Biologia 66/2: 258—265, 2011
Section Botany
DOI: 10.2478/s11756-011-0002-9

Phytogeochemical, geographical and vulnerability study of the Paleosubtropical element *Notholaena marantae* subsp. *marantae* (Sinopteridaceae) at the western edge of its range

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Abstract: Serpentine outcrops are distinct from their background geological context, as they are often isolated from one another, and provide a home to a significant number of rare or endemic taxa. The aim of this study was to assess the Portuguese populations of one such taxon, *Notholaena marantae* subsp. *marantae*, including information on its biogeography, habitat, geographical range and conservation status. A detailed study was also made of the endemic association *Notholaenetum marantae*, a member of the *Phagnalo saxatilis-Cheilanthion maderensis* alliance, a type of vegetation included in the EU Habitats Directive 92/43/ECC. The distribution of *Notholaena marantae* was highly fragmented, with two sites accounting for over 50% of the total number of individuals recorded. The presence of *N. marantae* is associated with that of ultramafic rocks, which show specific geochemical features. The conservation status of the species is a cause for concern. We propose that the species be considered 'vulnerable' according to the criteria of the IUCN (2001), and that areas where its populations grow be protected. This study confirms the endemic status of *N. marantae* subsp. *marantae* in Portugal and the importance of the conservation of its serpentine habitats.

Key words: *Notholaena*; biodiversity; conservation practice; extinction risk; Iberian Peninsula; geochemistry; protected areas; relict populations; serpentine outcrops; Portugal

Introduction

Conservation strategies are increasingly aimed at minimizing the impacts of habitat fragmentation and other processes that isolate natural communities (Noss & Csuti 1994). Patchy habitats are intriguing to both ecologists and evolutionary biologists. The distinctive flora of serpentine, with its anciently discontinuous distribution, would seem a prime target for such diversity-enhancing effects (Harrison et al. 2000).

Soils developed from different types of ophiolithic bedrock have long been known to represent hostile environments for plant life, as a consequence of their chemical and physical properties (Selvi 2007). These soils generally have a low Ca:Mg molar ratio, low macronutrient concentrations, especially of nitrogen and phosphorus, and elevated concentrations of cobalt, chromium and particularly nickel (Brooks 1987; Proctor & Nagy 1992). In contrast to soils developed from other siliceous rocks, the pH of serpentine substrates varies from basic to ultrabasic (pH 6.5–8). The flora growing in such areas therefore contains basiphilous-calcifugal taxa of great taxonomic, phytogeographic and ecological interest.

One such taxon is *Notholaena marantae* (L.) Desv. subsp. *marantae* (Sinopteridaceae). The distribution of this fern extends from the west of the Mediterranean Basin to the Himalayas. The Azores Islands, the Canary Islands and Madeira are home to the *Notholaena marantae* subsp. *subcordata* (Cav.) G. Kunkel (Muñoz Garmendia 1986). *Notholaena marantae* subsp. *marantae* is found in ultramafic sites across Southern Europe (Brooks 1987). In Spain it is rare, and usually found in areas isolated from one another in Andalusia, Castilla-La Mancha, Catalonia, the Valencia Region and Galicia. It is only protected, however, in Catalonia, it has the status of 'strictly protected species' (Anonymous 1993), and in Castilla-La Mancha, it has the status of 'special interest' (Anonymous 2001).

In Portugal its distribution is even more reduced; here it grows only over a few outcrops of ultrabasic rocks in the Vinhais-Bragança and Morais Massifs in the northeastern Province of Trás-os-Montes. These ultramafic rocks are rich in Mg (Fuente et al. 2007) and total soil Ni concentrations of 851–2962 mg kg^{-1} have been reported (Peterson et al. 2003). It is probable that the serpentine areas of Trás-os-Montes, which remained

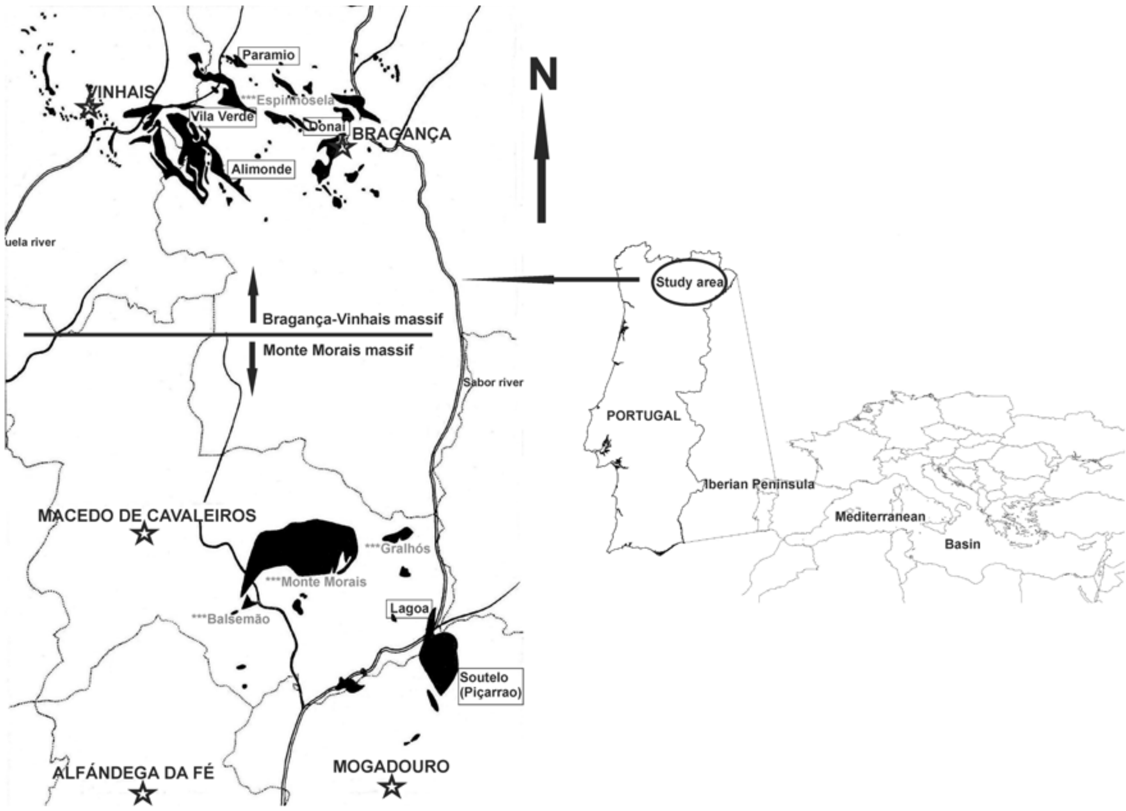

Fig. 1. Map showing the investigated populations and localities of *Notholaena marantae* in the North of Portugal.

free of ice during the last ice age, acted as refugio for certain northern species, and that from these areas sprang postglacial neo-endemics (e.g. *Alyssum serpyllifolium* susbp. *lusitanicum*, *Anthyllis vulneraria* subsp. *sampaioana*, *Armeria eriophylla* and *Dianthus laricifolius* subsp. *marizii*) via a process of particularly intense speciation (Pichi-Sermolli 1948; Löve 1963; Pignatti 1964). *Notholaena marantae* is a 'serpentine relict' of pre-glacial (probably Lower Pliocene) origin (Pichi Sermolli 1948; Pinto da Silva 1970) that continues to grow in serpentine habitats.

Serpentine outcrops typically are discrete and highly variable in extent and isolation. These areas support a distinctive flora with numerous uncommon or rare endemic taxa. Such qualities make this landscape an excellent area for studying subject of conservation (e.g. Harrison et al. 2000; Wolf 2001, Stevanović et al. 2003; Selvi 2007). Given the biogeographical and ecological interest of *N. marantae* in the context of European biodiversity, the aim of this study was to assess its populations in Portugal, recording information on the taxon's habitat, geographical range and conservation status.

Material and methods

Study area

The northeast of Portugal (between 41°25′ N and 41°54′ N) is home to serpentine outcrops which occupy an area of 80 km^2 (Fig. 1): 33 km^2 (41%) lie in the Vinhais-Bragança Massif towards the north, and 47 km^2 (59%) Morais Massif towards the south (Pinto da Silva 1970). The petrology of both massifs is extremely complex with the majority of the rocks of being either basic or ultrabasic, rare types of rock in Portugal (Aguiar 2001).

The Morais-Vinhais-Bragança complex is related to the Cabo Ortegal complex in Galicia (NW Spain). The peculiarities of the latter are described by Pereira et al. (2008) and references therein.

The mean annual temperature in the study area is 12.4 °C and mean annual precipitation is 720 mm (Carballeira et al. 1983).

Description and generic delimitation of Notholaena marantae

A detailed description of the family Sinopteridaceae and of *N. marantae* is provided by Muñoz Garmendia (1986). However, defining the generic limits within this family of ferns is not easy, with the genus *Notholaena* presenting the most difficulties in this respect (reviewed by Yatskievych & Smith 2003). However, irrespective of the genus to which it might belong (possibly *Gymnopteris*, *Notholaena*, *Paraceterach* or*Paragymnopteris*; see Yatskievych & Smith 2003), what is currently known as *N. marantae* is the only taxon of any of these genera to be found in the Iberian Peninsula, the rest of Europe or North Africa.

Data gathering and habitat description

The data collected for the present study were based on examination of collections, the literature, and fieldwork carried out between 2005 and 2008.

To assess the habitat of the species we studied biotope, community and bioclimatic traits. At the community level, we studied the phytosociological behaviour of the species according to the Zürich-Montpellier school methods (Braun-Blanquet 1979; Westhoff & van der Maarel 1973; Gehú &

Table 1. Major elements composition (%) of twelve rock samples in the studied area.

Samples	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MgO	MnO	CaO	Na_2O	K_2O	P_2O_5	LOI
Espinhosela	39.97	0.02	1.79	8.37	39.32	0.10	0.16	0.01	b.d.l.	b.d.l.	13.86
Donai*	37.62	0.02	1.66	8.39	39.13	0.12	0.26	0.04	b.d.l.	0.01	13.70
Paramio*	34.83	0.01	0.44	8.44	39.32	0.09	0.10	0.02	b.d.l.	0.01	13.21
Alimonde*	39.22	0.01	0.85	7.61	38.84	0.09	0.23	0.05	0.01	0.01	12.90
Soutelo-Piçarrao*	41.72	0.04	1.58	7.90	36.43	0.09	0.65	0.05	0.01	0.01	12.80
Vila Verde*	37.99	0.01	0.42	8.61	38.84	0.09	0.07	0.02	b.d.l.	b.d.l.	12.46
Gralhós	35.96	0.12	1.90	10.83	38.06	0.10	0.28	0.01	b.d.l.	0.03	11.98
Chacim-Balsemao	39.02	0.05	1.41	8.96	38.16	0.11	0.84	0.04	b.d.l.	0.01	11.77
Lagoa*	38.21	0.21	3.67	9.03	34.42	0.10	2.24	0.34	0.03	0.03	9.25
Vila Verde – river Tuela	50.05	0.15	3.23	7.15	24.36	0.14	9.68	0.52	0.02	0.01	2.76
Lagoa – river Sabor	48.38	1.43	15.16	9.65	9.23	0.15	10.26	2.94	0.13	0.10	2.49
Monte Morais	46.17	0.94	16.47	7.57	9.58	0.12	13.69	1.73	0.09	0.04	1.60

(b.d.l) below detection limit (*) plant has been found.

Rivas-Martínez 1981). Conservation status was assessed to document the occurrence of populations in protected areas.

Nomenclature and population estimates

Nomenclature follows Castroviejo et al. (1986–2008), except for *Alyssum serpyllifolium* subsp. *lusitanicum* T.R. Dudley & P. Silva in Agron. Lusit. 28: 72 (1967); *Linaria aeruginea* var. *simplex* P. Silva in Agron. Lusit. 30: 222 (1970); and *Antirrhinum rothmaleri* (Pinto da Silva) Amich et al. inéd. Sintaxa nomenclature follow Rivas Martínez et al. (2001, 2002).

Censuses were taken by directly counting (with marking to avoid recounting) all potentially reproductive individuals, or, given the problems of estimating the numbers of rupicolous which often grow in inaccessible areas, were estimated using the visual units method (García et al. 2001; Iriondo 2003). Since *N. marantae* subsp. *marantae* grows clonally, individuals were defined as one or more plants based on whether they were visible as easily identifiable units.

When the species was detected, we recorded geographical location, habitat, and phenological data (e.g. Bernardos et al. 2006a, b). A Garmin e-map GPS was used to geographically locate the populations using 1 × 1 km coordinates.

Sampling for rock analyses

Rock samples were taken at the sites of all the populations of *N. marantae* subsp. *marantae* encountered (in Alimonde, Donai, Lagoa, Paramio, Soutelo-Piçarrao and Vila Verde), as well as from other areas where the taxon was not found (Chacim-Balsemao, Espinhosela, Gralhós, Monte Morais, Lagoa-Sabor River and Vila Verde-River Tuela dam).

We determined major and trace elements using an acid treatment (HNO_3 plus HF) under pressure in an Ethos Sel microwave digester of Milestone. The diluted solution was buffered with boric acid. Determinations were made using an Ultima 2 Jobin Yvon optical emission plasma spectrometer at the Chemical Analysis Laboratory, University of Salamanca. Loss on Ignition was calculated by measuring few grams of the rock powder in a tared crucible, heating it under 950 ℃ in an oven, allowing water, and other volatiles to escape. After cooling in a controlled atmosphere, mass is redetermined and the difference is the LOI value.

Soil pH was determined in a soil:water (1:2.5) suspension using a pH meter.

Taxonomic sampling for analyses of major and trace elements in leaves dry matter

Leaves were collected from 25 plants from each population for the analysis of their major and trace element contents. No samples were collected from the Donai and Vila Verde populations owing to their very small populations. All samples were air-dried and ground. Around 0.1 g of homogenised material was wet ashed with 2 mL of concentrated HNO_3 in a Teflon pressure vessel at 150 ℃ for 10 h. The digest was then made up to 10 mL in MilliQ water. The solutions were then analysed for Ca, K, Fe, Mg, and Ni by flame atomic absorption spectrophotometry using a Pekin-Elmer 2380 apparatus (Aslin 1976; Fletcher 1981; Kabata-Pendias 2001) at the University of Coimbra. The same leaves were analyzed for trace elements by ICP-MS, at the Chemical Analysis Laboratory, University of Salamanca.

Results

Distribution and chorology in Portugal

The presence of *N. marantae* in northeast of Portugal is strongly associated with the area's ultramafic outcrops, although not all such outcrops harbor the taxon (Fig. 1). The petrology of these outcrops is very complex (Ribeiro 1974). Populations were confirmed to be restricted to six isolated sites in the ultramafic massifs of Vinhais-Bragança and Morais (Fig. 1). Other serpentine outcrops in the area with similar bioclimatic, biogeographic and geomorphological characteristics were visited but the taxon was not encountered.

Rocks parameters variability

Twelve samples of mafic and ultramafic rocks were collected. A clear shift in their composition was observed, related to the progression of serpentinization (Table 1). The degree of serpentinization is marked by the variable "loss on ignition" (LOI), a marker of the H_2O content in serpentine structure (≈13% H_2O), as described by Kyser et al. (1999) and D'Antonio & Kristensen (2004). The samples consisted of seven serpentinites (Espinhosela, Donai, Paramio, Alimonde, Soutelo-Piçarrao, Vila Verde, and Gralhós), one serpentinized peridotite (Chacim-Balsemao), one slightly serpentinized peridotite (Lagoa), and three amphibolites (Vila Verde-River Tuela, Lagoa-River Sabor and Monte Morais) made up mainly of amphibole and plagioclase with rather low LOI values. Tables 1 and 2 show the major and trace element geochemistry of these samples,

Table 2. Composition in terms of trace-element concentration (ppm) of twelve rock samples in the studied area.

Samples	Ba	Co	Cr	Cu	Ni	Pb	Sr	Zn
Espinhosela	b.d.l.	63	1955	b.d.l.	2185	b.d.l.	62	23
Donai*	b.d.l.	61	1127	b.d.l.	2555	b.d.l.	63	33
Paramio*	b.d.l.	73	353	b.d.l.	2616	b.d.l.	63	11
Alimonde*	11	66	778	b.d.l.	2215	b.d.l.	70	21
Sotelo-Piçarrao*	b.d.l.	50	1755	b.d.l.	2210	b.d.l.	70	21
Vila Verde*	b.d.l.	62	376	213	1396	b.d.l.	63	17
Gralhós	b.d.l.	63	2264	b.d.l.	1835	b.d.l.	63	22
Chacim-Balsemao	b.d.l.	61	2536	b.d.l.	1961	b.d.l.	64	32
Lagoa*	b.d.l.	2	313	16	83	8	169	41
Vila Verde-river Tuela	0.4	16	2416	143	536	b.d.l.	91	0.6
Lagoa-river Sabor	35	58	2276	25	1749	b.d.l.	120	31
Monte Morais	23	3	351	2	179	13	235	27

(b.d.l) below detection limit (*) plant has been found.

Table 3. pH value of several localities of serpentines.

Localities	Value
Bragança-Vinahis massif (BV)	
Alimonde*	7.40
Donai*	7.46
Espinhosela	7.29
Paramio*	7.28
Vila Verde*	6.83
Monte Morais massif (MM)	
Chacim-Balsemao	7.38
Gralhós	7.33
Lagoa*	7.18
Monte Morais	7.22
Soutelo-Piçarrao*	6.84

(*) plant has been found

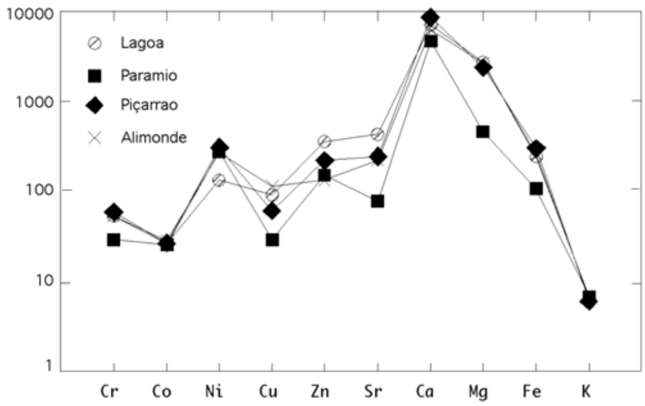

Fig. 2. Comparison of chemistry of *Notholaena marantae* leaves in the different sampling areas.

and whether the taxon under study was found at the collection sites.

The serpentinites were rich in MgO but depleted in other major elements. The peridotite had a similar composition, but with a lower MgO and a higher Al_2O_3 content. The amphibolites had higher Al_2O_3 and CaO contents while their K_2O and P_2O_5 contents were commonly below the detection limit. With respect to trace elements, all had high contents of Cr and Ni, but most were depleted in Ba, Cu and Pb (Tables 1 and 2).

The value of the soil pH in water varied between 6.83 and 7.46 where *N. marantae* subsp. *marantae* was present and between 7.22 and 7.38 where it was not found (Table 3).

Leaf analyses

The leaves showed a rather high content of toxic elements (Table 4). The Paramio samples showed the lowest mean Cr, Cu, Sr, Ca, Mg and Fe contents. The Lagoa leaf samples showed the lowest mean Ni content but the highest Zn and Sr content. The Alimonde leaf samples showed the highest Cu and the lowest K contents.

We looked for correlation among elements using the multipurpose statistical package SYSTAT. A strong correlation was found between the Zn and Sr contents ($R = 0.88$) and the Cu and Co contents ($R = 0.93$). Figure 2 shows that the content of the different elements differed among samples; even the trace elements content varied. The only geographic area where the Ni, Cr and Co contents correlated well was in Alimonde. Surprisingly, a perfect negative correlation was seen between the Cr, Ni and Sr contents of the Lagoa leaf samples and the rock content of these elements.

Ecology and plant communities

The plant communities associated with *Notholaena marantae* subsp. *marantae* are representative of the diversity of the serpentine plant communities of Mediterranean Basin. Portuguese populations exclusively inhabit serpentine outcrops of Vinhais-Bragança and Morais Massifs. According to the species composition recorded in the *relevés* examined (Table 5), the community of *Notholaena marantae* (*Notholaenaetum marantae* P. Silva 1970) belongs to the *Phagnalo saxatilis-Cheilanthion maderensis* alliance (order *Cheilanthetalia maranto-maderensis*, class *Asplenietea trichomanis*), which represents the characteristic vegetation of serpentine outcrops in the western Mediterranean. This association is impoverished compared to the Levantine Mediterranean (eastern Iberian Peninsula) vicariant *Cheilantho maderensis-Notholaenetum marantae* O.

Table 4. Mean values of major and trace elements in leaf tissue of *Notholaena marantae* subsp. *marantae*. (Results of trace elements are in ppb, Ca and Mg are in %; Fe and K are in ppm).

Samples	n	Cr	Co	Ni	Cu	Zn	Sr	Ca	Mg	Mg/Ca	Fe	K
Lagoa	2	49	25	119	84	317	377	0.58	0.23	0.40	220	6.40
Paramio	3	27	24	248	27	137	71	0.39	0.04	0.10	97	6.55
Soutelo-Piçarrao	6	54	25	274	56	195	217	0.70	0.20	0.28	267	5.78
Alimonde	3	50	26	241	104	119	195	0.52	0.22	0.42	231	17.9

(n) number of populations analyzed in each locality.

Table 5. *Notholaenetum marantae* P. Silva 1970 (*Phagnalo saxatilis -Cheilanthion maderensis, Cheilanthetalia maranto-maderensis, Asplenietea trichomanis*)

Relevé numbers	1	2	3	4	5	6	7
Area (m^2)	5	5	10	15	5	3	4
Coverage (%)	25	15	20	20	20	50	40
Slope (°)	45	80	25	50	70	80	80
Altitude (m)	795	475	505	730	880	300	550
Exposure	S	S	NW	E	S	W	S
Association and higher syntaxa features							
Notholaena marantae subsp. *marantae*	3	3	4	3	2	4	4
Asplenium adiantum-nigrum var. *corunnense*	.	.	2	2	2	.	.
Ceterach officinarum	.	1	1	.	1	1	.
Cheilanthes tinaei	.	.	.	.	.	+	+
Accompanying species							
Alyssum serpyllifolium subsp. *lusitanicum*	2	2	2	1	2	1	2
Centaurea cf. *paniculata*	.	1	1	1	1	.	1
Annogramma leptophylla	.	1	2	.	.	.	+
Umbilicus rupestris	.	1	1	.	.	1	+
Linaria aeruginea var. *simplex*	.	1	1	1	.	.	+
Anthyllis vulnearia subsp. *sampaioana*	3	.	.	2	.	.	.
Plantago holosteum	2	.	.	.	2	.	.
Allium sardoum	.	.	.	1	1	.	.
Linaria saxatilis	.	.	1	1	.	1	1
Antirrhinum rothmaleri	.	.	.	2	.	.	.
Saxifraga fragosoi	.	.	.	2	.	.	.
Erysimum linifolium	.	.	.	1	.	1	.
Cleome violacea	.	.	1	.	.	1	.

Localities (all of them in Trás-os-Montes province, Portugal):
1. Vinhais, Paramio, 29TPG7741, 30.5.2007, Aguiar, Amich & García-Barriuso
2. Mogadouro, Soutelo, Piçarrao, 29TPF8787, 31.5.2007, Amich & García-Barriuso
3. Mogadouro, Soutelo, Piçarrao, 29TPF8787, 18.7.2007, Amich, Bernardos & García-Barriuso
4. Bragança, Alimonde, 29TPG7429, 16.7.2007, Amich, Bernardos & García-Barriuso
5. Bragança, Donai, 29TPG8234, 16.7.2007, Amich, Bernardos & García-Barriuso
6. Macedo de Cavaleiros, Lagoa, 29TPF8386, 31.5.2007, Amich & García-Barriuso
7. Vinahis, Vila Verde, 29TPG7737, 16.7.2007, Amich, Bernardos & García-Barriuso

Bolòs 1956. Since *Notholaena marantae* is the only characteristic taxon of the order *Cheilanthetalia maranto-maderensis* in the study area, it may represent a basal community of the order. Towards more shaded habitats it makes contact with formations of *Asplenietum corunnensis* Rivas-Martínez et al. 2002, and towards the edges of rupicolous sites it makes contact with phytocoenoses of *Armerietum eriophyllae* P. Silva 1965. The *Phagnalo saxatilis-Cheilanthion maderensis* alliance is included in the EU Habitats Directive 92/43/ECC; in the study area its distribution is very restricted compared to the very amply distributed alliance *Rumici indurati-Dianthion lusitani* (Bernardos et al. 2004).

Size and altitude range

The Red List criteria (IUCN 2001) define Extent of Occurrence (EOO) as “the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon”. The Extent of Occurrence is extremely reduced (< 100 km^2), since we only found six main populations. The Area of Occupancy (AOO) of *N. marantae* subsp. *marantae* is also reduced ($< 175{,}000$ m^2) (Table 6) and has limited protected status. The Donai, Paramio and Vila Verde populations are found within the Monteshino Natural Park, but the Alimonde, Lagoa and Soutelo populations, the largest, are not on protected land.

All the studies samples from the different localities present fronds with spores, and the number of young individuals is high.

Table 6. Location, altitude, presence in protected areas, and size of Portuguese populations of *Notholaena marantae* subsp. *marantae*. Abbreviations: BV, Bragança-Vinhais massif; MM, Morais massif; RD, direct recount; E, estimation (visual units).

Portuguese localities	N° Population	Geographic coordinates (UTM)	Altitude (m)	Protected Area (Natural Park)	Estimate area of serpentine outcrops (m^2)	N° individuals
Bragança: Alimonde (BV)	1	29TPG7429	730	—	1200	800–1000 (RD + E)
Bragança: Donai (BV)	2	29TPG8234	885	Montesinho	15000	40–50 (RD)
Macedo de Cavaleiros: Lagoa (MM)	3	29TPF8386	275	—	25000	1500–2000 (RD + E)
Mogadouro: Soutelo, Piçarrao (MM)	4	29TPF8787	460	—	60000	3000–3500 RD + E)
Vinhais: Paramio (BV)	5	29TPG7741	795	Montesinho	12000	2000–2500 (RD + E)
Vinhais: Vila Verde (BV)	6	29TPG7737	610	Montesinho	45000	300–500 (RD + E)
TOTAL	6	Between 275 and 885			< 150.000	7.600–9.500

Notholaena marantae subsp. *marantae* populations appear from 275 m (Macedo de Cavaleiros: Lagoa) to 885 m (Bragança: Donai) of altitude, although 4 out of 6 populations (67 %) grow 600 m a.s.l. (Table 6).

Discussion

The ecological attributes described above for Portuguese populations of *Notholaena marantae* subsp. *marantae* agree with other areas of the world. Serpentine outcrops of NE Portugal represent similar edaphic conditions described for other areas, including the Rif Mountains in Morocco (Ater et al. 2000), Serbia (Vicentijevic-Markovic 2004) and Tuscany in Italy (Selvi 2007).

Although it is not easy to identify a general pattern for the ecological characteristics of the Portuguese populations of*N. marantae* subsp. *marantae* it appears to grow in serpentine and/or peridotite areas with either low or high Cr content (Tables 1, 2). However, if the Cr and Mg contents of the rocks are both high (> 1,750 ppm and > 25 ppm respectively) (Tables 1, 2), the taxon is not found. The combination of high Cr and Mg content would seem to be a limiting factor for the presence of *N. marantae* subsp. *marantae.* A particular case is represented at Monte Morais where low rock contents of Cr and Mg are found, but where *N. marantae* subsp. *marantae* does not appear to grow.

Comparing the geochemistry of the rock with the chemistry of the plant, we have observed that, in general, as the elements increase in the rock, they increase in the plant as well. There is a clear correlation between rock and plant, although this correlation differs for the different elements. Only the samples from Lagoa contain Cu, both in the rock and in the plant. We will analyze soils from the same sampling places to compare their chemistry with both plant and rock, and see if some of the elements are retained in the soil instead of going into the plant.

The Portuguese populations of *N. marantae* subsp. *marantae* are extremely isolated from one another. The preservation of isolated populations and knowledge of the geographic range of species is an important factor in conservation biology (i.e. Rojas 1992; Scott et al. 2000).

In the study areas where *N. marantae* subsp. *marantae* reaches its western limit (Muñoz Garmendia 1986), it may represent genetically distinct populations. Studies suggest that populations on the periphery of their ranges may be the most genetically variable and thus the most evolutionarily valuable when confronted with contracting ranges since they may be less vulnerable to anthropogenically-induced or natural changes (Lesica & Allendorf 1995). Thus, protection of populations at range limits is of great importance.

Applying the Red List criteria

Notholaena marantae subsp. *marantae* is not currently protected in Portugal. The results of this study suggest, however, that it should be regarded as "vulnerable", according to the Red List criteria of Gärdenfors et al. (2001) and the IUCN (2001) (VU B1ab(ii, iii); B2ab(ii, iii); D2):

Populations with a very restricted AOO (typically less than 2,000 km^2; in this case < 175,000 m^2), having a very restricted extent of occupancy (EOO) (typically less than 20,000 km^2; in this case < 100 km^2), to have severely fragmented populations, to grow in a reduced number of locations (typically five or fewer; in the present case 6 localities, although one of these, site 2, had a very small number of individuals), and to be prone to the effects of human activity or stochastic events.

In this step, the IUCN Red List Criteria are applied to the regional population of the species, and all the data used in this initial assessment (i.e., number of individuals, reduction, fragmentation) correspond to the regional population (IUCN 2003). However, since no significant immigration of propagules capable of reproducing in the region was recorded, and in agreement with the conceptual scheme of the procedure for assigning an IUCN Red List Category at the Regional level (IUCN 2003), no change in its preliminary categorisation is required.

As reported by other authors (Broennimann et al. 2005), species with conservation priority live in rare habitats but do not necessarily have small populations, as is the case of *N. marantae.* Indeed, geographic distribution is presently the most important criterion for deciding whether a species is endangered and reflects the overriding importance given to EOO and AOO criteria in the definition of IUCN categories (IUCN 1994, 2001).

The particular environmental requirements of the taxon mean that its populations are dispersed and geographically separated from one another. This isolation, along with the alteration of the stone walls and rocky ground where the species is found (owing to road construction, building projects and mining, etc.), are without doubt the major threats faced by the species. However, no historic data are available to show whether populations have not been declining over recent years, nor have we yet had the time to perform demographic studies of these populations.

Given the distance of the Portuguese populations from those of Spain, and the scarcity of the habitats this plant can colonize, the arrival of new propagules from Spanish territories that might help the taxon recover in Portugal after a hypothetical disappearance seems unlikely. The Portuguese populations can therefore be understood as true endemics of the study area (Gärdenfors et al. 2001).

Conclusions and recommendations

The present study provides some interesting insights into the threats faced by *N. marantae* subsp. *marantae.* The simple categorization of species according to their vulnerability to extinction in the Red List does not, on its own, represent an effective measure for preserving biodiversity. In this case, the areas presently protected in northeast Portugal must be reviewed, and to include those where *N. marantae* subsp. *marantae* populations grow if the conservation of the taxon is to be made possible. The present data show that protection of *N. marantae* subsp. *marantae* needs to be closely linked to the protection of serpentine sites.

The following conservation activities are therefore recommended:

1) Although it is recommended that the species be classed as "vulnerable" there is no immediate need to initiate intensive *in situ* or *ex situ* management. The most urgent priority is the control of anthropogenic activities.

2) The search for new individuals and populations in northern Portugal should continue, focusing efforts on ultramafic areas, especially in the Morais Massif.

3) Studies should be undertaken on the habitat preferences and breeding requirements of the taxon.

4) Detailed studies on the genetic structure of its populations should also be performed.

Acknowledgements

This work was partially supported by a grant from the Comunidad Autónoma de Castilla y León (Consejería de Educación, project SA060A07). Very gratefully acknowledge Dr. C. Aguiar (Technical College of Bragança, Portugal) for kindly showing us the serpentine areas in the Trás-os-Montes region, Dr. A. Crespí (University of Trás-os-Montes, Portugal) for his interest and advice, and Dr. P. Alonso Rojo (University of Salamanca, Spain) for helping with the analyses of soil pH.

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Received February 8, 2010
Accepted April 28, 2010