Article

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Contribution to the lichen flora of the Alvados valley, Natural Park of Serra de Aire e Candeeiros (Central-western Portugal)

Abstract

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Quercus faginea woods in Alvados valley are remnants of ancient forest within a strongly fragmented and diverse human-made matrix. In this contribution, new data concerning lichen diversity characterizing this area are presented, including two species new to Portugal. More than 20% of the species are typical of undisturbed, natural environments confirming these woods to be important areas for preservation of specific lichen flora.

Key words: Floristics, Lichenized *Ascomycetes*, Portuguese oak woods.

Introduction

Although lichen studies devoted to specific areas (e.g. Carvalho & al. 2002; Hespanhol & Marques 2023), pollution monitoring activities (e.g. Munzi & al. 2014; Sergio & al. 2016) and citizen science activities (e.g. Munzi & al. 2023) exist for Portugal, extensive, systematic surveys aimed at documenting the country's lichen flora have never been done. Many regions are still unexplored from the lichen point of view and the discovery of species new to science in the country is not uncommon (e.g. van den Boom 2005; Sanders & Llop 2020).

In June 2022, the COST Action Bottoms-Up organized a Training School on "Field sampling for multi-taxon biodiversity studies in European forests", in Portugal. This training school was meant to illustrate standard approaches for forest multi-taxa monitoring and provide the opportunity to apply some of the methodologies in the field with experts in the topic. Lichens were one of the taxa objects of the training, allowing the collection and the record of several lichen species in Alvados, the training area.

In this area, previous research has detected 161 lichen species (Hespanhol & Marques 2023; Pinho unpublished). Alvados is a civil parish located in the natural park of Serra de Aire e Candeeiros in central Portugal. The Aire and Candeeiros mountains are the most important repository of limestone formations in Portugal that, together with its Karst mor-

phology, nature of the vegetation cover, network of underground watercourses, and cave fauna, earned them the designation of a Natural Park. Three depressions formed by significant fractures - the Mendiga depression, the Minde-Mira polje, and the Alvados depression - characterize the region.

The climate in the Natural Park of Serras de Aire e Candeeiros is characterized by the transition between Mediterranean and Atlantic conditions, making it humid with average temperatures and a significant lack of water in the summer. While water is scarce in the surface, it is abundant underground, forming one of the largest reservoirs of underground freshwater in Portugal (ICNF 2023).

To date, around 600 plant species are known to grow in the 39,000 hectares of the park, corresponding approximately to one-fifth of the plant species occurring in the country. The landscape, shaped by centuries of fire, agriculture and grazing, is dominated by olive groves with significant clusters of *Quercus faginea* Lam. forest and limited clusters of *Q. rotundifolia* Lam., *Q. suber* L., *Ulmus* spp., and *Castanea sativa* Mill.. In terms of spontaneous vegetation, there is a prevalence of shrubby areas with kermes oak (*Quercus coccifera* L.) and sub-shrubby areas with rosemary (*Rosmarinus officinalis* L.) (ICNF, 2023). Relict *Q. faginea* forests are restricted to a very few isolated localities in Portugal. They are considered habitat of Community interest (9240 - *Quercus faginea* and *Quercus canariensis* Iberian woods) and "forest types of unique character in Europe and of extreme biological importance" (EUNIS 2023).

In order to contribute to the knowledge of the biodiversity of these woods, this work presents a preliminary checklist of lichen species for the study area and the floristic analysis.

Materials and methods

The lichen survey methodology employed adheres to the guidelines outlined in the handbook published by the COST Action Bottoms-up (Burrascano & al. 2021), aligning with standard methodologies commonly used for lichen biomonitoring (Giordani & al. 2020).

Three plots were identified in a *Q. faginea* cluster (Fig. 1) along a walking path near Alvados: plot 1 with coordinates 39.54436507, -8.77430666, plot 2 with coordinates 39.5443418, -8.77562866, and plot 3 with coordinates 39.54409309, -8.77647355.

In each plot, three *Q. faginea* trees were selected avoiding over-mature/dying trees, trees close to forest gaps, and trunks completely covered by bryophytes. On each trunk, four 10×50 cm sampling grids (each split into five 10×10 cm quadrats) were located at the four cardinal directions, between 100 and 150 cm from the ground. The frequency of each species present on each side of the grid was recorded for the nine trees. The list of species here presented is therefore not the total lichen diversity of the area but includes only the species falling in the portions of trees identified by the grid.

Lichens have been identified using a stereo microscope for macroscopic structures (such as reproductive and vegetative structures) and usual chemical spot tests K (a solution of 10% potassium hydroxide), C (sodium hypochlorite solution), and KC. A light microscope was used for identification of microscopic characters, such as paraphyses, asci and spores. For the identification of the lichen species several keys were used, mainly Smith & al. (2019) and online identification keys on ITALIC 7.0 (Martellos & al. 2023). Growth

Fig. 1. *Quercus faginea* forest in the study area in Alvados (Portugal) (credits: Silvana Munzi).

forms, photobionts and reproductive strategies were considered to detect the characteristics of lichen colonization. Nomenclature and bio-ecological characterization of the species followed Nimis (2016).

Results

The annotated floristic list includes 43 species, three of which recognized at genus level. Among them, 2 are new to Portugal (marched with # in the list), 30 are new to Alvados valley (marked with * in the list). For each taxon, the collection plots and bio-ecological traits (growth form, photobiont, reproductive strategy, poleotollerance, and nitrophilia) following Nimis (2016), are given. For shortness, the following abbreviations will be used: Fol.b (foliose, broad lobed); Fol.n (foliose, narrow lobed); Sq (squamulose); Frut (fruticose); Cr (crustose); Lp (leprose); Ch (lichens with green-algae, other than *Trentepohlia*); Tr (lichens with *Trentepohlia*); Cy (lichens with cyanobacteria); As.s (mainly asexual, by soredia, or soredia-like structures); As.i (mainly asexual, by isidia, or isidia-like structures); S (sexual); +S (sexual reproduction observed in the field in species adopting mainly asexual reproduction); P (Poleotolerance); N (Nitrophilia). A short note about the distribution in Portugal is reported for a few uncommon species.

**Acrocordia gemmata* (Ach.) A. Massal. – Plots: 1, 2, 3. Cr, Tr, S, P=1, N=1.

**Alyxoria varia* (Pers.) Ertz & Tehler – Plots: 1, 2, 3. Cr, Tr, S, P=1-2, N=1-2.

**Bacidia laurocerasi* (Duby) Zahlbr. – Plots: 3. Cr, Ch, S, P=0, N=1.

Cladonia sp. – Plots: 1. Frut, Ch.

**Coenogonium pineti* (Ach.) Lücking & Lumbsch – Plots: 2. Cr, Tr, S, P=1-2, N=1-2.

**Collema subflaccidum* Degel. – Plots: 2, 3. Fol.b, Cy, As.i, P=1-2, N=2-3.
Collema subnigrescens Degel. – Plots: 1. Fol.b, Cy, S, P=1-2, N=2-3.
Crocodia aurata (Ach.) Link – Plots: 2. Fol.b, Ch, As.s, P=0, N=1.
**Dendrographa decolorans* (Sm.) Ertz & Tehler – Plots: 3. Cr, Tr, As.s, P=1-2, N=2-3.
Flavoparmelia caperata (L.) Hale – Plots: 2, 3. Fol.b, Ch, As.s +S, P=1-2, N=1-3.
**Fuscopannaria mediterranea* (Tav.) P.M. Jørg. – Plots: 1, 3. Sq, Cy, As.s, P=1, N=1-2.
**Gyalecta derivata* (Nyl.) H. Olivier – Plots: 1, 3. Cr, Tr, S, P=1, N=1-2.
**Gyalecta truncigena* (Ach.) Hepp – Plots: 1, 2. Cr, Tr, S, P=0, N=1-2.
This species is characterized by a thin or inconspicuous grey thallus and urceolate apothecia with an orange-brown disc. It can be easily differentiated from other epiphytic species of the genus by ascospores muriform, oblong-fusiform, without conical tips, (14-)17-28(-31) x 5-9 µm. In Portugal, this species seems to by rarely collected, mostly in the western area (e.g. Sales & Hedge 2000; Alvarez Andrés & López de Silanes 2002; Van den Boom 2005).
**Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt – Plots: 2, 3. Fol.n, Ch, As.s, P=1-3, N=3-5.
**Lecanora* sp. – Plots: 2. Cr, Ch, S.
**Lepra albescens* (Huds.) Hafellner – Plots: 1. Cr, Ch, As.s, P=1-2, N=1-3.
Lepra amara (Ach.) Hafellner – Plots: 2. Cr, Ch, As.s, P=1-3, N=1-2.
**Lepra multipuncta* (Turner) Hafellner – Plots: 1. Cr, Ch, As.s, P=1, N=1-2.
**Lepraria* sp. – Plots: 1, 2, 3. Lp, Ch, As.s.
**Leptogium brebissonii* Mont. – Plots: 2, 3. Fol.b, Cy, As.i, P=1, N=1-2.
*#*Leptogium burnetiae* C.W. Dodge – Plots: 3. Fol.b, Cy, As.i, P=1, N=2-3.
This species is characterized by a tomentose shiny and slightly swollen, translucent blue-grey thallus, turning to dark olive-green when wet and by the presence of cylindrical to coralloid isidia, usually concolorous with lobes (Fig. 2). It is a mild-temperate species, widely distributed in the tropics.
**Leptogium furfuraceum* (Harm.) Sierk – Plots: 3. Fol.b, Cy, As.i, P=1, N=1-2.
Lobaria pulmonaria (L.) Hoffm. – Plots: 1, 2, 3. Fol.b, Ch, As.s, P=1, N=1-2.
Nephroma laevigatum Ach. – Plots: 1, 2. Fol.b, Cy, S, P=1, N=1.
**Normandina pulchella* (Borrer) Nyl. – Plots: 1, 2, 3. Sq, Ch, As.s, P=1-2, N=1-3.
**Ochrolechia subviridis* (Høeg) Erichsen – Plots: 1, 2. Cr, Ch, As.s, P=1, N=1.
** *Pannaria conoplea* (Ach.) Bory – Plot 3. Fol.n, Cy, S, P=0, N=1.
Pannaria rubiginosa (Ach.) Bory – Plots: 1, 2, 3. Fol.n, Cy, S, P=0, N=1.
Parmelia sulcata Taylor – Plots: 1, 2, 3. Fol.b, Ch, As.s + S, P=1-3, N=1-3.
**Parmotrema reticulatum* (Taylor) M. Choisy – Plots: 1, 2, 3. Fol.b, Ch, As.s, P=1-2, N=1-2.
Pectenia atlantica (Degel.) P.M. Jørg., L. Lindblom, Wedin & S. Ekman – Plots: 1. Fol.n, Cy, As.i, P=0, N=1.
**Peltigera praetextata* (Sommerf.) Zopf – Plots: 1, 3. Fol.b, Cy, As.i, P=1-2, N=1-2.
**Pertusaria leioplaca* (Ach.) DC. – Plots: 2. Cr, Ch, S, P=1, N=1-2.
**Pertusaria pertusa* (L.) Tuck. var. *pertusa* – Plots: 2. Cr, Ch, S, P=1-2, N=1-2.
**Pyrenula nitidella* (Schaer.) Müll. Arg. – Plots: 3. Cr, Tr, S, P=1, N=1.
This species is characterized by small perithecia (0.2-0.4 mm diam) and hymenium with orange-brown pigments reacting K+ purple-red. It is usually found on the bark

of deciduous trees in open, humid woodlands (Nimis 2016), very rarely outside Europe. In Portugal seems to by rarely collected (Consortium of Lichen Herbaria 2023).

**Ramalina calicaris* (L.) Fr. – Plots: 2. Frut, Ch, S, P=1, N=1-2.

Ramalina farinacea (L.) Ach. – Plots: 2. Frut, Ch, As.s, P=1-2, N=1-2.

**Ramalina implectens* Nyl. – Plots: 2. Frut, Ch, S, P=1, N=1-2.

Ricasolia amplissima (Scop.) De Not. – Plots: 1. Fol.b, Ch, S, P=0, N=1-2.

Ricasolia virens (With.) H.H. Blom. & Tønsberg – Plots: 1. Fol.b, Ch, S, P=0, N=1.

#**Scutula circumspecta* (Vain.) Kistenich, Timdal, Bendiksby & S.Ekman– Plot 3. Cr, Ch, S, P=1, N=1-3.

This species is characterized by an inconspicuous crustose whitish thallus and small (<0.7 mm diam.) black, marginate apothecia, usually inhabiting the bark of mature deciduous trees (Fig. 3). It is distinguished from similar taxa by different chemotaxonomic reactions and ± bacilliform ascospores.

**Scytinium lichenoides* (L.) Otálora, P.M. Jørg. & Wedin – Plots: 1, 2, 3. Sq, Cy, S, P=1-2, N=1-3.

**Usnea* sp. – Plots: 1, 2, 3. Frut, Ch.

Fig. 2. *Leptogium burnetiae* (Silvered Vinyl Lichen Wikimedia Commons, uploaded by Amada 44 https://italic.units.it/flora/images/species/images/LeptogiumburnetiaeCWDodge1620896814.jpg. Accessed on: 11.24.2023).

Discussion

The lichen flora of the *Quercus faginea* woods in Alvados Valley is dominated by crustose (37%) and foliose, broad lobed species (33%) most of which have green algae as photobiont (56% + 16% with *Trentepohlia*) and reproduce asexually (56%) (Fig. 4).

Fig. 4 shows the functional and morphological characterization of the lichen flora of the *Quercus faginea* woods in Alvados Valley. The lower percentage of narrow-lobed lichens (9%) confirms that this growth form is mainly associated with disturbed environments, i.e. from disturbed forests (Benítez & al. 2018) to urbanized areas (e.g. Koch & al. 2019). According to Aragón & al. (2016) and Koch & al. (2019), broad-lobed foliose and inconspicuous crustose lichens are light-demanding species adapted to open forests, consistently with our results. Epiphytic fruticose lichens occurred with low percentage frequencies (12%) usually preferring more sun-exposed niches, e.g. branches or higher parts of a bole. Moreover, typical species from shaded environments, such as leprose and squamulose lichens, are frequent in denser and more preserved forest areas (Asplund & Wardle 2016).

Lichens with green algae can photosynthesize with minimum thallus water content and reactivate photosynthesis from air humidity, so they usually occur with the higher percentage. However, in this habitat we find important contributes by both lichens with *Trentepohlia* and nitrogen-fixer cyanobacteria. Similar percentage values of *Trentepohlia* as photobiont are observed in *Quercus suber* woods of the Iberian Peninsula (Fos 1998)

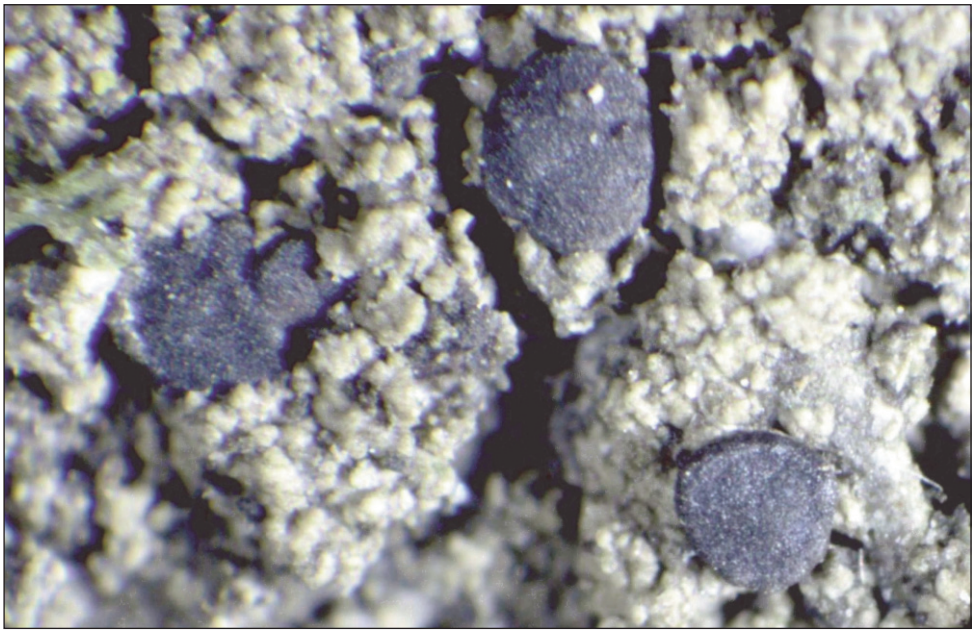

Fig. 3. *Scutula circumspecta* (Photo P.L. Nimis https://italic.units.it/flora/images/species/images/ScutulacircumspectaVainKistenichTimdalBendiksbySEkman1625569351.jpg. Accessed on: 11.24.2023.).

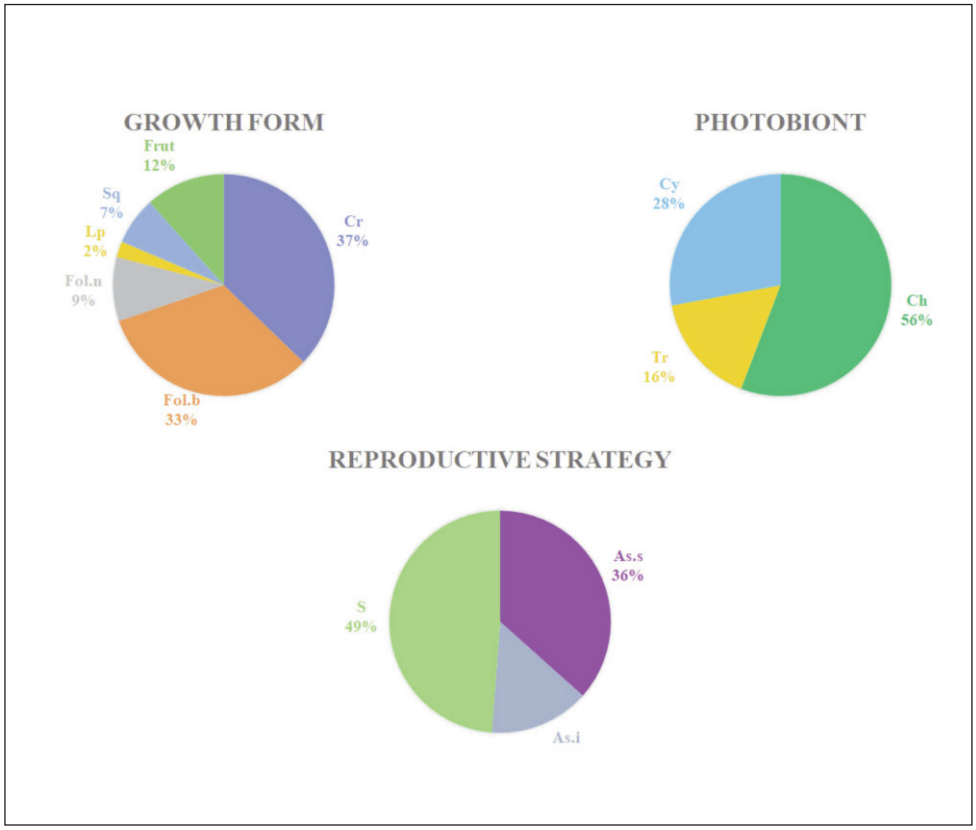

Fig. 4. Functional and morphological characterization of the lichen flora of the *Quercus faginea* woods in Alvados Valley: occurrence (%) of growth forms, photobionts, reproductive strategies. Fol.b: foliose, broad lobed; Fol.n: foliose, narrow lobed; Sq: squamulose; Frut: fruticose; Cr: crustose; Lp: leprose; Ch: lichens with green-algae, other than *Trentepohlia*; Tr: lichens with *Trentepohlia*; Cy: lichens with cyanobacteria; As.s: mainly asexual, by soredia, or soredia-like structures; As.i: mainly asexual, by isidia, or isidia-like structures; S: mainly sexual.

and in oak forests of Sardinia (Zedda 2002), i.e. 12% and 13%, respectively, suggesting adaptation to warm and humid conditions (Aptroot & Van Herk 2007; Matos & al. 2015; Kosecka & al. 2020). Moreover, a high percentage of cyanolichens (28%), especially with broad-lobed thalli, appears to be favored by the truly humid character of the Portuguese *Quercus faginea* forests, requiring for liquid water in photosynthesis.

Lichens can reproduce both sexually (ascospores) and/or asexually (conidia, thallus fragments i.e. soredia, isidia, goniocysts). Ascospores are generally smaller than vegetative structures and they are also usually actively discharged, to be dispersed over longer distances (Seymour & al. 2005). Reproductive structures are directly related to microclimatic factors associated with forest structure (e.g. canopy cover, and tree age) and abiotic factors

as humidity, temperature and light availability. Moreover, the availability of trees of different ages and of micro-habitats can be the main factors conditioning the most appropriate reproductive strategies (Giordani & al. 2012; Martínez & al. 2012). In these woods, lichens with mainly sexual and with mainly asexual reproduction are equally represented (49% vs. 51%) with a few of the latter (i.e. *Flavoparmelia caperata*, *Parmelia sulcata* and *Lobaria pulmonaria*) showing both apothecia and soredia (non-corticated propagules containing both fungal hyphae and algal cells). Considering sexual reproduction allows the genetic turnover of the populations, these observations are of considerable interest with respect to the importance of these woods for the conservation of lichen diversity in this area.

Fig. 5 shows the incidence of the species in the ecological groups. The flora is characterized by its adaptation to substrata with low eutrophication reflecting the scarcity of dust and nitrogen deposition. This is also confirmed by the occurrence of more than a fifth of the species (21%) which are known to exclusively occur on old trees in ancient, undisturbed forests (P = 0).

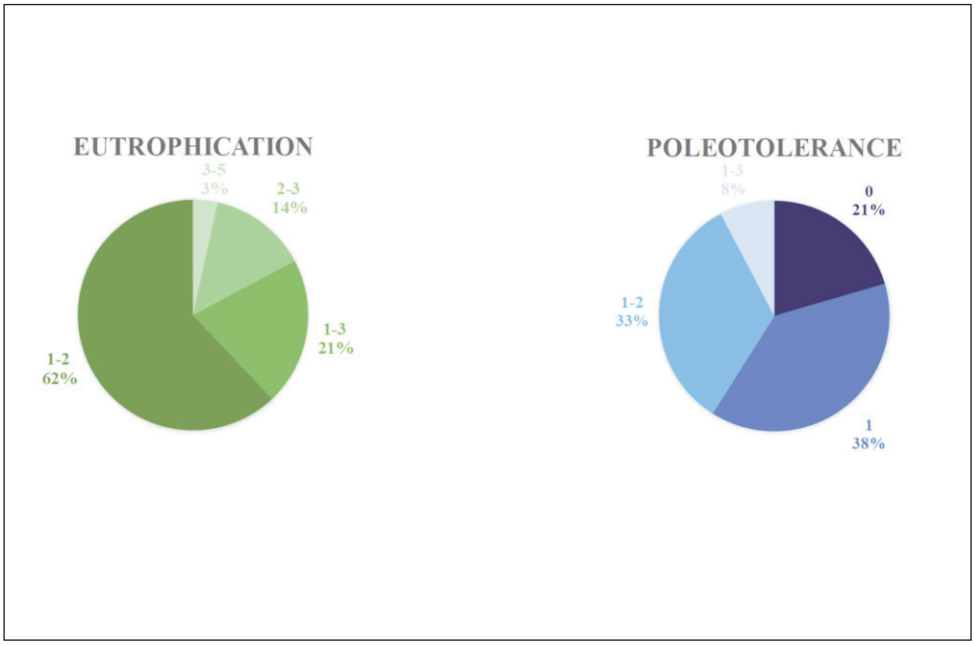

Fig. 5. Ecological characterization of the lichen flora of the *Quercus faginea* woods in Alvados Valley, following Nimis (2016). Eutrophication index is grouped in five classes: 1 = no eutrophication, 2= very weak eutrophication, 3 = weak eutrophication, 4 = rather high eutrophication, 5 = very high eutrophication. The poleophoby index is grouped in four classes: 3 = species occurring in heavily disturbed areas, incl. large towns; 2 = species occurring in moderately disturbed areas (agricultural areas, small settlements etc.); 1 = species occurring in natural or semi-natural habitats; 0 = species which exclusively occur on old trees in ancient, undisturbed forests. A species can belong to one or more classes depending on the width of its ecological range.

Conclusion

The field activities carried out in the *Quercus faginea* woods of Alvados valley highlighted a valuable epiphytic lichen flora, which includes 2 rarely collected (*Gyalecta truncigena* and *Pyrenula nitidella*) and two new species (*Leptogium burnetiae* and *Scutula circumspecta*) to Portugal. Furthermore, high percentages of sensitive species with higher habitat quality requirements (i.e. cyanolichens, anitrophile and poleophobic species) were detected. This interesting contingent of species seems to find in these relict forests, despite the study area being included in a highly fragmented anthropogenic matrix, favorable environmental conditions confirming, also from a lichenological point of view, the importance of this habitat for the purposes of biodiversity conservation.

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