. Accumulation of anthocyanins in leaf margins and inflorescences was detected in more than 72% of evaluated plants. Calyx colour varied among green, maroon, and a combination of these, showing maroon on the margins and green on the central part of the calyces. The combination of green and maroon was the most frequent colour (62.77%) followed by the one with complete green calyx (34.04%). Colour of pedicels was predominantly green (57.45%), but maroon pedicels were also observed (41.49%). Colour of bell-shaped flowers was characteristically pink-white to almost fuchsia (75.53%), whereas some flowers were

completely white (24.47%). Pubescence in mid-sections of stems was present for 62.77% plants and absent for 37.23% plants assessed in the study (Table 4).

Fruit colour was purple (43.62%) or black violet (56.38%), while no fruits were found with a the characteristically blue colour of *V. corymbosum* or *V. myrtillosum*, that may vary from blue to almost black. However, some mature berries of *V. meridionale* had white and light rose colour, which may be the possible mutants of structural or regulatory genes involved in anthocyanin synthesis, as it was shown for *V. myrtillosum* (Jaakola *et al.* 2002).

Some characters of fruit morphology, such as wax presence on fruit surface (90.43%) or the form of petiole insertion, and for which the major form was slightly sunken (85.17%), had very low variation. Persistence of sepals was a constant feature for mature fruits in all evaluated populations, with 100% plants grouped in the category "present" (Table 4).

## DISCUSSION

Our results indicated that natural populations of *V. meridionale* might be found under highly heterogeneous climate and microclimate conditions in a tropical mountain forest, in different geographic areas of Colombia. The location of wild populations of *V. meridionale* in the studied zones corresponded to the habitats previously described by Luteyn (2002) who mentioned preference of neotropical *Vaccinium* spp. to humid and undisturbed mountain habitats. The plants of *V. meridionale* were found growing under partial shade or free solar exposure as well as in canopy gaps suggesting high rates of adaptability to different micro-environments including those of solar radiation within a same study area.

Plant height, stem diameter and foliar density were highly variable morphological traits (Table 3), and, while plant height is a subject of phenotypic variation in wild clones of *Vaccinium* spp. (Gustavsson 2001), in our study, this variable might not provide a consistent comparison across the plants because their

ages were unknown. High variability of the above mentioned descriptors may be due to the fact that, in wild populations, there were plants of various age stages, reproduction strategies, light, water, and nutrient regimes - all these differences, when combined with occasional distortion of plant habitats by tree lodging, generated populations highly heterogeneous in plant height, stem diameter and foliar density. On the contrary, a low variation in fruit length and fruit width of *V. meridionale* (Table 3), indicates a low phenotypic plasticity of these characters and could be attributed to a low environmental effect over the fruit size that, in turn, implies that characterization of fruit morphology *in situ* could be used to estimate the levels of genetic diversity (Vargas & Blanco 2000). Fruit size in *Vaccinium* sp. is known to be affected both by genetic and environmental factors; among the latest, pollination (Eck 1988) and irrigation regimes (Hicklenton 2000, Mingeau 2001); a reduction in leaf size with increasing altitude, which is commonly observed in deciduous *Vaccinium* spp., that could be due to withstanding nitrogen limitation and low temperatures (Woodward 1986). Inflorescence length and number of flowers per inflorescence might also serve as indicators of plant adaptation to certain environments, as in the case of *V. corymbosum*, for which the flower number was reduced under moderate water stress (Mingeau 2001). These characters describe a reproductive state of the species; however, as in case of fruit or leaf size, low or moderate variation in inflorescence length and number of flowers per inflorescence (CV<30.2%, Table 3) indicates their low phenotypic plasticity.

In general, different environments that represented microclimates, influenced the variation of quantitative characters. Our findings are consistent with the pioneer studies of Medina & Lobo (2004) of wild populations of *V. meridionale* in the Province of Antioquia, Colombia, who revealed that plant height and stem diameter were highly variable morphological characters. The positive correlation established between plant height and stem diameter might be explained by plant age. Additionally,

in Ericaceae, an environment might have a significant effect over the stem diameter, such as the trends in decreasing stem diameter with increasing altitude of plant habitat (Woodward 1986, Merev & Yavuz 2000) or microclimate changes including low availability of soil water (Mingeau 2001).

The PCA revealed that fruit length, fruit width, number of flowers per inflorescence, and inflorescence length were the main loading characters that accounted for a total variance (Fig. 1). These characters, especially fruit size, are useful in characterization of sexual reproductive potential of *V. meridionale*, taking into account that the largest seeds (up to 1.9mm length and 1.4mm width) have the highest germination rates, and could be obtained from the largest fruits (Valencia & Ramírez 1993). The latest data may have an importance for sexual recruitment of the species in the wild, however, the rates of seedling establishment in natural habitats, in case of *V. meridionale*, are unknown, and, in case of other *Vaccinium* spp. (Eriksson 1989), might be negligible. However, it is worth mentioning that propagation by seeds, as well as somatic mutagenesis and initial genetic diversity in a given population, are the main factors that increase a genetic variation in clonal populations of *Vaccinium* spp. (Persson & Gustavsson 2001, Albert *et al.* 2005). The results of the PCA demonstrated a genetic diversity of *V. meridionale* within the metapopulation as well as an intrapopulation variation in plant morphology for some populations.

The morphological traits that, in the majority of plants, respond to the environmental stimuli changes are elongation of stem and foliar growth (Fordyce 2006). Thus, within a given population, the tallest plants of *V. meridionale* were frequently found in partial shade, while those of arbustive prostrate growth were typical of open sites; as shown for other Ericaceae (Dawson & Heenan 2004). Additionally, *V. meridionale* is a plant of clonal propagation through stolons and rhizomes (McDonald *et al.* 2003), and variation in form of growth in clonal plants also represents a particular case

of plasticity (Navas & Garnier 2002) observable in other *Vaccinium* spp.; as for example, a reduction in layering stem growth under intense radiation (Moola & Mallik 1998) or during fruit production (Elle 1996, Gustavsson 2001). The fact that plants were studied in highly heterogeneous environments makes it feasible to conduct further studies on the contribution of phenotypic plasticity to morphological variability of *V. meridionale*.

Several qualitative characters of species morphology were highly variable, which at least partially, could be attributed to phenotypic plasticity. Phenotypic plasticity related to changes in plant height and shape may be directed, in particular, to optimize light use efficiency in natural populations (Dawson & Heenan 2004, Wang *et al.* 2006). This could explain a high variability in height (plant height, stem diameter) and shape (growth habit, ramification density) characters of *V. meridionale*, and, indirectly, indicate that plants in the studied metapopulation experienced a certain environmental pressure. At the same time, some qualitative characters of fruit or leaf morphology had very low (wax presence on fruit surface, form of petiole insertion) or null (persistence of sepals on mature fruits) variation demonstrating that these characters were genetically determined.

The variables that characterize colour of stems, flowers and leaves in *Vaccinium* depend on anthocyanins contents in these plant organs (Eck 1988). Phenotypic variation in fruit colour, as the one shown in our study, is readily apparent among wild clones of lowbush blueberry (Kalt *et al.* 2001) and attributed to tissue levels of anthocyanins and other flavonoid compounds (Eck 1988). Synthesis of anthocyanins in leaves and fruits of *Vaccinium* is developmentally regulated (Witzell *et al.* 2003) and could be highly variable when stimulated by numerous environmental factors, including light, UV radiation, fungal infection, wounding, drought or low nitrogen status (Jaakola *et al.* 2004, Witzell & Shevtsova 2004). Direct sunlight activates the synthesis of anthocyanins in leaves, fruits and flowers of *V. myrtillus* (Jaakola *et al.* 2004 and references therein), so

that tissue concentrations of anthocyanins tend to increase conferring a higher desiccation tolerance and photoprotection of cells, especially at low temperatures (Tahkokorpi *et al.* 2007), the normal conditions with increasing altitudes in plant habitat. While the current relationship may not be always recognizable (Rieger *et al.* 2008), in our study, the highest levels of anthocyanins in leaves, fruits and flowers were scored mostly on plants growing in the Provinces of Boyacá and Santander that are characterized with the highest solar radiation levels (Table 1). Flowers colour variation among the populations might also have an effect on the reproductive traits and genetic diversity. Thus, in temperate climates, plants of lingonberry with more pinkish corollas began flowering later than plants with white flowers (Gustavsson 2001); this feature, under dry-rainy seasonality conditions in a tropical mountain forest, might be used to synchronize flowering and fructification with cycles of pollinators and frugivores, respectively (Hilty 1980).

In general, our studies revealed a wide morphological variation in the metapopulation of *V. meridionale* across the study areas. Several quantitative (plant height, stem diameter) and qualitative (growth habit, ramification density, presence of anthocyanins in stem) descriptors of the species morphology were highly influenced by the environment. Our results suggested that an important genetic resource exists for *V. meridionale* in the wild that could provide a basis for introduction of this species into world agriculture. Thus, low variation in fruit size (CV<18.02%), which constitutes a target trait for plant breeders, might be useful for the development of breeding programs aiming to the selection of cultivars of *V. meridionale*. Additionally, the studies on genetic diversity in *V. meridionale* at the molecular level would help to implement a conservation strategy for this species. Alteration of the species habitat results in reduction of population size that, in turn, could diminish a degree the morphological variation within and among the populations, the process that would further determine ecotypes formation of *V. meridionale*. While some

habitats of natural populations of *V. meridionale* in Santander and, especially, Nariño, remain undisturbed and practically inaccessible in the near future, fragmentation of a mountain forest, and reduction in populations size are increasing in other regions, such as Cundinamarca and Boyacá, so that the development of strategies to prevent genetic erosion of *V. meridionale* in its natural habitats should be addressed.

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## RESUMEN

*Vaccinium meridionale* es una planta promisoría para la región Andina de Sudamérica y está disponible actualmente sólo en forma silvestre. Las poblaciones espontáneas de esta planta se encuentran en las montañas colombianas y existen muy pocos reportes publicados respecto a su morfología. Se realizó un estudio de zonificación de *V. meridionale* en cuatro áreas principales de un bosque de baja montaña de Colombia (Provincias de Boyacá, Cundinamarca, Santander y Nariño) en 2007. Un total de 20 poblaciones y 100 plantas de *V. meridionale* fueron individualmente caracterizadas usando una lista de 26 descriptores de variables morfológicas (9 caracteres cuantitativos y 17 caracteres cualitativos). Nuestros resultados indicaron que las poblaciones naturales de *V. meridionale* podrían ser encontradas en el bosque tropical bajo unas condiciones de clima y microclima muy heterogéneas, en regiones de montaña diferentes entre 2 357 y 3 168masl. Los arbustos de *V. meridionale* presentaron un nivel alto de la variación intrapoblacional en varios caracteres morfológicos cuantitativos (altura de planta, diámetro de tallo) y cualitativos (hábito de crecimiento, densidad de ramificación, presencia

de antocianinas en tallos), sugiriendo una plasticidad fenotípica ambientalmente inducida. La altura de planta, el diámetro de tallo y la densidad foliar fueron los rasgos morfológicos más variables, con coeficientes de variación superior al 50%. Sin embargo, varios caracteres cuantitativos de su potencial reproductivo, como dimensiones de baya, longitud de raquis y número de flores por inflorescencia, presentaron plasticidad baja con coeficientes de la variación inferior al 30.2%, indicando que estos caracteres fueron genéticamente determinados. Los coeficientes de correlación más altos ( $p < 0.05$ ) fueron para longitud de fruto y ancho de fruto (0.90), longitud de hoja y ancho de hoja (0.78), altura de planta y diámetro de tallo (0.60), y longitud de inflorescencia y número de flores por inflorescencia (0.57). Los resultados sugieren que en condiciones naturales existe un recurso genético importante para esta especie. La poca variación en el tamaño de fruto, puede ser útil para la selección de cultivares de *V. meridionale* y se constituye un rasgo objetivo para fitomejoradores. Los resultados de este estudio también pueden ser aplicados en programas de conservación para proteger estas poblaciones diversas en los bosques de montaña de Colombia.

**Palabras clave:** caracteres morfológicos de planta, *Vaccinium meridionale*, bosque tropical, in situ caracterización, región Andina, Colombia.

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