Hybridization and conservation status of Echium portosanctensis: first results using ISSR markers

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INTRODUCTION
Echium L. (Boraginaceae) is represented in the Madeira Archipelago by three endemic woody taxa: E. nervosum W.T. Aiton (Madeira Island, Porto Santo Island and Deserta - Big Island) and E. portosanctensis (Madeira Island) and E. portosanciensis J.C.Carvalho, Pontes, Bat.-Marques & R.Jardim (Porto Santo Island, Fig. 3). In the island of Madeira E. canadensis inhabits high-altitude sites in complete isolation of E. nervosum. The hybridic species of the Echium portosanciensis therefore occurring in low altitude habitats. In the Porto Santo Island E. portosanciensis occurs in a few and scattered populations usually in sea cliffs and in the higher peaks of the island, that correspond to the Siderito multiflorae-Echietum portosanctensis community. The native status of E. nervosum is not clear in Porto Santo, even though there are some references for the existence of a native population in a restricted geographic location, but most E. nervosum in lower altitude and ruderal habitats seem to result from garden escapes (i.e. from plants of Madeiran origin). Interspecific hybridization resulting from the introduction of non-indigenous co-generic plants is recognized as a threat to endemic taxa (Rhymes & Simberloff, 1996). To this hybridization process are associated several problems, especially when heterotic hybrids emerge. The loss of genetic variability in endemic taxa is verified, as former separate genetic pools are allowed to fuse, due to the disruption of reproductive barriers between species. This process can lead to the extinction of native flora and fauna, since the new hybrid taxon competes directly with the native species (Todesco et al., 2016; Quilodrán et al., 2018). The occurrence of morphological putative hybrids (Fig. 4) seems to support an undergoing hybridization process between E. portosanciensis and E. nervosum, and for this reason E. portosanciensis is included in the IUCN list of threatened species as critically endangered (Rivers & Menezes de Sequeira, 2017). Aiming to evaluate existing hybridization processes between these species, a preliminary molecular study was carried out using ISSR markers.

MATERIAL AND METHODS
In this study twenty-two individuals were sampled from E. nervosum, E. portosanciensis and putative hybrids E. portosanciensis x E. nervosum hybrids (Table 1). Leaves, harvested between 2012 and 2018, were dried in silica gel and ca. 20 mg used in the extraction of total DNA by the CTAB method (Doyle & Doyle, 1990) with minor modifications. PCR reactions were executed with 20-30 ng of DNA, 0.3 µl UBC primers (British University of Columbia, Canada) and 1x of MyTaq-Plant polymerase PCR mix (BioLine, UK). The reactions were carried in an Applied Biosystems 2720 Thermal Cycler using the following program: 94°C for 2´, 35 Cycles [94°C for 30´´; 52°C (UBC 890) / 55°C UBC 888/889) for 30´´; 72°C for 2´] and a final extension for 5´ at 72°C. PCR products were visualized in 1% agarose gels in TAE 1X containing ethidium bromide. Binary matrices were built grounded on the presence/absence of bands. A dendrogram for the sampled individuals using Jaccard's similarity coefficient was performed using version 2.20e (Rolf, 2005). A dendrogram for the sampled individuals using Jaccard’s similarity coefficient, and upgma as clustering method, was used to access genetic proximity.

RESULTS AND DISCUSSION
The amplification of DNA generated in total 56 loci of which 53 were polymorphic. Despite the high polymorphic variation detected by primers (95% to 96%), it was not recognized a specific species locus. Figure 5 reveals high intra- and inter-population genetic variability in the Madeiran endemic taxa. The putative hybrids (Table 1) based on upgma analysis of ISSR polymorphisms using Jaccard coefficient, does not support any clear segregation of Echium samples according to their origin or even recognized species. Although some segregation of E. portosanciensis and E. nervosum from Ponta de S. Lourenço can be observed it is evident the dispersal of putative hybrids along the graphic, such dispersion could be assigned to several events of hybridization (e.g. introgressive hybridization) and result in complex mixtures due to transfer of parental genes mediated primarily by backcrossing, but results so far obtained do not support any clear taxonomic/geographical structure. The putative hybrid individuals might belong to distinct hybridization processes and pools integration. ISSR data suggest that the putative hybrid from Pico Juliana shows more similarities to E. portosanciensis putative individuals. Putative individuals of Pico do Facho population are all possibly related to E. nervosum from the Porto Santo Island while Ribeiro Cochoim individual resembles E. nervosum from Madeira Island. Nonetheless the dispersal of putative hybrids is coherent with the fully naturalization of E. nervosum in the Porto Santo Island, probably due to its introduction as an ornamental garden plant. In fact the references to E. nervosum as native of Porto Santo are all possibly related to E. portosanciensis and need further study. Moreover, results suggest that E. portosanciensis is genetically closer to the E. nervosum populations in the east side of the Madeiran archipelago (Ponta de S. Lourenço) the geographical site on the Island closest to Porto Santo. But similarity with one individual of E. nervosum from Garajau, is hard to explain (Garajau population showed the highest genetic variability).

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