Identification and characterization of polymorphic microsatellite loci in Mortiño (*Vaccinium floribundum* Kunth)

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Abstract

Mortiño (*Vaccinium floribundum* Kuth) is a perennial shrub from the Ericaceae family. This wild species is endemic to the high Andes of South America, and is found exclusively at altitudes between 1400 masl and 4350 masl. Mortiño plants produce edible berries which hold a special ethnobotanic significance for indigenous and rural communities, related to their use in ceremonial beverages, medicinal preparations and other food preparations. The species is classified as Vulnerable by the IUCN, mainly due to the fragmentation of its habitat. This prompts the need for genetic diversity and population structure studies, which can provide valuable information about a species and aid in the development of conservation and sustainable agriculture strategies. However, genetic studies are limited and molecular tools are not yet expanded in mortiño. In this study, we identify and characterize six polymorphic microsatellite loci as tools for future genetic analysis. The six loci show allelic richness values of 3-8 alleles per locus, observed PIC values ranging from 0.36 to 0.69, and expected heterozygosity values from 0.41 to 0.72. These species-specific polymorphic microsatellite markers will be used for the genetic characterization of *V. floribundum* populations across the Ecuadorian highlands.

Introduction

Fig 1. Shrub and fruit of Mortiño (*V. floribundum K*unth)

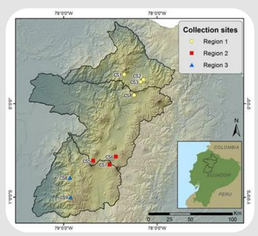
Mortiño (*Vaccinium floribundum* Kunth) (Fig 1.) is a native, wild diploid species from the high Andes of South America. It is distributed in Perú, Ecuador, Colombia and Venezuela, mainly in mountainous zones between 1400 and 4350 masl, where temperatures range from 8 to 16°C (also known as *páramos*). In Ecuador, this plant is spread across the highland region from the northernmost province of Carchi to the southernmost province of Loja (Trujillo, 2009; Coba et al., 2012).

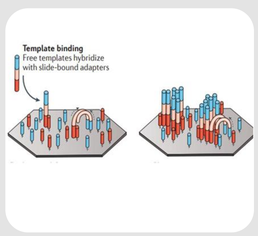
This species has important characteristics for the local ecosystems, native wildlife and indigenous communities. Because of its regeneration capacity, mortiño is essential for the *páramo* reforestation (Aguilar, 2009). In addition, it represents a food source for wild animals such as squirrels and pigeons (Echeverría et al., 2009).

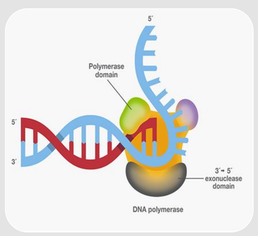
Due to its nutritional fruit, mortiño is consumed by Andean communities as juice, jams, sweets and ceremonial beverages (Coba et al., 2012). This plant has a high content of polyphenols that are used to treat different metabolic disorders (Schreckinger et al., 2010).

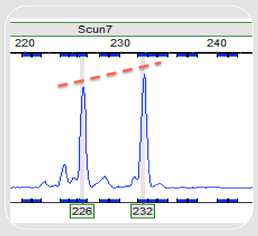
Owing to various agricultural issues, *V. floribundum*'s habitat has shown significant fragmentation, representing a considerable risk for the species' conservation status (Coba et al., 2012). For this reason, it is important to understand the diversity and population structure of the mortiño wild populations, in order to develop conservation strategies and sustainable agricultural programs (Cobo et al., 2016). However, the lack of information about this endemic species limits the development of adequate management schemes. The use of genetic tools would help improve the ability to define population structure and describe the existing genetic diversity of a species (Bertolotti et al., 2015). In this study, we present a set of preliminary polymorphic microsatellites markers developed specifically for this species, which can be used to study the genetic diversity and population structure among *V. floribundum* populations.

Methods









1) *Sampling*:

60 samples were collected from 9 locations among the provinces of Pichincha, Imbabura and Cotopaxi between the years 2012 and 2013.

2) *Microsatellite markers development:*

The microsatellite markers were developed by Jennifer Rowntree (Griffiths et al., 2016). 30 microstellites were selected and synthesized.

3) *PCR amplification:*

30 primers were standardized. Primers were labeled with VIC, 6-FAM, NED and PET using the three primers system-Tail A (Blacket et al., 2012).

4) *Data analysis:*

6 loci were analyzed. Allele peaks were scored using GeneMarker Software, and the *adegenet* and *polysat* packages in R were used to obtain He, Ho, PIC, Na.

Results

Table 1. Characterization of six preliminary polymorphic microsatellite loci for *Vaccinium floribundum* Kunth

Locus	Primer sequence (5´-3´)	Repeat motif	Dye	Ta (°C)	Size range (bp)	Na	Ho	He	PIC
M001	F: AACCTGTACAAGTCTACCCCTACCG R: TAATAACAGAACATCAGTGCAAGGC	TTCCTG (48)	VIC	58	327-369	8	0.54	0.72	0.69
M002	F: CAAAATAACCCTCAAACACACACC R: TTTATCATTATCCTACAGCGTCACC	TTTGG(50)	PET	58	158-163	3	0.34	0.43	0.38
M007	F: GAAGCCTGGTCAGTCCTTTCC R: CACTAGGAGTCTGACTTTCCTCTGC	TGC (24)	PET	63	285-294	3	0.33	0.42	0.37
M008	F: ACTACCCTGCCACTCTCACTACC R: CGGACCCAGAGTTAGGATAATACC	ACC (27)	VIC	63	336-366	8	0.31	0.48	0.45
M009	F: TATTCTTATGTTCGTCCTCGTAGGC R: TTTCCTGCTAGCTGTTGTTGTAACG	AGT (24)	NED	63	402-408	3	0.44	0.41	0.36
M010	F: TAGACAACCACTTTCTTTGGTTTCC R: AATAAGTCTTGCTTTGTACCTTGCC	TTC (30)	NED	63	292-307	6	0.4	0.45	0.41

A total of 31 alleles across the 60 individuals were registered by the six polymorphic primer pairs. The levels of polymorphism range from 3 to 8 alleles per locus, and estimates of expected heterozygocity lie between 0.41 and 0.72. PIC values range from 0.36 to 0.69.

Discussion

PIC and heterozygocity values are important descriptors of the genetic diversity at the interspecific and intraspecific levels. Particularly, the PIC index shows a marker's ability to detect population polymorphisms depending of the number and frequency of the alleles (Chesnokov & Artemyeva, 2015). Our results show a wide distribution of PIC values across loci ranging from 0.36 to 0.69. Locus M001 seems to be the more informative from the six loci (PIC=0.69), followed by M008 and M010 (PIC values of 0.45 and 0.41 respectively). The three remaining loci (M002, M007 and M009) are the less informative, with values between 0.36 and 0.38 (Table 1).

On the other hand, allele numbers for each locus (3-8 alleles) (Table 1) are relatively low compared to other species, such as highbush and lowbush blueberries (Boches et al., 2006; Debnath et al., 2014). This result is in accordance with those of Cobo et al. (2016), where 2-14 alleles per locus were reported using heterologous SSR markers. The expected heterozygocity in this study (He=0.485, var=0.013) also sides with the previously reported heterozygocity of He= 0.493, var=0.084 (Cobo et al., 2016). This limited allelic number and low expected heterozygocity can be explained by the small sampling range used in both studies, which reduces the probability of rare alleles being detected (Kalinowski, 2004).

According to the PIC and heterozygocity values, there is a moderate degree of the genetic diversity in our sample. Low values of heterozygosity could be explained by high rates of self-pollination in mortiño, compared to other *Vaccinium* species such as lingonberry, (which commonly shows cross-pollination), as well as its potential to propagate clonally (Luteyn, 2002). In addition, mutations at the SSR primer binding sites could also lead to reduced rates of alleles, calling null alleles and loss of codominance from the markers (Boches, 2005).

Conclusions

We report SSRs markers specifically designed for *Vaccinium floribundum* for the first time.

The six markers tested until now display high levels of polymorphism, making them informative for genetic analysis.

These markers will be used to expand the genetic characterization of *V. floribundum* populations in the Ecuadorian highlands.

Knowing about the genetic diversity and population structure of *V. floribundum* can help us to develop conservation programs that preserve this important biological resources from the Andean Regions.

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