Euphorbia piscatoria first approach to its molecular diversity using ISSR markers

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INTRODUCTION

The genus *Euphorbia* L. is represented in Macaronesia by 36 taxa, including one Macaronesian endemic (*Euphorbia mellifera* Aiton shared between Madeira and Canary Islands) and 14 "single-archipelago" endemics (Azores 2, Madeira 1, Selvagens 1, Canary islands 9 and Cape Verde 1).
Madeira Archipelago tree spurges taxa include *Euphorbia mellifera*, restricted to Madeira Island (also present in La Palma, Gomera and Tenerife), and *E. piscatoria* Aiton as endemic present in Madeira, Desertas and Porto Santo islands. *E. piscatoria* is included in *Euphorbia* subg. *Esula* Pers. sect. *Aphyllis* Webb & Berthel. subsect. *Macaronesicae* Molero & Bares.
Euphorbia piscatoria is a characteristic species of the *Euphorbietum piscatoriae* Sjögren (Madeira and Desertas, Fig. 1) and the *Gennario diphyllae-Euphorbietum piscatoriae* Jardim, Capelo, Sequeira, Aguiar & J.C. Costa (Porto Santo, Fig. 2) (Costa *et al.* 2012).
This study aims to search for patterns of genetic diversity related both to populations of Madeira and Porto Santo islands using inter-simple sequence repeat (ISSR) markers.

MATERIALS AND METHODS

Samples of *E. piscatoria* were collected in Porto Santo (3 populations) and in Madeira Island (8 populations) (Fig. 3). In total, young, healthy leaves of 34 individuals were collected and stored in silica gel. Silica dried leaves were ground to powder in liquid nitrogen. Genomic DNA was isolated using a CTAB based protocol. The concentration and quality of the DNA were assessed by agarose gel electrophoresis and ImageJ program. PCR amplifications were carried out in 15 μL using 20 ng of template DNA, 1x *My Taq* PCR mix (Bioline) and 0.3 μM ISSR primers (Table 1). The PCR cycling procedure was according to Gouveia *et al.* (2014). Duplicate amplifications as well as of two different DNA extractions were performed randomly for 10% of the samples to assess the consistency of ISSR profiles. PCR products were resolved by electrophoresis on 1.5% agarose gels in 1x TAE buffer stained with ethidium bromide and photographed under ultraviolet light. Clear, unambiguous and reproducible loci were scored visually in a size range from 0.2 to 2.0 kb for presence (1) or absence (0) on two independent occasions and compiled into a data matrix. ISSR data was analyzed using the NTSYSpc version 2.20e (Rolf 2005), Popgene version 1.32 (Yeh *et al.* 1997) and Arlequin (Excoffier L & Lischer 2010).

RESULTS AND DISCUSSION

All primers generated complex band profiles. Eighty four ISSR markers were scored of which 67 (79%) were polymorphic *loci* (Table 1). The most informative band profile was generated by primer UBC 814 as it was able to distinguish the samples from Porto Santo Island from those of Madeira Island (Fig. 4).
The dendrogram (Fig. 5A), based on UPGMA analysis of ISSR polymorphisms using DICE similarity coefficient, grouped all individuals from Porto Santo, whereas unpaired segregation was obtained from *E. piscatoria* collected in the N/S of Madeira Island. A similar result was obtained by Principal Coordinate Analysis based on Simple Matching (Fig. 5B).

ISSR markers used to determine the genetic diversity and genetic structure of *E. piscatoria* showed that all estimated parameters were lower in the population of Porto Santo (Table 2) when compared to those from Madeira Island.

Genetic analysis showed a limited genetic diversity (*Hs*) within populations (Table 3). The distribution of genetic diversity among and within populations showed that genetic diversity was higher in total than within populations, being highest in the north cost of Madeira Island. The coefficient of gene differentiation (*Gst*) was estimated to be 0.289, indicating that only 28.9% of the genetic variation was distributed among populations and 71.1% of the variation existed within populations, supporting local differentiation of populations.
In Madeira genetic variability of northern *Euphorbia* populations could be explained by their small size and discontinuous distribution (only a few small populations are known). In contrast, southern Madeira populations have a continuous distribution due to the extent of their potential distribution area (from sea level to 250 m a.s.l.) and because they are dominant in secondary vegetation (*Euphorbietum piscatoriae,* expanded due to agriculture abandonment), therefore potentially constituting a panmictic population.
Porto Santo represents a very distinct situation due to the overwhelming degree of past landscape change/destruction and to the maintenance of stress factors such as herbivory by rabbits or grazing by goats. In fact, *E. piscatoria* is represented by a small number of individuals per site, and due to the small size of the island it could genetically correspond to a single population (further studies are needed to check local genetic variation).
Morphological differences observed for Porto Santo plants (such as size, shape and colour of stems, leaves, cyathium, etc.) could correspond to distinct biogeographical histories and/or ecological conditions (Porto Santo is older than Madeira, 14 Ma vs 5.6 Ma, and more xeric).
In what concerns Madeira versus Porto Santo our results clearly support Barres *et al.* (2017) using AFLP and cpDNA. Morphological data so far obtained are coherent with molecular data and support the acceptance of two Evolutionary Significant Units as proposed by Barres *et al.* (2017) and consequently a taxonomical segregation.

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Figure 1. *Euphorbietum piscatoriae*, Achadas da Cruz, Madeira Island.

Figure 2. *Gennario diphyllo – Euphorbietum piscatoriae*, Portela, Porto Santo Island.

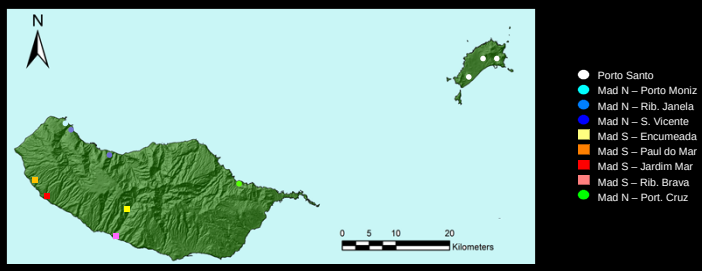

Figure 3. Madeira and Porto Santo maps with approximate locations of sample sites represented by dots.

Table 1. The characteristics of ISSR primers used and the polymorphism detected in *E. piscatoria*.

Primer	Sequence 5' - 3'	Annealing temperature (°C)	Nº scored bands	Nº polymorphic bands	Percentage of polymorphic loci
UBC 814	$(CT)_8A$	51	9	6	66.7
UBC 841	$(GA)_8YC$	52	15	12	80.0
UBC 888	$BDB(CA)_7$	55	7	6	85.7
UBC 889	$DBD(AC)_7$	55	19	18	94.7
UBC 890	$HVH(GT)_7$	52	17	10	58.8
UBC 891	$HVH(TG)_7$	55	17	15	88.2
Total			84	67	79.0

Y = (C, T); B = (C, G, T); D = (A, G, T); H = (A, C, T); V = (A, C, G)

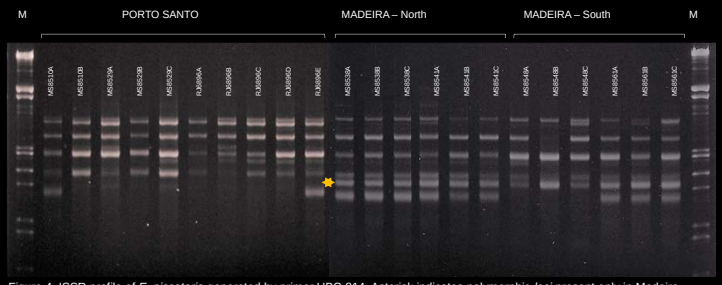

Figure 4. ISSR profile of *E. piscatoria* generated by primer UBC 814. Asterisk indicates polymorphic *loci* present only in Madeira samples. M = λ x *Eco*RI/*Hin*dIII

Table 2. Estimates of genetic variation in *E. piscatoria* from Porto Santo and Madeira islands using ISSR markers. Percentage of polymorphic loci (%P), effective number of alleles (Ne), Nei's gene diversity (He) and Shannon's index of phenotypic diversity (I).

Population	Sample size	% P	Ne	He	I
Porto Santo	10	39.3	1.2367 ± 0.3509	0.1379 ± 0.1924	0.2059 ± 0.2776
Madeira - North	12	54.7	1.2952 ± 0.3344	0.1807 ± 0.1878	0.2753 ± 0.2739
Madeira - South	12	40.5	1.2375 ± 0.3419	0.1410 ± 0.1886	0.2123 ± 0.2744
Overall	34	79.8	1.3488 ± 0.3396	0.2160 ± 0.1739	0.3392 ± 0.2402

Table 3. Estimates of genetic diversity in *E. piscatoria* from Porto Santo and Madeira islands. Total gene diversity (Ht), gene diversity within population (Hs) and genetic differentiation (Gst).

Population	Sample size	Ht	Hs	Gst
Porto Santo	10	0.128 ± 0.034	0.064 ± 0.011	0.502
Madeira - North	12	0.181 ± 0.035	0.044 ± 0.004	0.757
Madeira - South	12	0.141 ± 0.036	0.066 ± 0.010	0.533
Overall	34	0.214 ± 0.031	0.152 ± 0.016	0.289

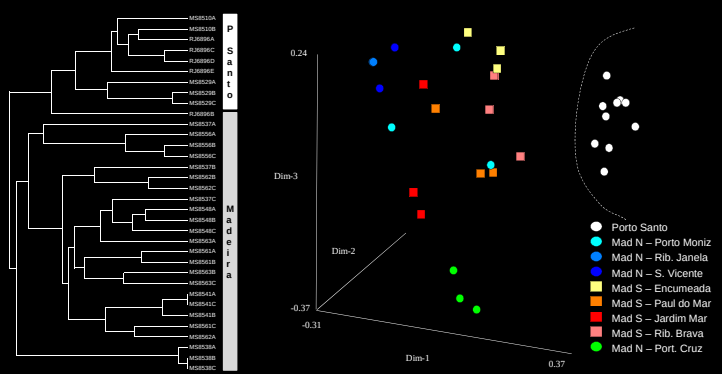

Figure 5. A, Dendrogram of 34 individuals of *E. piscatoria* Island based on UPGMA analysis of ISSR polymorphisms using DICE similarity coefficient. B, Principal Coordinate Analysis based on SM.