# Patterns of lichen diversity in coastal sand-dunes of northern Portugal

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Coastal sand-dunes of northern Portugal are dynamic ecosystems under natural and human disturbances. Still, they constitute an interesting subject for ecological research, since they spread over a main biogeographic transition between the Eurosiberian and the Mediterranean regions. Despite the awareness of lichen contribution to grey dune stability and protection, there are no recent published studies focusing on their lichen flora. Therefore, the overarching goal of this research was to evaluate the patterns of lichen diversity along biogeographic - and coastal dynamics - related gradients in northern Portugal. A catalogue with 17 species is presented, and includes a new species for Portugal, *Cladonia rei* Schaer. Patterns of lichen diversity were analysed through Mann-Whitney tests and Analysis of Similarity (ANOSIM), which showed distinct signals when considering different diversity components and scales of analysis. These results have strong implications for conservation and monitoring strategies of this type of ecosystem.

Keywords: biogeography, Cladonia rei, coastal dynamics, grey dunes, Iberian Peninsula.

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Las dunas costeras del norte de Portugal son dinámicas con perturbaciones naturales y humanas. Sin embargo, constituyen un tema interesante para la investigación ecológica, ya que se distribuyen a través de la transición biogeográfica entre las regiones Eurosiberiana y Mediterránea. A pesar de la contribución de los líquenes por la estabilidad y protección de las dunas-grises, no hay estudios recientes sobre su flora liquénica. Lo objetivo de esta investigación es evaluar los patrones de diversidad liquénica a lo largo de gradientes biogeográficos y de dinámica costera del norte de Portugal. Se presenta un catálogo con 17 especies, con una nueva especie para Portugal, *Cladonia rei* Schaer. Los patrones de diversidad fueron analizados a través de análisis de Mann-Whitney y de similitud, y muestran resultados distintos al considerar los diferentes componentes de la diversidad y escalas de análisis. Los resultados tienen importantes implicaciones para las estrategias de conservación y monitoreo de este tipo de ecosistema.

Palabras clave: Biogeografía, Cladonia rei, dinámica costera, dunas grises, Península Ibérica.

#### INTRODUCTION

Coastal sand-dunes are characterised by high levels of biological diversity and the occurrence of peculiar habitats (Acosta *et al.* 2005), providing several environmental services with both ecological and economic relevance (Martínez *et al.* 2007, Wilson *et al.* 2005). However, coastal sanddunes are within one of the most threatened ecosystems in the world due to natural cycles, global climate changes, and many other disturbance types, usually worsened by human occupation (Coelho *et al.* 2009, EEA 2006, MEA 2005). The negative effects of natural cyclic dynamics on coastal sand-dunes along northern Portugal have been locally enhanced by the construction of many coastal defence infrastructures (Granja *et al.* 2008). Also, the effect of climate change is expected to contribute to changes in sedimentary drift processes and to the acceleration of coastline regression (Coelho *et al.* 2009). In fact, the northern Portugal coastline is nowadays mostly under transgressive dynamics (Coelho *et al.* 2009), even though (meta-)stable sand-dunes can be observed due to natural coastline features, and localised effects of the former

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Fig. 1– Location of the study area, with the position of sampling sites indicated on the left (see Table 1 for details on site codes). The dotted line in the middle represents the border between the Eurosiberian (at north) and the Mediterranean (at south) biogeographic regions (EEA 2006).

defence infrastructures (Granja & Carvalho 1992, Honrado *et al.* 2010, Loureiro & Granja 2001).

Concerns about the impacts of coastal dynamics on sand-dune vegetation have been the focus of a series of scientific research regarding the interactions among sand-dune species, and between these and their surrounding environment (Honrado *et al.* 2010, Lomba *et al.* 2008, Maltez-Mouro *et al.* 2010, Vaz *et al.* 2013), namely the transition between the Eurosiberian and the Mediterranean biogeographic regions (Fidalgo *et al.* 2012, Macedo *et al.* 2010).

Lichens are recognised as an important feature of sand-dune vegetation, known to protect the dune surface against several disturbances, such as water and wind erosions (Cogoni *et al.* 2011). Nevertheless, only few studies on the lichen flora of Portuguese coastal sanddunes have been published to date (Alonso & Egea 1995), in contrast to other European countries, namely Spain (Fernández & Barradas 1997), Italy (Cogoni *et al.* 2011), France (Jun & Rozé 2005), England (Rhind *et al.* 2006) and Denmark (Christensen & Johsen 2001).

In such context, the main purposes of this study are to provide information on the lichen flora of coastal sanddunes in northern Portugal, and 2) to assess the patterns of lichen diversity along key environmental gradients, related to the biogeographic context and coastal-dynamics of the targeted sand-dunes.

#### MATERIALS AND METHODS

This study was conducted in the coastline of northern Portugal, a 200 km long area, circumscribed by the river Minho at north, and the Mondego Cape at south (Fig. 1). Climatically, the area is characterised by a sub-Mediterranean to Mediterranean climate with oceanic influences (Honrado *et al.* 2010, Rivas-Martínez 2004), with mean annual temperatures of ca. 14-15°C, and total annual rainfall ranging from ca. 800 mm (in the south) to 1500 mm (in the north; Vaz *et al.* 2013). The vascular vegetation of the study area is characterised by the occurrence of narrow endemics and a mixture of *taxa* from two biogeographic regions, Eurosiberian and Mediterranean. The study area further includes several national and international protected areas (EU 'Natura 2000' network; Lomba *et al.* 2008, Rivas-Martínez 2004, Neto *et al.* 2007, Vaz *et al.* 2013).

Sampling design. A total of 10 sampling sites were selected based on their biogeographic context, Eurosiberian versus Mediterranean regions, and prevailing coastal dynamics, (meta-)stable versus transgressive dunes (Table 1). At each site, three 25 m<sup>2</sup> square plots (A, B, and C) were placed in secondary ("grey") dunes and arranged perpendicularly to the coastline, with the seaward plot (A) placed immediately in an inland position from foredunes. Species abundance was measured as percentage cover through a scale with eight classes: (1) <1%, (2) 1-5%, (3) 5-15%, (4) 15-25%, (5) 25-50%, (6) 50-75%, (7) 75-90%, (8) 90-100%. Field surveys were conducted from 2007 to 2011.

**Data analysis.** Lichen specimens surveyed were identified in the laboratory by applying the usual stereomicroscopy and microscopy techniques, chemical spot tests and thin-layer chroma-

Table 1
Geographic coordinates, biogeographic context, and prevailing costal dynamics of the ten sampling sites considered
for assessing lichen flora and respective diversity patterns

Site	Code	Latitude (N)	Longitude (W)	Biogeography	Coastal dynamics
Cabedelo	CAB	41°40'50.70"	08°50'2.72"	Eurosiberian	transgressive
Amorosa	AMO	41°38'57.19"	08°49'32.78"	Eurosiberian	(meta-)stable
Foz do Neiva	FNE	41°36'20.19"	08°48'25.81"	Eurosiberian	transgressive
Apúlia	APU	41°28'30.40"	08°46'29.50"	Eurosiberian	transgressive
Aguçadoura	AGU	41°26'34.58"	08°46'42.94"	Eurosiberian	(meta-)stable
Furadouro	FUR	40°51'58.80"	08°40'39.51"	Mediterranean	transgressive
Torreira	TOR	40°45'30.53"	08°42'53.60"	Mediterranean	transgressive
São Jacinto	SJA	40°40'22.35"	08°44'36.17"	Mediterranean	(meta-)stable
Tocha	TOC	40°19'25.44"	08°50'46.58"	Mediterranean	(meta-)stable
Quiaios	QUI	40°13'20.32"	08°53'28.68"	Mediterranean	transgressive

tography (Orange et al. 2001). All specimens were kept in the Herbarium of the University of Porto (PO). Lichen nomenclature follows Hladun & Llimona (2002). Specimens were further characterised based on ecological indices concerning their known bioclimatic distributions (Burgaz & Ahti 2009, Rivas-Martínez 2004, Wirth 1995), and habitat preference regarding pH of the substratum, aridity and photophily (Nimis & Martellos 2008). Statistical analyses were applied in order to test for significant differences between lichen diversity (considering both community species richness and composition) at the regional (across biogeographic regions) and the local scale (across coastal dynamic context, and among the three 25 m<sup>2</sup> plots). Differences in lichen species richness were tested through Mann-Whitney U tests using SPSS software (SPSS, Inc). Differences in lichen species composition were tested through Analysis of Similarity (ANOSIM) implemented in software Primer 5 (Clarke & Gorley 2006).

## **RESULTS AND DISCUSSION**

Species are listed alphabetically with reference to their geographic occurrence (Fig. 1), ecological indices (A = aridity, P = photophily and pH = pH of the substratum), biogeographic preference (B) and herbarium number (PO). Among the 17 lichen species recorded, *Cladonia rei* is a first record for Portugal, and was found in two of the five Eurosiberian sites. *Collema limosum* (Ach.) Ach. is a first record for the provinces of Minho, Beira Litoral and Douro Litoral.

## Cladonia convoluta (Lamkey) Cout.

AMO; AGU; FUR; TOC. A = 3-4; P = 4-5; pH = 4-5; B = Holarctic. P08895-L; P08941-L

Morphologically similar to *Cladonia foliacea*, *C. convoluta* is easily recognisable in the study area by its larger and darker thallus, although the distinction between these two species is not supported by molecular data (Pino-Bodas *et al.* 2010b).

#### Cladonia foliacea (Huds.) Willd.

AMO; AGU; FUR; TOR; TOC. A = 3; P = 4-5; pH = 2-3; B = Holarctic. PO8903-L; PO8907-L; PO8919-L; PO8921-L; PO8922-L; PO8923-L; PO8962-L; PO8963-L; PO8964-L; PO8965-L.

#### Cladonia furcata (Huds.) Schrad.

CAB; AMO; APU; AGU; FUR; TOR; TOC. A = 3; P = 3-4; pH = 2-4; B = Temperate. PO8893-L; PO8940-L; PO8943-L; PO8948-L; PO8966-L; PO8967-L; PO8990-L.

## Cladonia humilis (With.) J. R. Laundon

CAB; AMO; FNE; TOR; TOC. A = 3; P = 3; pH = 2-3; B = Temperate. PO8904-L; PO8906-L; PO8910-L; PO8912-L; PO8913-L; PO8914-L; PO8925-L; PO8933-L; PO8946-L; PO8953-L; PO8958-L; PO8968-L; PO8982-L.

#### Cladonia mediterranea P. A. Duvign. & Abbayes

FUR; TOR. A = 2; P = 3-4; pH = 2-3; B = Temperate. PO8918-L; PO8927-L; PO8969-L; PO8970-L; PO8971-L; PO8972-L.

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According to Burgaz & Ahti (2009), *C. mediterranea* should be considered a threatened species in the Iberian Peninsula.

## Cladonia mitis Sandst.

TOR. A = 3; P = 3-5; pH = 1-2; B = Temperate to Mediterranean. PO8973-L; PO8974-L.

#### Cladonia ramulosa (With.) J. R. Laundon

CAB; AMO; AGU; TOR. A = 2-3; P = 3-4; pH = 1-2; B = (Sub)Boreal to Temperate. PO8894-L; PO8932-L.

#### Cladonia rangiferina (L.) Weber ex F. H. Wigg

TOR. A = 3; P = 4-5; pH = 1-3; B = Temperate to (Supra)Mediterranean. PO8961-L; PO8975-L.

#### Cladonia rangiformis Hoffm.

CAB; AMO; FNE; APU; AGU; FUR; TOR; QUI. A = 3; P = 4-5; pH = 3-5; B = Oceanic to Mediterranean. PO8896-L; PO8899-L; PO8901-L; PO8902-L; PO8905-L; PO8908-L; PO8915-L; PO8916-L; PO8917-L; PO8920-L; PO8924-L; PO8926-L; PO8929-L; PO8939-L; PO8944-L; PO8947-L; PO8955-L; PO8959-L; PO8960-L; PO8976-L; PO8977-L; PO8978-L; PO8978-L; PO8978-L.

*C. rangiformis* is the most common *Cladonia* species in the Iberian Peninsula (Burgaz & Ahti 2009) as well as in the study area.

#### Cladonia rei Schaer.

FNE; AMO. A = 3; P = 3; pH = 2- 3; B = Temperate. PO8897-L; PO8900-L; PO8909-L; PO8911-L; PO8934-L; PO8935-L; PO8936-L; PO8982-L.

*C. rei* is here reported as a new species for Portugal. The species can be confused with *C. subulata* (L.) Weber ex F. H. Wigg., as well as with *C. ochrochlora* Flörke., but differs in secondary chemistry. Despite having been considered a chemotype of *C. subulata* (Spier & Aptroot 2007) its distinction from the latter species is currently supported by molecular, chemical and morphological data (Dolnik *et al.* 2010, Pino-Bodas *et al.* 2010a). Collema limosum (Ach.) Ach.

CAB; AMO; FNE; APU; TOR; TOC. A = 3; P = 3 - 4; pH = 3 - 4; B = Temperate to (Sub)Mediterranean. PO8928-L; PO8954-L; PO8956-L; PO8957-L.

This species is rarely collected in Portugal and is a first record for the provinces of Minho, Douro Litoral and Beira Litoral.

#### Diploschistes interpediens (Nyl.) Zahlbr.

CAB. A = 3-4; P = 3-5; pH = 2-3; B = Boreal to Mediterranean. PO8945-L.

#### Leptogium cyanescens (Pers.) Körb.

CAB; AMO. A = 1-2; P = 2-3; pH = 3; B = Boreal to (Sub)Oceanic-Mediterranean. PO8898-L; PO8958-L.

#### Parmotrema perlatum (Huds.) M. Choisy

CAB. A = 2-3; P = 3-4; pH = 2; B = Temperate to (Sub)Oceanic-Mediterranean. PO8949-L.

#### Peltigera canina (L.) Willd.

CAB; AMO; FNE; APU; TOC. A = 2-3; P = 3; pH = 3-4; B = Oceanic-Temperate to Mediterranean. PO8930-L; PO8931-L; PO8937-L; PO8942-L; PO8950-L.

#### Peltigera didactyla (With.) J. R. Laundon

CAB. A = 3-4; P = 4; pH = 2-3; B = (Supra)Mediterranean to Temperate. PO8951-L; PO8952-L.

#### Xanthoparmelia protomatrae (Gyeln.) Hale

TOR. A = 4; P = 4; pH = 2-3; B = Boreal to Mediterranean. PO8981-L.

**Patterns of lichen diversity.** The most frequent genus surveyed was *Cladonia* (10 species), occurring in nine of the 10 sampling sites and in 80% of the total set of sampling plots. The high frequency and abundance values found for shrubby *Cladonia* species, namely *C. rangiformis* and *C. furcata*, characteristic of older stages of ecological succession, suggests a signal of great maturity of the studied sand-dunes (Ketner-Oostrova & Sýkora



Fig. 2- Ecological preferences of surveyed lichen species regarding. a: biogeography (Hol, Holarctic; Temp, Temperate; Med, Mediterranean; Bor, Boreal). b: hygrophily (1, hygrophytic; 2, rather hygrophytic; 3, mesophytic; 4, xerophytic). c: pH preference (1, very acid substrata; 2, acid substrata; 3, (sub)acid to (sub)neutral substrata; 4, slightly basic substrata; 5, basic substrata).
d: photophily (2, shaded sites; 3, sites with plenty of diffuse light but scarce direct solar irradiation; 4, sun-exposed sites, but avoiding extreme solar irradiation; 5, sites with very high direct solar irradiation).

2000, Jun & Rozé 2005). Additionally, 30% of the recorded lichen species consisted of cyanolichens (*i.e.* lichens containing cyanobacteria), including *Peltigera canina*, *P. didactyla*, *Collema limosum* and *Leptogium cyanescens*. In agreement to Jun & Rozé (2005) and Rhind *et al.* (2006), such results highlight the contribution of lichen vegetation to nitrogen fixation in this nutrient-poor habitat.

The analysis of ecological preferences within the recorded lichen flora showed the dominance of species with temperate affinities (Fig. 2a), and a considerable amount of mesophytic species (Fig. 2b). Such suggests a major preference for humid conditions, in concordance to what was reported by Cogoni *et al.* (2011) for Mediterranean areas of Italy. Regarding pH preferences, lichen species are mainly related with acid to subneutral substrates (Fig. 2c). Also, most species showed preferences for sites with plenty of diffuse light, including scarce to high direct solar irradiation (Fig. 2d).

For the 10 surveyed sites, Mann-Whitney tests resulted in no significant differences for lichen species richness between the biogeographic regions (p-value = 0.683) and no sigA. S. Vaz et al.



Fig. 3– Patterns of lichen species richness (a) and abundance (b) along the ocean-inland direction (*i.e.*, along the sampling plots, A-C). Values are expressed as median with indication of the minimum and maximum values obtained. Abundances of the most frequent lichen species, *Cladonia furcata* (rhomboids) and *C. rangiformis* (squares) are highlighted in the figure. Abundances of the remaining species are shown in the background.

nificant effect of coastal dynamics (p-value = 0.325). Also, along the three sampling plots (A, B and C) surveyed within each site, the only significant difference (p-value = 0.043) for lichens species richness was found between plots A (seaward position) and C (inland position). These results suggest a trend for increasing values of lichen species richness along the local ocean-inland gradient (Fig. 3a), in agreement to the results reported by Ketner-Oostra & Sýkora (2000) in The Netherlands.

Regarding lichen community composition (Fig. 3b), there were no significant differences between plots (A-C). Still, ANOSIM analysis resulted in significant differences between the biogeographic regions (R = 0.127, *p*-value = 0.035) and coastal dynamic context (R = 0.257, *p*value = 0.014). ANOSIM analyses considered only for the three sampling plots of each biogeographic region and each coastal dynamic type, resulted in only one significant difference between plots A and C in (meta-)stable dunes (R = 0.542, *p*-value = 0.09). Still, a general trend for an increase in species abundances along this local gradient was perceived (Fig. 4). These results suggest that the effect of coastal erosion over lichen community structure in transgressive dunes may therefore be homogenised across the local ocean-inland gradient.

## CONCLUSIONS

The analyses of diversity patterns considered in our research show that different signals can be obtained depending on whether the analyses are performed over lichen species richness or lichen species composition. Moreover, these distinct responses of diversity measures can yield complementary indications useful to devise and set-up conservation and monitoring programs for coastal sand-dunes in northern Portugal. In this regard, patterns and drivers of diversity at both regional and local scales should be taken into consideration, as suggested by Forey *et al.* (2008) and Honrado *et al.* (2010).

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Fig. 4- Patterns of lichen species abundance along the ocean-inland direction (from sampling plot A to plot C). Sites are grouped according to their biogeographic context: (a) Eurosiberian sites; (b) Mediterranean sites; and prevailing coastal dynamics: (c) (meta-) stable sites; (d) transgressive sites. Values are expressed as median with indication of the minimum and maximum values obtained. Abundances of the most frequent lichen species, *Cladonia furcata* (rhomboids) and *C. rangiformis* (squares) are highlighted in the figure. Abundances of the remaining species are shown in the background.

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