A framework for assessing the vulnerability of exposed schist surfaces to lichen-induced weathering in the Upper Douro region (NE Portugal)

Joana Maria Mendonça Marques
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Biology Department
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Supervisor
Prof. Dr. Rubim Almeida, Auxilliary Professor, Faculty of Sciences (Biology Department), University of Porto

Co-supervisor
Prof. Dr. Graciela Paz-Bermúdez, Profesor Contratado Doctor, E.U.E. Forestal, University of Vigo

Foreword

In accordance with article nº 7, point 3 of the Regulation of the Doctoral Programme in Biology at the Faculty of Sciences, University of Porto, which is in line with the Portuguese Law (Decree-Law nº 74/2006), the present thesis is composed of six articles, listed below, written in collaboration with co-authors. The candidate declares that she contributed in conceiving the ideas, compiling and producing the databases and analyzing the data, and also declares that she led the writing of all Chapters.

List of papers

Marques J., Schultz M., Paz-Bermúdez G. (2013) A *Peltula* Nyl. diversity hotspot in north-east Portugal, with one species new to science and three species new to mainland Europe. The Lichenologist 45 (4): 483-496 – **Chapter 6.1**

Marques J., Paz-Bermúdez G. (in review) New and interesting lichen records for the Portuguese flora from the Upper Douro region (NE Portugal). Mycotaxon – **Chapter 6.3**

Marques J., Hespanhol H., Paz-Bermúdez G., Almeida R. (in review) Choosing between sides in the battle for pioneer colonization of schist in the Côa Valley Archaeological Park: a community ecology perspective. Journal of Archaeological Science – **Chapter 7.1**

Marques J., Paz-Bermúdez G., Almeida R. (submitted) Searching for surrogates of lichen diversity for rock-art conservation monitoring. Journal of Cultural Heritage – **Chapter 7.2**

Marques J., Vázquez-Nion D., Paz-Bermúdez G., Prieto B. (submitted) Assessing the susceptibility of weathered versus unweathered schist to biological colonization in the Côa Valley Archaeological Park (north-east Portugal). The Science of the Total Environment – **Chapter 8.1**

Marques J., Gonçalves J., Paz-Bermúdez G., Almeida R., Prieto B. (submitted) Evaluation of lichen-induced weathering of exposed schist surfaces under contrasting microclimates. International Biodeterioration and Biodegradation – **Chapter 8.2**

Additional contributions

López de Silanes M. E., Paz-Bermúdez G, Carballal R., Marques J. (2012) The genus *Leptogium* (Ach) Gray (Collemataceae, Ascomycotina) in mainland Portugal. Sydowia 64 (1): 67-102.

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1 Abstract | Resumo

Exposed rocks are subjected to the continuous and synergic action of several biotic and abiotic weathering agents. Although it seems contradictory, lichens may constitute a defence against those agents and simultaneously represent a serious threat to outcrop stability. Recent reviews on the weathering promoted by lichen growth and development on rock surfaces agree on two ways by which saxicolous lichens act on their substrate: hyphal penetration through pre-existing fissures, cracks and other areas of least resistance is the most important mechanism of lichen-induced physical weathering, whereas the production of a wide array of substances frequently referred to as “lichen acids” is accountable for a great part of the lichen-induced chemical weathering. Lichen impact on rock surfaces is however still the subject of some debate regarding its extent, relative contribution to rock weathering in face of other weathering agents and dependence on environmental constraints, but there is a general consensus around the idea that lichen-induced weathering is a reality and that conservation of open-air rock-art on schist might be impaired by lichen colonization.

A great body of research has been carried out into the effects of lichens on limestone and marble as well as granite, reflecting the geographical origin of the target rock type and the importance of the respective stone in terms of commercial or cultural use. The weathering role of lichens on schist is by far less documented. Concern about all aspects of schist weathering has increased since the discovery of one of the world’s largest collection of open-air rock-art in the Côa River Valley (north-east Portugal), engraved on massive vertical schist surfaces along the slopes of what is now the Côa Valley Archaeological Park. Efforts are being made to characterize the weathering dynamics affecting the Côa Valley’s rock-art, integrating biological, geophysico-chemical and environmental data in order to prevent major damages.

This project focused on the analysis of lichen-induced weathering of exposed schist surfaces in the Côa Valley Archaeological Park and aimed at introducing an ecological perspective into the assessment of rock-art conservation status by means of: 1) detailed taxonomic identification of saxicolous and terricolous lichens occurring on schist surfaces in the Côa River Valley; 2) data collection on the local-scale patterns of lichen diversity upon those surfaces; 3) selection of representative target species to be analysed in terms of lichen-induced rock weathering; 4) definition of lichen diversity surrogates and evaluation of their relevance for rock-art conservation monitoring in the study area. The dynamics and timing of biological colonization were also contemplated in order to evaluate the risks of biodeterioration and support effective management decisions for rock-art conservation. A broader area outside the boundaries of the Park roughly corresponding to what is known as the Upper Douro region was also prospected in terms of lichen diversity given the almost complete absence of information about its lichen flora and the strong suspiction of its lichenological importance, thus providing additional data that can be used in the future to assess the impact of lichens on rock-art located outside the park.

A total of 205 saxicolous and terricolous lichen taxa are here reported from schist outcrops in the Upper Douro region, including one species new to science (*Peltula lobata*), four species new to mainland Europe (*Peltula bolanderi*, *P. farinosa*, *P. zahlbruckneri*, *Trapeliopsis gymnidiata*), three species new to the Iberian Peninsula (*Acarospora boulderensis*, *Dermatocarpon deminuens*, *Endocarpon simplicatum*) and the first records of twenty four lichen taxa for mainland Portugal with data on their currently known distribution and regional ecology. *Agonimia opuntiella*, *Caloplaca grimmiae*, *Epiphloea terrena*, *Glyphopeltis ligustica*, *Squamarina concrescens*, *Toninia squalida*, *Placidium imbecillum*, *Psora gresinonis* and *Toninia cinereovirens* are second records for Portugal. *Acarospora macrospora* subsp. *murorum*, *Epiphloea terrena*, *Porocyphus coccodes* and *Squamarina concrescens* were previously known to occur in Portugal but were last mentioned in literature by the late 19th or early 20th centuries.

A key uncertainty in the assumptions on the relationship between lichen colonization and long-term rock-art deterioration in the Park is in the way that lichens act under different weathering environments. Different species are expected to act differently on the surface upon which they grow and even the same species can change its performance under the influence of environmental change. If little was known about the lichen diversity of schist surfaces in the Upper Douro region even less was known about the composition of lichen assemblages upon those surfaces. Structural and compositional variation in these assemblages associated with surface orientation was significant enough to require separate consideration in the analysis of lichen-induced weathering. Based on their frequency and abundance at opposite slopes in the Côa Valley, one squamulose (*Peltula euploca*) and three crustose lichens (*Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Lecanora pseudistera*) were selected to address the phenomenon of lichen-induced weathering under the effect of major environmental constraints. A strongly reduced subset of surrogate species and species functional groups based on selected vegetative traits, accurately reproduced overall lichen species richness. Surrogate species and species functional groups may constitute a good alternative to exhaustive species surveys in the study area and are expected to facilitate cost-effective monitoring of lichen-induced weathering in the Côa Valley Archaeological Park and the Upper Douro region.

The few observations that existed in the literature as to how schist surfaces are affected by lichen activity in the Côa Valley were mostly speculative. The link between the observed differential weathering in north-west versus south-east facing schist surfaces and differential weathering activity of lichens came from controlled experiments with individual species tested against bare rock controls. Changes in the weathering degree of the bare parent rock were shown to increase its bioreceptivity. Analysis by X-ray microdiffraction and Raman spectroscopy revealed the presence of kaolinite and halloysite, two common products of schist weathering, in both colonized and bare rock control schist samples irrespective of the orientation of the source outcrop. Of particular interest is the presence of calcium oxalates detected by both techniques on samples colonized by the four target species. Unlike kaolinite and halloysite, however, calcium oxalate seems to be produced more frequently in south-east than in north-west facing surfaces which contradicts certain assumptions of a causal relationship between lichen-induced weathering and rock-art deterioration in the Côa Valley. Surface orientation produced no detectable effect on the extent of hyphal penetration of individual species. However some evidence was found for differences between species in the extent of hyphal penetration, with physical impact of *Lecanora pseudistera* seeming higher than that of the remaining three target species. It is possible that lichen-induced physical weathering ends up being higher at north-west facing surfaces than at south-east facing surfaces due to the abundance of more physically aggressive species, whereas the contrary happens with lichen-induced chemical weathering. This matter needs to be investigated further as the abundance of physically aggressive species may depend on the availability of a more vulnerable rock substrate at north-west facing surfaces due to the previous action of other weathering agents.

The spatial pattern and population dynamics of these lichens may also need to be considered. Much of the required information for such approach has been here provided. While much remains to be done, this work has laid the groundwork for further research into the problem of lichen colonization of open-air rock-art on schist.

Keywords

Lichens, Taxonomy, Ecology, Saxicolous Communities, Rock Weathering, Bioreceptivity, Biodeterioration, Preventive Conservation, Rock-art conservation

As superficies rochosas expostas estão sujeitas à acção continua e sinérgica de diversos agentes meteóricos bióticos e abióticos. Embora aparentemente contraditória, a acção dos líquenes pode resultar na defesa contra esses agentes e simultaneamente representar uma séria ameaça à estabilidade dos afloramentos. De acordo com estudos recentes sobre os processos de meteorização da rocha promovidos pelo crescimento e desenvolvimento dos líquenes em superfícies rochosas, existem dois mecanismos através dos quais os líquenes saxícolas actuam sobre o substrato: a penetração de hifas através de fissuras pré-existentes e outras áreas de menor resistência é o mecanismo mais importante de meteorização física, enquanto que a produção de uma grande variedade de substâncias, frequentemente designadas por "ácidos liquénicos", é responsável por uma grande parte da meteorização química induzida por líquenes. O impacto dos líquenes sobre a rocha, no entanto, continua a ser objecto de debate nomeadamente no que respeita a extensão desse impacto, contribuição relativa para a meteorização da rocha face outros agentes e dependência de factores ambientais extrínsecos, mas existe um consenso em torno da ideia de que a meteorização induzida por líquenes é uma realidade e que a conservação da arte rupestre ao ar livre em xisto pode ser de alguma forma prejudicada pela colonização liquénica.

A maior parte da investigação acerca dos efeitos meteóricos dos líquenes tem incidido sobre rochas calcárias, mármores e granitos, reflectindo a origem geográfica da rocha em causa e a importância da respectiva pedra do ponto de vista comercial ou cultural. O papel dos líquenes na meteorização do xisto está muito menos documentado. A preocupação com todos os aspectos da meteorização do xisto tem aumentado desde a descoberta de uma das maiores colecções mundiais de arte rupestre ao ar livre no vale do rio Côa (nordeste de Portugal). Os motivos foram gravados sobre extensas superfícies verticais de xisto situadas ao longo das encostas de uma área actualmente delimitada pelo Parque Arqueológico do Vale do Côa. Actualmente estão a ser empregues esforços no sentido de caracterizar as dinâmicas de meteorização que afetam a arte rupestre do Vale do Côa, integrando dados biológicos, geo-físico-químicos e ambientais, a fim de evitar grandes danos.

Este projecto está focado na análise da meteorização de superfícies de xisto induzida por líquenes no Parque Arqueológico do Vale do Côa e visa introduzir uma perspectiva ecológica no processo de avaliação do estado de conservação da arte rupestre através da: 1) identificação taxonómica detalhada dos líquenes saxícolas e terrícolas presentes nas superfícies de xisto do vale do rio Côa; 2) colheita de dados sobre os padrões de diversidade liquénica nessas superfícies à escala local; 3) selecção de espécies representativas para uso como alvo da análise da meteorização da rocha induzida pelos líquenes; 4) definição de substitutos da diversidade liquénica e avaliação da sua relevância para a monitorização da conservação da arte rupestre na área de estudo. A dinâmica e tempo de colonização biológica também foram contempladas, de forma a avaliar os riscos de biodeterioração e apoiar decisões de gestão eficazes do ponto de vista da conservação da arte rupestre. Foi também prospectada uma área mais ampla, correspondendo essencialmente àquela que é conhecida por região do Douro Superior, dada a quase total ausência de informação sobre a flora liquénica dessa região e a forte suspeição do seu interesse liquenológico, proporcionando assim a obtenção de dados que poderão ser

utilizados no futuro para avaliar o impacto dos líquenes sobre a arte rupestre localizada para além dos limites do Parque.

É aqui reportada a ocorrência de 205 *taxa* de líquenes saxícolas e terrícolas sobre afloramentos de xisto na região do Douro Superior, incluindo uma espécie nova para a ciência (*Peltula lobata*), quatro espécies novas para a Europa continental (*Peltula bolanderi*, *P*. *farinosa*, *P*. *zahlbruckneri*, *Trapeliopsis gymnidiata*), três espécies novas para a Península Ibérica (*Acarospora boulderensis*, *Dermatocarpon deminuens*, *Endocarpon simplicatum*) e os primeiros registros de vinte quatro taxa liquénicos para Portugal continental com dados sobre a sua distribuição, tal como é actualmente conhecida, e ecologia regional. *Agonimia opuntiella*, *Caloplaca grimmiae*, *Epiphloea terrena*, *Glyphopeltis ligustica*, *Squamarina concrescens*, *Toninia squalida*, *Placidium imbecillum*, *Psora gresinonis* e *Toninia cinereovirens* constituem segundos registos para Portugal. A ocorrência de *Acarospora macrospora* subsp. *murorum*, *Epiphloea terrena*, *Porocyphus coccodes* e *Squamarina concrescens* em Portugal era já conhecida, mas tinha sido referida pela última vez na literatura em finais do século XIX ou início do século XX.

A principal incerteza em torno das relações entre a colonização liquénica e a deterioração a longo prazo da arte rupestre no Parque Arqueológico do Vale do Côa está na forma como os líquenes actuam sob o efeito de diferentes ambientes de meteorização. É provável que cada espécie actue de forma diferente sobre a superfície que coloniza, e a mesma espécie pode ver alterada a sua forma de actuação sob influência de alterações ambientais. Se era escasso o conhecimento sobre a riqueza liquénica das superfícies de xisto na região do Douro Superior menor era ainda o conhecimento sobre a composição das respectivas comunidades. A resposta estrutural e composicional destas comunidades em função da orientação das superfícies é suficientemente variável para justificar uma análise independente do efeito da meteorização induzida pelos líquenes. Com base na sua frequência e abundância em encostas de exposição noroeste e sudeste no Vale do Côa, foram selecionadas três espécies alvo, uma do tipo escamoso (*Peltula euploca*) e três do tipo crustáceo (*Aspicilia hoffmanniana*, *Caloplaca subsoluta* e *Lecanora pseudistera*), para abordar o fenómeno da meteorização induzida por líquenes sob o efeito de factores ambientais predominantes. Foram selecionados subconjuntos fortemente reduzidos do ponto de vista da riqueza específica, compostos por espécies substitutas e grupos funcionais baseados em caracteres vegetativos, que reproduziram de forma fiel a riqueza específica total. O recurso a espécies substitutas e grupos funcionais pode constituir uma boa alternativa aos inventários exaustivos das espécies de líquenes presentes na área de estudo e dessa forma facilitar a monitorização eficaz da meteorização induzida por líquenes no Parque Arqueológico do Vale do Côa e região do Douro Superior.

As escassas referências à forma como as superfícies de xisto são afectadas pela actividade liquénica no Vale do Côa eram meramente especulativas. Neste estudo foi estabelecida uma relação entre a meteorização diferencial observada nas superfícies de xisto das encostas expostas a noroeste versus encostas expostas a sudeste, através de experiências controladas envolvendo amostras de xisto colonizadas pelas espécies alvo seleccionadas e amostras controlo, não colonizadas. Ficou também aqui demonstrado que a meteorização sofrida pela rocha-mãe após a exposição, aumenta a sua biorreceptividade. As análises de microdifracção de raios-X e espectroscopia Raman revelaram a presença de caulinite e haloisite, dois produtos comuns da meteorização do xisto, em amostras colonizadas e não colonizadas, independentemente da orientação da superfície de origem. A presença de oxalato de cálcio, detectada por ambas as técnicas em amostras colonizadas pelas quatro espécies alvo é particularmente relevante. Ao contrário da caulinite e da haloisite, no entanto, a presença de oxalato de cálcio parece ser mais frequente nas superfícies expostas a sudeste do que a noroeste, also que contraria o pressuposto de uma relação causal entre a meteorização induzida por líquenes e a deterioração da arte rupestre no Vale do Côa. A orientação da superfície não produziu qualquer efeito detectável sobre a profundidade de penetração das hifas das espécies a nível individual. No entanto, foram encontradas algumas evidências para a existência de diferenças entre espécies no que respeita a extensão dessa penetração, sendo o impacto físico de *Lecanora pseudistera* aparentemente mais elevado do que o das restantes espécies alvo. É possível que a meteorização física induzida por líquenes seja mais elevada em superfícies expostas a noroeste do que em superfícies expostas a sudeste, devido à abundância de espécies mais agressivas do ponto de vista físico, e se verifique o contrário ao nível da meteorização química. Esta hipótese requer uma avaliação mais profunda dado que a abundância de espécies mais agressivas do ponto de vista físico pode depender da disponibilidade de substratos rochosos mais bioreceptivos a Noroeste devido à acção prévia de outros agentes de meteorização.

O padrão de distribuição espacial e dinâmica populacional destes líquenes também deverão ser tidos em conta. Uma parte considerável da informação necessária para tal abordagem foi aqui fornecida. Embora longe de ser definitivo, este estudo constitui a base para futuras investigações em torno do problema da colonização liquénica de arte rupestre em xisto.

Palavras-chave

Líquenes, Taxonomia, Ecologia, Comunidades saxícolas, Meteorização da rocha, Bioreceptividade, Biodeterioração, Conservação preventiva, Arte Rupestre.

2 Introduction

2.1 On the persistence of lichens

A common and fundamental goal of ecology and systematics is to understand how organisms are divided into species, the basic rank of biological classification, and how those species interact with their surroundings. In fungi, the recognition of species remains controversial. Although many fungi develop microscopically diagnostic structures, phenotypic divergence is often minimal, and the relationship between phenotype and species genetic markers is sometimes difficult to establish. Lichen-forming fungi are not an exception and present a unique exercise in the study of the species definition problem (Thomson 1963).

Lichens are actually one of those enigmatic organisms that highlight the inspiration we can get from nature to improve ourselves as (social) human beings, as they constitute a living example of how quite different microorganisms working closely together are able to develop into a much successfull form of macroorganism. The vegetative body, known as the lichen thallus, is the result of a symbiotic association between an heterotrophic fungus (called mycobiont) and at least one unicellular (sometimes coenobial), photosynthetic partner (called photobiont) that can either be a green algae or a cyanobacteria (*e.g.* Nash 1996). Mycobiont and photobiont form such an intimate association, that once the lichen is formed it behaves as a stable and self-supporting individual, *i.e.* nutricionally independent and able to pass its specific characteristics to the descendants. In fact, the lichen thallus exhibits great morphological, physiological and adaptative originality when compared with the isolated components – naturally free-living in certain environments in the case of algae and cyanobacteria, or artificially cultivated in the case of the fungi (Fig. 2.1.1).

This association has been such an evolutionary success that lichens can be found in most terrestrial ecosystems of the world. Their biomass contribution is sometimes fairly small, but in certain biomes, such as the taiga, the tropical and temperate rainforests and the deserts, they contribute substantially to aboveground productive biomass and are particularly important for ecosystem functioning (Galloway 1996).

Wherever lichens proliferate, they often provide a colourful appearance to their substrate. The distinct colours of many lichens are due to the massive accumulation of diverse secondary metabolites, often called ‘lichen acids’ although not all of them are in fact acids. These are small but chemically complex substances belonging to five main groups according to their chemical structures (Huneck & Yoshimura 1996): depsides (*e.g.* atranorin, lecanoric acid, squamatic acid, gyrophoric acid), depsidones (*e.g.* fumarprotocetraric acid, norstictic acid, psoromic acid, salazinic acid, stictic acid), dibenzofuranes (*e.g.* usnic acid), pulvinic acid derivatives (*e.g.* rhizocarpic acid) and quinones (*e.g.* parietin). Lichens adopted diverse biosynthetic pathways to produce such chemical diversity: acetate-polymalonate, shikimic acid and mevalonic acid pathways. Substances found in lichens additionally include the photosynthetic products of the photobiont (sugar alcohols, mono- and polysaccharides, carotenoids and vitamins).

Fig. 2.1.1. The *Cladonia grayi* G. Merr. ex Sandst. mycobiont can be isolated in culture, a feature that not all lichens share. In pure culture on agar medium aposymbiotic lichen mycobionts form thallus-like colonies (A) with no morphological resemblance to the symbiotic phenotype of young *Cladonia grayi* growing on soil (B). Source: http://genome.jgi.doe.gov/Clagr2/Clagr2.home.html.

In general, the lichen thallus has a strict organization with different functional layers, typically including an upper cortex (usually containing only the mycobiont), an algal layer (containing the photobiont), a medullary layer (containing the mycobiont) and, sometimes, a lower cortex (see Fig. 6.1.2). A simplistic classification of lichens usually distinguishes three major lichen growth forms: the fruticose form (which is roughly circular in section, usually highly ramified, and attached to the substratum by a single holdfast); the foliose form (that comprises a series of radially arranged leaf-like lobes attached to the substratum directly through the lower cortex or by means of a more or less dense net of rhizines or tomentum); and the crustose form (lacking a lower cortex and tightly attached to the substratum, sometimes almost completely immersed on it). Lichen substances are often expressed differentially in these layers, and typical cortical substances can be distinguished from compounds usually found only in the medulla. This could be linked with their biological function: the cortical compounds are externally visible crystallized and non-crystallized pigments regarded as a kind of protective filter against UV radiation, while the compounds below the algal layers are usually colourless and may be involved in processes of substrate weathering. In addition to the differences within the lichen thallus (Edwards *et al.* 2005) or between species (*e.g.* Edwards *et al.* 1998), the presence of substances can also differ among individuals of the same species, according to geographic, altitudinal or microhabitat conditions (e. g. Edwards *et al.* 1995, Modenesi *et al.* 1998). Besides chemical composition, the internal structure of apothecia and perithecia, the types of spores, their size, the types of pycnidia, the shapes of soredia and isidia, among many others, have provided the characters for the delimitation of lichen species.

Although their fossil record is limited and fragmentary, the biogeography of lichens unquestionably points to their evolutionary antiquity, believed to be as old as 600 million years (Yuan *et al.* 2005). Algae, cyanobacteria and fungi do have ancient origins (Lücking 2009, Schirrmeister *et al.* 2011) and there is no reason to doubt that lichens were formed relatively soon after their appearance, especially since the symbiotic strategy apparently increased their ability to cope with extreme environments (Seaward 1996).

Since the mycobiont is usually unique of each symbiotic association and the dominant partner, lichens are traditionally classified as a life-form of fungi according to the rules of the International Code of Nomenclature for algae, fungi and plants adopted in July 2011 by the Eighteenth International Botanical Congress at Melbourne, Australia (McNeill *et al*. 2012). The lichen-forming habit is maintained by one-fifth of all fungi, the majority of which belonging to the Ascomycota. Lichenization is much rarer among the Basidiomycota, with only 50 lichen species known to date, while 200 species are currently assigned to Deuteromycota. So far almost 19.000 different lichen species have been described worldwide. A precise estimate for the global number of currently recognized lichens is hampered by several factors, including the unsettled species concepts within several groups and numerous unresolved taxonomic problems (Feuerer & Hawksworth 2007). Because many regions of the world have been poorly collected, an even higher estimate may well be more reasonable. Recent molecular work on lichens suggests that many presently defined species actually comprise several cryptic species that have not yet been distinguished with current phenotypic characters (Crespo & Pérez-Ortega 2009). Whereas cryptic species have been extensively investigated in some groups of lichens such as the family Parmeliaceae or Graphidaceae (*e.g.* Leavitt *et al.* 2013, Molina *et al.* 2011, Rivas Plata *et al.* 2013) in others, our current knowledge of cryptic diversity is still poorly known. Furthermore, it has been demonstrated that mutations are a frequent phenomenon increasing with age of the lichen, so that older thalli can in fact be a mosaic of genotypes.

2.2 Recent progress in the knowledge of lichen flora in mainland Portugal

The beginnings of Portuguese lichenology, dating back to the mid 17th century, were similar to those of other countries in the way that they closely followed the steps of botany. The earliest reference to Portuguese lichens is found in Gabriel Grisley's Viridarium Lusitanicum, first published in 1661 and including at least four lichen species (Grisley 1661). This work was later re-edited by Domenico Vandelli in 1789, adapting the pre-Linnaean names in Viridarium Lusitanicum to the Linnaean classification system (Vandelli 1789), and can be considered the first Portuguese flora since, according to Vandelli (*apud* Rómulo de Carvalho 1987), it was the only source of information about Portuguese plants at the time. Grisley's work needed a good time to be followed by that of Félix de Avellar Brotero (1744-1828), adding 74 lichen species to the previous list under the genus name *Lichen* in his Flora Lusitanica (Brotero 1804). By that time Erik Acharius (1757-1819) had just published the highly influential Methodus Lichenum which set the pattern that led to the emergence of lichenology as an independent discipline, but it was not until 1868 that the german lichenologist Ferdinand Christian Gustav Arnold (1828-1901) published the first work fully devoted to Portuguese lichens which he entitled Lichenes Lusitaniae (Arnold 1868), consisting of the description of 84 lichen species collected by Graf Reinhard zu Solms-Laubach (1801-1870) in the Algarve. Similar works were being produced almost simultaneously by Miguel Colmeiro (1816-1901) and Mariano del Amo y Mora (1809-1896) on the cryptogams hitherto known to occur in the Iberian Peninsula. Colmeiro (1867) included 86 species for the Portuguese lichen flora whereas an almost four times higher number of lichen species was there reported to occur in Spain.

The late 19th century marked the beginning of a golden period for the study of Portuguese lichens that progressed remarkably throughout the first half of the 20th century thanks to the contributions of **Friedrich Martin Josef Welwitsch** (Austria, 1806-1872) whose avid collections, partially identified by the finnish lichenologist **Wilhelm Nylander** (1822-1899) and the French abbot **Julien Harmand** (1844-1915), were kept at the Natural History Museum of South Kensington in London (Cabral 2009, Nylander 1888); **Júlio Augusto Henriques** (1838-1928) at the University of Coimbra, initially assisted by Nylander, on the lichens of Serra da Estrela, Gerês and Bussaco; **Isaac Newton** (1840-1906) at the Polytechnic Academy of Porto, also assisted by Nylander, mainly on the lichen flora of the north of the country; Nylander himself when already living in Paris, based on specimens sent to him by Newton (Nylander 1888); the Jesuit priest **Valério Aleixo Cordeiro** (1877-1940) at the College of S. Fiel in Setúbal, assisted by Harmand after some exchange of correspondence and specimens between the two clergyman (Harmand 1906); **António Xavier Pereira Coutinho** (1851-1939) at the Polytechnic School of Lisbon; and **Gonçalo Sampaio** (1865-1937) at the Polytechnic Academy of Porto. The latter acquired a solid grounding in lichenology under the guidance of some of the most renowned lichenologists of his time, including **Maurice Bouly de Lesdain** (France, 1869-1965), **Henri Olivier** (France, 1849-1923), **Adolf Hugo Magnusson** (Sweden, 1885-1964) and **Alexander Zahlbruckner** (Austria, 1860-1938) with whom he maintained solid correspondence. It was with Bouly de Lesdain that Sampaio established the most extensive specimen exchanges, though. In the period between 1916 and 1923 Sampaio published 14 contributions adding numerous records to the flora of Portugal and describing 142 new lichen species as well as the lichen genus *Carlosia* (Paz-Bermúdez *et al.* 2002). Among Sampaio's students was **Ricardo Jorge** (1858-1939), best-known in Portugal for his medical research, whose travellings around the country greatly contributed to the Portuguese lichen flora, under the guidance of Sampaio. By the end of this period the number of lichens known to occur in mainland Portugal had reached approximately 600 species.

In the mid 20th century Portugal finally saw the rise of the first important modern Iberian lichenologist and one of the most important lichenologists of his time, **Carlos das Neves Tavares** (1914-1972). Tavares published several contributions on the lichens of mainland Portugal and Macaronesia and embarked on the hard work of disentangling the taxonomy of many Mediterranean taxa. It should be noted that by that time lichenology in Mediterranean Europe was not at its best: the information and the herbaria for the study of Mediterranean lichens were spread and difficult to access, with Tavares in Portugal, Camillo Sbarbaro (1888-1967) in Italy and Roger-Guy Werner (1901-1977) in Morocco being perhaps the only three scientists working in this field (Llimona & Hladun 2001). Unfortunatly Tavares did not have the chance to train pupils and the study of Portuguese lichens in the late 20th century would have been completely abandoned if were not for the works led by foreigh researchers, including the British **Maurice Pugh Jones** (Carvalho & Jones 1997, Jones 1980, 1999) and the Dutch **André Aptroot** (Aptroot *et al.* 1992, Aptroot & van den Boom 1995) and **Pieter van den Boom** (van den Boom *et al.* 1990, van den Boom & Giralt 1996, van den Boom & Giralt 1999), just to name a few, during the eighties and the nineties. Their contributions were nevertheless scarce and fragmentary since they were based on sporadic visits to the country mostly during summer vacations.

In recent years, in parallel with the progress of lichenology in neighbouring Spain, the literature on Portuguese lichens has grown to a considerable extent, mainly owing to the contributions of **Palmira Carvalho** at the University of Lisbon (*e.g.* Carvalho 2009), Pieter van den Boom in the Netherlands (*e.g.* van den Boom 2003, 2005, 2006) and the Spanish lichenologist **Graciela Paz-Bermúdez** who stayed at the University of Porto between 2001 and 2004 (*e.g.* Paz-Bermúdez & Carballal 2005, Paz-Bermúdez & Elix 2004), followed most recently by the contributions resulting from the PhD of **Sandrina Rodrigues** at the University of Aveiro (Rodrigues *et al.* 2007, 2011a, 2011b).

Since 2001, the Spanish lichenologists Xavier Llimona and Nestor Hladun from the University of Barcelona have been undertaking the task of inputing all available information on the occurrence of Iberian lichens (and lichenicolous fungi) into a web-based checklist (Hladun & Llimona 2002-2007). Despite providing the most complete picture to date of the lichen flora in the Iberian Peninsula, this checklist is already lacking some information as further records have been published since it was last updated, in 2007. Besides, Portuguese and Spanish records are hardly discriminated from each other. In the period between 2007 and 2012, at least 90 lichen taxa have been reported for the first time in mainland Portugal (Culberson *et al.* 2011), of which 10 corresponded to the description of new species to science (Giralt *et al.* 2011, Paz-Bermúdez *et al.* 2009a, Rodrigues *et al.* 2011b, van den Boom 2010, van den Boom & Brand 2007, 2008, van de Boom & Giralt 2012), 9 were novelties for mainland Europe (Paz-Bermúdez & Giralt 2010, van den Boom & Giralt 2012) and 4 were new records for the Iberian Peninsula (Paz-Bermúdez *et al.* 2009b, Paz-Bermúdez & Giralt 2010, Rodrigues *et al.* 2007). According to the checklist by Feuerer (2012), the Portuguese lichen flora currently comprises 804 taxa in mainland territory. This value is merely indicative since it is based on few evaluated publications and several other taxa can be added to this list. A more detailed checklist of Portuguese lichens is planned for the near future by Palmira Carvalho, currently responsible for the curation of the lichen collections at the National Museum of Natural History in Lisbon.

Epiphytic lichens have been studied most extensively both because they present fewer taxonomic problems (Nimis 1991) and because of their link to biomonitoring projects (e. g. Rodrigues *et al.* 2007, 2011a, 2011b). Several recent papers dealing with the taxonomic treatment of a specific family or genus in the Iberian Peninsula end up finding new species among the previously overlooked collections of Portuguese herbaria (e. g. Boqueras & Llimona 2003, Burgaz 2010, Prieto *et al.* 2010).

Although Sampaio had planned to write a Lichen Flora of Portugal (Cabral 2009) he was not able to accomplish this task. In fact, still today there is no comprehensive source of information on Portuguese (or Iberian) lichens. In 1989, funded by the Spanish Government, the Spanish Lichenological Society undertook the ambitious project of an Iberian Lichen

Flora which resulted in 10 volumes on the taxonomic treatment of 39 genera published between 2003 and 2012. Unfortunately the project is currently stopped due to lack of funding.

2.3 A bird's eye view of open-air rock-art in the study area

Before the recognition of the Mazouco open-air rock-art (Freixo de Espada a Cinta, north-east Portugal) as contemporaneous of Palaeolithic representations in caves (Jorge *et al.* 1981) general thought was that rock-art of this period could not be preserved out of caves or rock-shelters (Aubry *et al.* 2010). Other open-air rock-art surfaces were later discovered in the Iberian Peninsula and southern France, and attributed to the Palaeolithic period including Siega Verde in Salamanca, Domingo Garcia in central Spain, Piedras Blancas in Almeria and Fornols-Haut in the eastern French Pyrenees (Bicho *et al.* 2007) the latter being the only open-air Palaeolithic rock-art site known to the north of the Pyrenees (Aubry *et al.* 2010). All of these were produced on schist surfaces of similar origin and mineralogical composition, belonging to the so-called schist-greywacke complex (see Chapter 4.4).

In Portugal, open-air rock-art dating from the Palaeolithic has also been invariably found on the same kind of schist outcrops (Baptista 1999) located along the slopes and banks of some of the main tributaries of River Douro: the Côa, the Sabor and, to a lesser extent, the Tua Rivers (Baptista 1999, 2001a, 2012); as well as of the two right margin tributaries of River Tagus: the Ocreza (Baptista 2001b) and the Zêzere (Baptista 2004). Open-air rock-art of the Côa, the Sabor and the Tua River Valleys is nowadays considered as part of a regional group in the Iberian Peninsula that forms some of the oldest and largest galleries in the world and also includes the sites of Mazouco, located only 40 km upstream along the Douro, near the border with Spain; and Siega Verde, on the left bank of the Águeda River, another tributary of the Douro, in Spain. In addition, it is said that many other engravings exist along the banks of River Douro, nowadays submerged by the water reservoirs of successive dams (Baptista 1999).

Although ongoing research may result in the identification of other sites, it is currently accepted that the Upper Douro region holds the world's largest concentration of open-air rock-art sites from this period along with important representations of rock-art dating from the Iron Age and Neolithic, Chalcolithic and the Bronze Age (Baptista 1999) in what is considered the longest rock-art cycle known in Western Europe (Baptista 2001b). Arguably the most well known are located in the Côa valley, internationally acknowledged since its inclusion in the UNESCO's World Heritage List back in December 2nd, 1998. Even though the first Upper Palaeolithic engravings in the Côa valley are likely to have been known much earlier, they were only revealed to the public by the end of 1994, followed by unprecedent controversy regarding their age and importance. The first published studies followed almost immediately (Rebanda 1995, Zilhão *et al*. 1997) and surveys are still in progress throughout the region. The Côa Valley Archaeological Park, named after that main river crossing the classified area, was created in 1996 to ensure the preservation of open-air rock-art within its natural context; to involve local community in heritage management and conservation; to develop education services for visitors; and to support scientific research at a national and international level. Traditionally, individual markings are called motifs and groups of motifs are known as panels. Agglomerative panel sequences are treated as archaeological sites. Such artificial division is kept for the convenience of people who study these sites but has become less popular among archaeologists as they are fully aware of its unlikely relevance for the art's creators. Even the word 'art' carries with it many modern preconceptions about the purpose of the engraved motifs that are not necessarily true - for an interesting discussion on the philosophy surrounding the definition of art in the context of Prehistoric to contemporary human manifestations see Fernandes (2012). The geographic and cenic space of the Côa long rupestrian cycles (between the Upper Palaeolithic and the present) extends well beyond the demarcated area, although the main sites are in fact located inside the Park, stretching in an arc from east of the Douro River Valley, inland to the Côa River. Up to the time of writing (August 2013), the Park comprised approximately 1000 rock outcrops featuring rock-art from all periods.

Despite the significant increase in the detection rate of open-air rock-art in recent years, open-air rock-art still constitutes only a small percentage of all rock-art assigned to the Palaeolithic (Aubry *et al.* 2010). Some researchers consider this proportion to reflect some Human preference in the Palaeolithic for cave and rock-sheltered settings. Others argue that current proportion is not representative of what existed in the past when open-air images were a common and permanent component of the Palaeolithic monumental art (Bahn 1995, Baptista & García Díez 2002), but were mostly destroyed because of their direct exposure to weathering (Zilhão *et al*. 1997).

2.4 Setting the scene for the study of lichen colonization of open-air rock-art

From the biological point of view engraved surfaces are like any other exposed rock surface available for the establishment of pioneer organisms. The colonization of rock starts early beneath the ground, owing to the action of chemoautotrophic organisms, such as particular types of bacteria, which are able to draw energy from inorganic substances in the absence of light. Once the rock is exposed, it becomes available for the establishment of air and waterborne propagules presumably deposited by gravity, and a series of photosynthetic organisms start to develop, including microscopic algae and cyanobacteria, which often constitute the major biomass on rock surfaces of both tropical and desert environments (Büdel 1999, Warren-Rhodes *et al.* 2007). These are followed by lichens, bryophytes, pterydophytes and finally vascular plants. Photosynthetic organisms use the substratum as a support to reach light, their primary source of energy. Heterotrophic organisms arrive later because they need to feed on the organic substances produced by the autotrophs. The process of orderly and predictable species additions and replacements over time (Lawrey 1991), in straight dependence on a variety of interacting physical, chemical and biological phenomena until a balance is established between the species assemblages and their environmental surroundings is known in community ecology as ecological succession.

Lichens are certainly the most conspicuous and important colonizers of rock-art surfaces in the Upper Douro region. Lichen colonization of engraved surfaces is obviously similar to that of newly exposed rock surfaces in nature and, as in any ecosystem, must be essentially conditioned by factors such as temperature, moisture, mineral nutrients and substratum characteristics, as well as by the adaptability of the species and the efficiency of their mode of reproduction. Ecological succession also takes place among the members of all the three major lichen growth forms (crustose, foliose and fruticose) and habits (saxicolous and terricolous) associated with the niches that start forming on the rock surface. Crustose lichens are usually dominant on smooth rock surfaces, especially at earlier stages of ecological succession. Growth of crustose lichens is often very slow – not more than a fraction of a millimeter per year – and indeterminate, *i.e.* they grow until they meet other lichens or some other kind of environmental obstacle (Jettestuen *et al.* 2010). Their fruiting bodies, however, protrude on the surface and, at least on calcareous rocks, might leave small holes as they fall, that constitute typical weak spots for all sorts of weathering actions. Many foliose species also grow radially but much more quicly than crustose lichens, with rates between 2 and 5 mm per year, which contributes to their superior competitive ability in face of crustose species (Woolhouse *et al.* 1985). Fruticose lichens are usually more abundant at late successional stages, growing upon other lichens and bryophytes as well. If some sort of disturbance occurs (*e.g.* cleaning in the context of rock-art conservation but it can be related to pollution, climate change or animal grazing, just to mention a few examples) a reversion in the community takes place, whose effects will depend on the type and extent of the disturbance and on the community resilience.

Under the influence of the action of colonizing organisms, and inherent to the process of ecological succession, is therefore a series of geophysical and geochemical processes leading to changes in the properties of the rock. When occuring on stonework and other man-made materials, such changes have been, since the late-60s, grouped under the terms ‘biodegradation’ or ‘biodeterioration’ (Hueck 1965) inheriting the terminology applied to some biologically induced processes of change in materials (such as wood decay brought about by fungi). The difference between the two concepts, if it exists, might be related with the scale of the processes involved in that change: the word ‘biodegradation’ seems to be applied more specifically to the transformation of substances or chemical compounds, while ‘biodeterioration’ is more commonly used for the changes produced in more complex and structured materials. Both can be distinguished from other fields of study such as corrosion and wear of materials, which relate to undesirable changes in the properties of those materials brought about by chemical, mechanical, and physical agents. However, these definitions somehow assume that biological, chemical and physical phenomena are independent, discrete processes while it is obvious that changes occurring on exposed rocks are ultimately either physical (mechanical) or chemical in nature, with biological phenomena being one of the main drivers of both processes (Hall *et al.* 2012).

A more accurate use of terminology in the context of open-air rock-art conservation should therefore also consider the difference between rock biodeterioration and biologically induced rock weathering. Deterioration, understood as the ‘undesirable changes in the properties of a material brought about by biological, chemical and physical influences that result in loss of quality or value’ (Vergès-Belmin 2008), seems to be more adequate to stones used in buildings or to those surfaces that hold some sort of human manifestation that is worth preserving. Weathering, ‘regarded as any chemical or mechanical process by which rocks exposed to the weather undergo changes in their properties’ (Vergès-Belmin 2008), is a more general concept that applies to all exposed surfaces. The debate is still on, since as Gorbushina & Krumbein (2005) remembered, it might be awkward using a term derived from weather, therefore totally environmental, when much of the physical and chemical processes operate under the direct or indirect control of biological colonization. The term ‘biodeterioration’ will be used throughout this text only to refer specifically to the changes occurring on stonework or, in this particular case, on the engraved surfaces, thus incorporating the idea of value loss.

Despite the recent demand among geomorphologists (Hall *et al.* 2012, Viles 2001) for an updated terminology and scalling-up perspective in the subject of rock weathering, the still consensual framework brilliantly discussed by Hall *et al.* (2012) continues to pay a good service for the time being and especially for the purpose of this research, given that many of the small scale processes that concern both ecologists and archaeologists or conservators are still unknown and far from being solved: we are still largely ignorant, for example, about the relative contributions of individual species and species assemblages to weathering, and about how their action is influenced by environmental change.

There are other available terms in literature frequently used as synonyms of rock weathering and rock deterioration, which we will refrain from using, such as decay, alteration, abrasion, denudation, among many others (Gorbushina & Krumbein 2005) whose subtle differences are more relevant in the field of stone conservation. However a note is left here that, given that these works are, by nature, interdisciplinary, agreement between experts is strongly needed to avoid confusion. Some terms that have emerged in the framework of plant ecology such as epilith, chasmolith and endolith have been adopted by geomorphologists, archaeologists and conservators for the purpose of describing biological colonization on exposed rocks, building stones and artifacts, and are becoming more usual in conservation science, whereas terms that used to be restricted to conservation science such as biofilm, biocrust or biorind (Viles 1995) are being introduced in the field of community ecology. The concept of bioreceptivity (Guillitte 1995) has appeared more recently to express the potential of a material to host living organisms, independently of the colonisation potential of the environment, and with it a whole new field of research on how, and to what extent, bioreceptivity interacts with climate and biota to produce weathering (and biodeterioration).

2.5 The current knowledge about the processes of lichen-induced rock weathering

Lichens have the ability to accumulate elements, such as nitrogen, phosphorus and sulphur, thereby increasing their potential bioavailability to successive life forms and creating suitable microenvironments for the growth of other organisms. Organic material accumulated underneath the lichen thallus, together with detached particles of the substratum and atmospherically-derived dusts trapped by thalli all contribute to the development of primitive soils. Such action on natural substrata is clearly of significance for pedogenesis but has been either exaggerated by many 18th and 19th century naturalists (Iskander & Syers 1971) or underestimated by those who considered it only in a geological time-scale (Seaward 1997). The subject has always been a controversial one, with every claim about the importance of lichens as agents of minerals and rock weathering being followed almost immediately by warnings about the danger of assuming such role on the basis of evidence obtained from lichen-covered rocks without information on the rates of weathering of comparable lichen-free rocks (Hawksworth & James 1974).

Such debates still occur and are far from being conclusive as current research is finally focusing on determining the weathering rates of different species on different lithotypes and the influence of climate and microclimatic factors in such rates, while controlling for the relative contribution of other weathering agents (Carter & Viles 2003, 2004, Scarciglia *et al.*

2012). Consensus is more or less limited to the facts that weathering will occur whether or not lichens are present, and that lichens are typically involved in both physical and chemical weathering mechanisms (Adamo & Violante 2000, Chen *et al.* 2000, Jones 1988, Jones & Wilson 1985, 1986, Saiz-Jimenez 1999, Seaward 1997, Viles & Pentecost 1994) even if they sometimes do so at such a slow rate that the opposite applies and rocks are in effect protected to a considerable extent against abiotic weathering agents by the presence of lichens (Ariño *et al.* 1995, Favero-Longo *et al.* 2009, Mottershead & Lucas 2000). Some authors have suggested the existence of a delicate balance between the biodeteriorative and the bioprotective actions of lichens on rock surfaces (Ariño *et al.* 1995, de la Rosa *et al.* 2013) whose thresholds probably hide the answer to the million dollar question in the field of conservation science: to clean or not to clean rock (or stone) surfaces.

2.5.1 Lichen-induced physical weathering

Fungal hyphae in contact with the rock surface tend to penetrate into the rock through pre-existing fissures or cracks and areas of least resistance. The depth of hyphal penetration is likely to depend on the characteristics of the substratum, the species growth form (crustose, foliose, fruticose, epilithic, endolithic), and life-strategy (early or late pioneer) (Sanders *et al*. 1994). Being rich in gelatinous and mucilaginous substances, their volume is strongly influenced by water content, thereby increasing the size of the fissures and cracks and decreasing the cohesion between minerals after several hydration-dehydration cycles (Seaward 1997). The same effect seems to be produced by the crystallisation of salts originated from the reactions of lichen acids with the minerals of the rock. Such internal tensions are accompanied by a superficial one, associated with the moisture-dependent expansion and contraction of the thallus subsequent to desiccation and rehydration, resulting in the lifting of the marginal part of the lichen and leading to the detachment of superficial mineral fragments (Fry 1924). The incorporation of mineral grains by lichen thalli is often observed (*e.g.* Ascaso *et al.* 1982, Edwards *et al.* 1998) but not restricted to thallus margins, occurring also at the thallus centre. Those grains do not necessarily derive from the activity of the lichen itself but may have been detached by other weathering agents. The swelling action of thallus, hyphae and lichen-derived salts is obviously worsened by freeze-thaw cycles verified in regions with extremely cold seasons. Few additional breakthroughs have been achieved in this subject since the reviews by Adamo & Violante (2000) and Chen *et al.* (2000). Exceptions may be the findings by Carter & Viles (2004), who described a differential effect of some species traits, namely colour and growth form, on the thermal response of limestone surfaces and therefore on their susceptibility to breakdown by temperature fluctuations; by Concha-Lozano *et al.* (2011), who discussed the possibility that lichen hyphae (and other sources of organic matter) act as barriers against water and pollutants by filling the superficial porous network of the rock; and by de la Rosa *et al.* (2013), who showed that susceptibility to weathering increases following hyphal decay caused by changes in microenvironmental conditions; after demonstrating the potential role of endolithic lichens in the development of some microscale morphologies (de la Rosa *et al.* 2012).

2.5.2 Lichen-induced chemical weathering

The chemical alterations lichens induce on rocks are thought to be due to the generation of respiratory carbon dioxide, excretion of oxalic acid and secretion of lichen acids.

Carbon dioxide, produced through respiration, changes into carbonic acid [H_2CO_3] in an aqueous environment. Although weak, carbonic acid can convert relatively insoluble salts such as calcium [$CaCO_3$] and magnesium [$MgCO_3$] carbonates, present in many rocks, into much more soluble bicarbonates such as calcium bicarbonate [$Ca(HCO_3)2$] and magnesium bicarbonate [$Mg(HCO_3)_2$] (Caneva *et al.* 1991). This process is common to all organisms, and in the case of lichens is not considered particularly relevant to rock weathering.

Lichen acids, on the contrary, are organic compounds secreted exclusively by lichens (Chapter 2.1) that have the ability to chelate metallic cations from the rock-forming minerals and therefore cause significative mineral dissolution. Naturally, lichens are not the only cause of metallic cation deplection from the minerals in rocks. Conclusions about the involvement of lichen activity in the deplection of elements like Al, Fe, K or Ca necessarily comes from the use of bare-rock controls.

Despite some experimental evidence for the formation of metal-organic complexes by the reaction of lichen acids with silicate minerals (Iskander & Syers 1972) most field-based reports on the neoformation of biogenic minerals directly attributed to lichen activity is surprisingly dominated by other kind of precipitations: those formed by the reaction of oxalic acid with major elements in rocks. Unlike lichen acids, oxalic acid is not an exclusive product of lichens. It is common in all living organisms as an intermediate of the Krebs cycle and is currently seen as the most active acid in the chelation of metallic cations. The type of oxalates found in lichens has been shown to be related with the chemical composition of the rock upon which they grow, thus revealing a certain dependence on the provision of the corresponding metallic cations from the substrate: magnesium oxalate dihydrate (glushinskite) has been found in lichens encrusting rocks of appropriate chemical composition such as serpentinite, mainly composed of magnesium silicate (Wilson *et al.* 1981); manganese oxalate in lichens growing on manganese rich rocks (Wilson & Jones 1984); copper oxalate in copper sulphide-bearing rocks (Purvis 1984); and calcium oxalate in lichens growing on limestone and marble, usually in two hydrated forms: monoclinic monohydrate (whewellite) and tetragonal dehydrate (weddellite).

Oxalates tend to accumulate at the lichen-rock interface, or inside the thallus, sometimes forming a crystalline layer on the upper surface of the thallus (Jackson 1981), that lichenologists have been used to call ‘pruina’. Such process is species-dependent as different lichen species have different oxalate production abilities and the occurrence of oxalate crystals in lichens is sometimes so constant within species or species groups that it became usefull as a differentiating character (an example can be seen in Fig. 6.1.4 but also Heidmarsson 1996). Intra-specific variation still occurs, however, and is probably environmental (Edwards *et al.* 1995, Wadsten & Moberg 1985).

Availability of metallic cations in rock does not necessarily imply, however, that the corresponding oxalates are formed, as proved by the exclusive accumulation of calcium oxalate in certain lichen thalli despite the significant presence of magnesium ions (Arocena *et al.* 2003, Edwards *et al.* 2003). Calcium oxalate is moreover formed on calcium-poor rocks such as granites (Prieto *et al.* 1997). Although species adapted to calcareous substrata have much higher calcium oxalate levels than species living on siliceous substrata, it seems that calcium oxalate production in lichens is not totally dependent on Ca uptake from the substrate as lichens are also able to use other sources such as airborne dust in the same manner producing calcium oxalate (de Oliveira 2002). As a result, calcium oxalate is by far the most common form of oxalate found in lichens. The monohydrate form of calcium oxalate is the major biodeterioration product at the lichen-rock interface and the ratio between this and the dihydrate form is also dependent on various environmental factors.

Chemical and physical weathering mechanisms are closely interrelated because biophysical weathering will increase the surface area/volume ratio of a rock or mineral grain, rendering it more susceptible to biochemical processes (Syers & Iskandar 1973).

2.6 The importance of studying the lichen-induced weathering of schist

The most immediate effect of lichen colonization on open-air rock-art, though, is of a chromatic nature: schist surfaces in the Upper Douro region are populated by extinct auroques, goats, dears and bird headed knights on horses (Luis 2008) whose noses are *Caloplaca* red, whose heads are *Acarospora* yellow and whose bodies are *Aspicilia* grey in a black and brown striped scenery. As Nimis (2001) would state, the result may not be that unpleasant, but is probably far from what the Palaeolithic artist had in mind (Fig. 2.6.1). Little is known about the identity and effects of the lichens that produce these colours (Romão 1999, Vänskä 2001) but since they are an important component of the biological colonization on the engraved surfaces, conservation plans which do not consider lichen-induced weathering are suspected to only partially achieve their role in rock-art conservation. Fortunately only a small part of the existing schist outcrops has been used as a support for rock-art and several other vertical schist surfaces are available, allowing for the direct assessment of biomechanical and biochemical weathering processes including the use of methods that require destructive sampling. Additionally the mineralogy and geochemistry have been previously characterised in detail allowing an accurate assessment of any changes which may have resulted from biological processes.

Fig. 2.6.1. Detail of auroque in a surface colonized by *Aspicilia contorta* subsp. *hoffmanniana* (creamy white) and *Caloplaca subsoluta* (orange). Rock 3 Canada do Inferno (Vila Nova de Foz Côa, Portugal). Photograph by Cristiana Vieira.

In Portugal, lichen-induced weathering has been traditionally approached under the general framework of stone pathologies (*e.g.* Begonha 1997, Magalhães 2000). Only in relatively recent times has the presence of lichens on monuments and archaeological sites been examined (Ascaso *et al.* 2002, Romão & Ratazzi 1996). Some studies have also investigated the damages to granite and limestone monuments caused by other types of organisms including biofilm-forming microorganisms (Miller 2009, Pereira de Oliveira *et al.* 2011) and vascular plants (Mouga 1997) following the great body of research having been carried out in other European countries. No study on the susceptibility of schist rocks to biological colonization are known to have been carried out to date despite the evidences of lichen activity on schist (Aghamiri *et al.* 2002, Cann 2012, Galvan *et al.* 1981, Fry 1924, 1927, Sanders *et al.* 1994) and the suggestion that metamorphic rocks undergo a more intense lichen weathering process than igneous rocks (Jones & Wilson 1985 *apud* Chen 2000) thanks to an easier water circulation through their fractures and the hygroscopic nature of clay mineral constituents (Rebelo & Cordeiro 1997).

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3 Scope, aims and thesis organization

This research project was concerned with the synergic influence of lichen species assemblages and environmental factors in the process of biodeterioration of rock-art on schist.

During the course of it, specific goals were accomplished:

- Data collection on the local-scale patterns of lichen diversity on exposed schist outcrops;
- Identification, through statistical ecology techniques, of useful lichen indicators for rock-art condition assessments;
- Characterization of schist in terms of bioreceptivity and related intrinsic properties;
- A preliminary evaluation of the role played by lichens in physical and chemical weathering of schist and rock-art deterioration.

This research project contributes to progress in Lichenology, by throwing some light into one of the least known lichen floras of Europe; and Community Ecology, by addressing the effect of major environmental constraints on the structure and composition of lichen species assemblages on vertical schist surfaces. The importance of this research also relates with its application in the field of Rock Conservation since rock conservation and restauration practices rely on previous knowledge about the identity, ecological requirements and biodeteriorative action of rock-dwelling organisms.

3.1 Conceptual structure and thesis outline

Assessing biologically induced rock weathering (and rock-art deterioration) requires integrated biological, environmental, and geophysico-chemical data. Therefore this thesis is divided into three levels of analysis:

Level 1 | Lichen diversity in the Upper Douro region (Chapter 6)

Taxonomical identification must always be performed since each species may be unique in its weathering ability. Chapter 6.1 is focused specifically on the taxonomic treatment of genus *Peltula* Nyl., proved to be the dominant among the colonizers of vertical schist surfaces in the study area and extremely rich in species, which motivated the preparation of the first identification key to the European species of the genus. A group of four lichen species previously unknown in the Iberian Peninsula, including one new to mainland Europe, are the subject of Chapter 6.2. A larger group composed of new and interesting lichens for the Portuguese flora, as well as some Iberian rarities, are treated in Chapter 6.3. Finally, a catalogue of all taxa identified in the study area is presented in Chapter 6.4 with some notes on their frequency and microhabitat preferences in the study area.

Level 2 | Ecological tools for rock-art condition assessment in the Upper Douro region (Chapter 7)

Studying the assemblage of species that colonize rock-art surfaces can help identify the factors that favour their growth and distribution, thus contributing to the improvement of rock-art conservation practices. Integrating ecological information about lichen species assemblages on rock surfaces is therefore fundamental for rock-art conservation. The case study in Chapter 7.1 specifyed the structure and composition of lichen assemblages on vertical schist surfaces. These assemblages may be easier assessed through functional groups or reduced subsets of representative species as defined in Chapter 7.2. An important subsidiary outcome of this level of analysis was the selection of the most appropriate species for the study of the specific role of lichens in rock weathering processes at the local scale.

Level 3 | Towards a comprehensive model of the mechanism of lichen-induced schist weathering (Chapter 8)

A key uncertainty in projections of the long-term effect of lichen growth and distribution on rock surfaces is how lichen-induced weathering varies under changing environmental conditions. Obviously, lichen colonization is a process depending on the capacity of the substrate to host different species. The physical properties and chemical composition of rock differ widely between lithotypes and even within the same lithotype. Different lithotypes are more or less bioreceptive and hence vulnerable to lichen colonization and is useful to know how bioreceptive the rock is in order to understand how it will contribute to biodeterioration. The experiment in Chapter 8.1 investigated the changes brought about by weathering in the susceptibility of schist to colonization by biofilm-forming cyanobacteria as a possible analogue for the susceptibility of schist to lichen colonization.

Lichen colonization is also imposed by microclimate conditions and these vary with surface orientation but their effect on lichen-induced weathering has rarely been discussed in detail and remains under-researched. A preliminary analysis of the physical and chemical changes produced by dominant lichen species on differently oriented schist surfaces was the focus of Chapter 8.2.

4 Characterization of the study area

4.1 Geographic delimitation

This project was first planned to respond to the demands of archaeologists in the Côa Valley Archaeological Park for an assessment of the potential effect of lichen growth on the deterioration of open-air rock-art on schist, in the framework of a broader conservation programme to be implemented in the Park (Fernandes 2004). Sampling strategy was therefore initially focused on the schistose areas of the Côa Valley (Fig. 4.1.1) where most rock-art sites are located. Soon it became clear that the neighbouring areas shared many characteristics in terms of climate, geology and landscape history, as well as lichenological interest. An empirical stratification of the region based on the known occurrence of rock-art of the same period (Bicho *et al.* 2007) and similar geology (Oliveira *et al.* 1992), biogeography (Franco 1994) and landscape (Andresen 2006) was used to define the limits of a broader study area that is roughly centred around the valley of River Douro and the valleys of its tributaries Côa, Sabor and Tua (Fig. 4.1.2). The westernmost part of the study area is approximately 200 km from the Atlantic coast and the easternmost areas border with Spain.

Although difficult to define in terms of administrative boundaries, the study area is focused on the hottest and driest region of the former province of Trás-os-Montes e Alto Douro (north-east Portugal), and includes several municipalities of the current Douro region (NUTS III) - Alijó, Carrazeda de Ansiães, Murça, Torre de Moncorvo and Vila Nova de Foz Côa - plus a few from Alto Trás-os-Montes region (NUTS III) - Alfândega da Fé, Mirandela, Mogadouro and Vila Flor. For simplicity reasons, throughout this document the study area will be referred to as the Upper Douro region, although it should not be confused with several other existing local administrative groupings with the same designation (see Table 4.1.1 at the end of this Chapter for a comparison between the study area and other conventional divisions of the territory that may also be translated into English as "Upper Douro").

The task of characterizing the patterns of lichen-induced rock-art deterioration is nevertheless limited in detail to specific areas inside the Côa Valley Archaeological Park where most rock-art is concentrated, as a means of identifying a general trend for the Upper Douro region without having to resort to a full analytical approach. Given the time constraints imposed by this project and the amount of work involved in assessing such an immense and diverse area, studying the impact of lichen colonization more precisely would have taken longer than the time available to complete it.

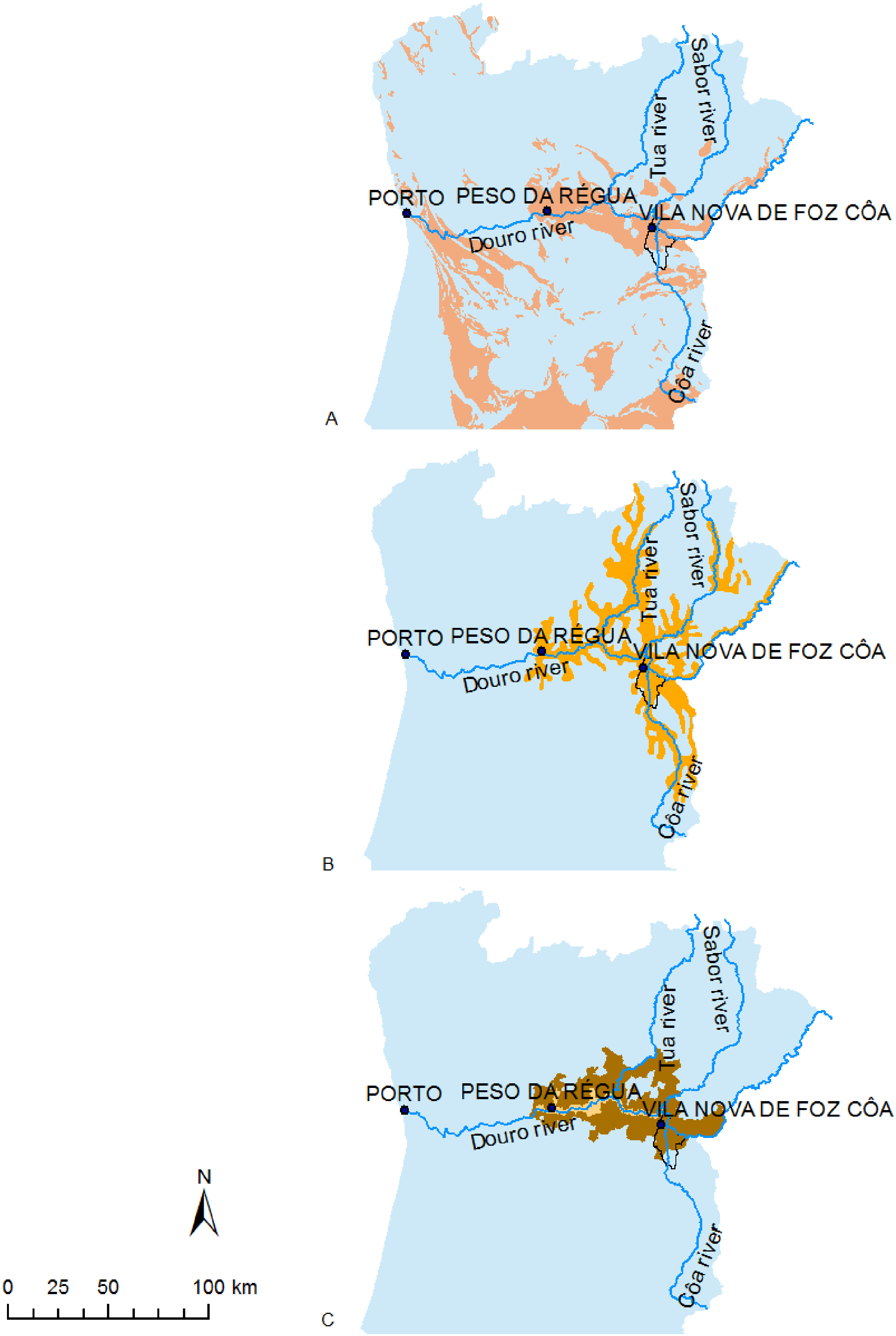

Fig. 4.1.1. The definition of the study area took into account several criteria which included the limits of the schist-greywacke complex (A), the limits of a major phytogeographical zone called “Terra Quente” (B), and the limits of the two Unesco World Heritage Sites in the region (C). Maps produced in ArcGIS 10.1 (ESRI, Redlands (CA) USA) by Cláudia Oliveira.

Table 4.1.1. The Upper Douro region in its various presentations. Municipalities that are included in the study area are signalled with an asterisk (*).

Name in English	Name in Portuguese	Description	Municipalities
Alto Douro Wine Region	Alto Douro Vinhateiro	Unesco World Heritage Site since 2001, occasionally referred to as Upper Douro wine region	Alijó*, Armamar, Carrazeda de Ansiães*, Lamego, Mesão Frio, Murça*, Peso da, Régua, Sabrosa, Santa Marta de Penaguião, São João da Pesqueira, Tabuaço, Torre de Moncorvo*, Vila Real, Vila Nova de Foz Côa*
Upper Douro	Douro Superior	The easternmost sub-region of the demarcated region of the Douro, with approximately 110 000 ha but only 9.3% of it occupied by vineyards	Carrazeda de Ansiães*, Freixo de Espada à Cinta, Torre de Moncorvo *, Vila Flor*, Vila Nova de Foz Côa*
Upper Douro	Douro Superior	Municipality Association	Freixo de Espada à Cinta, Miranda do Douro, Mogadouro*, Torre de Moncorvo*, Vila Nova de Foz Côa*
Upper Douro	Douro Superior	Development Association	Freixo de Espada à Cinta, Mogadouro*, Torre de Moncorvo*, Vila Nova de Foz Côa*
Upper Douro	Douro Superior	Study area	Alfândega da Fé*, Alijó*, Carrazeda de Ansiães*, Mirandela*, Mogadouro*, Murça*, Torre de Moncorvo*, Vila Flor*, Vila Nova de Foz Côa*

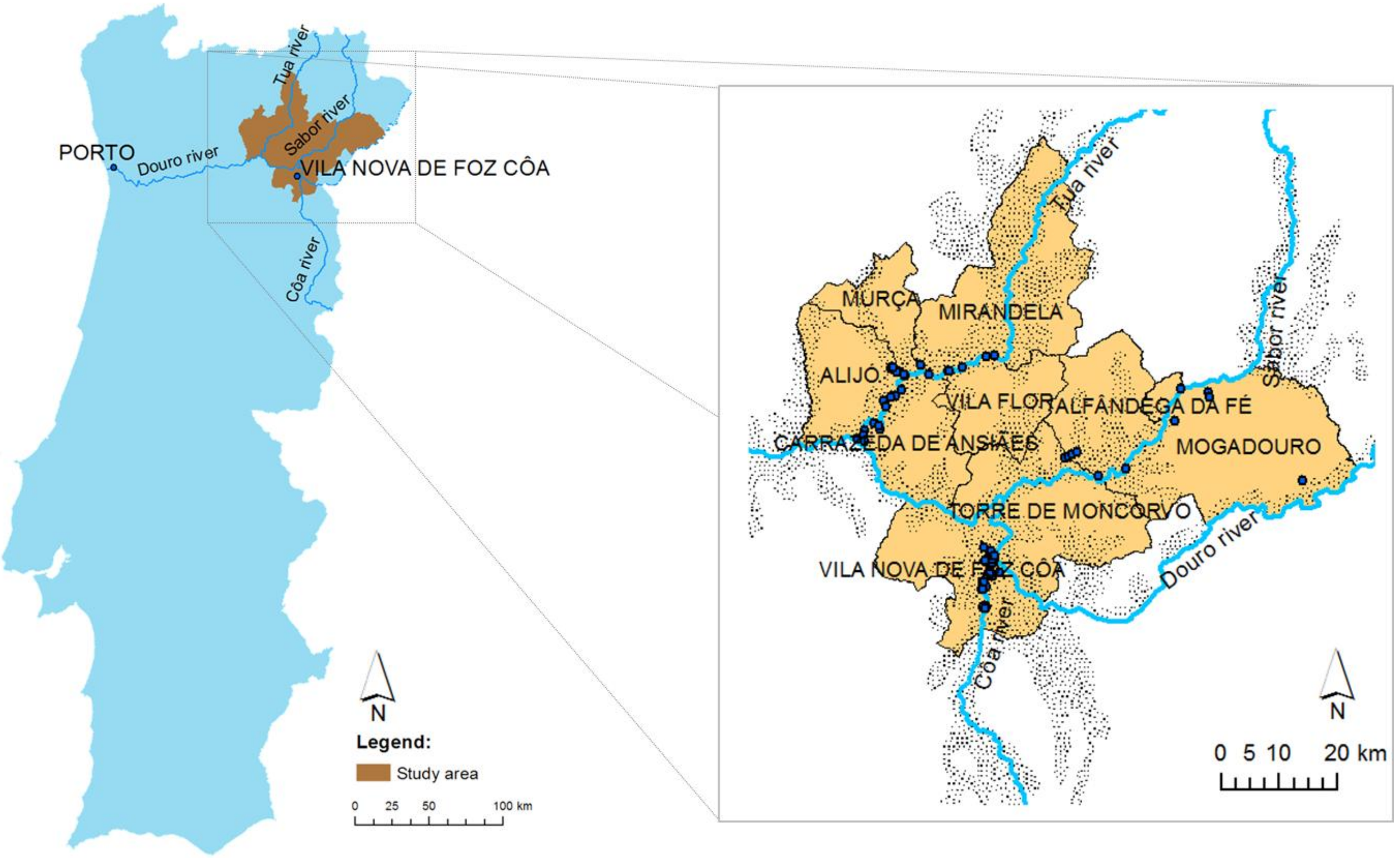

Fig. 4.1.2. Location of sampling sites (●) within the study area. The dotted area represents the domain of the "Terra Quente" phytogeographical zone. Map produced in ArcGIS 10.1 (ESRI, Redlands, California, USA) by Cláudia Oliveira.

4.2 Climate

The climate of the Upper Douro region is characterized by cold winters and extremely hot, dry summers. The singularity of this type of climate in northwestern Iberian Peninsula (Fig. 4.2.1) is highly influenced by the predominance of low elevations and by the mountains of Marão-Alvão (1415 m, to the east), Montemuro (1380 m to the south) and Montesinho (1500 m, to the north), which provide a moderate barrier effect against the penetration of moist winds from the Atlantic ocean (Föhen effect): when moist air hits the mountains, it is lifted up over those mountains and progressively dehydrated so that when it descends on the other side, it has little to no moisture, compresses and heats. The climatic continentality increases to the east under the influence of these dry and warm winds (Föhen winds).

Mean annual temperatures in the Upper Douro region range between 11.8 and 16.5 ºC (Jones 2013) but absolute values may easily reach up to 47 ºC (António Fernandes, pers. comm.). In fact, the region has been registering the highest mean annual temperatures in the country, specifically at the lower slopes of the Douro and its main tributaries in the region: Tua, Sabor and Côa (Jones 2013, Pereira *et al.* 2000). The highest mean maximum temperature of the warmest month registered in the study area reached 34.5 ºC at Vila Nova de Foz Côa, which ranks the summer of this municipality in the heart of the Côa Valley Archaeological Park as the hottest of the country. The continental character of the climate in this region is well reflected by its thermal contrasts and xerothermic indicators (Pereira *et al.* 2000). The highest thermal amplitude in the country, when considering its division into municipalities, has been verified in the neighbouring Pinhel (33.5 ºC), where a small southern portion of the Côa Valley Archaeological Park is located. Vila Nova de Foz Côa registered the highest value for the xerothermic indicator (4.5) and the highest thermal amplitude in the study area (33 ºC). The latter is the second highest thermal amplitude of the country together with that of Figueira de Castelo Rodrigo, located to the south-east of the study area.

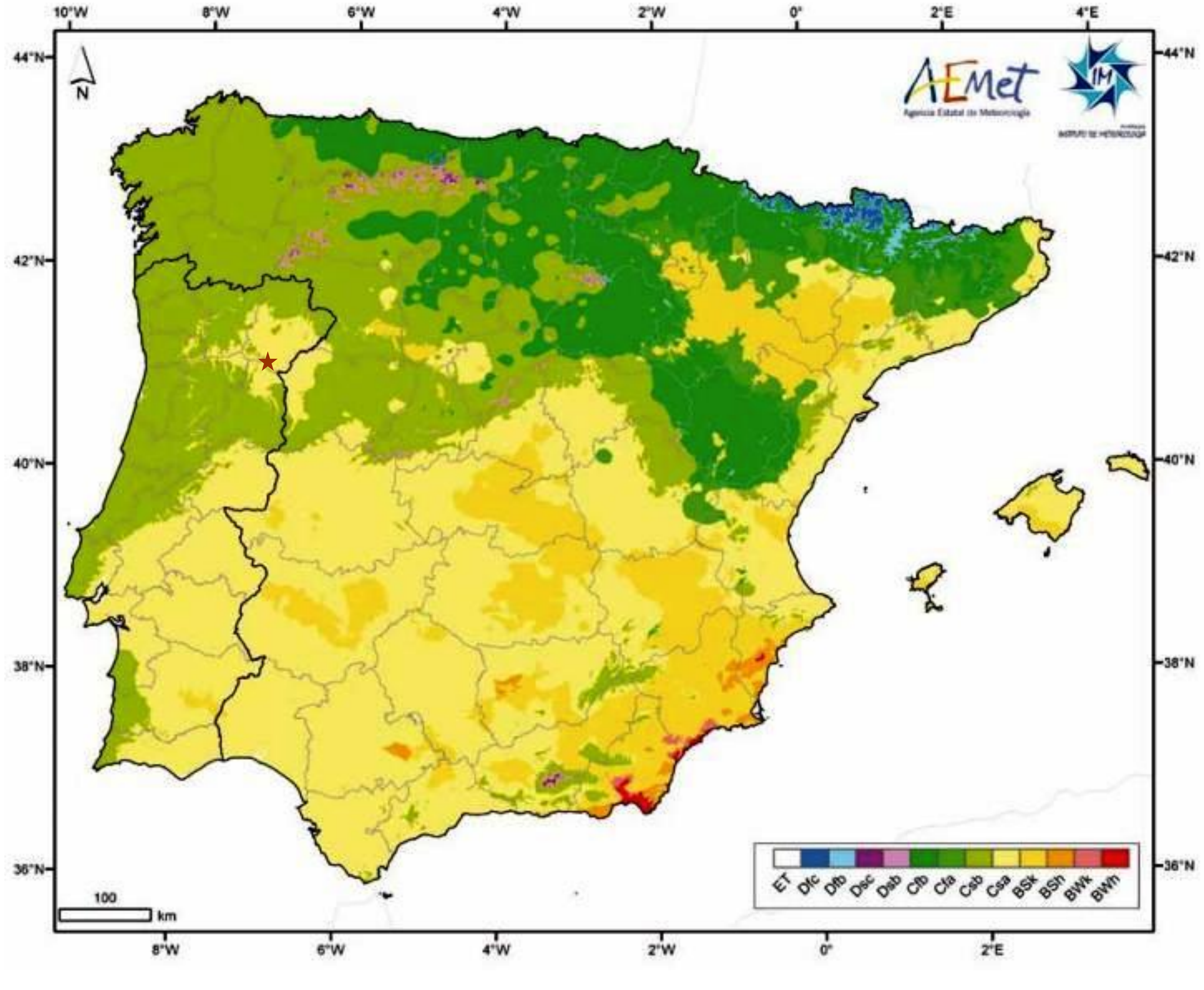

Fig. 4.2.1. Köppen-Geiger climate type map of the Iberian Peninsula. The location of the study area is signalled with a brown star. Csa: Mediterranean (hot summer); Csb: Mediterranean (cold summer); The meaning of the remaining symbols is provided by Peel *et al.* (2007). Source: http://www.aemet.es/documentos/es/conocermas/publicaciones/Atlas-climatologico/Atlas.pdf.

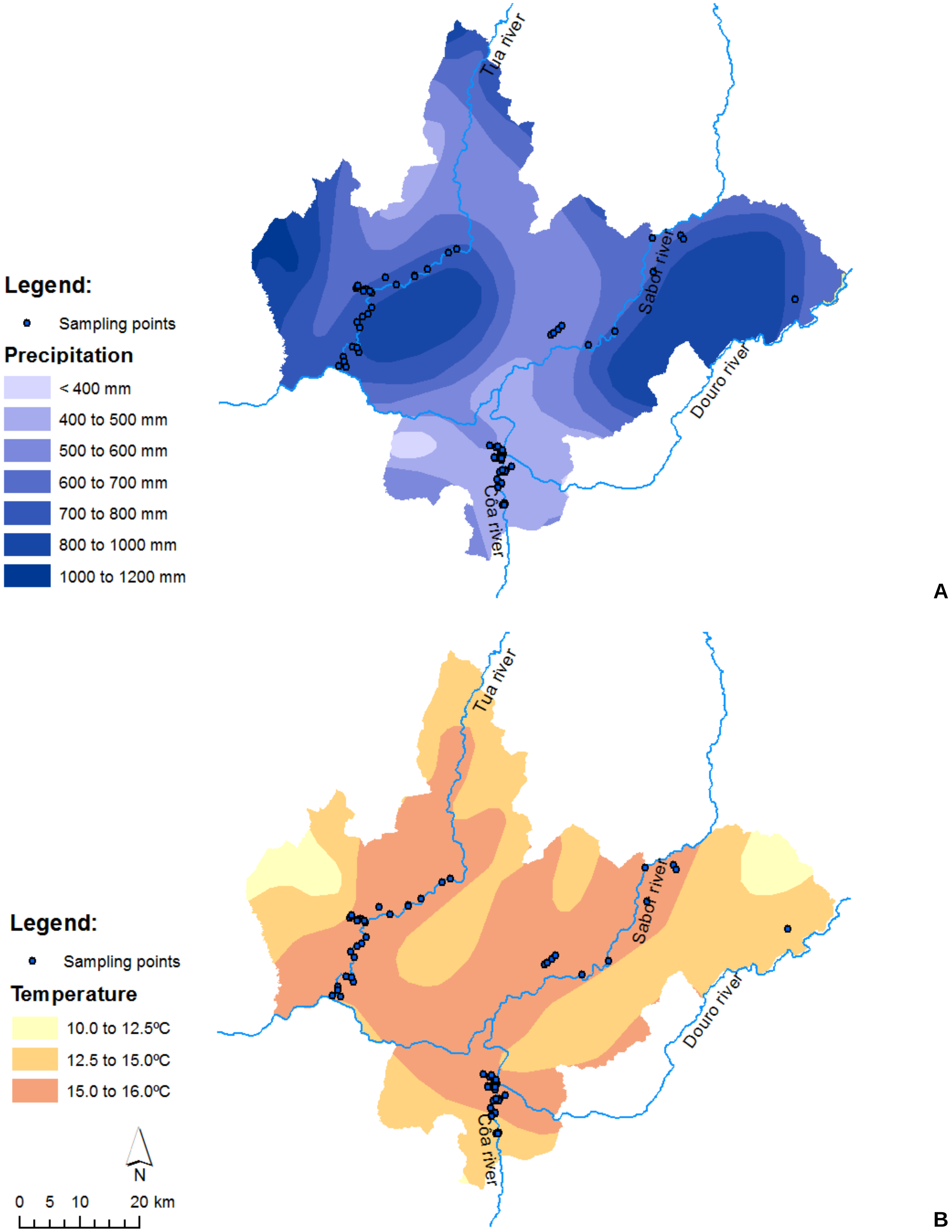

Fig. 4.2.2. Location of sampling sites within the boundaries of the study area, stratified by (A) total precipitation and (B) daily mean air temperature.

Rainfall is asymmetrically distributed but varies regularly throughout the year, with higher values in December and January (in some locations in March), and lower values in July or August. Mean annual precipitation ranges between 1000 mm (Mogadouro) and 400 mm (Vila Nova de Foz Côa), and general tendency is of a decrease from the west to the border with Spain (380 mm registered to the east of the study area in Barca d'Alva). Similar precipitation regimes (450 mm) are registered in the south of the Iberian Peninsula and along the Sahel, which is indicative of the climatic similarity between this region and other semi-arid regions in the world.

Slope aspect is of great importance in characterizing the climate of any region, and has gained renewed interest within the framework of rock-art conservation in the study area, as it allows a better understanding of the small-scale weathering dynamics produced in the main valleys. South facing slopes are under the influence of dry winds from the south and the east, with the north facing slopes exposed to cold and moist northerly winds, and less solar radiation. Air temperature is usually higher in places exposed to the south than in places exposed to the north (Pereira *et al*. 2000).

The hydrographical network is dense, a feature that distinguishes this region from similar areas in terms of climate to the south, and includes the lower Côa (with Ribeira de Piscos and Massueime as the main tributary streams), the lower Tua (with Tinhela as the main tributary river) and the lower Sabor (with small tributary streams being the Azibo, Juncaínhos and Vilariça) as well as the smaller tributaries of the Douro itself, such as Vale da Veiga and Aguiar. If was not for the influence of existing dams along the Douro (and very soon the lower Tua and lower Sabor), all these watercourses would be highly seasonal, drying almost completely in summer, as is typical of Mediterranean rivers.

4.3 Flora and vegetation

The Upper Douro region is bioclimatologically characterized by a mediterranean pluvistational oceanic bioclimate due to the strong seasonality of precipitations but moderate thermal contrasts. According to Aguiar (2001) the regional transition from the supra-mediterranean to the meso-mediterranean bioclimatic belts occurs roughly at 700 m, corresponding well to the transition from the predominance of marcescent Pyrenean oak (*Quercus pyrenaica* Willd.) forests to the predominance of evergreen sclerophyllous forests of class *Quercetea ilicis* Br.-Bl.. All sampling sites are situated well below that altitude, and the study area completely within the meso-mediterranean thermotype ranging from meso-mediterranean superior at higher elevations, roughly above 500 m, to meso-mediterranean inferior along the steep slopes of the valleys of Rivers Douro, Tua, Sabor and Côa, and including a topographic form of thermo-mediterranean at the rocky floodplains of the most deep, narrow river valleys (Costa *et al.* 1998). Ombrotype varies from sub-humid inferior (within meso-mediterranean superior) to dry or semi-arid (within meso-mediterranean inferior) (Aguiar 2001, Caldas *et al.* 2002). Biogeographically, the study area falls perfectly within the Ribaduriense subsector (Aguiar 2001) which belongs to the Lusitano-Duriense sector of the Carpetan-Leonese subprovince and Mediterranean-Iberoatlantic province as described by Aguiar (2001) based on the earlier works of Costa *et al.* (1998) and Rivas-Martínez & Loidi (1999).

The potential natural vegetation in the meso-mediterranean superior sub-humid belt consists of evergreen broadleaf sclerophyllous forests dominated by cork oak (*Quercus suber* L.) [*Physospermo cornubiensis-Quercetum suberis* subas. *quercetosum fagineae*] frequently associated with *Q. faginea* Lam*.* Cork oak forests are replaced by holm oak (*Quercus rotundifolia* Lam.) forests [*Genisto hystricis-Quercetum rotundifoliae* subas. *pistacietosum terebinthi ined*.] (Aguiar 2001) on steep slopes of the river valleys, typically occupying edaphoxerophilous positions. *Arbutus unedo* L., *Daphne gnidium* L., *Erica arborea* L., *Erica scoparia* L., *Phillyrea angustifolia* L. and *Viburnum tinus* L. constitute subserial shrubby or woody formations [*Erico scopariae-Arbutetum unedonis* Ortiz, Amigo & Izco 1991] within the climacic domain of cork oak. Broom communities consisting of *Cytisus striatus* (Hill) Rothm., *Cytisus multiflorus* (L'Hér.) Sweet and *Lavandula stoechas* L. subsp. *sampaiana* [*Lavandulo sampaianae-Cytisetum multiflori*] complete the cork oak shrub series in the study area.

The meso-mediterranean inferior belt is characterized by the occurrence of juniper (*Juniperus oxycedrus* L.) which is mixed with cork oak in the meso-mediterranean inferior dry superior [*Junipero oxycedri-Quercetum suberis* Rivas-Martínez *ined*.] (Aguiar 2001) or holm oak [*Junipero oxycedri-Quercetum rotundifoliae* Rivas-Martínez 1964] in the meso-mediterranean dry inferior belts. Subserial stages of mixed forests with juniper are characterized by the presence of *Retama sphaerocarpa* (L.) Boiss. and *Cytisus multiflorus* [*Cytiso multiflori-Retametum sphaerocarpae* Rivas-Martínez]. The most common dwarf shrub formations are composed by *Cistus ladanifer* L., *Euphorbia oxyphylla* Boiss., *Halimium umbellatum* (L.) Spach subsp*. viscosum* (Willk.) O. Bolòs et Vigo, *Helichrysum stoechas* (L.) Moench, *Lavandula stoechas* subsp. *sampaiana* and *Thymus mastichina* L. [*Euphorbio oxyphyllae-Cistetum ladaniferae* Aguiar & Penas *ined*.] (Aguiar 2001, Costa *et al.* 1998). The association *Lavandulo sampaioanae-Cistetum albidi* M. T. Santos may also occur in basiphilous situations.

Under the influence of a semi-arid ombrotype produced in the lower slopes of Rivers Sabor and Côa, as well as in a portion of the Douro between Barca d'Alva and Pocinho (Aguiar 2001, Caldas *et al.* 2002), forests are always absent and the potential vegetation is limited to shrubby formations of *Pistacio lentisci-Rhamnetalia alaterni* Rivas-Martínez [*Asparago albi-*

Rhamnion oleoidis Rivas Goday] characterized by the species *Asparagus albus* L. and *Rhamnus lycioides* L. subsp. *oleoides* (L.) Jahand. et Maire.

Fig. 4.3.1. The River Côa at Penascosa. Photograph by Cristiana Vieira.

Edaphohygrophyllous forests occur on the moist soils of riparian habitats and are highly diverse in terms of their composition, mainly of three types (Caldas *et al*. 2002): alder (*Alnus glutinosa* L.) forests on alluvial soils, when the watercourse is roughly permanent and stable and currents are weak; ash (*Fraxinus angustifolia* Vahl.) forests replacing alder forests along those portions of the valleys whose watercourses are more affected by the seasons (rainfall); mixed forests of *Salix atrocinerea* Brot., *Salix fragilis* L., *S*. *salviifolia* and *Populus nigra* L. in narrow valleys fed by small streams that completely dry in summer but maintain a certain level of moisture due to great depth and narrowness of the slopes. Where the valley is open, the watercourse is slower and small rocks and sand accumulate, the riparian forest is enriched or totally replaced by sage-leaf willow (*Salix salviifolia* Brot.) shrublands on sandy or *Flueggea tinctoria* (L.) G. L. Webster on rocky riverbeds.

The rocky floodplains of the deeper and narrower portions (topographic thermo-mediterranean belt) of the main right bank tributaries of the Douro, hold one of the most original plant formation in the study area, dominated by mosaic communities of *Buxus sempervirens* L. with pioneer communities of chamaephytes [*Erico arboreae-Buxetum sempervirentis* Aguiar, Esteves & Penas 1999] which is endemic of the study area (Aguiar 2001).

Wide crevices of schist outcrops located on the floodplains are, according to Aguiar (2001), dominated by the grass *Festuca duriotagana* Franco & Rocha Afonso [*Centaureo ornatae-Festucetum duriotaganae* Capelo, Aguiar, Penas, J. C. Costa & Lousã 1998]. Earth banks between schist outcrops along the slopes of these valleys are typically occupied by *Anarrhinum bellidifolium* (L.) Willd., *Antirrhinum graniticum* Rothm., *Galium teres* Merino, *Melica ciliata* subsp. *magnolii* (Gren. & Godron) Husnot, *Phagnalon saxatile* (L.) Cass., *Rumex induratus* Boiss & Reut. and *Silene coutinhoi* Rothm. & P. Silva [*Phagnalo saxatilis-Rumicetum indurati* Rivas-Martínez *ined*.] (Aguiar 2001). Chasmophytic vegetation on narrow crevices of these schist outcrops is mainly composed of small ferns such as *Asplenium billotii* F. W. Schultz and *Cheilanthes hispanica* Mett. [*Asplenio billotii-Cheilanthetum hispanicae* Rivas Goday].

4.4 Geological setting

The study area is geologically situated in a northwestern sector of the Hesperian or Iberian massif named Central Iberian Zone (CIZ). The geology of the CIZ has its main starting point in the northern Gondwana margin that resulted from the Cadomian orogeny in the late Neoproterozoic to the early Cambrian (650-530 Ma). This margin suffered several extensional events during Cambro-Ordovician and Silurian times and was strongly influenced by the Variscan (or Hercynian) orogeny, which was active between the Middle Devonian (about 390 Ma) and the Late Westphalian (about 310 Ma) when collision of Laurasia and Gondwana took place to form the supercontinent of Pangaea. A relatively continuous Variscan belt extending for more than 3000 km, from Morocco to northeastern Bohemia, has been inferred by numerous structural, petrological, geochemical, geophysical and geochronological studies (Charoy & Noronha 1999). While presenting only a slightly sinous trend in central Europe, the Variscan belt is highly distorted at its western end, giving rise to the so-called Ibero-Armorican arc (Fig. 4.4.1) which is the main Variscan structure in western Europe and is well preserved in the central Iberian terranes (Dias & Ribeiro 1995).

The CIZ is characterized by the occurrence of essentially two lithotypes: metasedimentary rocks resulting from the metamorphic episodes undergone by the sediments accumulated in the Cadomian and Variscan cycles; and granitoids of varying type and size that intruded the metassedimentary basement during the late Variscan orogeny (Noronha *et al.* 2012). Particular attention is here given to the metasediments since it is upon these lithotypes that most rock-art has been produced.

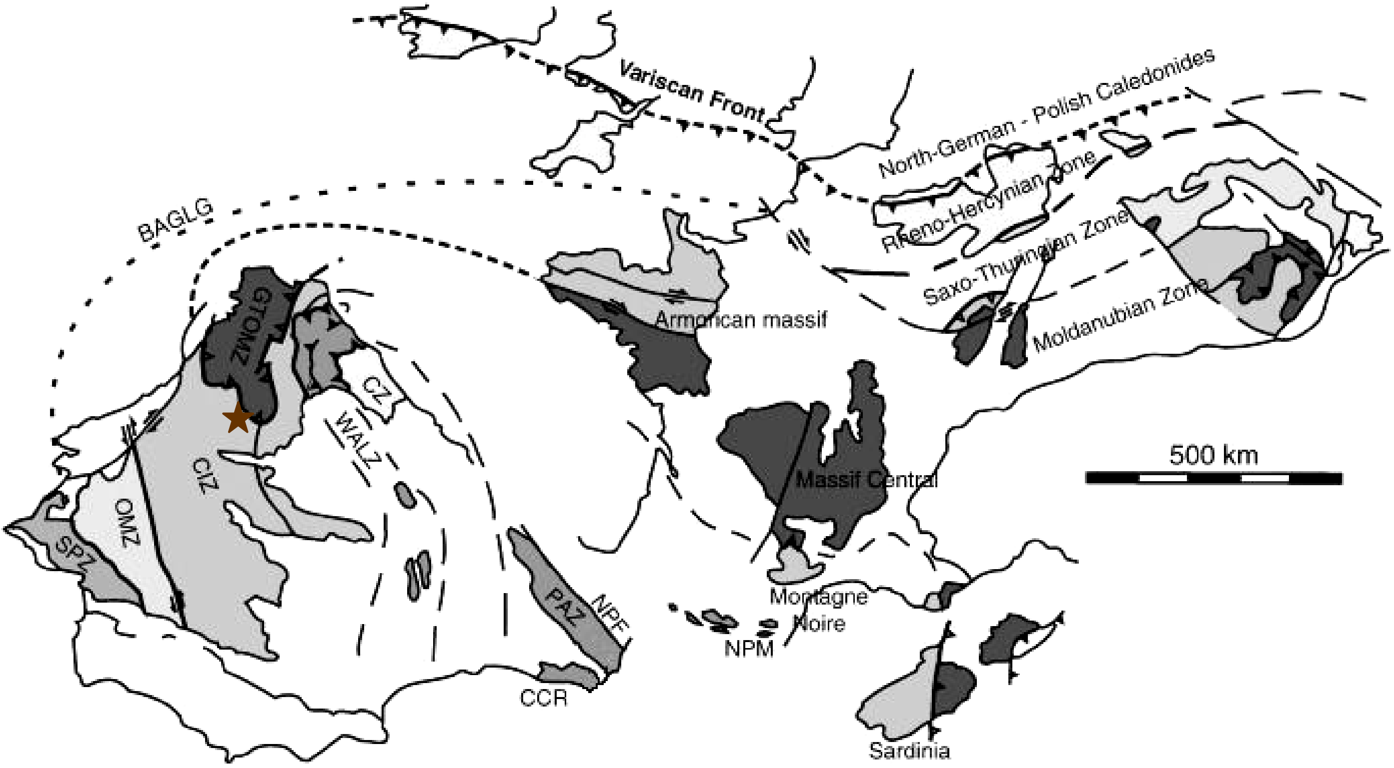

Fig. 4.4.1. Variscan massifs in southwestern and central Europe with the location of the study area (brown star). CZ: Cantabrian Zone; WALZ: West Asturian Leonese Zone; GTOMZ: Galicia-Trás-os-Montes Zone; CIZ: Central Iberian Zone; OMZ: Ossa Morena Zone; SPZ: South Portuguese Zone; PAZ: Pyrenean Axial Zone; CCR: Catalan Coastal Ranges; NPF: Northern Pyrenean Fault; NPM: North Pyrenean Massifs; BAGLG: Beja-Acebuches-Galicia-Lizard-Giesen oceanic suture Zone. Adapted from Robardet (2002).

Two main lithostratigraphic units are recognized within the autochthonous central Iberian metasediments: the Douro group (Sousa 1982) - a subdivision of the Dúrico-Beirão supergroup (formerly known as the schist-greywacke complex) - characterized by the occurrence of calc-turbidites (Moreira *et al.* 2010) in a sequence with an origin set in the Neoproterozoic and evolution through the Palaeozoic; and the Quartzitic group of Trás-os-Montes (Sá *et al.* 2005), consisting of a post-Cambrian (Ordovician to Silurian) sequence whose name derives from the predominant quartzitic nature of its formations. Both units are partially covered by Mesozoic to Cenozoic sediments (Fig. 4.4.2).

The Douro group is composed of four formations (Moreira *et al.* 2010), namely (from bottom to top): Bateiras formation (= Rio Pinhão formation), Ervedosa formation (= Pinhão formation), Desejosa formation and S. Domingos formation - that have several correspondences with formations described for other parts of the Variscan orogen in Western Europe. The Douro group is the most extensive lithostratigraphic unit in the Côa Valley

Archaeological Park but represented mainly by the Desejosa formation, and to a lesser extent also by the Rio Pinhão and Pinhão formations. Differences between these units within the Douro group are based on their sedimentary content, variation in the percentage of sand and clay minerals, and the nature and relative importance of turbidite deposits in the original sedimentary basement. The Desejosa formation is characterized by the predominance of finely banded phyllites, intercalated by metagreywackes, calc-silicates and less frequently metacalcareous rocks (Búrcio 2004; Sousa 1982). Banding in the phyllites is caused by alternating dark-coloured pelitic (mica-rich) and light-coloured psammitic (quartz-rich) layers (Aires *et al.* 2011). The Desejosa formation in the Côa Valley Archaeological Park extends from Vale de Cabrões, in the slopes of the Douro River Valley, as far as Quinta da Barca in the Côa River Valley. Here the territory of the Pinhão formation begins and tags along the course of the Côa River for just about 2 km. The Pinhão formation consists of a green-coloured, thin beding sequence containing alternating levels of green (chlorite-rich) phyllites and metagreywackes (Sousa 1982). The Rio Pinhão formation is the one that follows the Pinhão formation and is characterized by the predominance of metagreywackes, usually intercalated by thin layers of banded phyllites (Sousa 1982). It extends towards the south of the Park only to be interrupted by the granites of Tomadia and Meda, which mark the southern limit of the study area.

The rocks of the Quartzitic group of Trás-os-Montes crop out extensively outside the boundaries of the Park, encircling the parautochthonous and allochthonous terranes of the Galicia-Trás-os-Montes Zone (GTMZ) (Figs. 4.4.1-2). The litostratigraphical sequence within this group lies unconformably on the Cambrian Desejosa formation (Douro group) and starts with conglomerates and volcano–sedimentary rocks transitionally followed by the ubiquitous Marão formation (also known as the Armorican Quartzites formation), which is composed of quartzite and black slate beds with important iron deposits towards the top (da Silva & González Clavijo 2010; Sá *et al.* 2005). This formation also occurs within the boundaries of the Park at Monte de São Gabriel, breaking the lithological monotony of the Douro group in this area. Overlying the quartzites is the monotonous black slate sequence of Moncorvo formation (Dias da Silva & González Clavijo 2010). The higher units are represented by the sequence of marls and grey schists with quartzite intercalations of Chão do Amieiral formation or by a sandstone, quartzite and black slate sequence of Maceiras formation, underlined in both cases by a thick ironstone horizon (Sá *et al.* 2005). In some limited areas, foliated marbles intercalated with basic metavolcanites (greenschist and amphibolite) of the Santo Adrião formation lie unconformably over the black slates of the Moncorvo formation.

Great metamorphic and magmatic heterogeneity is added by the existence of the allochthonous crustal block of the Galicia-Trás-os-Montes Zone (GTMZ). The allochthonous of the GTMZ is in fact a complex series of stacking of exotic units thrusted over the Central

Iberian Zone (Moreira *et al.* 2010) due to the collisional event leading to the formation of Pangaea during the Variscan orogeny. It is nowadays crossed by the valleys of Rivers Sabor and Tua and defines the northern limit of the study area (Fig. 4.4.2).

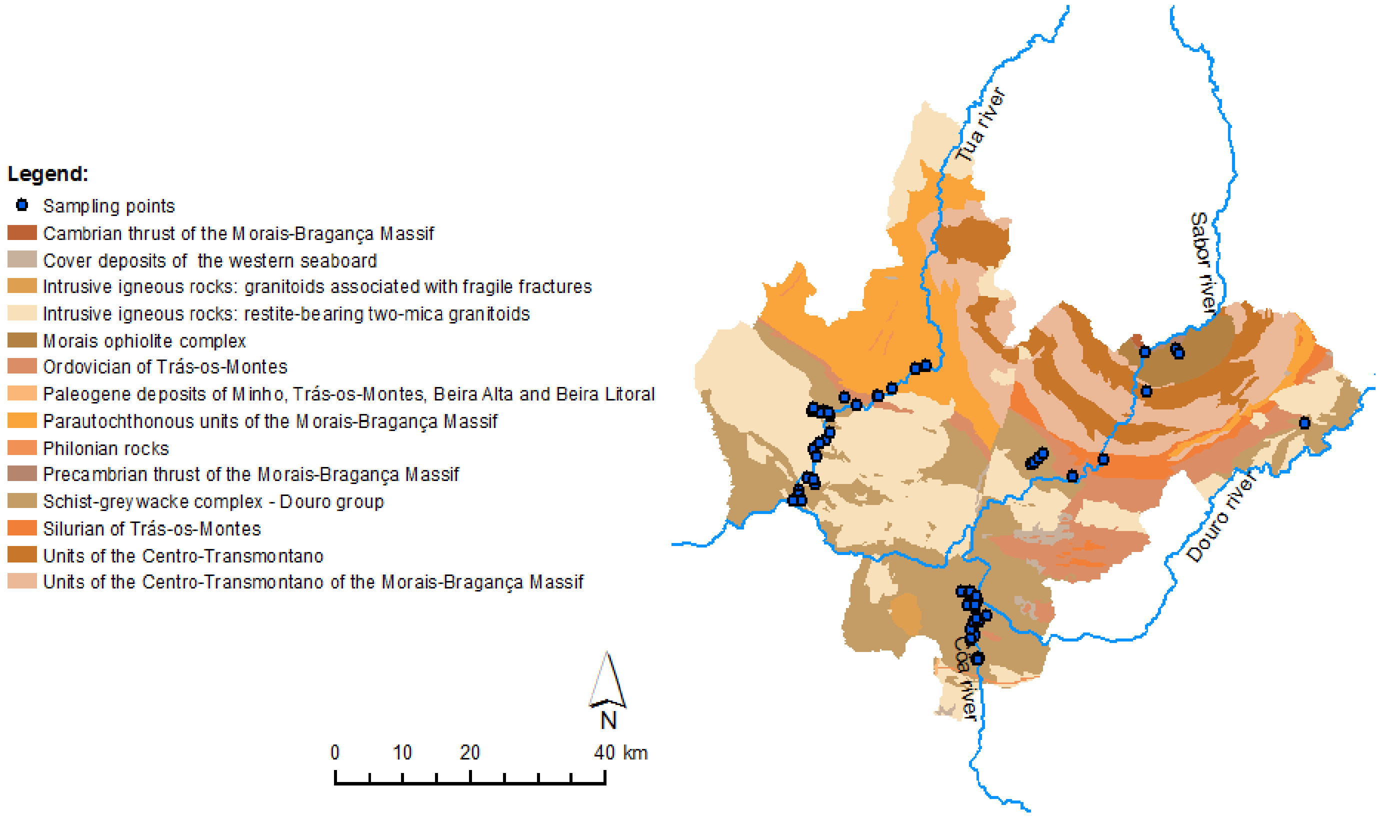

Fig. 4.4.2. Geological background of the study area with location of sampling sites. Map produced in ArcGIS 10.1 (ESRI, Redlands (CA), USA) by Cláudia Oliveira.

The traces of the Variscan orogeny are also present in the tectonic structure of both the autochthonous and the allochthonous terranes, owing to three main episodes of Variscan deformation: D1, D2 and D3 (Noronha *et al.* 2012). Most structures in the upper levels of the autochthonous, including the Poio and Vila Nova de Foz Côa synclines, are the result of the first phase of Variscan deformation (D1), of probable Upper Devonian age (Mateus & Barriga 1991), and consist of a dense network of subvertical folds oriented W-E to WNW-ESE, following the typical pattern of the southern branch of the Ibero-Armorican virgation. Cleavage (S1) is axial planar to the folds and perpendicular to the stratification plane (S0) in the synforms, but penetrative towards the antiforms, becoming parallel to S0 and producing true schistosity (Búrcio 2004). Penetrative bedding-parallel cleavage is here usually associated with the occurrence of extensive strike-slip faults roughly NW-SE oriented, *i.e.* parallel to the axial plane of the D1 folds, caused by syn-D1 shearing forces (Fig. 4.4.3). The second phase of Variscan deformation (D2), with an age ranging from the upper Devonian to Namurian, is a localized episode related with the formation of shear zones associated with the NW-SE thrust stacking of the allochthonous units, and reactivation of D1 shear zones in some sections of the autochthonous (Mateus & Barriga 1991, Noronha *et al.* 2012). First generation folds (D1) were transposed by D2 near the thrusts giving rise to a regional schistosity S2 that is mainly observed in allochthonous complexes and often transposes S1. Second generation (D_2) folds are well developed in the allochthonous and sometimes in the parautochthonous overlying the autochthonous sequence but are poorly developed in the autochthonous sequence itself (Noronha *et al.* 2012). The third phase of Variscan deformation (D3), of Upper Carboniferous age, covered the autochthonous, allochthonous and parautochthonous and is responsible for the generation of tight and upright folds oriented WNW-ESE, refolding the earlier structures, and of two sets of strike-slip shear zones (ENE-WSW sinistral, and NW-SE dextral) (Mateus & Barriga 1991). Cleavage (S3) ends up being perpendicular to S0 and S1 in the antiform and parallel to S0 in the synform. In late Variscan times the basement was strongly fractured by the intrusion of granitoids, leading to the development of a profuse set of faults whose geometry was locally constrained by D3 shear zones, and affected all the Variscan structures in the CIZ and GTMZ. A reactivation of all the more ancient crustal lineaments occurred during the Alpine orogeny with sinistral strike-slip.

The most significant fault system currently extends in the NNE-SSW direction, perpendicular to D1 structures, associated with the late Variscan orogeny (The Vilariça fault, passing 1 km from Vila Nova de Foz Côa takes this direction), conjugated with a NNW-SSE one, possibly associated with the syn-D1 shear corridors, which are sometimes filled by veins of basic and calc-silicate rocks of variable thickness (Ribeiro 2001).

4.5 Geomorphology and landscape

The landscape of the Upper Douro region is strongly determined by the Douro drainage basin, the widest in the Iberian Peninsula. When the Douro reaches Portugal it has already crossed almost 600 km of Spanish territory, including 150 km of the Iberian Meseta, with important contributions from the Spanish Rivers Pisuerga to the north and Tormes to the south. For about 120 km, the course of the Douro changes from the usual E-W to a NE-SW direction, influenced by the main orientation of a Tardi-Variscan fault (see previous Chapter), and forms the border between the two countries along this way. The fluvial canyons and steep cliffs in this portion of the Douro Valley are important elements of the regional landscape, alternating between the Variscan granites and the metasedimentary rocks of interest to this study. Occasionally, the course of the Douro is under the control of NW-SE, N-S and E-W oriented faults (Alves *et al.* 2002), which causes the deeply embanked meanders for which the Douro River Valley is locally well-known. Near Barca d'Alva the

Douro returns to its normal E-W course, enjoying an easy way through the metasedimentary basement (Girão 1960) into Portuguese territory, towards the study area.

The course of the Douro divides the study area into two asymmetrical northern and southern flanks since the Ribaduriense sector (Chapter 4.3) is considerably more extensive at its major right bank tributaries, Sabor and Tua, than at its major left bank tributary, the Côa. Additional asymmetry across the study area is either due to different amplitudes of block displacement along the major tectonic faults or to the lithological nature of the substrate.

At the mouth of River Sabor into the Douro, geomorphology is conditioned by the NNE-SSW oriented Vilariça strike-slip fault, clearly responsible for an approximately 6.5 km long trench of the Douro and Sabor Rivers to the north of the Côa Valley Archaeological Park. This fault, of horizontal displacement estimated at 5.5 km, divides the study area into a western block, lifted approximately 300 to 400 m after the Vilafranquian (Ribeiro, 1991) and nowadays extremely eroded; and an eastern block dominated by the Côa Valley. The courses of the Côa and the Tua take advantage of similarly NE-SW oriented faults.

Remains of the Iberian Meseta can be seen to the north-east and north-west limits of the study area, at the Miranda and Carrazeda plateaus, respectively. However, as schists are less resistant to erosion than granites or quartzites, most part of the metasedimentary basement has been deeply incised by these rivers and their dense network of tributary streams (Aubry *et al.* 2012). The higher plateau areas are excluded from this study and besides schistose environments, only the residual ridges of Armorican quartzite and occasional granitic outcrops were considered, at the border of the schist synclines.

A great part of the study area is therefore hilly terrain rising from almost 100 m to slightly bellow 600 m, mostly occupied by vineyard or olive and almond tree plantations since prior to the expansion of the Roman Empire, which add to the uniqueness of the landscape in the region. Natural vegetation is extremely modified by human activities (agriculture, pasture) and by the effects of fire directly or indirectly associated with these practices. The natural forests are reduced to small fragments between a mosaic of shrub, meadow and grassland communities, especially around some massive outcrops at higher locations, probably kept to provide shelter for grazing animals against the extreme summer heat. Regeneration of spontaneous vegetation has been favoured in a few places by the abandonment of vineyards during the *Phylloxera* epidemic at the end of the 19th century.

Occasionally the Sabor, the Tua and the Côa River Valleys widen into small alluvial and colluvial plains only to become narrow again until their confluence with the Douro. The surface of the steeper slopes is mainly composed of countless schist outcrops (Figs. 4.5.1 and 7.1.1) that have resisted weathering and human intervention.

Fig. 4.5.1. Schist outcrops (on the left) are a distinctive feature of the landscape in certain areas of the Upper Douro region. They are blocks of harder rock that remain after softer rock has been weathered away and, in the case of the Upper Douro region, where slopes were not used for vineyard or olive and almond tree plantations. Photograph by Cristiana Vieira.

The formation of schist outcrops in the study area is also tectonically controlled through the ocurrence of a dense system of parallel fractures (Fig. 4.5.2) and corresponding joints, the most significant of which are vertical to subvertical and NE-SW oriented. These joints frequently reach more than 10 cm and are therefore filled with quartz, calcite and pirite, or clay material (Búrcio 2004). In the vast majority of cases, the engravings were produced not on schistosity or bedding planes of schist outcrops, but precisely on the preserved and naturally exposed surfaces of these joints (Fernandes & Delgado 2008, Rebelo & Cordeiro 1997) after splitting and gravity-induced block displacement along the slopes of the valleys. Another characteristic form of block displacement affecting the stability of schist outcrops in the study area is named toppling (Fernandes & Delgado 2008) and consists of a progressive, horizontal advance of the upper layers of the outcrops, parallel to the schistosity plane.

The resulting surfaces bear numerous cracks and crevices of different sizes, some widespread splintering and alveolization, besides carbonate efflorescences and intense oxidation (Fernandes 2012). According to Rebelo & Cordeiro (1997) heavily weathered surfaces must have undergone multiple freeze-thaw cycles during the cold period of the late glacial maximum (approximately 13000 to 10000 yBP) whereas smoother and reasonably well preserved surfaces, have most probably been covered by a protective, impermeabilizing clay coating from periglacial colluvial deposits, and lately uncovered thanks to the progressive erosion of those deposits.

Fig. 4.5.2. The intense parallel fracturing/jointing of schist outcrops in the Côa Valley progressively exposed thousands of fracture joint planes as pieces of a primitive blackboard on which Palaeolithic (and modern) engravings were made. Photograph by Cristiana Vieira.

4.6 Mineralogy of unweathered schist

In order to assess the impact of lichen colonization on rock-art, it is firstly necessary to understand the character of the unweathered rock type (or lithotype). As previously stated (Chapter 4.1) the main focus of this study was the Côa Valley Archaeological Park, where the highest concentration of engraved surfaces is found. The dominant lithotype is the Desejosa schist but some of the most emblematic rock-art sites, such as Penascosa and Quinta da Barca, are located in the domain of the Pinhão formation. The mineralogy of the unweathered schist of the Desejosa formation has been described by people such as Aires *et al.* (2011), Noronha *el al*. (2010, 2012) and Sousa (1982) based on X-ray difractometry and thin section observations under the petrographic microscope. In the course of this study another mineralogical characterization had to be carried out on the unweathered schist in order to control for the effect of lichen colonization (see Chapter 8). Nevertheless the following data was used as guidance.

Unless otherwise stated, the term schist will be taken in a broad sense throughout this text to include several types of metasedimentary rocks, which are schistose or exhibit schistosity, *i.e.* well-marked foliation roughly parallel to the stratification. By this definition schist is a broad term, and slates and phyllites are also types of schists. 'True' schists are medium-grade metasedimentary rocks. The studied 'schist' is in fact a low-grade metamorphosed (greenshist facies) calcareous phyllite or metaquartzwacke, depending on the degree of contribution of the psammitic component. Phyllites are mainly composed of quartz, sericite and/or muscovite, chlorite and biotite minerals, with variable amounts of plagioclase feldspars depending of the psammitic contribution showing a lepidoblastic texture (quartz phyllites) and thin granulometry (Sousa 1982). Chlorite occurs in lenticular porphyroblasts parallel oriented to the first-generation cleavage S1 (Sousa 1982). Plagioclase feldspars are mostly albite, with some having been partially replaced by sericite, and less frequently anorthite (Aires *et al.* 2011, Sousa 1982). Sericite gives the characteristic sheen to phyllite and is a common alteration mineral of plagioclase feldspars. Muscovite can also be a secondary mineral resulting from the alteration of feldspar. The metagreywackes are mainly feldspathic with inclusions of other minerals such as quartz and albite. The matrix is composed by quartz, chlorite, muscovite and very often calcite (Sousa 1982). Calcite is in fact usually present in the matrix of all formations of the Douro group (Aires *et al.* 2011, Sousa 1982) – the reason why these rocks are sometimes refered to as calc-turbidites - and Desejosa is no exception. Hand samples can contain enough calcite crystals to be reactive to acids (Sousa 1982). Accessory minerals found in the matrix of both phyllites and metaquartzwackes are illite, kaolinite, pyrite, montmorillonite, graphite, turmaline, zircon, apatite, epidote, hematite, leucoxene and some alkali feldspars, such as microcline and orthoclase (Aires *et al.* 2011, Gomes & Almeida 2003, Solicel 2003, Sousa 1982). Illite occurs as an alteration product of muscovite and feldspar or it may be a component of sericite. Magnetite and, more sporadically, pyrite crystals are present in both the psamitic and pelitic levels as accessory constituents (Sousa 1982, Noronha *el al*. 2010) in the schist of the Pinhão formation (Fernandes *et al.* 2006). Biotite is scarce and chlorite more abundant at this formation.

4.7 References

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5 Methodological notes

5.1 Lichen sampling

The gathering of information about the lichen diversity on the engraved schist surfaces in the Upper Douro region occurred in two separate phases: the first one was dedicated to the collection of as many taxa as possible in all microhabitats of the studied schist outcrops, and took more than four years as the lichen flora of this region was almost completely unknown, except for the somewhat short list by Romão (1999); the second was specifically focused on the inventory of lichen assemblages on vertical schist surfaces of the same kind as those used for the engravings (Table 5.1.1). Such inventories were necessary for the case studies in Chapters 7.1 and 7.2 but could only be accomplished after sufficient knowledge was acquired about the identity of the specimens to ensure that most species could be reliably identified in the field. Nevertheless, dubious specimens were always collected for later identification in the laboratory.

Table 5.1.1. Main characteristics of sampling sites. Those used for lichen inventories are signalled with an asterisk (*)

Code	Main site	River valley	Latitude	Longitude	Altitude (m)	Aspect	Slope (%)
Açude15	Ribeiro Juncaínhos	Sabor	41.351895	-6.802354	473	North-west	7
AF	Alto da Fraga	Sabor	41.405931	-6.747802	580	West	48
*AHFT5	Ponte do Tinhela	Tua	41.327954	-7.366301	204	North	20
AHFT6	Vale do Moinho	Tua	41.335388	-7.391351	200	East	32
AHFT7	Praia Fluvial	Tua	41.242640	-7.392989	136	North	30
AHFT8	Vilarinho das Azenhas	Tua	41.384114	-7.208999	206	North-west	13
AHFT9	Ponte de Abreiro	Tua	41.349237	-7.277102	203	West	15
AHFT10	Codeçais	Tua	41.337541	-7.314484	296	North	52
AHFT11	Caldas do Carlão	Tua	41.330483	-7.373690	200	North	6
AHFT12	Foz do Tinhela	Tua	41.325385	-7.363223	181	South-east	12
AHFT13	Rio Tinhela (praia fluvial)	Tua	41.329493	-7.378798	225	North	21
AHFT14	Quinta da Ribeira	Tua	41.228816	-7.419916	132	East	5
AHFT15	Castanheiro do Norte	Tua	41.236083	-7.389506	378	North-east	46
AHFT17	Amieiro	Tua	41.280652	-7.393656	153	East	21
AHFT18	S. Lourenço	Tua	41.293145	-7.371809	188	North-west	18
AHFT19	S. Lourenço (jusante)	Tua	41.289890	-7.381107	156	North-west	22
AHFT21	Amieiro (jusante)	Tua	41.272311	-7.386736	186	West	18
AHFT22	Longra	Tua	41.359153	-7.252246	219	East	31
*AHFT23	Serra de Valverde	Tua	41.389210	-7.192760	219	South	6
AHFT24	Alto das Eirinhas	Tua	41.347916	-7.335425	260	South-east	32
AHFT25	Louzedo	Tua	41.328097	-7.379904	230	North	50
AHFT26	Alto do Vale de Moinho	Tua	41.331562	-7.394017	324	South-east	36
AHFT27	Vale de Moinho (praia fluvial)	Tua	41.334942	-7.391693	203	East	32

Code	Main site	River valley	Latitude	Longitude	Altitude (m)	Aspect	Slope (%)
AHFT30	Fiolhal	Tua	41.222842	-7.419543	203	West	62
AHFT31	Ferrado (praia fluvial)	Tua	41.303338	-7.363148	153	North	8
AHFT34	Barcos	Tua	41.243785	-7.402821	182	North	36
AHFT35	Quinta da Pendurada	Tua	41.349828	-7.277686	200	South-east	24
AHFT-RA1	Fiolhal	Tua	41.213514	-7.415312	308	South	35
AP	Alto da Penascosa	Côa	41.006135	-7.102552	270	South-west	38
*CA1	Canada do Amendoal	Côa	41.055240	-7.102690	310	West	25
*CA2	Canada do Amendoal	Côa	41.055590	-7.103590	318	West	23
*CA3	Canada do Amendoal	Côa	41.055020	-7.104230	300	South-west	18
CA4	Canada do Amendoal	Côa	41.057560	-7.108050	190	North-west	25
CA5	Canada do Amendoal	Côa	41.055530	-7.103490	315	West	23
CA-RA1	Canada do Amendoal	Côa	41.057322	-7.100753	344	South	15
CA-RA2	Canada do Amendoal	Côa	41.055979	-7.102962	330	South-west	31
CA-RA3	Canada do Amendoal	Côa	41.055318	-7.102924	315	West	25
CA-RA4	Canada do Amendoal	Côa	41.055564	-7.102987	318	West	23
CA-RA5	Canada do Amendoal	Côa	41.055325	-7.103874	309	West	23
CA-RA6	Canada do Amendoal	Côa	41.054609	-7.102150	348	West	28
*CI1	Canada do Inferno	Côa	41.054580	-7.112400	126	South-east	2
*CI2	Canada do Inferno	Côa	41.054650	-7.112390	126	South-east	2
CI3	Canada do Inferno	Côa	41.054760	-7.112310	126	South-east	2
*CI13	Canada do Inferno	Côa	41.054886	-7.112641	130	East	17
*CI14	Canada do Inferno	Côa	41.054760	-7.112660	129	East	17
CI15	Canada do Inferno	Côa	41.054846	-7.112710	131	East	17
CI-RA1	Canada do Inferno	Côa	41.054630	-7.112540	127	East	17
*CM-RA1	Canada da Moreira	Côa	41.062680	-7.089050	316	North	25
ECT3	Ribeira de Relvas	Sabor	41.259134	-7.007183	259	East	34
ECT4	Cardanha	Sabor	41.261353	-7.001736	301	West	21
*EP954	Cabeço do Aguilhão	Sabor	41.263355	-6.880222	175	East	13
F11	Fariseu	Côa	41.037188	-7.109885	127	North-east	10
F8	Fariseu	Côa	41.036138	-7.109586	129	East	12
F9	Fariseu	Côa	41.037054	-7.109698	126	East	14
FAIA	Faia da Água Alta	Douro	41.306092	-6.522189	514	South-east	35
*FC16	Foz do Côa	Côa	41.081350	-7.105220	125	East	33
*FC93	Foz do Côa	Côa	41.078540	-7.109560	216	South-east	57
*FC139/FCS1	Foz do Côa	Côa	41.079735	-7.108112	187	South-east	57
F-RA1	Fariseu	Côa	41.037129	-7.109860	127	North-east	10
MF	Monte do Fariseu	Côa	41.032979	-7.117154	263	South-east	59
P2	Penascosa	Côa	41.005307	-7.104784	164	West	15
*P3	Penascosa	Côa	41.005304	-7.104882	164	West	15
*P4	Penascosa	Côa	41.005109	-7.105075	161	North-east	3
*P5	Penascosa	Côa	41.004969	-7.105037	156	North-east	3
*P6	Penascosa	Côa	41.004764	-7.104884	158	West	15
P7	Penascosa	Côa	41.004798	-7.104818	160	West	15
P8	Penascosa	Côa	41.004720	-7.104783	160	West	15
P-RA1	Penascosa	Côa	41.004724	-7.104715	162	West	15
*QB-RA1	Quinta da Barca	Côa	41.004780	-7.107387	150	East	13
QB-RA2	Quinta da Barca	Côa	41.004948	-7.106881	150	North-east	9
*QB-RT3	Quinta da Barca	Côa	41.004690	-7.107740	151	East	13
*QTN	Quinta das Tulhas	Côa	41.077780	-7.106654	136	South-west	2

Code	Main site	River valley	Latitude	Longitude	Altitude (m)	Aspect	Slope (%)
RP-RT2	Ribeira de Piscos	Côa	41.031010	-7.117360	131	South-east	23
RP-RT17	Ribeira de Piscos	Côa	41.031145	-7.117502	136	South-east	23
RP-RA1	Ribeira de Piscos	Côa	41.031484	-7.117134	130	South-east	23
*SAB	Santo Antão da Barca	Sabor	41.263087	-6.880113	175	North-west	0
SABOR	Ponte de Remondes	Sabor	41.402508	-6.803513	200	East	18
SOUT	Soutelo	Sabor	41.399441	-6.742316	580	South-west	9
*TD1	Tudão	Côa	41.094689	-7.132472	378	South	7
*V1	Vermelhosa	Douro	41.086730	-7.107190	158	East	32
*V2	Vermelhosa	Douro	41.086670	-7.107290	158	East	32
*V3	Vermelhosa	Douro	41.086740	-7.107820	168	East	32
*V-RA1	Vermelhosa	Douro	41.086704	-7.107553	147	East	32
*VC1	Vale Cabrões	Douro	41.092870	-7.116150	248	North	17
*VC4	Vale Cabrões	Douro	41.090641	-7.118795	301	East	7
*VC5	Vale Cabrões	Douro	41.090674	-7.118955	302	East	7
*VC-RA1	Vale Cabrões	Douro	41.090621	-7.118985	303	East	7
*VF1	Vale do Forno	Côa	41.076100	-7.123690	320	East	28
*VF9	Vale do Forno	Côa	41.076420	-7.123100	298	East	28
*VF-RA1	Vale do Forno	Côa	41.076563	-7.122904	297	East	28
*VF-RA4	Vale do Forno	Côa	41.075996	-7.123452	292	East	28
*VFN1	Vale do Forno	Côa	41.075705	-7.122240	258	North	23
*VFN2	Vale do Forno	Côa	41.076001	-7.121475	251	North-east	16
*VFN3	Vale do Forno	Côa	41.075379	-7.122438	270	North	23
*VFN4	Vale do Forno	Côa	41.074953	-7.121207	295	North	26
*VFIG-R10	Vale Figueira	Côa	41.043300	-7.118470	218	South-east	45
*VFIG-R11	Vale Figueira	Côa	41.043380	-7.118580	228	South-east	45
*VFIG-R12	Vale Figueira	Côa	41.043720	-7.118080	223	South-east	48
*VFIG-RA1	Vale Figueira	Côa	41.043911	-7.117777	200	South-east	48
VJE1-RT1	Vale de José Esteves	Côa	41.083818	-7.107016	161	North-east	26
VJE3	Vale de José Esteves	Côa	41.084045	-7.106469	146	East	23
VJE17	Vale de José Esteves	Côa	41.083699	-7.109734	184	North-east	17
*VJE18/VJES3	Vale de José Esteves	Côa	41.083770	-7.109910	188	North-east	17
*VJE22/VJES2	Vale de José Esteves	Côa	41.083810	-7.109920	190	North-east	17
*VJES1	Vale de José Esteves	Côa	41.084358	-7.109591	208	South-east	21
*VJES4	Vale de José Esteves	Côa	41.084110	-7.109490	196	South-east	21
*VJEN1/VJE16	Vale de José Esteves	Côa	41.083437	-7.109556	177	North-east	23
*VJEN	Vale de José Esteves	Côa	41.083250	-7.106469	178	North-east	26

Fieldwork was carried out between September 2006 and May 2012. Special attention was paid to the lower Côa River Valley, the main focus of this project, but also extended to the lower Tua and lower Sabor River Valleys as previously explained (Chapter 4). Specimens that grew directly on engraved surfaces were carefully collected with the aid of a scalpel whereas the rest of the specimens were collected with chisel and hammer if firmly attached to the rock, or with a pocket knife if easily detached from the substrate. Collected specimens were placed in paper bags properly labelled with basic information about its microhabitat, collection date, GPS location and a reference number or inventory code whenever applicable, after which they were carried to the laboratory for drying and processing.

It is worth noting that some engraved surfaces in the Côa Valley have been subjected to different management strategies since the creation of the Côa Valley Archaeological Park, including the mechanical removal of lichens for rock-art recording, while others haver never been cleaned. Some sampling sites are periodically subjected to flooding events due to the effect of the neighbouring dams. Surfaces affected parcially or entirely by these events where not considered for the purpose of this research project. There is either no control of phanerogams with herbicides or its effect was not considered significant at the sampling sites, nor has any effect of environmental pollution been observed in the vitality of dominant lichen species.

Vouchers of examined specimens were deposited in the Herbarium of the University of Porto (PO), unless stated otherwise.

5.2 Taxonomic determination of collected specimens

5.2.1 Classical taxonomy

All specimens were examined under a Nikon SMZ1000 stereomicroscope for detailed observation of thalline structures and routinely spot tested with potassium hydroxide 10% (K), sodium hypochlorite or commercial bleach (C) and alcohol solution of para-phenylenediamine (P), for the detection of taxonomically relevant chemical compounds. Whenever necessary, hand-cut sections and squash preparations of thallus and apothecia were mounted in water and/or 10% K for spore measurements and observation of other anatomical features by conventional optical microscopy in a Nikon Eclipse 50i microscope.

Lichen identification was first based on the dichotomous keys provided by general floras, including the lichen flora of western Europe (Clauzade & Roux 1985), the lichen flora of Great Britain and Ireland (Smith *et al.* 2009), the flora of Italy (Nimis 1993). Monographs and taxonomic treatments were then used for addressing particular taxa or group in the following genera: *Aspicilia* (Nordin *et al*. 2010, Rico 1999), *Acarospora* (Clauzade *et al.* 1981), *Buellia* (Scheidegger 1993), *Caloplaca* (Arup 2006, Arup & Grube 1999, Arup *et al.* 2013, Gaya 2009, Šoun *et al.* 2011, Vondrák *et al.* 2008, 2009, 2011, Wetmore 2003), *Cladonia* (Burgaz & Ahti 2009), *Collema* (Carvalho 2012), *Dermatocarpon* (Heidmarsson 1998, Orange 1998), *Diploschistes* (Lumbsch 1989, Fernández-Brime *et al*. 2013), *Endocarpon* (Breuss 2002, McCarthy 2001), *Fuscopannaria* (Carballal *et al*. 2010), *Koerberia* (Burgaz 2011, Burgaz & Martínez 2001), *Lecanora* (Brodo 1984), *Leptochidium* (Burgaz & Martínez 2001), *Leptogium* (López de Silanes *et al*. 2012), *Lichinella* (Moreno & Egea 1992, Schultz 2007, Schultz & Büdel 2002), *Massalongia* (Burgaz 2011, Burgaz & Martínez 2001), *Nephroma* (Burgaz &

Martínez 2003), *Peltigera* (Burgaz & Martínez 2003), *Pertusaria* (Boqueras & Llimona 2003), *Physcia* (Figueras & Hladun 2007, Moberg 1977), *Peltula* (Büdel 1987, Büdel 2001, Büdel & Nash 2002, Egea 1989), *Placidium* (Prieto *et al.* 2010), *Polychidium* (Burgaz 2011, Burgaz & Martínez 2001), *Psora* (Timdal 1984, 2010), *Psorotichia* (Moreno & Egea 1994), *Pterygiopsis* (Moreno & Egea 1994), *Toninia* (Timdal 1991), *Rhizocarpon* (Runemark 1956), *Rhizoplaca* (Gómez-Bolea & Barbero 2009), *Rinodina* (Giralt 2001), *Trapeliopsis* (Aptroot & Schumm 2012), *Vahliella* (Carballal *et al*. 2010), *Xanthoparmelia* (Hale 1990).

A new taxonomy of the family Teloschistaceae has been proposed recently by Arup *et al*. (2013) introducing radical changes to this family and splitting the genus *Caloplaca* into 28 genera. A conservative approach is taken in this study and the older names are maintained while the discussion is still open and ongoing (E. Gaya, pers. comm.). A mention to the new combinations can be found in front of the respective synonyms in Chapter 6.4.

5.2.2 Quimiotaxonomy

Identification of lichen substances was based on standardized Thin-Layer Chromatography (TLC) as proposed by Culberson & Kristinsson (1970) with later adaptations by Culberson (1972, 1974). Small portions of thallus and/or apothecia of each specimen to be tested were placed in individual 1.5 ml Eppendorf tubes and covered with acetone for extraction of lichen substances. A mixed extract of *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb. and *Pleurosticta acetabulum* (Neck.) Elix & Lumbsch, previously collected by the author, was also prepared to provide the pattern for atranorin and norstictic acid. Control and test extracts were spotted on three replicate silica-coated aluminium plates (Merck Millipore, Darmstadt, Germany) which were allowed to migrate separately in solvent systems A (toluene 180 ml : 1,4-dioxane 60 ml : glacial acetic acid 8 ml), B (hexane 130 ml : diethyl-ether 100 ml : formic acid 20 ml) and C (toluene 200 ml: glacial acetic acid 30 ml) until reaching the previously fixed height of 10 cm. The plates were then sprayed with 10% sulphuric acid and placed in a 110ºC oven until colours developed. TLC plates where finally divided into classes of Rf-values based on the known Rf-values of norstictic acid (4) and atranorin (7) contained in control extracts. The identification of lichen substances was based on the tables provided by White & James (1985). TLC-plates were scanned for permanent record since the colour of most spots changes or fades away with time.

In addition a subset of 29 *Caloplaca* specimens was analysed with the assistance of Dr. Ulrik Søchting (University of Copenhagen) through High Performance Liquid Chromatography (HPLC) according to Søchting (1997).

5.2.3 Molecular taxonomy of selected specimens

Sequences of the nuclear ribossomal internal transcribed spacer (ITS) region, including the 5.8S rRNA gene, were obtained from apothecial extracts of a subset of 44 *Caloplaca* specimens by a procedure named Direct PCR following Arup (2006) using the DNeasy Plant Mini Kit (Qiagen, Venlo, The Netherlands). This subset included nine specimens of *Caloplaca* aff. *aractina* (Fr.) Häyrén *s. lat.*, 15 specimens of *Caloplaca arenaria* (Pers.) Müll. Arg. *s. lat.*, one specimen of *Caloplaca cancarixiticola* Nav.-Ros., Egea & Llimona, one specimen of *Caloplaca crenulatella* (Nyl.) H. Olivier, one specimen of *Caloplaca epigaea* Søchting, Huneck & Etayo, one specimen of *Caloplaca flavocitrina* (Nyl.) H. Olivier, one specimen of *Caloplaca erythrocarpa* (Pers.) Zwackh, two specimens of *Caloplaca pellodella* (Nyl.) Hasse, nine specimens of *Caloplaca subsoluta* (Nyl.) Zahlbr. and four specimens of *Caloplaca xerica* Poelt et Vezda *s. lat*. Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). PCR cycling parameters followed Ekman (2001). The PCR products were purified using Nucleofast (Macherey-Nagel, Düren, Germany). Sequencing was performed by Macrogen Ltd. in Korea, using the same primers as for PCR amplification. Sequence chromatograms were edited and assembled using Sequencher software version 3.1.1 (GeneCodes, Ann Arbor (MI), USA). Sequences previously obtained from reference material belonging to Dr. Ulrik Søchting (Natural History Museum of Denmark in Copenhagen) were used for comparison. Sequence alignment was also performed with Sequencher and subsequently adjusted manually by visual inspection.

5.3 Characterization of microclimate in vertical schist surfaces

To characterize the thermal and hydric regimes at the scale of the colonized schist surfaces, 11 miniature iButtons Hygrochron dataloggers (Maxim Integrated, Sunnyvale (CA) USA) (Table 5.3.1) were placed on key vertical schist surfaces facing all major aspect classes in the Côa Valley Archaeological Park (Table 5.3.2).

Table 5.3.1. Technical details of the DS1923-F5 iButton Hygrochron temperature/humidity dataloggers.

Temperature range	Humidity range	Resolution (programmed)	Maximum number of readings
-20°C to +85°C	0% to 100%	0.5°C	4096

Placing of dataloggers was done in such manner that they would not affect any engraving or have any influence over lichen growth on the respective surfaces. Dataloggers were synchronized and set to record both temperature and relative humidity, at hourly or half-hourly intervals during a 3-year period from late September 2010 to late September 2013 (see Table 5.3.2).

Table 5.3.2. Location and some characteristics of dataloggers used for temperature and relative humidity measurements in the Côa Valley Archaeological Park.

Code	Rock-art site	Panel	Latitude	Longitude	Surface orientation	Starting date	Interval
CA1	Canada do Amendoal	1	41.055240	-7.102690	West	29-09-2010	hourly
CA-RA2	Canada do Amendoal		41.055590	-7.103590	South	29-09-2010	hourly
QB-RA1	Quinta da Barca		41.004690	-7.107740	South	31-10-2010	hourly
QT5-G	Quinta das Tulhas	5	41.077780	-7.106654	South	15-04-2011	hourly
QT5-NG	Quinta das Tulhas	5	41.077780	-7.106654	South	15-04-2011	hourly
QTN4	Quinta das Tulhas		41.077780	-7.106654	North	15-04-2011	hourly
VC1	Vale Cabrões	1	41.092870	-7.116150	East	31-10-2010	half-hourly
VC5	Vale Cabrões	5	41.090560	-7.118960	South	31-10-2010	hourly
VF1	Vale do Forno	1	41.076100	-7.123690	South	28-10-2010	half-hourly
VJE16-G	Vale de José Esteves	16	41.083573	-7.109619	North	15-04-2011	hourly
VJE16-NG	Vale de José Esteves	16	41.083573	-7.109619	North	15-04-2011	hourly
VJES	Vale de José Esteves		41.084354	-7.109596	South	02-07-2011	half-hourly

5.4 Laboratory essays

5.4.1 On the use of experiments with biofilm-forming cyanobacteria for the determination of schist bioreceptivity

Lichen colonization takes place after (and while) an assortment of microorganisms, including bacteria, cyanobacteria, algae, fungi and other organisms capable of adhering to the rock surface had already started the rock weathering process. An ideal assessment of schist bioreceptivity in the Côa Valley Archaeological Park would rely on artificial inoculations under controlled environmental conditions of the same organisms (or their diaspores) that occur in the target rock surfaces, followed by a quantification of the resulting biomass (Guillitte 1995, Guillitte & Dreesen 1995, Miller *et al*. 2012). Because biological colonization is usually part of a complex synecological mechanism, Guillite's original proposal to accomplish a good simulation of natural conditions in biorreceptivity accessments was to use a mixture of representatives of all major biological groups. This is not an easy task when considering lichen colonization.

Besides being slow growing, lichens are still very difficult to develop and maintain in axenic cultures, which precludes the use of lichens in long term laboratory-based biorreceptivity experiments, at least for the time being, contrary to what is referred by Miller *et al.* (2012) based on the work of Favero-Longo *et al*. (2009). These authors have in fact used the lichen mycobiont, isolated from collected lichen thalli, not the whole lichen. The mycobiont's behaviour on rock should be very different to the lichen's since it does not develop a well-structured thallus rather being more similar in terms of growth form to non-symbiotic fungi (see Chapter 2.1). Besides, cultured mycobionts do not usually produce most secondary metabolites found in the original lichen, which are presumably so important for the lichen-induced chemical weathering.

The current tendency is to use cultures of cosmopolitan, fast-growing and easily maintainable groups of photoautotrophic microorganisms such as microalgae and cyanobacteria (Miller *et al*. 2006, 2009, 2010, Prieto & Silva 2005, Prieto *et al*. 2006), while culturing methods of other taxonomic groups are not fully developed. The biomass that is quantified at the end of these experiments, as Guillite (1995) suggested, is mostly photosynthetic biomass. Tests carried out with a single type or limited set of organisms may therefore be somewhat atypical (Miller *et al.* 2012) but so are the controlled laboratory conditions under which they are carried on, based on the assumption that "the bioreceptivity of a material will be best expressed under maximum accessibility and optimal environmental conditions for the development of organisms" and related with the necessity of carrying studies "under experimental conditions that are as standard as possible to remove any subjectivity attached to the concept" (Guillite 1995). There is still no standardized protocol for rock bioreceptivity evaluations (Miller *et al*. 2012), controling for important aspects such as the type of organism (bacteria, fungi, algae, lichens, bryophytes, etc); grouping criteria (taxonomical, functional); measure of abundance (density, cover, biomass, etc); quantification techniques (direct observation, optical and electron microscopy, spectrophotometry, etc); sampling design (number of measurements, measuring area, time), among others. Conditions vary among studies, hampering the comparisons of results obtained by different authors and instruments. This is mainly due to the fact that bioreceptivity is still a young discipline within the framework of biodeterioration.

Many lichen communities, especially those that proliferate in the studied rock surfaces (see Chapter 4) are supposed to depend on the previous formation of an almost continuous cover of cyanobacteria-rich biofilms that are known to contribute to water and nutrient retention at the surface level (Llimona & Egea 1985) and eventually constitute an important source of photobionts for lichen establishment. In the words of its author, bioreceptivity is the "missing link" that is required in the adoption of an integrated approach to the biological colonization.

A stock culture of cyanobacteria containing two of the most common photobiont genera found in cyanolichens (Prieto & Silva 2005) was readily available providing almost immediate information on what can be considered a proxy to the susceptibility of the studied schist to lichen colonization. Both chlorophyll-a quantification and determination of total colour difference have been yielding reliable results (Prieto & Silva 2005). The experiments with bioreceptivity of schist in the Côa Valley (Chapter 7.1) are based on, or adapted from, previous assessments by Prieto & Silva (2005) and Prieto *et al*. (2004, 2006) as follows.

5.4.2 Bioreceptivity experiment

The origin of weathered and unweathered lithotypes

Schist samples came from a local quarry named Poio that has been active for centuries and is currently in the middle of the Côa Valley Archaeological Park. Blocks of each type of schist were cut into six cubes of 5x5x5 cm and twelve smaller square blocks of 4x4x1 cm for physical properties and bioreceptivity assessment, respectively. Surface textures of all samples were sawn smooth in order to avoid any influence of surface roughness in the results. All blocks were sterilized, oven dried at 65ºC for 24 hours and let cool down to room temperature in a plastic container with silica gel previous to their use in the following experiments.

Quantification of photosynthetic biomass

One of the most popular methods to estimate photosynthetic biomass in limnology and plant physiology has always been chlorophyll-a quantification, which involves the extraction of chlorophyll from disintegrated cells in an organic solvent and subsequent determination by spectrophotometry (Hiscox & Israelstam 1979, Lorenzen 1967). Only very recently has it been applied to estimate algal and cyanobacteral biomass on monuments (*e.g.* Ariño & Saiz-Jimenez 1996, Miller *et al*. 2008, Ortega-Calvo *et al.* 1995). Although destructive, it has the advantage of estimating the total biomass that is present both on and within the rock samples.

Cyanobacterial culture conditions

An aliquot of a stock culture containing the cyanobacterial strains *Nostoc* sp. PCC 9025, *Nostoc* sp. PCC9104 and *Scytonema* sp. CCC9801, was placed in fresh BG-11_0 liquid medium (Rippka *et al.* 1979) and allowed to reach the exponential growth phase in aerified Erlenmeyer flasks under the controlled environment of a plant growth chamber. Temperature was kept at 22ºC and relative humidity at 95% on a 12/12h day/night cycle (light intensity of 1,600 lx).

Quantification of chlorophyll-a content in the inoculum

The inoculum used in this experiment was harvested from the cyanobacterial culture mentioned above but prior to inoculation its chlorophyll-a content had to be estimated in order to evaluate the colonization success throughout the experiment.

Five replicates of 10 ml aliquots of the cyanobacterial culture in BG-11_0 liquid medium were vacuum filtered through cellulose nitrate membranes of 0.45 µm pore size (Merck Millipore, Darmstadt, Germany) and the chlorophyll extracted directly from the filter in dimethyl-sulfoxide (DMSO) at 63°C for 40 min. The absorbance of the extract was then measured by spectrophotometry at 665 and 649 nm wavelength as required by Wellburn (1994) in his proposed equation to determine the concentration of chlorophyll-a. DMSO extraction has the advantages associated with this solvent: it is easy to manipulate, has a greater extracting power than other solvents and if the extracts are stored in the fridge, the pigments remain stable for a long time (Ariño & Saiz-Jimenez 1996, Hiscox & Israelstam 1979).

Sample inoculation

The upper surface of each sample was inoculated with 10 ml of the prepared cyanobacterial mixture (above). Samples of each schist type (weathered and unweathered) as well as Blanco Cristal granite were taken in triplicate for chlorophyll-a quantification after 30, 60, 90 and 120 days of incubation. Water loss by evaporation was checked daily and compensated by the addition of sterilized distilled water whenever necessary.

Quantification of chlorophyll-a content in incubated rock samples

Every 30 days until the end of the experiment, a triplet of samples of each lithotype were placed onto individual Petri dishes containing 6,88 ml of DMSO (extrant/sample ratio expressed in volume of DMSO/sample surface cm2) and sonicated by inserting the narrow tip of an ultrasonic generator (UP200S; Dr Hielscher GmbH) into the extractant following the protocol described by Fernández-Silva *et al.* (2011) in order to release all cells from the rock samples into the DMSO bath. Sonication was for 5x30 s (0.5 duty cycle, 60% amplitude), with 30 s breaks to avoid overheating. Chlorophyll extraction was obtained accordingly by submiting the DMSO bath after sonication to 63°C for 40 min. The absorbance of the extract was then measured by spectrophotometry at 665 and 649 nm wavelength and chlorophyll-a concentration determined as previously stated (Welburn 1994).

Determination of total colour difference in CIE L*a*b* colour space

Colour derived from photosynthetic pigments has also become an important alternative for the purpose of photosynthetic biomass quantification (Prieto *et al*. 2002, 2004). Portable spectrophotometers (Fig. 5.4.1) allow measuring the colour of reflected light, which is detected even when it is almost invisible to the naked eye. These measurements are usually performed under the CIE L*a*b* (or CIELAB) colour space, a three-dimensional device-independent model of colour representation proposed by the International Commission on Illumination, the english name for the *Commission internationale de l'éclairage* (*CIE*). Other common alternative spaces of representation proposed by CIE are CIE-RGB (1931) based on the magnitude of red, green and blue as parameters of colour characterization. However, unlike the RGB and other previous models of colour representation, CIE L*a*b* is designed to mimic the nonlinear response of the human eye owing to the nonlinear relationships for *L**, *a**, and *b** thus including all perceivable colours. CIE L*a*b* is therefore the most perceptually uniform of the colour spaces (Prieto *et al*. 2004).

Fig. 5.4.1. Konica Minolta CM-700d Portable Spectrophotometer.

CIE L*a*b* colour space is defined by three parameters (or Cartesian coordinates) plotted along the three orthogonal axes of a Cartesian coordinate system: L* is for lightness or luminosity of colour, which varies from 0 (absolute black) to 100 (absolute white); and a* and b* are chromaticity coordinates associated with changes in redness/greenness and in yellowness/blueness, respectively: a* ranges from green (negative) to red (positive) and b* from blue (negative) to yellow (positive). Chromaticity coordinates can be plotted on a two-dimensional diagram that defines all the visible colours. The asterisk (*) after *L*, *a* and *b* are pronounced *star* and are part of the full name, since they represent *L**, *a** and *b**, to distinguish them from the analogous Hunter's *L*, *a*, and *b* colour space. Colour, being naturally heterogeneous, is by this means expressed through a strongly reduced set of parameters. There are delta values associated with this colour scale. ΔL*, Δa*, and Δb* indicate how much a control and a test sample differ from each other in L*, a*, and b*. Therefore these delta values can be used to analyse differences between treatments (before-and-after) or follow-up data. The total colour difference, ΔE* is a single value which takes into account the differences between the L*, a*, and b* of the test sample and the control.

The colour of an object can appear different because of differences in the level of specular reflectance. Portable spectrophotometers allow taking colour measurements in two modes: the mode of colour measurement that excludes the specular reflectance is named SCE (Specular Component Excluded); if the specular reflectance is included the mode is named SCI (Specular Component Included). In general, it is an accepted theory in colour science that the SCE mode correlates best to the way observers perceive the colour of an object whereas SCI mode is adequate for analyzing the intrinsic colour of objects.

5.4.3 Physical properties testing

Rocks and natural stones are frequently characterized by their physical properties, whose test methods lay down on the European standards (EN) adopted by the members of the European Committee for Standardization (CEN). The tests run in the framework of this project were those providing the most relevant rock physical properties for the evaluation of lichen-induced weathering processes occurring on the target schist, following what was recommended by the International Union of Laboratories and Experts in Construction Materials, Systems and Structures (RILEM) in 1980 (RILEM TC 25-PEM, 1980) and by several authors thereafter (*e.g.* Rivas 1996, Miller *et al*. 2009, Prieto & Silva 2005, Sanmartín *et al*. 2012), thus including: porosity (EN 1936 | RILEM Test no I.1), density (EN 1936 | RILEM Test no I.2), water absorption by capillarity (EN 1925 | RILEM Test no II.6), water absorption and de-absorption at atmospheric pressure (adapted from EN 13755 following Sanmartín 2007).

Physical properties are lithotype specific, but can vary depending on the weathering degree of the rock. All of the referred tests were applied to seven square blocks of both weathered and unweathered schist of approximate dimensions 5x5x5 cm. Unweathered schist samples came from the Poio quarry, which is located within the boundaries of the Côa Valley Archaeological Park. Weathered schist samples were taken from natural outcrops in a nearby rock-art site named Vale de José Esteves. Soiling and decay expressed by a different surface colour and some fissuration were visible but no biological colonization was evident on the surface of the block. Before each test, blocks were sterilized, oven dried at 65ºC for at least 24 hours and let cool down to room temperature in a plastic container with silica gel. Tests were performed at stable room conditions (temperature around 20ºC and 60% of relative humidity). A Precisa 600C balance (PAG OERLIKON AG, Zurich, Switzerland) was used for all weighings. Average values were obtained for weathered and unweathered schist considering the values of the respective blocks (or replicate samples).

Bulk density and open porosity

The abundance of pores, their size, shapes and conections (*i.e.* their capillary structure) determine the amount of water that the rock intakes and looses (as well as its kinetics) and are important factors in relation to weathering. Both EN and RILEM guidelines foresee the determination of real and bulk (or apparent) density and of open and total porosity. For dense, low porosity rocks, however, differences between bulk density and real density, as well as between total and open porosity are very small. In such cases it is sufficient to determine the bulk density and open porosity. These are obtained from the following formulas:

Bulk density (Kg/m^3 or g/cm^3)

Bulk density or apparent density is the ratio between the mass of the dry sample and its bulk volume, as determined by the sample's exterior surface:

$$\rho_b = \frac{m_d}{m_s - m_h} \times \rho_w$$

ρ_b: bulk density; m_d: mass of the dried sample; m_s: mass of the sample saturated with water under vaccum, weighed in the air; m_h: mass of the sample saturated with water under vaccum, weighed in water; ρ_w: density of water (998 Kg/m^3)

Open porosity (%)

Open porosity is the ratio of the pore space in rock that is accessible to water, to its bulk (or apparent) volume:

$$p_o = \frac{m_s - m_d}{m_s - m_h} \times 100$$

p_o: open porosity; m_d: mass of the dried sample; m_s: mass of the sample saturated with water under vaccum, weighed in the air; m_h: mass of the sample saturated with water under vaccum, weighed in water.

5.4.4 Abrasion pH

According to Grant (1969) the protocol goes as follows: 20 mg of previously washed and dryed rock samples are introduced in a Agata mortar with 40 ml of distilled water, grinded for 2.15 min and allowed to settle for another 2 min. 15 ml of the supernatant are extracted and used to measure pH.

5.5 Image analysis and classification

Analysis of physical weathering was based on the quantification of the depth and extent of hyphal penetration into the rock. Colonized schist samples were cut transversal, surface polished and stained using the periodic acid-Schiff method (PAS) according to Favero-Longo *et al.* (2005), to highlight the Hyphal Penetration Component (HPC). Microphotographs of the stained sections were acquired at ×10 magnification using a Nikon SMZ1000 stereomicroscope equipped with a Nikon DS Fi1 digital camera. The acquired images were submitted to a protocol of pixel-based supervised classification using colour and texture features, as follows.

5.5.1 Image pre-processing

Firstly, an *a priori* selection of images was conducted based on a visual inspection of available imagery. Images with better distinction of visual elements (based on different combinations of f-stop and exposure values) were selected. In order to speed-up image processing and reduce computation time, the selected images were resampled to 40% of the initial size (final size equal to 1024 x 768 pixels) using bicubic interpolation. Additionally the contrast and sharpening of the images was enhanced using a combination of automatic filters allowing a better discrimination of hyphae and other recognizable structures. These procedures were entirely performed in ImageJ image analysis software (Abramoff *et al.* 2004, Rasband 2012).

5.5.2 Feature extraction

Two types of features were calculated from the original image: colour (Maia *et al.* 2009) and texture features (Zhan *et al.* 2011) subsequently used for training a set of Random Forest classifiers (Breiman 2001, Liaw & Wiener 2002).

Colour features were extracted using package ‘adimpro’ (Polzehl & Tabelow 2007) in R by converting the colour space of the original images (RGB) into four different colour spaces: HSI, YUV, YIQ and XYZ. The numerical values obtained for each channel of the five colour spaces were used as input features. MaZda image analysis software (Szczypiński *et al.* 2009) was used to calculate features related to run-length and co-occurrence matrices, histogram and gradient features for two distinct kernels of 5x5 and 10x10 pixels (162 texture features calculated in total).

5.5.3 Calibration input data

ImageJ was used with the purpose of obtaining training data for the calibration of the Random Forest classifier. This step started by defining the appropriate legend to each image based on the visual identification of all structures in the image (*i.e.*, classes or labels). Then for each image/class several points were pickedon user input (generally more than 100 per-class) and the coordinates exported to a text file. The input-point selection used a semi-systematic unaligned sampling design for point collection thus allowing capturing the large diversity of each class in terms of colours and textures.

5.5.4 Random Forest classifier calibration and validation

With the purpose of obtaining a classifier able to predict the labels for the entire target image based on user training inputs, the Random Forest (Breiman 2001, Liaw and Wiener 2002) machine-learning algorithm (also implement in R statistical software) was used for this purpose. In the developed analysis framework, the classification algorithm used class labels as the response variable and texture and colour features as predictor variables.Random Forest has shown excellent performance in high dimensionality situations where sometimes the number of predictor variables is much larger than the number of observations, for their ability to handle with complex interaction structures as well as highly correlated variables and can provide measures of variable importance (Boulesteix *et al*. 2012).

A two-pass methodology was employed to develop the Random Forest classifiers. The first pass consisted in selecting the 20 most important features based on an initial algorithm calibration with all training points; based on this, Random Forest routines allow ranking features according to their relative predictive importance. By subsetting the initial train dataset to the 20 most important features, this allowed to enhance the signal-to-noise ratio, improving classifier performance (results not shown) and also to reduce the amount of required computer resources and processing time. The second pass consisted in the calibration and evaluation of the final classifiers based on the reduced dataset (obtained in the first pass). The following Random Forest parameters were changed to improve modelling performance: *ntree*= 200, *mtry*= 6 and *nodesize*= 5 and the remaining were kept as default.

Monte-Carlo cross-validation (Xu & Liang 2001, Xu *et al*. 2004) was used to evaluate the classifiers performance and generalization ability, considering a total of 100 test-rounds and a random splitting of the input data of 80% for training and 20% for testing. For each test-round the mean classification accuracy (*i.e.* across all classes) was calculated, as well as the confusion matrix.

From the 100 classifiers generated, the one with highest accuracy was selected to predict the class labels for the entire image. Descriptive statistics such as the frequency (number of pixels) and area of each class were calculated.

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6 Lichen diversity in the Upper Douro Region

6.1 A *Peltula* Nyl. diversity hotspot in north-east Portugal, with three new species to mainland Europe and one species new to science

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Abstract

Recent field work carried out for the study of the lichen flora in the Upper Douro region (north-east Portugal) has resulted in a remarkable collection of *Peltula* specimens, including three new species for mainland Europe (*Peltula bolanderi*, *Peltula farinosa* and *Peltula zahlbruckneri*) and seven new species for mainland Portugal, that are here reported with a few notes on their morphology and currently known distribution. *Peltula lobata* is described as new to science, based on the same collection. Thallus shape, size, presence of large, sessile apothecia with widely opened discs and complete absence of soredia separate *Peltula lobata* from any other known *Peltula* species. Identical specimens were also obtained from the nearby locality of Siega Verde in the province of Salamanca and from the southern locality of Abla in the province of Almeria, both in Spain. New insights into the morphological diversity of *Peltula euploca* and *Peltula bolanderi* are also provided. The region reveals to be a hotspot for *Peltula* diversity and a very promising area for further research on the morphological and molecular variation among the former species. A revised key to European species of the genus is presented at the end of this paper.

6.1.1 Introduction

Peltula Nyl. is a worldwide distributed lichen genus of *c*. 40 mostly soil and rock inhabiting species. It is most diverse and ecologically significant in arid and semi-arid regions or wherever arid microclimates are found (Büdel & Nash 2002) and particularly noticeable on seepage tracks of both acidic and basic rocks. The diversity of *Peltula* is particularly high in the Mediterranean basin, which hosts all of the species known so far from the European continent (Hladun & Llimona 2002-2007, Feuerer 2012). Egea (1989) first revised the genus in the Iberian Peninsula, accepting a total of seven species for Spain, of which two were also known to occur in Portugal namely *Peltula euploca* (Ach.) Poelt ex Ozenda & Clauzade and *Peltula omphaliza* (Nyl.) Wetmore.

During recent field studies in the Upper Douro region (north-east Portugal), it became apparent that *Peltula* had been largely overlooked in this somewhat neglected region of the Iberian Peninsula. In fact, few references exist of lichenological studies carried out in this region and these are mainly from the late 19th and early 20th centuries (*e.g.* Sampaio 1917), when many sites were still quite difficult to access. The entire country was scarcely visited by lichenologists in the past when compared to the neighbouring Spain. Contributions to the lichen flora of Portugal are mainly from the second half of the 20th century and have increased only very recently. These include several new records for Portugal (*e.g.* Carvalho & Jones 1997, van den Boom & Giralt 1999) and species new to science (*e.g.* Paz-Bermúdez & Elix 2004, Paz-Bermúdez *et al.* 2009b).

The Upper Douro region is located in the northern sector of the Central Iberian geotectonic zone (Ribeiro 2001) and revolves around the Douro hydrographical basin *c*. 200 km upstream from the River Douro estuary, in Porto. The lithology is dominated by intensely folded and faulted metasedimentary rocks (including schist, metagreywacke and quartzite) intruded by igneous rocks (granite) ranging in age from the Precambrian to the Ordovician (Ribeiro 2001, Aubry *et al.* 2012). The rigid tectonic, differential erosion and down-cutting by River Douro and its tributaries resulted in an irregular topography (Aubry *et al.* 2012) that has had a major influence on the landscape of the region. The climate is predominantly dry meso-Mediterranean (Costa *et al.* 1998), sheltered from the Atlantic influence by mountains to the north and the west. However, topographic thermo-Mediterranean microclimates are usually produced in most river valleys, where air temperature frequently reaches 47°C in summer and the mean annual precipitation is often bellow 400 mm. A special feature of the landscape is the widespread presence of vertical schist surfaces arranged in layers along the valley slopes, which have been exposed by a sequence of gravity-induced detachment of offset rock joints, perpendicularly to the schistosity plane, in the schist and metagreywacke basement (Aubry *et al.* 2012); and of massive quartzite outcrops along the residual hills of the original peneplain (Costa *et al.* 1998). Significant intercalations of metacalcareous rocks and calcosilicate veins (Ribeiro 2001) provide a source of carbonaceous dust which accumulates in most crevices and horizontal surfaces thanks to limited precipitation.

These features provide an ideal natural context for the occurrence of lichen communities rich in species of *Peltula*. The study by Paz-Bermúdez *et al.* (2009a) in the valley of River Sabor (a right-bank tributary of the Douro) was the first in the westernmost part of the Upper Douro region and lists the occurrence of *Peltula euploca*. The current study extends the prospection further east up to the Spanish border, including the valleys of Rivers Tua, Côa and Douro.

6.1.2 Material and methods

Approximately 155 specimens of *Peltula* from the Upper Douro region (north-east Portugal) and the provinces of Salamanca and Almeria (NW and SW Spain, respectively) were investigated and deposited in the herbaria of the University of Porto (PO), the University of Hamburg (HBG) and the University of Santiago de Compostela (SANT). Specimens provided by the herbaria ASU, BCN and CANB, and from the personal collection of M. Schultz were studied for comparison. The internal morphology of the lichen thallus and ascomata were studied from hand and cryotome sections, mounted in water and lactophenol-cotton blue, respectively. Ascus structure and amyloid reaction of the ascus wall were observed by adding Lugol's solution directly to sections and squash preparations mounted in water before and after treatment with K (10%). Photographs of sections were taken on an Olympus XC50 camera mounted on an Olympus BX51 compound microscope and using differential interference contrast (DIC). All habit photographs were taken on a Canon EOS 400D digital camera with a Zeiss Luminar 40 mm lens mounted on bellows.

6.1.3 Results

Peltula lobata **J. Marques, M. Schultz & Paz-Berm. sp. nov.**

MycoBank No.: MB 803401

Similis specie *Endocarpon adscendens* sed thallo pallido olivaceo, algiis ad ordinem Chroococcales, apotheciis grandis, laminalis, sessilis, discis apertis et ascis polysporis

Typus: Portugal, Vila Nova de Foz Côa, Foz do Côa, 29TPF589493, 186 m, on narrow crevices of schist outcrops, 03.05.2011, J. Marques (PO9021-L – holotypus); Portugal, Macedo de Cavaleiros, Casas, 29TPF8587, 345 m, on wide crevices of schist outcrops, 05.09.2006, J. Marques (PO9024-L – paratypus); Portugal, Vila Nova de Foz Côa, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (HBG-019526 – paratypus); Portugal, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 196 m, on vertical schist surfaces, 02.05.2011, J. Marques (HBG-019527 – paratypus); Portugal, Alijó, Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 07.03.2012, J. Marques (SANT11708 – paratypus); Portugal, Vila Nova de Foz Côa, Canada do Amendoal, 29TPF5946, 310 m, on vertical schist surfaces, 07.04.2011, J. Marques (SANT11709 – paratypus).

Thallus thick foliose to subfoliose, lobate, initially composed of simple, ascending squamules attached by lateral holdfasts, becoming deeply divided into loosely overlapping to strongly imbricate lobes in well-developed specimens, rising from a common holdfast that may produce a short flattened stalk, and forming extensive cushions up to 4 cm (or larger aggregates); lobes elongate, up to 5 mm long and 3 mm wide, flat to somewhat undulate and contorted, with margins usually bent downwards to occasionally revolute; upper surface olive-green to olive-brown, pale brown towards the base of the lobes, epruinose, matt to sometimes glossy, smooth to slightly rugose (Fig. 6.1.1 A), occasionally fissured (Fig. 6.1.1 B), without soredia or isidia; lower surface pale reddish brown to white in well-developed lobes, or darkened by substrate interaction (Fig. 6.1.1 C); thallus thickness 150-450 µm (up to 550 µm at the stalk); upper cortex usually present, 12.5-62.5 µm thick, of paraplectenchymatous cells varying in diameter from 5 to 25 µm, with a more or less well delimited continuous algal layer beneath, 50-120 µm thick; a pale yellow smooth epinecral layer 5-18 µm thick is usually present; medulla white, 50-250 µm thick, prosoplectenchymatous, composed of loosely to densely interwoven, elongated hyphae (Fig. 6.1.2 A) but sometimes more densely arranged and appearing paraplectenchymatous, namely in immature squamules as well as at thallus margins, and then difficult to distinguish from lower cortex (Fig. 6.1.2 B); lower cortex 35-125 µm thick, of paraplectenchymatous cells mostly 10 µm in diameter, sometimes becoming eroded at thallus margins; Apothecia rare, adnate, with a raised rim when mature (Fig. 6.1.1 D); disc: reddish brown, 0.3-1.3 mm in diameter; hymenium 100-200 µm tall, orange-brown, I^+ wine red; paraphyses moniliform with apices expanded to 6 µm, not branching but sometimes anostomosing; asci clavate to obclavate, with a lacerate gelatinous sheeth, ascus wall I^+ reddish brown, blue after pre-treatment with K, more than 100-spored (Fig. 6.1.2 C); ascospores hyaline, ellipsoid to slightly reniform, simple, 5.5-9.0 x 2.5-4.0 µm; Pycnidia immersed, cerebriform; conidia ellipsoid, hyaline, 2-3.2 x 0.8-1.5 µm; Spot tests all negative; Secondary metabolites not investigated.

Habitat and distribution

A frequent colonizer of vertical, sun-exposed rock crevices in riverbanks as well as of periodically submerged rock surfaces in temporary riverbeds. Although commonly found on schist it also colonizes quartzite and granite. So far it has only been observed in ten localities in north-east Portugal, where it is common and forms large, rather conspicuous brown patches, at low altitudes (between 130 and 500 m); and in two localities in the Spanish provinces of Salamanca (at 700 m) and Almeria (at 780 m), respectively (Fig. 6.1.3).

Peltula lobata is frequently the sole colonizer of the thinnest cracks or river-washed crevices but when these become wider and filled with soil other terricolous species occur, including *Endocarpon pusillum* Hedw. and *Peltula patellata* (Bagl.) Swinscow & Krog, among others. On rock surfaces, it is often associated with the siliceous rain-track lichen communities described by Llimona & Egea (1985) therefore accompanying other species of *Peltula*, such as *Peltula euploca* and *Peltula obscurans* (Nyl.) Gyeln., and other member of the Lichinales including for instance *Pyrenopsis triptococca* Nyl and *Lichinella stipatula* Nyl. *Caloplaca subsoluta* and *Aspicilia contorta* subsp. *hoffmanniana* S. Ekman & Fröberg are among the dominant species in the driest areas of those rock surfaces. When bryophytes such as

Grimmia laevigata (Brid.) Brid. and *Grimmia dissimulata* E. Maier are present, *Peltula lobata* occasionally overgrows them, becoming muscicolous.

The outcrops from which *Peltula lobata* was collected were always occurring in the sub-humid to semi-arid ombroclimate of the meso-Mediterranean to the topographically influenced thermo-Mediterranean belts of the Mediterranean Region (Costa *et al.* 1998) amongst *Quercus rotundifolia* Lam. and *Juniperus oxycedrus* L. or cultivated olive and vine.

Notes

In the field *Peltula lobata* closely resembles *Endocarpon adscendens* (Anzi) Müll. Arg. or *Endocarpon loscosii* Müll. Arg., with which it may share the same habitat near riverbanks, but is readily distinguished after a closer look, since the latter have perithecia and green algae as photobiont.

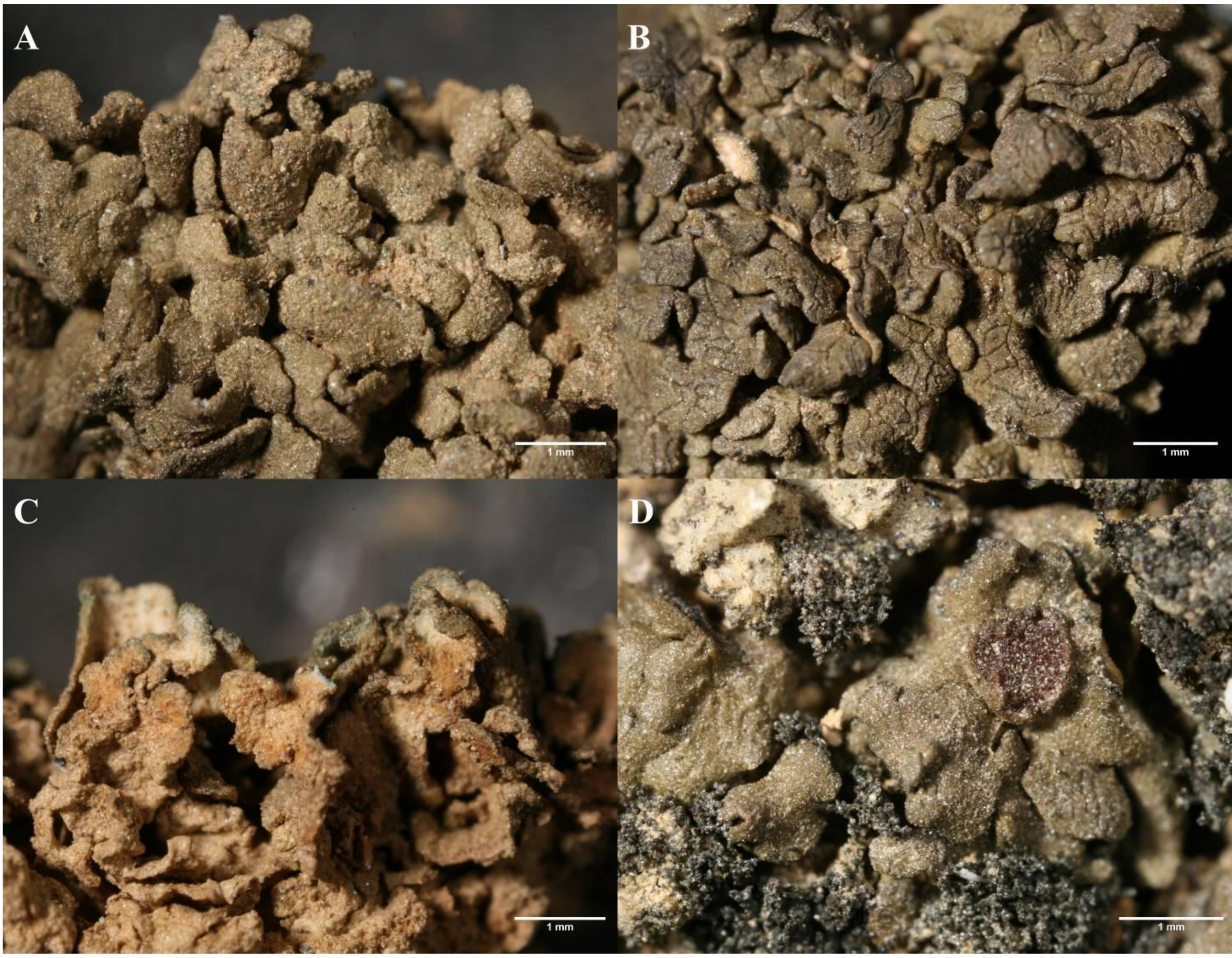

Fig. 6.1.1. *Peltula lobata* (habitus). A: Smooth upper surface (PO9040-L); B: Fissured upper surface (PO9040-L); C: Detail of lower surface (PO9040-L); D: Detail of upper surface with apothecia (HBG-019527 - paratype).

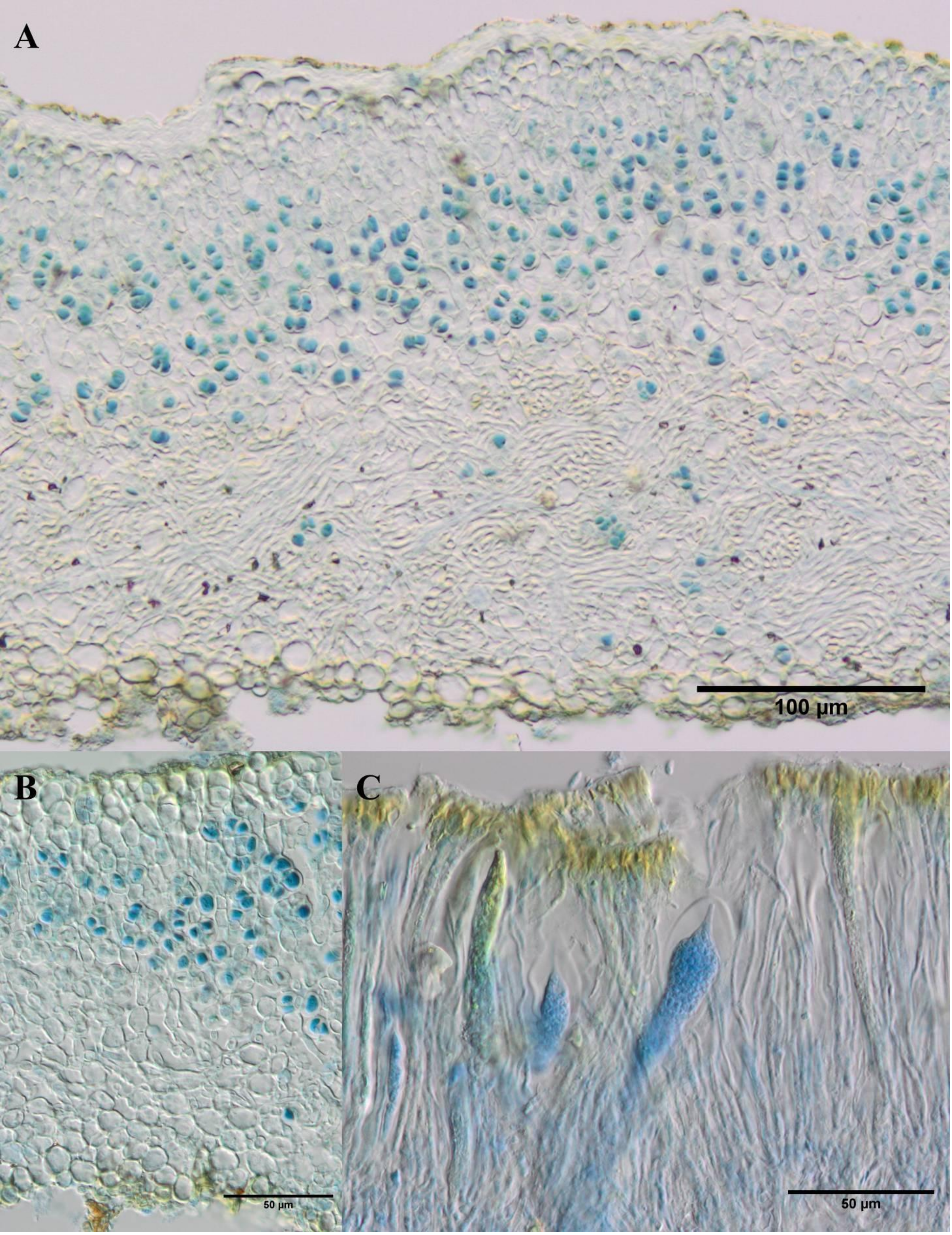
A
100 µm
B
50 µm
C
50 µm

Fig. 6.1.2. *Peltula lobata* anatomy. A: Thallus section of a mature lobe with a thick epinecral layer, upper and lower paraplectenchymatous cortex and prosoplectenchymatous medulla, composed of densely interwoven hyphae (PO9021-L – holotype); B: Thallus section of a juvenile lobe showing a partly paraplectenchymatous medulla (PO9021-L – holotype); C: Detail of polysporous ascus (PO9021-L – holotype).

Fig. 6.1.3. Currently known distribution of *Peltula lobata*.

Peltula lobata is also rather similar to species of *Heppia* but these have 8-spored, thin walled asci and a different thallus anatomy. Sterile specimens may, however, be difficult to distinguish from *Heppia*. *Peltula lobata* is distinguished from all other species of *Peltula* by the thick lobate, non-sorediate thallus and laminal, sessile apothecia with dark red, open discs. Fully developed specimens of *P. lobata* are characterized by multi-branched and strongly imbricate lobes and are outstanding in thallus size.

In this predominantly squamulose genus, only few species have been described with a lobate thallus. Based on the relationship between thallus growth form and ecological preferences among species of *Peltula* illustrated by Büdel (1987: 26, Fig. 3), *P. lobata* fits between the peltate type (“peltater Typ”) characteristic for species of seepage track communities and the lingulate type (“lingulater Typ”) common in species from periodically submerged habitats. This type of thallus development is also found in *P. corticola* Büdel & R. Sant, *P. congregata* (Nyl.) Swinscow & Krog, *P. imbricata* Filson and *P. auriculata* Büdel, M. Schultz & Gröger (Table 6.1.1). *Peltula lobata* and *P. corticola* are somewhat similar morphologically and anatomically, including the presence of a completely paraplectenchymous thallus (Büdel 1987) which may be observed in immature thalli of *P. lobata*, but the latter differs in having larger and thicker thallus, larger apothecia and spores, and absence of soredia. The type of attachment organ is also different. *Peltula corticola* is attached with tufts of thick-walled, anastomosing rhizohyphae (Büdel *et al.* 2007) while *P. lobata* is attached to the substratum by a rather flat stalk. Furthermore, *Peltula corticola* is exclusively corticolous while *P. lobata* is mainly saxicolous or sometimes terricolous. *Peltula lobata* is well distinguished from *Peltula congregata* by the thallus anatomy, namely the development of an upper cortex, the type of apothecia and spore size; and from *P. imbricata* by much larger thallus and apothecia (see Table 6.1.1). *Peltula auriculata* differs in the presence of characteristic dark-rimed, ear-shaped appendices along the margins of thallus squamules.

Specimens of co-occurring *Peltula obscurans* with somewhat lobulate squamules (var. *hassei sensu* Wetmore 1970), are never as strongly lobate and do not develop such a well-delimited upper cortex as in *Peltula lobata*. In fact, the occurrence of a distinct upper cortex in *P. lobata* is a rather unusual character within genus *Peltula*, and might be a structural adaptation to the harsh conditions in Mediterranean riverine habitats, increasing the mechanical strength of its large lobes against damage by water in winter and simultaneously providing protection in summer against drought and excessive solar radiation.

ADDITIONAL SPECIMENS EXAMINED – PORTUGAL: **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 24.03.2012, J. Marques (PO9022-L); Carlão, Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 7.03.2012, J. Marques (PO9023-L). **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques, (PO9037-L); Valverde, Serra de Valverde, 29TPF5183, 219 m, on narrow crevices of quartzite outcrops, 15.10.2011, J. Marques (PO9025-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques, PO9000-L; Soutelo, Alto da Fraga, 29TPF8886, 538 m, on wide crevices of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9026-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9027-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 367 m, on narrow crevices of schist outcrops, 14.03.2012, J. Marques (PO9028-L); Penascosa, 29TPF5941, 160 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9029-L); Penascosa, 29TPF5941, 164 m, on narrow crevices of schist outcrops, 15.05.2007, J. Marques (PO9039-L); Muxagata, Fariseu, 29TPF5844, 127 m, on narrow crevices of schist outcrops, 01.04.2010, J. Marques (PO9040-L); Ribeira de Piscos, 29TPF5843, 130 m, on narrow crevices of schist outcrops, 11.10.2010, J. Marques (PO9030-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on narrow crevices of schist outcrops, 08.02.2006, J. Marques (PO9041-L); Foz do Côa, 29TPF5849, 216 m, on vertical schist surfaces, 14.10.2012, J. Marques (PO9036-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9031-L); Vale do Forno, 29TPF5748, 301 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9033-L); Vale Figueira, 29TPF5845, 223 m, on vertical schist surfaces, 13.03.2012, J. Marques (PO9032-L). – SPAIN: **Almeria**, Abla, Las Juntas, 30SWG219115, 780 m, on vertical schist surfaces, 03.04.2012, J. Marques (PO9034-L; PO9038-L). **Salamanca**, Ciudad Rodrigo, Siega Verde, 29TPF976080, 588 m, 24.04.2012, J. Marques (PO9035-L).

Fig. 6.1.4. Thallus morphology of selected species of *Peltula* present in the Upper Douro region (north-east Portugal). A: *Peltula euploca* (typical form); B: *Peltula euploca* (polyphyllous form); C: *Peltula euploca* (small form); D: *Peltula euploca* (small effigurate form); E: *Peltula euploca* (esorediate form); F: *Peltula bolanderi*; G: *Peltula farinosa*; H: *Peltula zahlbruckneri*. Photographs by Matthias Schultz.


A
1 mm
B
1 mm
C
1 mm
D
1 mm
E
1 mm
F
1 mm
G
1 mm
H
1 mm

The species new to mainland Europe

Peltula bolanderi (Tuck.) Wetmore

This sorediate species is similar and presumably closely related to *P. euploca*, with which it has been partly confused, but is readily distinguished by its polyphyllous thallus formed of smaller and thinner squamules with markedly undulate margins (Büdel & Nash 2002) and carrying one to several apothecia (Fig. 6.1.4 F). These are totally immersed at first but become sessile with a small thalloid rim. The two species share a similar ecology in the study area, thriving on seasonally moist but highly exposed schist surfaces although *P. bolanderi* tends to show at least subtle differences in microhabitat preferences, colonizing the more shaded areas of these exposed surfaces, mainly associated with *Pyrenopsis triptococca*.

So far, *P. bolanderi* has been reported from Australia, SW North America, Brazil, southern Africa, the Middle East (Egea 1989, Büdel 2001), New Guinea (Aptroot 1998), the Baikal Region in Siberia (Makryi 1999) and the Canary Islands (Schultz & van den Boom 2007). It has probably been overlooked in the Iberian Peninsula (Fig. 6.1.5 A).

SPECIMENS EXAMINED – PORTUGAL: **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3363, 308 m, on vertical schist surfaces, 12.09.2011, J. Marques (PO8988-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO8994-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 163 m, on narrow crevices of schist outcrops, 31.03.2010, J. Marques (PO8984-L); Chãs, Quinta da Barca, 29TPF5940, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO8986-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical quartzite surfaces, 01.04.2010, J. Marques (PO8983-L); Vila Nova de Foz Côa, Vale Cabrões, 29TPF5850, 248 m, on vertical schist surfaces, 30.09.2011, J. Marques (PO8985-L). – SPAIN: **Teruel**, Bezas, 30TXK406659, 1260 m, on vertical sandstone surfaces, 03.09.2010, J. Marques (PO8875-L).

Peltula farinosa Büdel

Very similar to *P. euploca* but characterized by a larger thallus up to 22 mm in diam. and markedly pruinose thallus surface (Fig. 6.1.4 G). It was found only in two sites of the Côa River Valley, growing on sheltered to fully exposed schist surfaces. At these sites it seems to be associated with rain-track communities dominated by *P. euploca* and *Glyphopeltis ligustica* (de Lesd.) Timdal. The possibility of association of this species with calcium-rich secondary deposits as mentioned by Büdel & Nash (2002) is coherent with what was observed in the study area, where schist is usually covered with calcium precipitates (T. Aubry & L. Luis, pers. comm.). Previously known from South Africa, Pakistan and SW North America (Büdel & Nash 2002), Namibia (Schultz *et al.* 2009) and the Canary Islands (Schultz & van den Boom 2007) (Fig. 6.1.5 B).

SPECIMEN EXAMINED – PORTUGAL: **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale do Forno, 29TPF5749, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO8989-L).

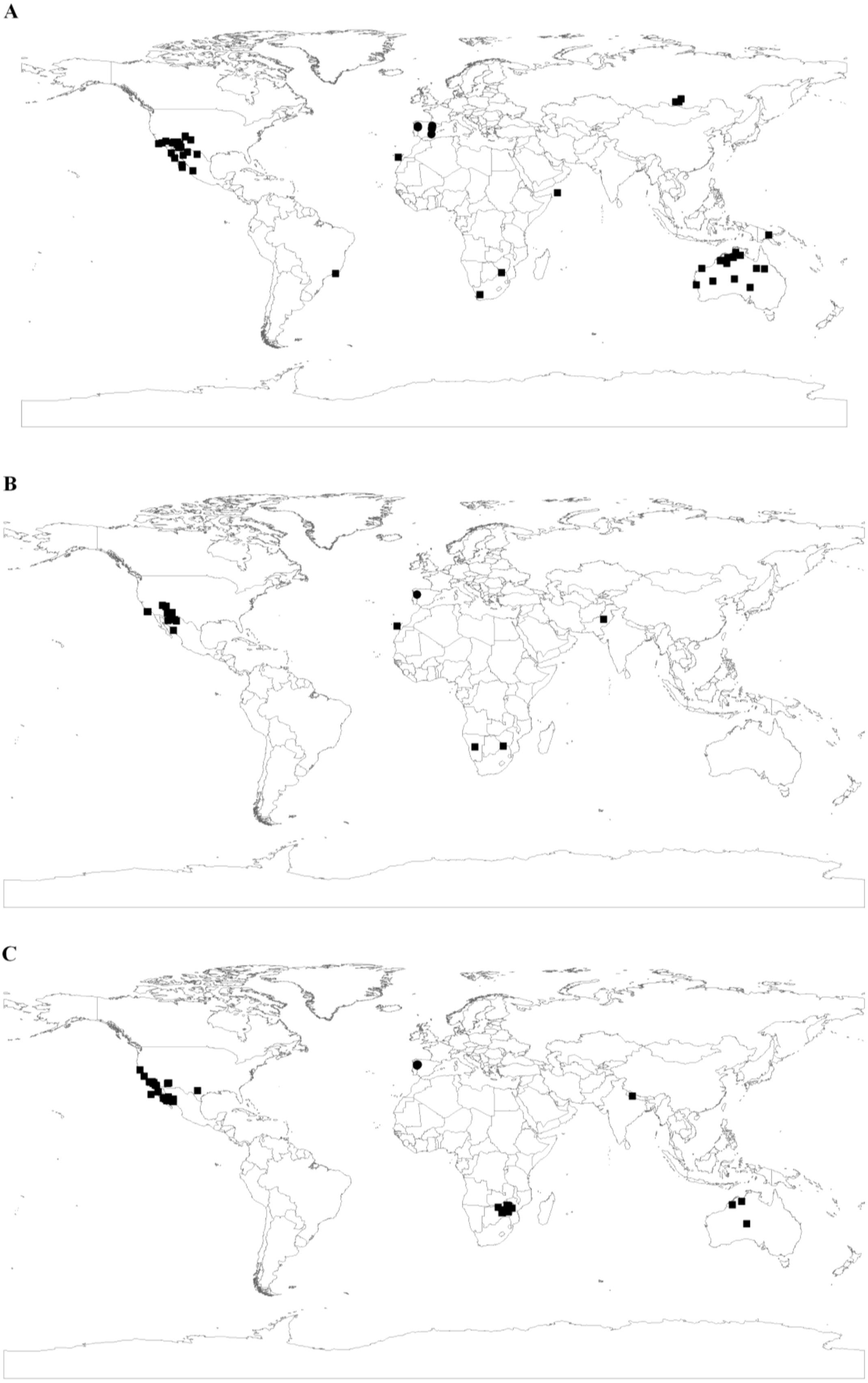

Fig. 6.1.5. World distribution of the three species of *Peltula* new to mainland Europe. A: *Peltula bolanderi*; B: *Peltula farinosa*; C: *Peltula zahlbruckneri*. Circles (●) represent new data resulting from this study. Squares (■) represent data based on the following literature: Büdel (1987), Büdel (2001), Büdel & Nash (2002), Schultz & van den Boom (2007) and Schultz *et al.* (2009).

Peltula zahlbruckneri (Hasse) Wetmore

Composed of distinctively swollen squamules, anchored by a short stalk or umbilicus, dark greenish brown surface with darker to almost black margin. One to several apothecia per squamule, initially punctiform and opening with age, showing a dark reddish brown to more usually black disc (Fig. 6.1.4 H).

One of the rarest species in the study area, an early colonizer of the hard schist and quartzite surfaces, in close contact with thermophilous and heliophilous lichens such as *Acarospora hilaris* (Dufour) Hue, *Dimelaena oreina* (Ach.) Norman, *Caloplaca carphinea* (Fr.) Jatta and others, frequently accompanied by *Peltula placodizans* (Zahlbr.) Wetmore. It was previously known from SW North America, southern Africa, India and Australia (Büdel 2001). The reference to the presence of *P. zahlbruckneri* in Sevilla (Spain) by Rowe & Egea (1985a, 1985b) is erroneous (J. M. Egea, pers. comm.) and was later corrected by Egea (1989), who excluded this species from the checklist of the Iberian Peninsula. However, the Portuguese specimens agree very well with material collected by one of the authors (MS) in California and also match the type of the species also from California which is preserved in herbarium of the Natural History Museum Vienna (W). Therefore, this is the first confirmed record of *P. zahlbruckneri* in Europe (Fig. 6.1.5 C).

SPECIMENS EXAMINED – PORTUGAL: **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on horizontal schist surfaces, 13.05.2009, J. Marques (PO8993-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210 m, on inclined quartzite surfaces, 15.10.2011, J. Marques (PO8994-L). **Vila Nova de Foz Côa**, Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9008-L); Ribeira de Piscos, 29TPF5843, 139 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO8991-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5749, 320 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO8992-L).

The species new for Portugal

Peltula obscurans (Nyl.) Gyeln.

Invariably terricolous in the study area, most collected specimens present sublobulate to lobulate, usually imbricate, squamules anchored by rhizinoids. These lobulate morphotypes closely resemble the description of *Peltula crispatula* (Nyl.) Egea by Egea (1989), namely in the anatomy of medullary tissue and algal layer, and the form of attachment to substrate. This author relies on the attachment organ to distinguish *P. obscurans*, with an umbilicus, from *P. crispatula*, with rhizinoids. The exclusive presence of an umbilicus in *P. obscurans* is not followed by Büdel & Nash (2002) or by Swinscow (1982), who considers that it can be “attached to substrate by umbilicus or hyphae or both”. Therefore, in the current study, the type of attachment organ was not considered a good character to differentiate the species, and is most likely a reflection of ecotypic differences. Additional differences between these species are, according to literature, the paraplectenchymatous medulla and downcurved margins in *P. obscurans*, while in *P. crispatula* the medulla is almost absent or invaded by algae and the margin is clearly ascending (Egea 1989). *Peltula crispatula* is a poorly known species from the northern Sahara and southern Spain and was never observed fertile. Comparisons between the specimens collected and those of *P. crispatula* from the J. M. Egea collection at the Herbarium of the University of Barcelona (BCN) were inconclusive regarding these characters. Therefore we refrain from using the name *P. crispatula* and rely on general thallus morphology and apothecial characters to assign the specimens collected to the name *P. obscurans*. The distinction of the varieties recognized by Wetmore (1970) within *P. obscurans* based on thallus morphology and epihymenium reaction with K, proved to be unfruitful since both varied in the same specimen, as observed by Egea (1989).

The ecology of *P. obscurans* in the study area is quite similar to *P. patellata* with which it is usually found on fully exposed compacted mineral soil. *Peltula obscurans* is distributed in southern Europe, Africa, North and South America, Asia and Australia (Büdel 2001). This is the most western reference of the species for the Iberian Peninsula.

SPECIMENS EXAMINED – PORTUGAL: **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3363, 308 m, on compacted soil between schist outcrops, 12.09.2011, J. Marques (PO8995-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210 m, on compacted soil between quartzite outcrops, 15.10.2011, J. Marques (PO9005-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9000-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on compacted soil between schist outcrops, 17.09.2011, J. Marques (PO9018-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on compacted soil between schist outcrops, 15.10.2010, J. Marques (PO8996-L); Penascosa, 29TPF5941, 163 m, on wide crevices of schist outcrops, 31.03.2010, J. Marques (PO8997-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5749, 320 m, on wide crevices of schist outcrops, 04.05.2011, J. Marques (PO8998-L).

Peltula patellata (Bagl.) Swinscow & Krog

Easily recognized by the orbicular to somewhat lobulate squamules, to about 4 mm diam., with a distinctive thickened rim along the margins and one to few apothecia, frequently occupying the whole squamule. This species is widely distributed in southern Europe, Africa, North and Central America, India and Australia (Büdel 2001). Prior to this study it had been rarely collected in the Iberian Peninsula, but was found to be quite common in the study area, growing on dry and exposed earth-filled crevices or compacted calcareous soil between rock outcrops, and less often spreading towards the neighbouring soft schist surfaces. These data extend the known distribution of the species in the Iberian Peninsula towards the north-west.

SPECIMENS EXAMINED – PORTUGAL: **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on wide crevices of schist outcrops, 13.05.2009, J. Marques (PO9004-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210 m, on compacted soil between quartzite outcrops, 15.10.2011, J. Marques (PO9005-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9006-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on compacted soil between schist outcrops, 17.09.2011, J. Marques (PO9007-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 163 m, on compacted soil between schist outcrops, 31.03.2010, J. Marques (PO8984-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5749, 320 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9020-L).

Peltula placodizans (Zahlbr.) Wetmore

This species is easily recognized by the small placodioid thallus and black capitates soralia on top of the central areoles. According to Egea (1989), it has its optimum in the arid and sub-arid areas of the western Mediterranean. In the Iberian Peninsula it is mainly distributed in south-east Spain, locally penetrating the Eurosiberian region, but never collected in mainland Portugal prior to this study. These records extend the known distribution of the species in the Iberian Peninsula towards the North-west. In the study area it seems to be restricted to the warmer and dryer areas of the Côa River Valley, spreading along fully exposed hard schist and quartzite surfaces.

SPECIMENS EXAMINED – PORTUGAL: **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 309m, on vertical schist surfaces, 14.03.2012, J. Marques (PO9010-L); Muxagata, Monte do Fariseu, 29TPF5844, 263m, on vertical quartzite surfaces, 01.04.2010, J. Marques (PO9008-L); Ribeira de Piscos, 29TPF5843, 139m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9009-L).

Other *Peltula* species in the study area

Peltula euploca (Ach.) Poelt ex Ozenda & Clauzade

Known from all continents apart from Antarctica, it is the most widespread species of the genus, and one of the few previously reported from Portugal, together with *Peltula omphaliza* (Hladun & Llimona 2002-2007). It is quite frequent in the study area, colonizing virtually all sheltered to fully exposed vertical schist surfaces as a dominant species of the rain-track communities described by Llimona & Egea 1985. Typically, a single peltate squamule is up to 12 mm wide (Fig. 6.1.4 A) (Büdel & Nash 2002), but large morphological variation has been described (Egea 1989, Büdel & Nash 2002) and also observed in the study area. These include: 1) a polyphyllous form with squamules that are repeatedly divided into elongated, somewhat to distinctly undulating lobules (Fig. 6.1.4 B); 2) a small form rather close to *P. bolanderi* with squamules around 2-4 mm wide and with dark, sorediate margins (Fig. 6.1.4 C) that is usually but not exclusively associated with typical *P. euploca*; 3) a particularly interesting form, composed of small, closely adnate, sorediate squamules showing a tendency to shape an effigurate thallus (Fig. 6.1.4 D), a phenomenon that has been described by Wetmore (1970) and interpreted as regenerating thalli after mature squamules have become detached from the substratum, frequently accompanied with the well-known placodioid species, *P. placodizans*, and growing on extremely flat and hard rock surfaces; and 4) a frequent form in the study area that shows general similarities with characteristic specimens of *P. euploca* but differs in the somewhat smaller thallus size, the complete absence of soralia and the regular formation of apothecia (Fig. 6.1.4 E).

This variation may be influenced by the developmental stage of the specimens or site-specific factors such as light intensity and moisture availability as well as rock characteristics (Büdel 1987), but a more detailed study on the morphological and genetic variability within this species is required and will be conducted in the near future.

SELECTED SPECIMENS EXAMINED – PORTUGAL: **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9014-L). **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3363, 308m, on vertical schist surfaces, 12.09.2011, J. Marques (PO8988-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9015-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9016-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204m, on vertical schist surfaces, 08.03.2012, J. Marques (PO9017-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 367m, on vertical schist surfaces, 14.03.2012, J. Marques (PO8990-L); Penascosa, 29TPF5941, 163m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9012-L); Vila Nova de Foz Côa, Vale Figueira, 29TPF5845, 218m, on vertical schist surfaces, 13.03.2012, J. Marques (PO8999-L); Vale do Forno, 29TPF5749, 320m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9013-L).

6.1.4 Discussion

As a result of this study, the number of *Peltula* species known in mainland Europe increases from eight to twelve (eleven in the Iberian Peninsula), including the new species, *Peltula lobata*, and *P. bolanderi*, *P. farinosa* and *P. zahbruckneri* as new additions. *Peltula bolanderi* and *P. lobata* were also found among Spanish material raising the number of *Peltula* species known from mainland Spain (Egea 1989) to nine. *Peltula obscurans*, *P. patellata* and *P. placodizans* are here reported for the first time from mainland Portugal. The checklist of Portuguese lichens is thus enlarged from two (Hladun & Llimona 2002-2007) to nine species of *Peltula*.

Given the biogeographical and ecological significance of these findings in the context of both Iberian and European lichen diversity, the Upper Douro region in North-east Portugal may be considered a hotspot for *Peltula* diversity in Europe. It is possible that the Iberian Peninsula, hosting approximately 25% of currently known species of *Peltula* and gathering almost the totality of European species, is a major speciation centre for this genus.

6.1.5 A revised key to the lichen genus *Peltula* in Europe

1. Thallus sorediate 2

1. Thallus lacking soredia 6

2. Thallus areolate, margin effigurate; soralia black or dark grey capitate .... *P. placodizans*

2. Thallus composed of scattered to confluent squamules; soralia different 3

3. Squamules large, (3-)4-22 mm in diameter.. 4

3. Squamules smaller, 1-3 mm in diameter 5

4. Upper surface olive to olive-brown, epruinose; thallus up to 12 mm in diameter with revolute and eventually somewhat lobulate margins; soralia bluish-grey, marginal, occasionally laminal to submarginal; apothecia numerous, with punctiform disks but often absent .... *P. euploca*

4. Upper surface distinctly greyish-pruinose; thallus up to 22 mm in diameter with wavy margins; soralia dark grey to black, marginal; apothecia absent .... *P. farinosa*

5. Squamules distinctly lobulate, fragile, lobules strongly undulate and ascending; margins dissolved into granular soralia .... *P. bolanderi*

5. Squamules roundish, robust, sometimes effigurate; margins regularly sorediate .... *P. euploca* (see discussion)

6. Thallus areolate-squamulose, squamules inflated, dull olive-brown, apothecia punctiform enlarging with age .... *P. zahlbruckneri*

6. Thallus squamulose, peltate or subfoliose, not inflated 7

7. Thallus subfoliose, of elongated, tongue-shaped, slightly branched, ascending and often imbricate lobes; apothecia rare, one or few per squamule, laminal and more ou less sessile, with distinct shallow thalline margin and widely opened, dark red disk .... *P. lobata*

7. Thallus squamulose to peltate, not divided into elongated, tongue-shaped lobes 8

8. Thallus peltate, attached to substrate by central umbilicus; apothecia numerous, punctiform 9

8. Thallus squamulose, attached to substrate by rhizohyphae; apothecia one or few per squamule, enlarging with age 10

9. Thallus of roundish and strongly convex squamules, up to 3 mm in diameter .... *P. omphaliza*

9. Thallus of larger and more or less flat squamules, deeply divided into 3-5 lobes .... *P. rodriguesii*

10. Squamules with a prominent raised and darkened rim .... *P. patellata*

10. Squamule lacking raised margins 11

11. Squamules lobulate, up to 1.5 mm in diameter, with ascending margins; lower cortex of 4-5 layers of large cells .... *P. crispatula*

11. Squamules not lobulate nor ascending; lower cortex of 1-2 layers of small cells 12
12. Spores ellipsoid to cylindrical .... *P. obscurans*
12. Spores globose to subglobose .... *P. obscuratula*

6.1.6 References

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Table 6.1.1. Main diagnostic characters to distinguish among lobate *Peltula* species.

Characters	***P. congregata***	***P. corticola***	***P. imbricata***	***P. auriculata***	***P. lobata***
Thallus	compound	foliose, lobate	lobate	squamulose to subfoliose, lobate	foliose, lobate
	indeterminate	5 cm	1 cm	indeterminate	4 cm
Lobes	subfruticose, sparcely branched	branched	imbricate	ear-like	multi-branched and strongly imbricate
	7.0 x 5.0 mm	2.0 x 0.5 mm	less than 1mm	2.5x1.5 mm	5.0 x 3.0 mm
Lobe margins	flat	bent upwards	slightly raised	with a distinctive rim	bent downwards
Soredia	absent	present	absent	absent	absent
Epinecral layer	present	present	absent	present	present
Upper cortex	absent	absent	present	present	present
Medulla	with numerous small cavities	paraplectenchymatous, of densely interwoven hyphae	of loosely to compact interwoven hyphae	of loosely interwoven hyphae with numerous air spaces, sometimes prosoplectenchymatous	prosoplectenchymatous, of loosely to densely interwoven hyphae, paraplectenchymatous at early stages
Attachment organ	stalk	rhizohyphae	rhizohyphae	umbilicus	stalk
Apothecia	numerous	numerous	numerous	rare	rare
	immersed	adnate	sessile	adnate	adnate
Apothecial disk	punctiform	wide	wide	wide	wide
	-	0.2-0.4 mm	up to 1mm	up to 0.5 mm	0.3-1.3 mm
Ascospores	globose to ellipsoid	ellipsoid to fusiform	ellipsoid	globose	ellipsoid
	3.5-5 x 2.5-3.5 µm	4-5.5 x 1.5-2.2 µm	6-7 x 3 µm	3-4x3-4 µm	6-9 x 3-4 µm
Ecology	saxicolous	corticolous	terricolous	saxicolous	saxicolous and terricolous
Distribution	Southern, Eastern and Western Africa, Korea, Australia	Eastern Africa (Kenya), Arabian Peninsula (Yemen), SW North America	Australia	Venezuela, Guyana, Mexico	Iberian Peninsula
References	Büdel (1987) Büdel (2001)	Büdel (1987) Büdel *et al.* (2007)	Büdel (2001)	Büdel (2002)	-

6.2 Four lichens new to the Iberian Peninsula

The following text will be complemented with information from the PhD thesis of Samantha Fernández-Brime (Fernández-Brime 2012), and adapted accordingly, to be submitted in a joint short communication to The Lichenologist. Only the information related with Portuguese material is therefore included.

6.2.1 Introduction

According to Hladun & Llimona (2002-2007) and Feuerer (2012), the Iberian Peninsula currently comprises 3515 lichen taxa. However, a considerable amount of lichen names can be added to this list as further taxonomical studies have been published since it was last updated (Culberson *et al*. 2011). In the period between 2007 and 2012, at least 33 lichen taxa have been reported for the first time in the Iberian Peninsula, of which 18 corresponded to the description of new species.

Vast areas of the Peninsula are still poorly known and species occurrence on some substrata remains under researched. Such is the case of the schistose environments in the Upper Douro region (north-east Portugal), whose lichenological interest has been demonstrated recently (Marques *et al*. 2013) by a remarkable collection that included one new species and three novelties in mainland Europe. Studies being run almost in parallel and focusing on the lichen diversity of schist outcrops in the Cap Creus peninsula (north-east Spain) have also revealed numerous interesting lichens, including six species previously unknown in the Iberian Peninsula (Fernandez-Brime 2012). The two locations share some important characteristics, including a typically Mediterranean climate and the predominance of intensely folded and faulted metasedimentary rock outcrops (including schist, metagreywacke and quartzite) of same age and similar composition (Druguet 1997, Sousa 1982).

The occurrence of *Acarospora boulderensis* H. Magn., *Dermatocarpon deminuens* Vain. and *Trapeliopsis gymnidiata* Aptroot & Schumm is here briefly updated with newly collected specimens from the Upper Douro region (Portugal). These species are new to the Iberian lichen flora. *Trapeliopsis gymnidiata* is also new to mainland Europe. *Endocarpon simplicatum* (Nyl.) Nyl. has been previously reported from Cap Creus by Fernández-Brime (2012) and together with Portuguese specimens adds to the list of Iberian novelties. Short notes on the main distinguishing characters, ecology and distribution of these species are provided.

6.2.2 The species

Acarospora boulderensis H. Magn.

A species recently resurrected from *Acarospora badiofusca* (Nyl.) Th. Fr., known to occur in central Europe and across temperate North America (Knudsen & Kocourková in press). Easily recognized by the light brown squamulose thallus, non-reactive to K, and wide, usually raised apothecia that sometimes occupy the entire squamule. According to Knudsen & Kocourková (in press) it is distinguished from *A*. *badiofusca* by the robust squamules and large red apothecial disks, besides being more frequently found at lower altitudes, whereas *A*. *badiofusca* is apparently restricted to higher elevations, above 1200 m. The examined specimen was found in one of the most peculiar situations in the Côa River Valley, on a massive north-west facing vertical schist surface probably enriched by secondary calcium deposits, as indicated by the accompanying species - a peculiar mixture of silicolous and calcicolous lichens including *Acarospora hilaris*, *Caloplaca arnoldii* (Wedd.) Zahlbr. ex Ginzb. subsp. *obliterata* (Pers.) Gaya, *Caloplaca flavescens* (Huds.) J. R. Laundon and in agreement with the ecology of the species as described by Knudsen & Kocourková (in press). This record greatly expands the currently known European distribution of the species further west. Rare in the study area, on vertical south-east facing surfaces.

SPECIMENS EXAMINED IN THE UPPER DOURO REGION – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9592-L).

Dermatocarpon deminuens Vain.

Dermatocarpon deminuens belongs to a limited group of long-spored *Dermatocarpon* species with pruinose upper surface (Heidmarsson 1998). Although *D*. *deminuens* is said to differ from the identical *Dermatocarpon bachmannii* Anders in thallus thickness, colour of lower surface, and size of medullary hyphae (Santesson 1939 *apud* Orange 1998), the only consensual character to distinguish between the two species seems to be the colour of lower surface (Orange 1998, Heidmarsson 1998), which is said to be dark brown to black in *D*. *bachmannii* and light to dark brown in *D*. *deminuens*. Full separation of these two species is nevertheless still uncertain (Orange 1998, Heidmarsson 1998). The examined specimens are characterized by a black lower surface, a character for *D*. *bachmannii*, but agree with *D*. *deminuens* (Heidmarsson 1998) in every other character, including lobe size, which can be much less than 10 mm, thallus thickness (0.26–0.38 mm) and completely smooth lower surface. Ecology of examined specimens is also in agreement with that of *D*. *deminuens*, on seepage tracks of large granite boulders in the floodplain of the Tua River (Upper Douro region, north-east Portugal), together with *Peltula zahlbruckneri* and *Pterygiopsis affinis* (A.

Massal.) Henssen. Neither *D*. *deminuens* nor *D*. *bachmannii* have been reported from the Iberian Peninsula before. *Dermatocarpon deminuens* was previously known to occur in Fennoscandia and the British Isles whereas *D*. *bachmannii* is known from northern and central Europe (Heidmarsson 1998, Orange 1998).

SPECIMENS EXAMINED IN THE UPPER DOURO REGION – **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3264, 203m, on vertical granite surfaces, 23.03.2012, J. Marques (PO9481-L). **Mirandela**, Abreiro, Quinta da Pendurada, 29TPF4479, 200m, on vertical granite surfaces, 09.03.2012, J. Marques (PO9482-L).

Fig. 6.2.1. The monosporous asci in *Endocarpon simplicatum*.

Endocarpon simplicatum Nyl.

This species is similar and closely related to *Endocarpon pusillum* with which it often occurs, but the monosporous asci (Fig. 6.2.1) are quite distinctive (Breuss 2002). The examined specimens were found on narrow and wide earth-filled crevices and earth banks between schist outcrops. In Europe, it was previously known only from the eastern Pyrenees and the regions of Languedoc and Provence in southern France (Clauzade & Roux 1985). Besides southern Europe, *Endocarpon simplicatum* also occurs in Australia and SW North America (Breuss 2002, McCarthy 2001).

SPECIMENS EXAMINED – **Alijó**, Carlão, Alto do Vale de Moinho, 29TPF3477, 324m, on wide crevices of schist outcrops, 07.03.2012, J. Marques (PO9593-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9594-L).

Trapeliopsis gymnidiata Aptroot & Schumm

Trapeliopsis gymnidiata is a recently described species from the Macaronesia (Aptroot & Schumm 2012) segregated from *Trapeliopsis wallrothii* (Flörke ex Spreng.) Hertel & Gotth. Schneid. based on differences in the type of propagules and ecological preferences. Instead of isidia, *T*. *gymnidiata* develops gymnidia, a particular type of decorticated propagules that leave crater-like scars on thallus surface after falling off. The examined specimens constitute first records of this species in mainland Europe, although specimens from low altitudes in other Mediterranean areas are likely to be *T*. *gymnidiata* instead of *T*. *wallrothii*. Most examined specimens are sterile but fertile specimens are relatively frequent in the study area. It is usually found terricolous on earth-filled crevices of schist outcrops and compacted mineral soil, overgrowing mosses and other lichens together with *Peltula obscurans* and *Squamarina concrescens* (Müll. Arg.) Poelt.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3264, 203 m, on wide crevices of schist outcrops, 23.03.2012, J. Marques (PO9595-L). **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 538 m, on wide crevices of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9577-L). **Torre de Moncorvo**, Cardanha, Ribeira de Relvas, 29TPF6669, 259 m, on earth banks between schist outcrops, 18.02.2011, J. Marques (PO9353-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9355-L); Canada da Moreira, 29TPF6047, 316 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9354-L); Penascosa, 29TPF5941, 160 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9596-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on horizontal schist surfaces, 11.10.2010, J. Marques (PO9567-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9476-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9356-L); Vale do Forno, 29TPF5748, 297 m, on horizontal schist surfaces, 14.04.2011, J. Marques (PO9514-L).

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6.3 New and interesting lichen records for the Portuguese flora from the Upper Douro region (north-east Portugal)

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Abstract

Thirty five saxicolous and terricolous lichens are here reported from the Upper Douro region (province of Trás-os-Montes e Alto Douro, north-east Portugal) with data on their currently known distribution and regional ecology. *Acarospora charidema*, *Acarospora macrospora* subsp. *murorum*, *Aspicilia crespiana*, *Aspicilia cupreoglauca*, *Aspicilia viridescens*, *Caloplaca arnoldii* subsp. *obliterata*, *Caloplaca rubelliana*, *Endocarpon adscendens*, *Endocarpon loscosii*, *Lichinella nigritella*, *Psorotichia schaereri*, *Pterygiopsis affinis*, *Rhizoplaca maheui*, *Rinodina vezdae*, *Toninia toepferii* and *Verrucaria geophila* are new for mainland Portugal.

6.3.1 Introduction

The Upper Douro region (province of Trás-os-Montes e Alto Douro, north-east Portugal) is well-known as the origin of Port wine production, whose beginnings are set in the late 18th century. The tradition of winemaking in the region is much longer, however, and can be traced back to Roman times. The vineyards have been planted on narrow platforms along the steep hillsides of the Douro and its tributary rivers, supported by walls made out of schist, after breaking of the original bedrock and part of the surrounding outcrops. The resulting landscape was finally classified as World Heritage by UNESCO in 2001. At the easternmost point of this region and extending nearly to the Spanish border, is an area that had previously been granted the same UNESCO classification, after the discovery of what is now considered to be one of the most important and extensive open-air prehistoric rock-art collections worldwide, carved in thousands of exposed vertical schist surfaces. The main area stretches within the boundaries of the Côa Valley Archaeological Park, named after that tributary of the Douro, along the last 22 km of the Côa River Valley and extending into a portion of the Douro River Valley. Some remnants of Prehistoric rock-art can also be found outside the Park, towards the north-west, along the Tua and Sabor River valleys, two main tributaries on the right margin of the Douro.

The Upper Douro region is also quite appealing to ecologists for its unique combination of a harsh climate, diverse geology and complex topography. However, many parts of its territory are yet unexplored by lichenologists, with few exceptions (Paz-Bermúdez *et al.* 2009). In the Côa Valley and surroundings, vineyards are less extensive and natural vegetation has been left more or less untouched between olive and almond plantations.

The effect of lichen growth in the process of open-air rock-art deterioration is currently under study in the Côa Valley Archaeological Park and provided an exceptional opportunity to further access the lichen diversity of the region. As a result of this study, a remarkable lichen collection has been reported recently (Marques *et al.* 2013), comprising three novelties for mainland Europe and one species new to science.

The purpose of this paper is to present additional information on new and interesting lichen records for the Portuguese flora as well as new data on the ecology and distribution of poorly known lichen species in the Iberian Peninsula. Schist outcrops were the main target of this study but some collections were also made in granite and quartzite, which outcrop throughout the region in the vicinity of schist. Calc-silicates may also occur as rather narrow intercalations within the schist and certainly play a major influence on the composition of these lichen assemblages, especially those occurring on crevices, where calcareous soil accumulates thanks to limited precipitation.

6.3.2 Material and methods

Fieldwork was carried out between September 2006 and May 2012 in the Upper Douro region (province of Trás-os-Montes e Alto Douro, north-east Portugal). Collected specimens were analysed morphologically and chemically, following the standard identification methods for lichenized fungi (Smith *et al.* 2009), and deposited in the herbaria of the University of Porto (PO). Specimens provided by the herbaria PO, SANT and BCN were used for comparison. Thallus morphology was examined under a Nikon SMZ1000 stereomicroscope. Internal morphology of the lichen thallus and ascomata were studied from hand cut sections mounted in water observed under a Nikon Eclipse 50i microscope. Ascus structure and amyloid reaction of the ascus wall were observed by adding Lugol's solution directly to sections and squash preparations mounted in water before and after treatment with K (10%). Chemical analysis followed standardized TLC methods (Orange *et al.* 2001). Data on accompanying species was obtained *in situ* or after examination of collections from the same microhabitat. Lichen names are according to Index Fungorum (2013).

6.3.3 The species

Since all specimens were collected in Portugal, province of Trás-os-Montes e Alto Douro, this information is omitted in the following paragraphs. Species that are new records to mainland Portugal are signalled with an asterisk (*). The remaining lichens are second records to the country, new records to the province and rare species in Portugal or the Iberian Peninsula.

Acarospora charidema (Colmeiro) Llimona

Acarospora charidema is a pioneer species described from south-east Spain that was later found in Morocco (Egea & Rowe 1987), formed by robust, moderately convex, squamules, up to 5(-6) mm wide; with numerous apothecia up to 0.5 mm wide (Clauzade *et al.* 1981). *Acarospora charidema* is usually distinguished from *Acarospora epithallina* H. Magn., a juvenile parasite of *A*. *hilaris*, on the basis of life habit, ecology and shape of spores, which are globose in *A*. *epithallina* and ovoid in *A*. *charidema* (Clauzade *et al.* 1981) but bears a possible resemblance to free-living squamules of *A. epithallina* (Crespo *et al.* 1976). Egea & Llimona (1982) also mention the striate margins of squamules for *A*. *charidema*, a character that is present in both examined specimens but not in the specimens assigned to *A*. *epithallina* (bellow). The occurrence of *Acarospora charidema* in the study area as well as in the nearby Spanish province of Zamora (Terrón-Alfonso *et al.* 2000) is quite rare and somewhat inconsistent with the thermo-mediterranean, semi-arid and pioneer character of *A*. *charidema* (Egea & Rowe 1987). *Acarospora epithallina*, on the contrary, is considered a meso-mediterranean species (Egea & Rowe 1987). Further clarification on the distinction between both taxa is needed (Víctor J. Rico, pers. comm.). In the study area, *A*. *charidema* was found on highly exposed vertical schist surfaces together with *Glyphopeltis ligustica*, *Lichinella cribellifera* (Nyl.) P. P. Moreno & Egea, *Peltula euploca*, *P*. *placodizans* and *P*. *zahlbruckneri*.

SPECIMENS EXAMINED – **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9042-L). **Vila Nova de Foz Côa**, Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9041-L).

Acarospora epithallina H. Magn.

Last reported from Portugal by Paz-Bermúdez *et al.* (2009) and relatively frequent in the study area where it can be easily recognized by the small, up to 3 mm wide, convex squamules always growing as a parasite on *Acarospora hilaris*. According to Crespo *et al.* (1976), the occurrence of this species could be indicative of more hydric and eutrophic microhabitats than typically occupied by the host *A*. *hilaris*. Our data is insufficient to support such distinction but, contrarily to what was described by these authors, specimens collected in the study area were not exclusively on horizontal surfaces, colonizing both vertical and horizontal schist and quartzite surfaces.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9049-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9046-L). **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9042-L); Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9047-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9048-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9043-L); Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9044-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9045-L).

Acarospora macrospora subsp. *murorum* (A. Massal.) Clauzade & Cl. Roux

Rare, on dry and exposed earth-filled crevices or compacted soil between schist outcrops. The large spores, thallus with the same colour as the substrate and prominent thalline margin in apothecia are distinctive characters (Clauzade *et al.* 1981). Before this study, it had only been reported in Portugal from the archipelago of the Azores by Tavares (1944).

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 330 m, on wide crevices of schist outcrops, 29.10.2010, J. Marques (PO9050-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on narrow crevices of schist outcrops, 09.05.2009, J. Marques (PO9051-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9052-L).

Agonimia opuntiella (Buschart & Poelt) Vězda

Easily overlooked due to its small size but quite frequent in the study area among chasmolithic and epigeic lichen communities. It was erroneously cited as a first record for Portugal by Paz-Bermúdez *et al.* (2009) as it had already been reported from Nazaré (van den Boom 2006). It is also known from several localities in the Algarve (van den Boom & Giralt 2012).

SELECTED SPECIMENS EXAMINED – **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9005-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 162 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO8997-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9057-L); Muxagata, Fariseu, 29TPF5844, 127 m, on wide crevices of schist outcrops, 01.04.2010, J. Marques (PO9053-L); Ribeira de Piscos, 29TPF5843, 150 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9054-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9055-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9056-L).

Aspicilia crespiana V. J. Rico

A species described by Rico (1999) from material collected in several Spanish provinces and in Sardinia (Italy), having some morphological affinities with *Aspicilia contorta* subsp. *hoffmanniana* but developing into a clearly squamulose thallus with a dense network of rhizomorphs, on saxicolous mosses and rock crevices of exposed siliceous rocks. In the study area it was found on both granite and schist but fertile specimens were only found in more humid situations, overgrowing mosses.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 13.10.2011, J. Marques (PO9078-L). **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on narrow crevices of schist outcrops, 13.05.2009, J. Marques (PO9074-L). **Carrazeda de Ansiães**, Castanheiro, Praia fluvial, 29TPF3466, 136 m, on vertical schist surfaces, 12.10.2012, J. Marques (PO9080-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9077-L). **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9081-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5846, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9075-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9076-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 196 m, on wide crevices of schist outcrops, 02.05.2011, J. Marques (PO9082-L).

Aspicilia cupreoglauca B. de Lesd.

Rare in the study area but readily distinguished by the orange-brown colour of the thallus reacting K^+ yellow to red.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9073-L).

Aspicilia viridescens (A. Massal.) Hue

A member of the *Aspicilia contorta* group similar to *A. contorta* subsp. *hoffmanniana* in the development of a continuous thallus. Although not widely accepted as a separate species, according to Ozenda & Clauzade (1970) it differs from typical *A. contorta* subsp. *hoffmanniana* in the colour of thallus, which is greyish green or yellowish green and intensified by the action of acids. Relatively rare in the study area, on north- to north-west facing schist surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9083-L).

Caloplaca arnoldii (Wedd.) Zahlbr. ex Ginzb. subsp. *obliterata* (Pers.) Gaya

A new combination recently incorporated in the *Caloplaca saxicola* group (Gaya 2009). The examined specimen falls within the range of the "*Caloplaca saxicola* subsp. *obliterata*" morphotype, as defined by the same author, including a slightly pruinose thallus that is dusty orange to ochraceous, or deep orange where the pruina is absent; formed by well delimited rosettes, with well developed marginal lobes broadened at the apices; and crowded apothecia where the colour of margin is lighter than disk. Found only once in the study area, on a vertical schist surface facing south-east, close to a small watercourse.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on inclined schist surfaces, 30.03.2010, J. Marques (PO9084-L).

Caloplaca grimmiae (Nyl.) H. Olivier

The only record of this species for Portugal, previous to this study, is the one by van den Boom & Jansen (2002), as *Caloplaca congrediens* Steiner, found at the higher altitudes of Serra da Estrela. Most collected specimens in the study area were growing as parasites on *Candelariella vitellina* (Hoffm.) Müll. Arg., but one (PO9086-L) was also growing upon *Candelariella coralliza* (Nyl.) H. Magn. The following records considerably extend the known distribution of the species in Portuguese territory.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on *Candelariella coralliza* on horizontal schist surfaces, 13.05.2009, J. Marques (PO9086-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on *C. vitellina* on vertical schist surfaces, 15.05.2007, J. Marques (PO9537-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on *C. vitellina* on vertical schist surfaces, 09.05.2009, J. Marques (PO9375-L).

Caloplaca rubelliana (Ach.) Lojka

Readily distinguished by the dark orange apothecia sunken in a thin salmon orange thallus. Rare in the study area, found only once on a south-east facing schist surface.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5941, 156 m, on vertical schist surfaces, 05.07.2012, J. Marques (PO9085-L).

Caloplaca squamuloisidiata van den Boom & V. J. Rico

This is a recently described species, known only from the Iberian Peninsula (van den Boom & Rico 2006, Paz-Bermúdez *et al.* 2009) with an unsettled phylogenetic position although morphologically fitting *Caloplaca cerina* group (Šoun *et al.* 2011). It can be recognized by the greyish green subsquamulose to weakly placodioid thallus with abundant branched coralloid isidia appearing from small erect squamules. It is easily overlooked due to small size, discrete thallus colour and peculiar habitat, on sheltered and often slightly overhung schist, quartzite and granite surfaces. The assemblage of accompanying species in the Upper Douro region is more similar to the one mentioned for the type locality in Spain than to the previously described from northern Portugal (van den Boom & Rico 2006), and includes *Acarospora epithallina*, *A. hilaris*, *Caloplaca carphinea*, *Caloplaca demissa* (Flot.) Arup & Grube and *Solenopsora vulturiensis* A. Massal.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical schist surfaces, 14.10.2011, J. Marques (PO9091-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9092-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Amendoal, 29TPF5846, 330 m, on vertical schist surfaces, 29.09.2010, J. Marques (PO9090-L).

Endocarpon adscendens (Anzi) Müll. Arg.

Composed of broadly ascending to imbricate squamules loosely attached to substrate through a pale brown lower surface that lacks rhizomorphs. Very rare in the study area, overgrowing mosses on vertical schist surfaces along the riverbanks of the main watercourses.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9093-L).

Endocarpon loscosii Müll. Arg.

This species is similar to *Endocarpon adscendens* in thallus shape and colour of lower surface, but differs in the development of pale robust rhizomorphs. Rare in the study area, overgrowing mosses on periodically submerged granite boulders associated with temporary riverbeds together with *Solenopsora vulturiensis* and *Peltula lobata*. The examined specimens constitute second records for the Iberian Peninsula.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical schist surfaces, 24.03.2012, J. Marques (PO9095-L); Carlão, Vale do Moinho, 29TPF3477, 203 m, on vertical schist surfaces, 7.03.2012, J. Marques (PO9096-L). **Carrazeda de Ansiães**, Castanheiro, Praia fluvial, 29TPF3466, 136 m, on vertical schist surfaces, 12.10.2012, J. Marques (PO9097-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2012, J. Marques (PO9094-L).

Epiphloea terrena (Nyl.) Trevis.

This species belongs to a poorly-known genus of family Heppiaceae characterized by muriform spores and comprising only two species formerly included in Collemataceae. *Epiphloea terrena* differs from the similar *Epiphloea byssina* (Hoffm.) Henssen & P. M. Jørg. in the well-developed, squamulose thallus that is paraplectenchymatous in section. Easily overlooked due to its small size, it was twice reported from Portugal by Sampaio (1921) as *Amphidium terrenum* Nyl. and more recently by van den Boom & Giralt (2012). It is elsewhere known only from a few localities in the mediterranean region (Nimis 1993), the closest to the study area being from south-east Spain (Hladun & Llimona 2002-2007). It is highly dependent on soil stability and relatively rare in the study area, on horizontal schist surfaces where soil accumulates and earth banks between schist outcrops.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on earth banks between schist outcrops, 24.03.2012, J. Marques (PO9127-L); Carlão, Alto do Vale de Moinho, 29TPF3477, 324 m, on earth banks between schist outcrops, 07.03.2012, J. Marques (PO9128-L). **Torre de Moncorvo**, Cardanha, Ribeira de Relvas, 29TPF6669, 259 m, on earth banks between schist outcrops, 18.02.2011, J. Marques (PO9129-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 302 m, on wide crevices of schist outcrops, 30.09.2011, J. Marques (PO9130-L); Vale do Forno, 29TPF5748, 297 m, on horizontal schist surfaces with soil accumulation, 30.03.2010, J. Marques (PO9131-L).

Glyphopeltis ligustica (B. de Lesd.) Timdal

Previously included in genus *Psora*, it is characterized by the large, pale brown squamules, up to 10 mm wide, centrally attached by an umbilicus; and the black marginal apothecia. In the study area it is quite frequent, growing directly on exposed vertical schist surfaces as a characteristic species of rain-track communities dominated by *Peltula euploca*. It has been reported from the Mediterranean region, Madeira, Morocco and South Africa (Nimis 1993) but this is only the second record from mainland Portugal, after having been found in the valley of River Sabor (Paz-Bermúdez *et al.* 2009).

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9133-L). **Macedo de Cavaleiros**, Lagoa, Casas, 29TPF8587, 345 m, on vertical schist surfaces, 05.09.2006, J. Marques (PO9134-L). **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9135-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques, (PO9136-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques, (PO9137-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical quartzite surfaces, 01.04.2010, J. Marques (PO9008-L); Ribeira de Piscos, 29TPF5843, 150 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9138-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9139-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9140-L); Vale do Forno, 29TPF5748, 301 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9013-L); Vale de José Esteves, 29TPF5949, 146 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9141-L).

Koerberia sonomensis (Tuck.) Henssen

Easily distinguished by the rosulate thallus with narrow lacinia and striate surface. Apothecia are dark brown to black with a persistent thalline margin. Once reported from Póvoa de Lanhoso in the province of Minho by Sampaio (1924) as *Koerberia lusitanica* Samp. and later from Serra da Estrela by Jansen (1993). However, van den Boom & Jansen (2002) realized that the specimen from Serra da Estrela had been mistakenly identified and actually belonged to *Polychidium muscicola* (Sw.) Gray. The references provided by Burgaz & Martinez (2003) for the province of Trás-os-Montes e Alto Douro are also erroneous as they refer to *Koerberia biformis* A. Massal. *Koerberia sonomensis* is one of the rarest species in the study area, growing on vertical schist surfaces facing south. The examined specimen therefore represents a second record for Portugal and the first for the province of Trás-os-Montes e Alto Douro, extending the currently known distribution of the species in Portugal further north-east.

SPECIMENS EXAMINED – **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9132-L).

Lecanora pseudistera Nyl.

This species had been said by Sampaio (1917) to occur in the nearby locality of Barca d'Alva, was recently found in Nazaré (van den Boom 2006), the Algarve (van den Boom & Giralt 2012) and is quite frequent in the study area colonizing both north-west and south-east facing vertical schist surfaces. It often proliferates towards the narrow crevices of south-east facing schist surfaces, together with *Caloplaca subsoluta* (*C*. *irrubescens* misapplied by auct. iber.) and *C*. *pellodella*, in addition to *Aspicilia crespiana*, *Peltula obscurans* and *Toninia cinereovirens* (Schaer.) A. Massal.

SPECIMENS EXAMINED – **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9149-L). **Vila Nova de Foz Côa**, Castelo melhor, Penascosa, 29TPF5941, 164 m, on narrow crevices of schist outcrops, 15.05.2007, J. Marques (PO9142-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on narrow crevices of schist outcrops, 09.05.2009, J. Marques (PO9143-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5941, 110 m, on wide crevices of schist outcrops, 08.02.2009, J. Marques (PO9144-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9145-L); Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9146-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal surfaces above schist outcrops, 08.05.2009, J. Marques (PO9147-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9148-L).

Lichinella cribellifera (Nyl.) P. P. Moreno & Egea

Characterized by the foliose, polyphyllous thallus with radial folds in the upper surface and attached to the substrate by an umbilicus. According to Moreno & Egea (1992) it is one of the most typical species of rain-track communities in exposed siliceous rocks. It was previously reported for Portugal, from the province of Minho (Moreno & Egea 1992). The examined specimens represent new records for the province of Trás-os-Montes e Alto Douro, extending the currently known distribution of the species in Portugal further east.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 24.03.2012, J. Marques (PO9099-L); Carlão, Praia fluvial do Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 07.03.2012, J. Marques (PO9100-L). **Mirandela**, Abreiro, Quinta da Pendurada, 29TPF4479, 200 m, on vertical granite surfaces, 09.03.2012, J. Marques (PO9101-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 309 m, on vertical schist surfaces, 14.03.2012, J. Marques (PO9102-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO8983-L); Ribeira de Piscos, 29TPF5843, 136 m, on inclined schist surfaces, 09.05.2009, J. Marques (PO9589-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on wide horizontal crevices of schist outcrops, 03.05.2011, J. Marques (PO9150-L); Vale de José Esteves, 29TPF5949, 161 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9104-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9103-L).

Lichinella nigritella (Lettau) P. P. Moreno & Egea

Similar to *L. cribellifera*, however the deeply branched lobes, often dissected into small lobules and globose isidia are distinctive (Moreno & Egea 1992).

SPECIMENS EXAMINED – **Alijó**, Carlão, Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 07.03.2012, J. Marques (PO9100-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 330 m, on vertical schist surfaces, 14.03.2012, J. Marques (PO9105-L); Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9106-L); Vila nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9107-L).

Lichinella stipatula Nyl.

This is the most ubiquitous species of the genus (Schultz 2007), and a recurrent element of rain-track communities on both siliceous and calcareous rocks (Moreno & Egea 1992). In the study area it also occurs among the terricolous lichen communities that colonize the narrow crevices of vertical schist surfaces. It is separated from *Lichinella robusta* Henssen, known to occur in the southerly province of Alentejo (Henssen *et al.* 1985), by the absence of a robust hyphal strand in the centre of the thallus and by forming compact, irregularly branched, dwarf-fruticose cushions (Moreno & Egea 1992). *Lichinella stipatula* was recently reported from Algarve by van den Boom & Giralt (2012) and is here reported for the first time from the province of Trás-os-Montes e Alto Douro thus extending the known distribution of this species in Portugal considerably further north.

SPECIMENS EXAMINED – **Mogadouro**, Brunhoso, Ribeiro de Juncaínhos, 29TPF8380, 473 m, on vertical surfaces of basic vulcanite rock outcrops, 17.02.2011, J. Marques (PO9171-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9172-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9173-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 310 m, on vertical schist surfaces, 07.04.2011, J. Marques (PO9174-L); Penascosa, 29TPF5846, 164 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9175-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9176-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on wide horizontal crevices of schist outcrops, 09.05.2009, J. Marques (PO9177-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide horizontal crevices of schist outcrops, 08.02.2006, J. Marques (PO9055-L); Foz do Côa, 29TPF5849, 187 m, on wide horizontal crevices of schist outcrops, 03.05.2011, J. Marques (PO9150-L).

Placidium imbecillum (Breuss) Breuss

Although very common in the Iberian Peninsula it was not known to occur in Portugal until Prieto *et al.* (2010) reported it for the first time based on material collected in the province of Beira Litoral (north-west Portugal). Specimens with intermediate features are very difficult to distinguish from *P. subrufescens* (Breuss) Breuss but we relied on the length of conidia (never exceeding 5 μm) to confirm the identity of examined specimens. These constitute second records for Portugal and first records for the province of Trás-os-Montes e Alto

Douro, confirming the presence of *P. imbecillum* at the westernmost areas of the Iberian Peninsula. It was found growing on soil and horizontal crevices together with *Squamarina concrescens* and *Massalongia carnosa* (Dicks.) Körb.

SPECIMENS EXAMINED – **Torre de Moncorvo**, Felgar, Ribeira da Sardinha, 29TPF5946, 268 m, on narrow crevices of schist outcrops, 25.04.2009, J. Marques (PO9151-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 315 m, on earth banks between schist outcrops, 07.04.2011, J. Marques (PO9152-L); Vila Nova de Foz Côa, Quinta das Tulhas, 29TPF5949, 135 m, on earth banks between schist outcrops, 17.03.2012, J. Marques (PO9153-L); Vale Cabrões, 29TPF5750, 302 m, on earth banks between schist outcrops, 30.09.2011, J. Marques (PO9130-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9154-L).

Porocyphus coccodes (Flot.) Körb.

According to Moreno & Egea (1994), the genus *Porocyphus* is in need of a thorough revision, given its immense variability. *Porocyphus coccodes* is characterized by the combination of a dark green-olive thallus, composed of very uneven and coralloid areoles, apothecia that are initially immersed and globose and finally stalked with a thalline margin; and 8-spored asci. Last reported from Portugal by Sampaio (1922) as *Porocyphus furfurellus* (Nyl.) Forssel. New for the province of Trás-os-Montes e Alto Douro.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9108-L).

Psora gresinonis B. de Lesd.

A rare species known to occur in mediterranean Europe and Macaronesia, as well as central Asia (Timdal 2010). The small, concave, dark olivaceous brown squamules, with smooth to crenulate edges, paler than thallus, and the presence of norstictic acid in the medulla are diagnostic (Timdal 1984, 2010). It was previously reported from Portugal by van den Boom & Giralt (1996, 2012). In the study area it is relatively frequent on the compacted mineral soil that accumulates on horizontal surfaces and stable earthbanks, usually acompanied by *Peltula obscurans*, *Endocarpon pusillum* and, less frequently, *Epiphloea terrena*.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo melhor, Canada do Amendoal, 29TPF5846, 344 m, on horizontal schist surfaces, 30.03.2012, J. Marques (PO9541-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on horizontal schist surfaces, 11.10.2010, J. Marques (PO9543-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 320 m, on horizontal schist surfaces, 15.10.2010, J. Marques (PO9494-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9109-L; PO9479-L); Vermelhosa, 29TPF5950, 147 m, on horizontal schist surfaces, 14.10.2010, J. Marques (PO9542-L).

Psorotichia schaereri (A. Massal.) Arnold

A widespread species known to occur in the European and American continents, but rare in the study area, found only in one locality on vertical schist surfaces facing south-east, and among other lichens of rain-track communities. The granulose-areolate thallus and apothecia with prominent margins are diagnostic. In the Iberian Peninsula this species is known to occur in several Spanish provinces (Moreno & Egea 1994) but not in Portugal, before this study.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9098-L).

Pterygiopsis affinis (A. Massal.) Henssen

This species is easily distinguished from any other crustose Lichinaceae by the effigurate, lobulate thallus, multispored asci and small globose to ellipsoid spores (Moreno & Egea 1994). A pink pigment is usually present in the epithecium and himenium. In the study area it was found colonizing slightly inclined to fully vertical and exposed rain-track surfaces, of both schist and granite outcrops. In the Iberian Peninsula this species was previously reported only from the Spanish province of Murcia (Hladun & Llimona 2002-2007).

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9014-L). **Alijó**, Amieiro, Amieiro, 29TPF3471, 310 m, on inclined granite surfaces, 24.03.2012, J. Marques (PO9110-L). **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3264, 203 m, on vertical schist surfaces, 23.03.2012, J. Marques (PO9111-L). **Mirandela**, Abreiro, Quinta da Pendurada, 200 m, 29TPF4479, on inclined granite surfaces, 09.03.2012, J. Marques (PO9112-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 310 m, on vertical schist surfaces, 07.04.2012, J. Marques, PO9113-L; Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9108-L).

Rhizoplaca maheui (Hue) Gómez-Bolea & M. Barbero

A very detailed description of this species, originally described from Spain as *Polycauliona maheui* Hue and conspecific with *Omphalodina bullata* Follmann & A. Crespo, is provided by Gomez-Bolea & Barbero (2009). Fully developed specimens are typically pulvinate and attached to substrate by an umbilicus. Examined specimens and field observations show that, at early stages of development, the thallus is somewhat placodioid with characteristic bullate lobes in the centre and marginal lobes slightly inflated. Both marginal and central lobes are weakly attached to the substrate and either deformed by contact with each other or completely obscured by clusters of apothecia. Usually accompanying *Acarospora hilaris* and *Caloplaca demissa* in vertical schist and granite surfaces, but also found on a remarkably large granite boulder among rain track communities dominated by *Pterygiopsis affinis* and *Peltula zahlbruckneri*.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on inclined granite surfaces, 13.10.2011, J. Marques (PO9155-L). **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9158-L). **Mirandela**, Abreiro, Quinta da Pendurada, 29TPF4479, 200 m, on inclined granite surfaces, 09.03.2012, J. Marques (PO9157-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9156-L).

Rinodina vezdae H. Mayrhofer

Morphological and chemically identical to *Rinodina oxydata* (A. Massal.) A. Massal., with which it often occurs on vertical south-east facing schist surfaces and respective crevices. It differs in the slightly larger spores, reaching 30-16 µm in size but doubts remain regarding the separation of both species (Giralt 2001).

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9011-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 160 m, on wide crevices of schist outcrops, 15.05.2007, J. Marques (PO9305-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on wide crevices of schist outcrops, 08.06.2006, J. Marques (PO9306-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 05.07.2012, J. Marques (PO9307-L); Vale de José Esteves, 29TPF5949, 190 m, on narrow crevices of schist outcrops, 08.05.2009, J. Marques (PO9308-L).

Squamarina concrescens (Müll. Arg.) Poelt

This isidiate species is one of the most conspicuous terricolous lichen in the study area but was last reported from Portugal by Tavares (1956 *apud* Hladun & Llimona 2002-2007) as *Lecanora concrescens* (Müll. Arg.) Zahlbr. At present it is also known from other arid regions in the Algarve (van den Boom & Giralt 2012), Spain (Málaga) and Italy (Timdal 1991). It grows on the compacted soil that accumulates in wide crevices and earth banks between schist outcrops.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on wide horizontal crevices of schist outcrops, 13.05.2009, J. Marques (PO9159-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9160-L); Brunhoso, Ribeiro de Juncaínhos, 29TPF8380, 473 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9161-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9162-L); Ponte de Remondes, 29TPF8385, 200 m, on wide crevices of schist outcrops, 11.05.2009, J. Marques (PO9163-L). **Vila Nova de Foz Côa**, Castelo melhor, Canada do Amendoal, 29TPF5846, 344 m, on horizontal surfaces above schist outcrops, 30.03.2010, J. Marques (PO9164-L); Penascosa, 29TPF5941, 160 m, on wide crevices of schist outcrops, 15.05.2007, J. Marques (PO9165-L); Muxagata, Fariseu, 29TPF5844, 127 m, on wide crevices of schist outcrops, 01.04.2010, J. Marques (PO9166-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9167-L); Vale de José Esteves, 29TPF5949, 161 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9168-L).

Toninia opuntioides (Vill.) Timdal

Erroneously cited as a first record for Portugal by Paz-Bermúdez *et al.* (2009) since Timdal (1991) had already referred several specimens found in Lisbon area. It is separated from the closely related *Toninia sedifolia* (Scop.) Timdal by the flat, rounded squamules, resembling the cladodes of *Opuntia* plants. Relatively rare in the study area on wide crevices of schist outcrops and schist walls.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9115-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 301 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9114-L).

Toninia cinereovirens (Schaer.) A. Massal.

Erroneously cited as a first record for mainland Portugal (Paz-Bermúdez *et al.* 2009) since Coutinho (1916) had already referred several Portuguese herbarium specimens, but very common in the study area on narrow crevices of both vertical and inclined schist surfaces facing south, together with *Aspicilia crespiana*, *Caloplaca subsoluta* (*C*. *irrubescens* misapplied by auct. iber.) and *Peltula obscurans*.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on narrow crevices of schist surfaces, 13.05.2009, J. Marques (PO9120-L). **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on wide crevices of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9121-L). **Vila Nova de Foz Côa**, Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9122-L); Ribeira de Piscos, 29TPF5843, 130 m, on narrow crevices of quartzite outcrops, 09.05.2009, J. Marques (PO9123-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9125-L); Penascosa, 29TPF5941, 160 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9124-L).

Toninia squalida (Ach.) A. Massal.

Very similar to *Toninia cinereovirens* but the uniform thallus colour and large acicular spores are distinctive (Timdal 1991). In fact, in the study area it acts as an eco-vicariant species of *Toninia cinereovirens*, colonizing more sheltered schist outcrops, usually facing north, directly on rock or overgrowing saxicolous mosses. Last reported from Portugal by van den Boom & Jansen (2002) and van den Boom & Giralt (2012) and from the province of Trás-os-Montes e Alto Douro by Timdal (1991).

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, on wide crevices of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9116-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9094-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 344 m, on narrow crevices of schist outcrops, 30.03.2010, J. Marques (PO9117-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9118-L); Vale do Forno, 29TPF5748, 258 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9119-L).

Toninia toepfferi (Stein) Navàs

Reported here for the first time from mainland Portugal, it was previously known from Macaronesia, including Madeira and the Azores (Barreno *et al.* 1984), and from a few places along the Mediterranean basin, namely in southern Spain (Barreno *et al.* 1984), Italy (Nimis 1993), Greece (Breuss 1989) and eastern Turkey (Breuss & John 2004). In the study area, it grows on stable compacted soil accumulated in the wide crevices of schist outcrops and schist walls built in the vicinity of these outcrops. A few specimens are moderately pruinose in some parts of thallus and apothecia, a character for *Toninia physaroides* (Opiz) Zahlbr., the other pseudocyphellate species of the genus. In such cases, the distinct, white punctiform pseudocyphellae in contrast with the brown-olive bullate squamules, as well as the dark reddish brown colour of the upper part of hypothecium and inner part of exciple (Timdal 1991) were considered distinctive.

SPECIMENS EXAMINED – **Alijó**, Carlão, Alto do Vale de Moinho, 29TPF3477, 324 m, on vertical schist surfaces, 07.03.2012, J. Marques (PO9072-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2011, J. Marques (PO9067-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9069-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9063-L); Soutelo, 29TPF8886, on schist walls, 11.05.2009, J. Marques (PO9064-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 344 m, on horizontal surfaces of schist outcrops, 30.03.2010, J. Marques (PO9068-L); Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9058-L); Ribeira de Piscos, 29TPF5843, 136 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9062-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9065-L); Foz do Côa, 29TPF5849, 187 m, on wide crevices of schist outcrops, 03.05.2011, J. Marques (PO9070-L); Vale Cabrões, 29TPF5750, on earth banks between schist outcrops, 30.09.2011, J. Marques (PO9126-L); Vale de José Esteves, 29TPF5949, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9071-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9066-L).

Toninia tristis (Th. Fr.) Th. Fr. subsp. *pseudotabacina* Timdal

The species is easily distinguished by the dark brown, bullate squamules with pores on the surface. The subspecies is characterized by simple ellipsoid spores, green epithecium and terricolous habit. In mainland Portugal it was previously known only from Coimbra (Timdal 1991) and the Algarve (van den Boom & Giralt 2012) and is quite rare in the study area, on earth-filled crevices or compacted mineral soil between schist outcrops.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 330 m, on wide crevices of schist outcrops, 29.09.2010, J. Marques (PO9060-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 196 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9061-L).

Vahliella leucophaea (Vahl) P. M. Jørg.

Last reported from Portugal by Tavares (1965) as *Pannaria microphylla* (Sw.) Nyl. and known to occur in the Atlantic provinces of Minho and Douro Litoral (Carballal *et al.* 2010) but very rare in the study area, found at very low altitudes, on moist and shaded vertical schist surfaces in the riverbanks of River Tua possibly compensating for the low relative humidity and high temperatures that characterize the main valleys. New for the province of Trás-os-Montes e Alto Douro.

SPECIMENS EXAMINED – **Alijó**, Carlão, Vale do Moinho, 29TPF3477, 200 m, on vertical schist surfaces, 18.09.2011, J. Marques (PO9169-L). **Carrazeda de Ansiães**, Castanheiro, Praia fluvial, 29TPF3466, 136 m, on vertical schist surfaces, 12.10.2012, J. Marques (PO9170-L).

Verrucaria geophila Zahlbr.

A terricolous species with most currently known localities in southern Europe (Breuss 1989, Breuss & John 2004, Nimis 1993, Spribille *et al.* 2006) but also found in central Europe and Fennoscandia (Pykälä 2007) as a rare colonizer of calcareous soils. Among the terricolous species of the genus it differs in the combination of large ovoid spores and development of an involucrellum that completely encloses the excipulum (Breuss 1989, Clauzade & Roux 1985). The collected specimens are characterized by relatively large perithecia (ca 0.35–0.5 mm) and ascospores of 25–35 ×10–15 µm, and by a well developed light brown, cracked-areolate thallus. Rare in the study area but probably overlooked since it is well disguised among the brown soil with *Endocarpon pusillum*.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on wide crevices of schist outcrops, 01.04.2010, J. Marques (PO9058-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9059-L).

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6.4 Annotated catalogue of the saxicolous and terricolous lichens on rock outcrops in the Upper Douro region

The following pages provide a complete list of the lichen taxa found on the rock outcrops of the study area as a result of field collections carried between September 2006 and May 2012. A total of 205 lichen taxa were identified among a collection of 1500 specimens which are now deposited in the herbarium of the University of Porto (PO), greatly adding to the knowledge of the still relatively incompletely known Portuguese lichen flora. These taxa belong to 67 genera, among which the most species-rich is by far *Caloplaca* represented by 21 taxa, followed by *Lecanora*, *Leptogium* and *Xanthoparmelia*, represented by 10 taxa each. From the systematic point of view, important genera were found to be *Acarospora*, *Caloplaca* and *Peltula* with numerous novelties of Iberian and European relevance: *Peltula lobata* is described as new to science (Chapter 6.1); *Peltula bolanderi*, *P. farinosa*, *P. zahlbruckneri* and *Trapeliopsis gymnidiata* are reported for the first time from mainland Europe (Chapters 6.1 and 6.2). *Acarospora boulderensis*, *Endocarpon simplicatum* and *Dermatocarpon deminuens* are new for the Iberian Peninsula (Chapter 6.2). *Acarospora charidema*, *Aspicilia crespiana*, *A. cupreoglauca*, *A. viridescens*, *Caloplaca arnoldii* subsp. *obliterata*, *C. cancarixiticola*, *C. crenulatella*, *C. rubelliana*, *Endocarpon adscendens*, *E. loscosii*, *Leptogium plicatile*, *Lichinella nigritella*, *Peltula obscurans*, *P. patellata*, *Pertusaria flavicans* Lamy, *Pertusaria leucosora* Nyl., *Psorotichia schaereri*, *Pterygiopsis affinis*, *Rhizoplaca maheui*, *Rinodina vezdae*, *Verrucaria geophila* are new for Portugal (Chapters 6.2 and 6.3). *Acarospora macrospora* subsp. *murorum*, *Peltula placodizans* and *Toninia toepferii* are new for mainland Portugal (Chapters 6.1 and 6.3). Some additional unreported species were recognized among the examined material, especially in the genera *Acarospora*, *Caloplaca* and *Pterygiopsis*, but these have been left out due to their uncertain identity, until further studies.

Taxa are listed alphabetically, with references to microhabitat according to the scheme presented in Fig. 6.4.1, as well as preferred orientation and frequency in the study area. The collecting was quite extensive, and a considerably larger amount of material was examined than the rather short selection of examined specimens would indicate. Information about species frequency is complemented by field observations and may bring some subjectivity attached to it. The most interesting and noteworthy taxa, including rare and new species, were already treated in previous chapters with notes on the most distinguishing characters and are therefore referred briefly. Notes on the ecology and distribution of taxa not treated in those chapters are additionally provided. Schistose environments were the main target of this study but some exceptional specimens collected from granite and quartzite occurring in the vicinity of schist outcrops, are also presented.

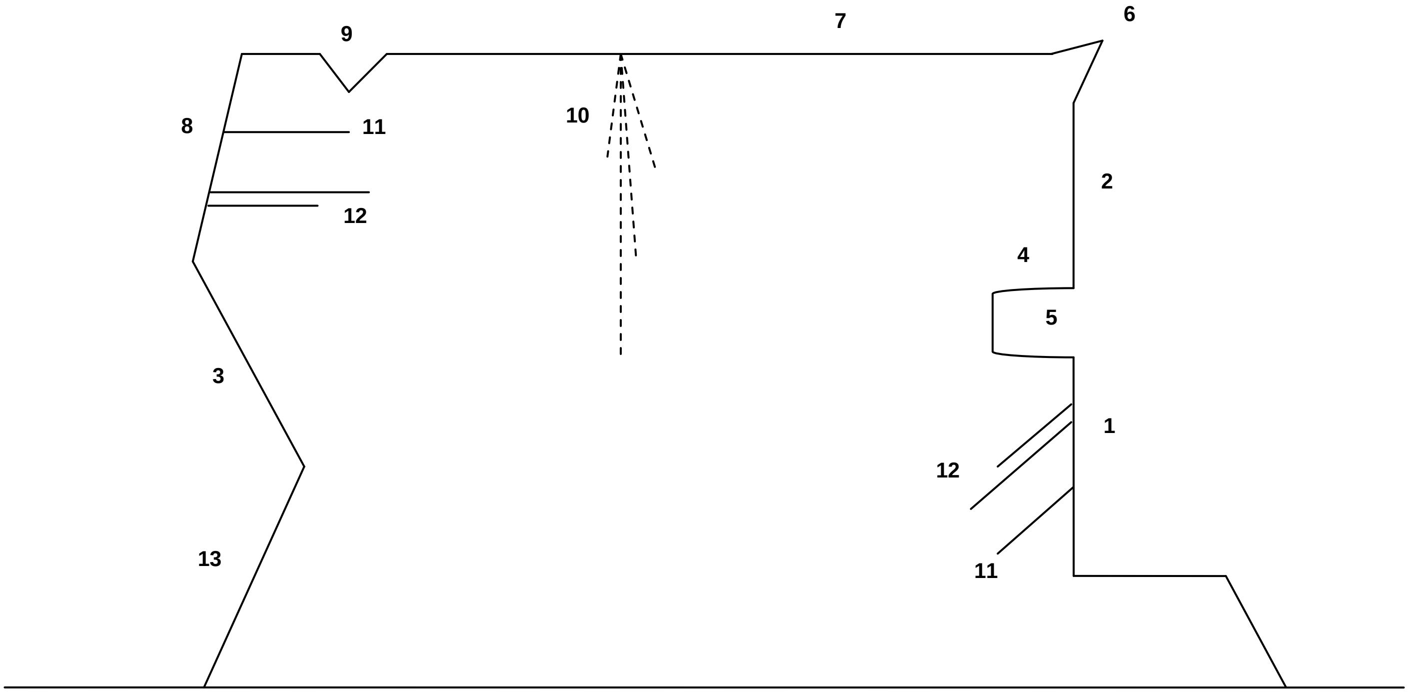

Fig. 6.4.1. Microhabitats found on the schist outcrops in the study area. 1: Vertical lower surfaces; 2: Vertical upper surfaces; 3: Overhangs; 4: Roofs; 5: Pits; 6: Ledges; 7: Horizontal upper surfaces; 8: Inclined upper surfaces; 9: Rock pools; 10: Rain-tracks; 11: Narrow crevices; 12: Wide crevices; 13: Inclined lower surfaces. Adapted from Egea & Llimona (1983).

The nomenclature follows the *Index Fungorum* (2013), except in the case of recently described species or taxonomic treatments not yet contemplated by that checklist, when we refer to Aptroot & Schumm (2012) for *Trapeliopsis gymnidiata*, Otálora *et al.* 2008 for *Leptogium pulvinatum* (Hoffm.) Otálora, Marques *et al.* (2013) for *Peltula lobata* and Nordin *et al*. (2010) for *Lobothallia recedens* (Taylor) A. Nordin, S. Savić & Tibell. Synonyms for *Caloplaca* spp. are according to Arup *et al*. (2013).

6.4.1 Taxa

Acarospora A. Massal.

Selected specimens revised by: Dr. Kerry Knudsen (University of California Riverside)

Acarospora boulderensis H. Magn.

Rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.2. **New for the Iberian Peninsula**.

Fig. 6.4.2. *Acarospora charidema*. Photograph by Cristiana Vieira.

Acarospora aff. *bullata* Anzi

Examined specimens are characterized by an indeterminate to more usually effigurate thallus, reacting KC^{+} red, with inflated to somewhat bullate central areoles and scabrid apothecial disks. These specimens superficially resemble *Acarospora bullata* Anzi but apothecia lack the epihymenial accretions, known as umbos, which are typically found in this species. The lighter colour and thinness of thallus, as well as the somewhat slender lobules preclude from assigning such specimens to either *A*. *bullata* or the closely related *A*. *rosulata* H. Magn. (Kerry Knudsen, pers. comm.). Ongoing taxonomic treatments of European and North American species in *A*. *bullata* group will hopefully help to clarify the identity of the examined specimens. In the Iberian Peninsula, *A*. *bullata* is known only from south-east Spain (Egea & Llimona 1981). *Acarospora rosulata* is not known to occur in the Iberian Peninsula. This species is rare in the study area, on poorly colonized vertical south facing schist and granite surfaces, together with *Acarospora hilaris*, *Caloplaca demissa* and *Rhizoplaca maheui*.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9049-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9001-L); Rio Tinhela, 29TPF3576, 225 m, on vertical granite surfaces, 18.09.2011, J. Marques (PO9002-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9003-L).

Acarospora charidema (Colmeiro) Llimona

Rare, on south-east facing vertical schist surfaces (Fig. 6.1.2). Further notes on Chapter 6.3. **New for Portugal**.

Acarospora epithallina H. Magn.

Relatively rare, always growing as a parasite on *A. hilaris*. Further notes on Chapter 6.3.

Acarospora hilaris (Dufour) Arnold

Relatively frequent, on vertical to slightly overhang but fully exposed south facing schist, quartzite and granite surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 25.04.2009, J. Marques (PO9183-L). **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9049-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9046-L). **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9042-L); Foz do Tinhela, 29TPF3676, 181 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9047-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9048-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9043-L); Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9044-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surface, 14.10.2010, J. Marques (PO9373-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9045-L).

Acarospora macrospora subsp. *murorum* (A. Massal.) Clauzade & Cl. Roux

Rare, on wide and narrow crevices. Further notes on Chapter 6.3. **New to mainland Portugal**.

Acarospora cf. *nitrophila* H. Magn.

Rare, on vertical south-east facing schist surfaces with *Caloplaca subsoluta*, *Peltula euploca* and *Glyphopeltis ligustica*, among others. Thallus of examined specimens is formed by tightly adpressed areoles covered by a thick and irregular epinecral layer that gives the thallus a heavily fissured appearance. Although found spreading abundantly on the respective surfaces both examined specimens were sterile. Their identity remains uncertain due to lack of material to compare them with, until further notice.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9185-L); Vale Cabrões, 29TPF5750, 302 m, on vertical schist surfacecs, 15.10.2010, J. Marques (PO9184-L).

Acarospora umbilicata Bagl.

Frequent, on horizontal surfaces and narrow crevices with different orientations but always fully exposed to direct sunlight. Examined specimens present the typical areolate to squamulose thallus of *A. umbilicata*, reacting KC$^+$ red, but considerable variation has been observed. The tendency of apothecia to develop between the areoles or squamules seems to be a good character for *A. umbilicata* (Clauzade *et al.* 1981, Fletcher *et al.* 2009) as is the size of apothecial disks which, when fully developed, are flat and rugulose and usually reach over 1 mm. Most observed specimens are only slightly pruinose to less frequently totally epruinose and could be mistaken for *A. fuscata* (Nyl.) Arnold especially when sterile or immature. Clauzade *et al.* (1981) relies on the presence of pruina to distinguish *A. umbilicata* from *A. fuscata* but this is contradicted by observations by other authors (Fletcher *et al.* 2009), who found that *A. fuscata* can also be slightly pruinose. The presence of pruina is therefore not a good character to distinguish between these species and is more likely dependent on calcium availability in the substrate or surrounding environment. Additional differences between these species are, according to literature, the homogenous thallus colour and flat areoles in *A. fuscata* while in *A. umbilicata* the large squamules are distinctively lighter coloured and areoles are distinctively convex (Clauzade *et al.* 1981, Fletcher *et al.* 2009). Some squamulose epruinose morphotypes closely resemble the description of *A. gallica* H. Magn. namely in the pale lower surface and development of imbricate, stipitate squamules interconnected by rhyzomorphs, but lack the characteristic predominance of small areoles with a single apothecium observed by Knudsen & Kocourková (2012). *Acarospora gallica* is a poorly known species from central and western Europe and some parts of Asia (Knudsen & Kocourková 2012) overlooked until quite recently due to a superficial resemblance with *A. fuscata* but was never reported from Portugal. A thorough revision of European specimens of *A. fuscata* group, including *A. fuscata*, *A. umbilicata* and *A. gallica*, is therefore required (Kerry Knudsen pers. comm.) and will be the subject of a forthcoming study.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9011-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on horizontal schist surfaces, 12.10.2011, J. Marques (PO9019-L). **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9053-L); Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9057-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9079-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9186-L). **Torre de Moncorvo**, Cardanha, Cardanha, 29TPF6769, 301 m, on vertical schist surfaces, 26.04.2011, J. Marques (PO9088-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9089-L); Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9138-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.09.2010, J. Marques (PO9139-L); Muxagata. Ribeira de Piscos, 29TPF5843, 130 m, on narrow crevices of schist outcrops, 09.05.2009, J. Marques (PO9179-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9180-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9181-L); Vale de José Esteves, 29TPF5949, 161 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9182-L).

Acarospora veronensis A. Massal.

Rare, on vertical south facing schist surfaces with *Acarospora hilaris*.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9046-L).

Agonimia Zahlbr.

Agonimia opuntiella (Buschart & Poelt) Vězda

Frequent, on narrow and wide crevices. Further notes on Chapter 6.3.

Aspicilia A. Massal.

Specimens revised by: Dr. Víctor J. Rico (Complutense University of Madrid)

Aspicilia caesiocinerea (Nyl.) Arnold

Relatively frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9488-L).

Aspicilia contorta (Hoffm.) Kremp. subsp. *hoffmanniana* S. Ekman & Fröberg

Extremely frequent, on vertical to slightly inclined schist surfaces. Considerable morphological variation in thallus colour has been observed, from light greyish-green in more shaded and moist situations, to light brown and pruinose in fully exposed south facing surfaces. A dark green morphotype is referred bellow to as *A. viridescens* (A. Massal.) Kremp., following Nimis (1993).

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9531-L); Soutelo, Alto da Fraga, 29TPF8886, 538 m, on compacted soil between ultrabasic rocks outcrops, 11.05.2009, J. Marques (PO9532-L). **Torre de Moncorvo**, Cardanha, Cardanha, 29TPF6769, 301 m, on vertical schist surfaces, 26.04.2011, J. Marques (PO9088-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9537-L); Penascosa, 29TPF5941, 163 m, on narrow crevices of schist outcrops, 15.05.2007, J. Marques (PO9387-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9041-L); Ribeira de Piscos, 29TPF5843, 136 m, on inclined schist surfaces, 09.05.2009, J. Marques (PO9589-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 127 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9583-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9295-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9588-L).

Aspicilia crespiana V. J. Rico

Frequent, on wide and narrow crevices usually overgrowing mosses or other lichens, in both schist and granite outcrops. Further notes on Chapter 6.3. **New for Portugal**.

Aspicilia cupreoglauca B. de Lesd.

Rare, on south facing schist surfaces. Further notes on Chapter 6.3. **New for Portugal**.

Aspicilia inornata Arnold

Rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9597-L).

Aspicilia intermutans (Nyl.) Arnold

Relatively frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9488-L); Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9491-L).

Aspicilia laevata (Ach.) Arnold

Very rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 188 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9533-L).

Aspicilia radiosa (Hoffm.) Poelt & Leuckert

Relatively frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9534-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on horizontal schist surfaces, 09.05.2009, J. Marques (PO9432-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03-05-2011, J. Marques (PO9535-L); Vale do Forno, 29TPF5748, 297 m, on inclined schist surfaces, 30.03.2010, J. Marques (PO9433-L).

Aspicilia viridescens (A. Massal.) Hue

Relatively rare in the study area, on north to north-west facing schist surfaces. Further notes on Chapter 6.3. **New for Portugal**.

Buellia De Not.

Buellia badia (Fr.) A. Massal.

Frequent, on narrow crevices and vertical schist surfaces of varying orientations, overgrowing other lichens.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 156 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9268-L); Canada da Moreira, 29TPF6047, 316 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9499-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9501-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 161 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9269-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9507-L).

Buellia dispersa A. Massal.

TLC: atranorin, 2’-O-methylperlatolic acid

Relatively frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9263-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 161 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9264-L).

Buellia spuria (Schaer.) Anzi

TLC: atranorin, norstictic, stictic acid complex

Relatively frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03-05-2011, J. Marques (PO9265-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques, (PO9266-L).

Buellia subsquamosa J. Steiner

TLC: atranorin, psoromic acid

Rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9263-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9267-L).

Caloplaca Th. Fr.

Specimens revised by: Dr. Ulrik Søchting (University of Copenhagen), Dr. Ester Gaya (Royal Botanical Gardens, Kew), Dr. Pere Navarro-Rosinés (University of Barcelona)

Caloplaca aff. *aractina* (Fr.) Häyrén

Clauzade & Roux (1985) and Wetmore (2007) have included *Caloplaca aractina* in the *Caloplaca cerina* (Hedw.) Th. Fr. group - whose members are characterised by a persistent thick grey thalline margin - but *C. aractina* differs in the presence of a distinct true exciple (Šoun *et al.* 2011). Examined specimens agree with *C. aractina* in apothecial anatomy but considerable variation in thallus morphology was observed, from light greenish-grey and distinctly cracked-areolate, to black and almost continuous. Their occurrence in the study area is also unusual, as currently known distribution of *C. aractina* is restricted to coastal areas, from the Mediterranean to the Scandinavian coast. The current delimitation of *C. aractina* is therefore based exclusively on a few phenotypic traits considered to be highly plastic. Morphological and molecular variability need to be thoroughly investigated to determine the real diversity hidden in this interesting lichen complex, a task that will be conducted in the near future. Relatively rare, on vertical south-east facing schist and granite surfaces.

SPECIMENS EXAMINED – **Mirandela**, Abreiro, Quinta da Pendurada, 29TPF4479, 200 m, on vertical granite surfaces, 09.03.2012, J. Marques (PO9210-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9211-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9213-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9212-L).

Caloplaca arenaria (Pers.) Müll. Arg. *s. lat.*

(= *Rufoplaca arenaria* (Pers.) Arup, Søchting & Froden)

Among the specimens here identified as *Caloplaca arenaria*, a few had been initially named *Caloplaca oxfordensis* Hedr. (apparently an overlooked homonym of *C. oxfordensis* Fink ex J. Hedrick) based on the differences pointed by Wetmore (1996), including the presence of lighter orange apothecia and a thicker grey thallus. Analysed specimens were in fact extremely variable in their ITS sequences and there was no clear distinction between them based on molecular data. *Caloplaca oxfordensis* is regarded as a synonym of *Caloplaca subpallida* H. Magn. by Wetmore (1996) and in the Iberian checklist by Hladun & Llimona (2002-2007). On the other hand, *Caloplaca subpallida* is usually mentioned as a synonym of

Caloplaca arenaria (Fletcher & Laundon 2009, Wetmore 1996) but not as so in the Iberian checklist by Hladun & Llimona (2002-2007). The name *Caloplaca arenaria s. lat.* is here assigned to all examined specimens of the *C. arenaria* complex until further revision of the group. Frequent, on horizontal to inclined schist surfaces, as well as wide crevices, facing south.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9214-L); Canada da Moreira, 29TPF6047, 316 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9215-L); Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9216-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on narrow crevices of schist outcrops, 09.05.2009, J. Marques (PO9217L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9218-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03-05-2011, J. Marques (PO9150-L); Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9219-L); Vale Cabrões, 29TPF5750, 301m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9220-L); Vale de José Esteves, 29TPF5949, 161 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9221-L).

Caloplaca arnoldii (Wedd.) Zahlbr. ex Ginzb. subsp. *obliterata* (Pers.) Gaya

(= *Calogaya arnoldii* (Wedd.) Arup, Froden & Søchting)

Rare, on vertical schist surfaces facing south. Further notes on Chapter 6.3. **New for Portugal**.

Caloplaca cancarixiticola Nav.-Ros., Egea & Llimona

(= *Variospora cancarixiticola* (Nav.-Ros., Egea & Llimona) Arup, Søchting & Froden)

This species was previously known only from the type locality (Navarro-Rosinés *et al.* 2000) and is here reported for the second time since the type description, colonizing vertical to slightly inclined south-east facing schist surfaces on the banks of seasonal streams together with *C. flavocitrina* and *C. flavescens*, among others. The identity of the examined specimens is supported by molecular data, based on the comparison of ITS sequences obtained from specimens PO9187-L and PO9598-L with that available on GenBank (EU639608). **New for Portugal**.

SPECIMENS EXAMINED – **Mogadouro**, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9187-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 330 m, on inclined schist surfaces, 29.09.2010, J. Marques (PO9188-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9189-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 05.07.2012, J. Marques (PO9598-L).

Caloplaca carphinea (Fr.) Jatta

(= *Usnochroma carphinea* (Fr.) Søchting, Arup & Froden)

Relatively rare, on vertical schist surfaces facing south.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9183-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 210 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9370-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9190-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9371-L).

Caloplaca conversa (Kremp.) Jatta var. *cravensis* Clauzade & Wunder

A former member of the *C. cerina* group, differing from the members of this group by the presence of a distinct true exciple (Šoun *et al.* 2011) and characterized by the dark brown to black apothecial disks at maturity. Very rare, on vertical schist surfaces facing north.

SPECIMENS EXAMINED – **Torre de Moncorvo**, Cardanha, Cardanha, 29TPF6769, 301 m, on vertical schist surfaces, 26.04.2011, J. Marques (PO9088-L).

Caloplaca crenularia (With.) J. R. Laundon

(= *Blastenia crenularia* (With.) Arup, Søchting & Froden)

Frequent, on vertical schist and granite surfaces of varying orientations. Also found growing on earth-filled crevices of schist outcrops (PO9201-L) and compacted soil between ultrabasic rock outcrops (PO9200-L).

SPECIMENS EXAMINED – **Mogadouro**, Alto da Fraga, 29TPF8886, 538 m, on compacted soil between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9200-L). **Murça**, Candedo, Vale do Moinho, 29TPF3477, 200 m, on vertical granite surfaces, 18.09.2011, J. Marques (PO9202-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 164 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9198-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9199-L); Vale de José Esteves, 29TPF5949, 161 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9201-L).

Caloplaca crenulatella (Nyl.) H. Olivier

(= *Xanthocarpia crenulatella* (Nyl.) Froden, Arup & Søchting)

Specimens currently ascribed to *C. crenulatella* belong to a still unresolved group (Vondrák *et al.* 2011) that is awaiting a more comprehensive delimitation. Nevertheless, the examined specimen shares the differentiating characters described in Navarro-Rosinés & Hladun (1996) for *C. crenulatella*, including the areolate to slightly subsquamulose epilithic thallus and zeorine apothecia with orange disks and contrasting yellow thallus margin. Very rare, on inclined south-east facing schist surfaces. **New for Portugal**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9203-L).

Caloplaca demissa (Flot.) Arup & Grube

Easily recognized by the sterile placodioid brown thallus that is often partially pruinose and covered by granulose soredia, especially towards the centre. Very frequent in the study area, on vertical schist and granite surfaces facing south.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3672, 188 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9599-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9534-L). **Murça**, Candedo, Rio Tinhela, 29TPF3576, 225 m, on vertical schist surfaces, 18.09.2011, J. Marques (PO9372-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9467-L); Penascosa, 29TPF5941, 158 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9374-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9373-L).

Caloplaca cf. *epigaea* Søchting, Huneck & Etayo

(= *Xanthocarpia epigaea* (Søchting, Huneck & Etayo) Froden, Arup & Søchting)

This species is supposed to belong to the *Caloplaca crenulatella* group (Vondrák *et al.* 2011) and is characterized by the thick areolate thallus gradually covered by numerous lecanorine to zeorin apothecia. According to Søchting *et al.* (2007) it is known only from a few localities of arid climate in central Germany and Spain, growing on soil and detritus. The specimen found in the Upper Douro region is morphological and anatomically consistent with one of the isotypes (C) and is here reported for the first time since the type description (Søchting *et al.* 2007) on somewhat intermediate ecological conditions, growing terricolous on compacted soil and among other calciphilous lichens such as *Psora decipiens*.

Vondrák *et al.* (2011) questioned its identity given the occurrence of the diagnostic characters of this species in other members of the *C. crenulatella* group but was unable to include a molecular analysis due to the lack of material. Preliminary accessment of ITS sequences places this specimen within *C*. *crenulatella* group but retrived no close match, the closest being in fact *C*. *epigaea*. Further studies based on ITS sequences of the examined specimen and other members of the broader *C*. *lactea* (A. Massal.) Zahlbr. group (Navarro-Rosinés & Hladun 1996) are being carried out and expected to eludidate the identity of the examined specimen.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9568-L).

Caloplaca erythrocarpa (Pers.) Zwackh

Very rare, on schist walls facing south.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2010, J. Marques (PO9191-L).

Caloplaca flavescens (Huds.) J. R. Laundon

(= *Variospora flavescens* (Huds.) Arup, Froden & Søchting)

According to Gaya (2009), within *Caloplaca aurantia* (Pers.) J. Steiner group, this species is characterized by a placodioid thallus that is only partially covered by a thin layer of pruina and clearly distinguished by the scleroplectenchymatous upper cortex with a well developed layer of crystals beneath the pigmented outer layer. The ecology in the study area is somewhat similar to what is described by Gaya (2009), on shaded to fully exposed euthophic schist surfaces near water courses together with *Lecanora albescens* (Hoffm.) Branth & Rostr., *Verrucaria nigrescens* Pers. and others.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9206-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9204-L); Quinta das Tulhas, 29TPF5941, 150 m, on vertical schist surfaces, 3.05.2011, J. Marques (PO9207-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9466-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9205-L); Vale Cabrões, 29TPR5750, 302 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9208-L).

Caloplaca flavocitrina (Nyl.) H. Olivier

(= *Flavoplaca flavocitrina* (Nyl.) Arup, Froden & Søchting)

Recognized either as a separate species or as a variety of *Caloplaca citrina* (Hoffm.) Th. Fr. *s. lat.*, the identity of this species is supported by morphological and molecular data and is probably overlooked in the Iberian Peninsula. Examined specimens present a cracked areolate thallus, with areoles lifted slightly at edge by marginal soredia, and adnate apothecia, as described by Arup (2006) and Vondrak *et al.* (2009).

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9187-L). **Vila Nova de Foz Côa**, Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9466-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9600-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9209-L).

Caloplaca fuscoatroides J. Steiner

Rare, on fully exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on vertical schist surface, 15.10.2010, J. Marques (PO9195-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9197-L).

Caloplaca grimmiae (Nyl.) H. Olivier

Very rare, on horizontal schist and granite surfaces parasitizing *Candelariella* spp. Further notes on Chapter 6.3.

Caloplaca lactea (A. Massal.) Zahlbr.

(= *Xanthocarpia lactea* (A. Massal.) A. Massal.)

According to Navarro-Rosinés & Hladun (1996) this species is distinguished from other members of the *Caloplaca crenulatella* group (Vondrák *et al.* 2011) by the smaller, up to 15 m long, ellipsoid ascospores and yellow pruinose apothecia with orange disks and yellow thalline margin. It has been recently reported for the first time in Portugal (Paz-Bermúdez *et al.* 2009) colonizing amphibolite outcrops. The examined specimen constitutes **a second record from Portugal**, on fully exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on horizontal schist surfaces, 07.05.2009, J. Marques (PO9569-L).

Caloplaca pellodella (Nyl.) Hasse

This species has been included either in the broad *Caloplaca sideritis* (Tuck.) Zahlbr. group (Wetmore 1996) - characterized by a well developed grey, whitish or brown thallus lacking anthraquinones, and lecanorine to zeorine apothecia with anthraquinones – or in the *Caloplaca cerina* group (Clauzade & Roux 1985, as *Caloplaca conglomerata* (Bagl.) Jatta) – morphologically characterized by lecanorine apothecia with strongly reduced, superficially invisible true exciple (Vondrák *el al*. 2008). Recent studies on the taxonomy and phylogeny of *C*. *cerina* group in Europe (Šoun *et al.* 2011) have concluded that *C. pellodella* differs from other species in this group in the zeorine apothecia, with brown to black true exciple devoid of anthraquinones, and a distinctly squamulose grey thallus. The likewise North American *Caloplaca amabilis* Zahlbr. is a widely accepted synonym (Wetmore 1996). The European *C. conglomerata* and respective synonym *C*. *peregrina* Samp. (Tavares 1961) have also been synonymised with *Caloplaca pellodella* (Paz-Bermúdez *et al.* 2006), despite the high degree of morphological variation among European specimens (Wetmore 1996). The current delimitation of *C*. *pellodella* is thus based on phenotypic traits that are suspected to be highly plastic, emphasizing the need to circumscribe *C*. *pellodella* through exhaustive and robust molecularly based phylogeny including material covering its entire range. The examined specimens presented a well developed greyish-green to olive and thick squamulose to sublobulate thallus and zeorin apothecia (although true exciple is frequently strongly reduced) with yellow to orange disks and a distinct and persistent thick thalline margin.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 24.03.2012, J. Marques (PO9225-L); Carlão, Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 07.03.2012, J. Marques (PO9224-L). **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9223-L); Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9222-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9016-L; PO9206-L; PO9226-L; PO9227-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 330 m, on vertical schist surfaces, 29.09.2010, J. Marques (PO9232-L); Penascosa, 29TPF5846, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9229-L; PO9230-L); Chãs, Quinta da Barca, 29TPF4479, 200 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9233-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9231-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9488-L).

Caloplaca rubelliana (Ach.) Lojka

Rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3. **New for Portugal**.

Caloplaca scotoplaca (Nyl.) H. Magn.

(= *Rufoplaca scotoplaca* (Nyl.) Arup, Søchting & Froden)

Rare, on fully-exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Canada da Moreira, 29TPF6047, 316 m, on compacted soil between schist outcrops, 15.10.2010, J. Marques (PO9195-L).

Caloplaca squamuloisidiata van den Boom & V. J. Rico

Rare, on sheltered and often slightly overhanging schist surfaces. Further notes on Chapter 6.3.

Caloplaca subsoluta (Nyl.) Zahlbr.

(= *Squamulea subsoluta* (Nyl.) Arup, Søchting & Froden)

This species has been synonymized with *Caloplaca irrubescens* (Nyl.) Zahlbr. by Wetmore (2003) but this synonymy has been overlooked in the Iberian Peninsula (Hladun & Llimona 2002-2007, Paz-Bermúdez *et al.* 2009). In the study area it is easily recognizable by the deep orange (pale yellow-orange in shade), areolate to subsquamulose thallus, paraplectenchymatous hypothecium and medulla (Wetmore 2003), and ascospores of the *Caloplaca citrina* type (Clauzade & Roux 1985). According to the description by Wetmore (2003), the subsquamulose specimens of *C. subsoluta* could be confused with the morphologically similar North American *Caloplaca squamosa* (B. de Lesd.) Zahlbr., but these were clearly separated by molecular data. *Caloplaca subsoluta* is one of the most characteristic species of vertical south-east facing schist surfaces in the study area.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9376-L). **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9377-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9016-L; PO9206-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9467-L); Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9378-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9373-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9008-L; PO9041-L); Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO8991-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9379-L); Tudão, 29TPF5650, 378 m, on overhanging schist surfaces, 08.05.2009, J. Marques (PO9380-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9220-L); Vale do Forno, 29TPF5748, 301 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9013-L); Vale de José Esteves, 29TPF5949, 146 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9587-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9580-L).

Caloplaca gr. *xerica* Poelt *et* Vězda

Species belonging to this still unresolved group (Vondrák *et al.* 2012) are characterized by a light to dark grey thallus, zeorine apothecia and secondary chemistry - chemosyndrom B according to Søchting (2001). Among the examined specimens, considerable morphological variation has been detected including: 1) a form with thick isidiate to blastidiate thallus and few apothecia, assignable to *Caloplaca xerica* s. str. (PO9192-L and PO9193-L), and rare on narrow crevices of south facing schist surfaces; 2) an unknown form characterized by thick squamulose thallus and apothecia with whitish thalline margin, found so far only in one location in the study area (PO9194-L), on fully-exposed horizontal schist surfaces, and 3) a form with relatively thick areolate thallus and abundant apothecia which was found on inclined to almost horizontal south facing schist surfaces. The later is morphologically close to *Caloplaca percocrata* (Arnold) J. Steiner., which was once reported from the study area by Sampaio (1918) as *Caloplaca percocrata* Jat., but whose type comes from the Tyrolean Alps (Austria) and is usually found at an altitude between 1500 and 2500 m a. s. l. in the Alps and Pyrenees (Claude Roux, pers. comm.). If confirmed, *C. xerica* is new for Portugal.

SPECIMENS EXAMINED – **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9196-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 309 m, on horizontal schist surfaces, 14.03.2012, J. Marques (PO9194-L); Penascosa, 29TPF5941, 161 m, on narrow crevices of schist outcrops, 15.05.2007, J. Marques (PO9192-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on narrow crevices of schist outcrops, 09.05.2009, J. Marques (PO9193-L).

Candelariella Müll. Arg.

Candelariella coralliza (Nyl.) H. Magn.

Rare, on inclined surfaces facing north.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on horizontal schist surfaces, 13.05.2009, J. Marques (PO9086-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9537-L).

Candelariella vitellina (Ehrh.) Müll. Arg.

Frequent, on fully-exposed horizontal and vertical schist surfaces facing south.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9089-L; PO9185-L); Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9234-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9139-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9381-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9600-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9400-L).

Cetraria Ach.

Cetraria aculeata (Schreb.) Fr.

Frequent, on fully-exposed horizontal schist and granite surfaces.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on horizontal schist surfaces, 12.05.2009, J. Marques (PO9237-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 156 m, on horizontal schist surfaces, 15.05.2007, J. Marques, (PO9236-L).

Cladonia P. Browne

Specimens revised by: Dr. Ana Rosa Burgaz (Complutense University of Madrid)

Cladonia cariosa (Ach.) Spreng.

TLC: atranorin, fumarprotocetraric acid

Rare, on earth banks between ultrabasic rock outcrops facing south.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo. Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9544-L).

Cladonia cervicornis (Ach.) Flot.

TLC: fumarprotocetraric acid

Frequent, on horizontal schist surfaces and earth banks between schist and ultrabasic rock outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo. Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9545-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9479-L).

Cladonia chlorophaea (Flörke ex Sommerf.) Spreng.

TLC: fumarprotocetraric acid

Frequent, on horizontal schist surfaces with soil accumulation.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on horizontal schist surfaces, 12.05.2009, J. Marques (PO9546-L).

Cladonia firma (Nyl.) Nyl.

TLC: atranorin, fumarprotocetraric acid

Frequent, on south-east facing inclined schist surfaces as well as earth banks between schist and ultrabasic rock outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo. Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9547-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on inclined schist surfaces, 09.05.2009, J. Marques (PO9475-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9476-L).

Cladonia foliacea (Huds.) Willd.

TLC: usnic and fumarprotocetratic acids

Frequent, on inclined south-east facing schist surfaces and earth-filled crevices of schist outcrops.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on inclined schist surfaces, 13.05.2009, J. Marques (PO9548-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on inclined schist surfaces, 09.05.2009, J. Marques (PO9478-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9477-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9479-L).

Cladonia iberica Burgaz & Ahti

TLC: atranorin

Relatively frequent, on horizontal schist surfaces with soil accumulation.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on horizontal schist surfaces, 12.05.2009, J. Marques (PO9474-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9479-L).

Cladonia rangiformis Hoffm.

TLC: atranorin, rangiformic acid

Frequent, on horizontal schist surfaces. Also found on north-east facing vertical schist surfaces overgrowing mosses.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on horizontal schist surfaces, 12.05.2009, J. Marques (PO9549-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9480-L).

Collema P. Browne

Specimens revised by: Dr. Palmira Carvalho (National Museum of Natural History and Science)

Collema auriforme (With.) Coppins & J. R. Laundon

Rare, on wide south facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9389-L).

Collema furfuraceum Du Rietz

Rare, on inclined schist surfaces facing south.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9391-L).

Collema polycarpon subsp. *corcyrense* (Arnold) Pišút

Rare, on vertical south-east facing schist surfaces with secondary calcium deposits.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Figueira, 29TPF5845, 223 m, on vertical schist surfaces, 13.03.2012, J. Marques (PO9390-L).

Collema ryssoleum (Tuck.) A. Schneid.

Frequent in the study area, on vertical south-east facing schist surfaces. When sterile it is difficult to distinguish this species from *Collema subnigrescens* Degel. as both are characterized by a pustulate and plicate thallus, but *Collema ryssoleum* produces ellipsoid to fusiform spores 25-45(-50) x (4-)5-8(-10) µm while *Collema subnigrescens* produces fusiform to acicular spores (35-)40-75 x (4-)4,5-6,5 (Carvalho 2012). Most collected specimens were sterile and could not be assigned specifically to any of these species.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9014-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9392-L). **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9393-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9394-L).

Collema subflaccidum Degel.

Rare, on vertical south-east facing schist surfaces with secondary calcium deposits.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Figueira, 29TPF5845, 223 m, on vertical schist surfaces, 13.03.2012, J. Marques (PO9390-L); Vale de José Esteves, 29TPF5849, 196 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9333-L).

Collema subnigrescens Degel.

Almost impossible to separate from *Collema ryssoleum* when sterile, but readily distinguished by spore size and shape. Further notes under *Collema ryssoleum*. Relatively frequent in the study area, on both north-west and south-east facing vertical schist surfaces.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, São Lourenço, 29TPF3672, 188 m, on vertical granite surfaces, 14.10.2012, J. Marques (PO9399-L). **Mogadouro**, Bemposta, Alto da Fraga, 29TPF8886, 580 m, on vertical surfaces of ultrabasic rock outcrops, 12.05.2009, J. Marques (PO9398-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on inclined schist surfaces, 30.03.2010, J. Marques (PO9397-L); Penascosa, 29TPF5941, 163 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9396-L); Quinta das Tulhas, 29TPF5949, 140 m, muscicolous on vertical schist surfaces, 03.05.2011, J. Marques (PO9317-L; PO9395-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9391-L).

Collema tenax (Sw.) Ach.

Rare in the study area, found only once growing in a small horizontal crevice of a vertical south-east facing schist surface.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9388-L).

Dermatocarpon Eschw.

Dermatocarpon deminuens Vain.

Rare, on inclined granite surfaces along the riverbanks of major rivers. Further notes on Chapter 6.2. **New for the Iberian Peninsula**.

Dermatocarpon luridum (Dill. ex With.) J. R. Laundon

Rare, on periodically submerged granite surfaces.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Barcos, 29TPF3367, 182 m, on vertical granite surfaces, 23.03.2012, J. Marques (PO9483-L).

Dermatocarpon miniatum (L.) W. Mann

Relatively frequent, on vertical north-west facing schist surfaces or associated with rain-track communities dominated by *Peltula euploca* on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 302 m, on vertical schist surfaces, 30.09.2011, J. Marques (PO9465-L).

Dimelaena Norman

Dimelaena oreina (Ach.) Norman

Relatively rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on the ledges of schist outcrops, 13.05.2009, J. Marques (PO9275-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9272-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9273-L); **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9044-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 05.07.2012, J. Marques (PO9274-L).

Diploicia A. Massal.

Diploicia canescens (Dicks.) A. Massal.

Rare, on vertical north-west facing schist surfaces, sheltered from direct sunlight.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Quinta das Tulhas, 29TPF5949, 140 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9271-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 251 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9270-L).

Diploschistes Norman

Diploschistes actinostomus (Ach.) Zahlbr.

Although it is usually characterized by a K- reacting thallus, specimens PO9261-L and PO9262-L presented a thallus reacting K+ yellow. These could not be assigned to any other known species of the group rather than *D. actinostomus* until further studies. Frequent on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9259-L); Quinta das Tulhas, 29TPF5949, 140 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9261-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9260-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9262-L).

Diploschistes diacapsis (Ach.) Lumbsch

According to Fernández-Brime *et al.* (2013), this species is distinguised from other members of the *D. scruposus* (Schreb.) Norman group (Lumbsch 1989) by the white to greyish-white thallus, 8-spored asci and ecology, growing exclusively on soil. Specimens found growing, at least in part, directly on rock were placed under *D. scruposus* (see bellow). Frequent on wide crevices of schist outcrops and fully-exposed horizontal schist surfaces, of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9247-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9246-L); Vale do Forno, 29TPF5748, 297 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9248-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9249-L).

Diploschistes muscorum (Scop.) R. Sant.

Assignment of examined specimens to *D. muscorum* is based on the characterization by Fernández-Brime *et al.* (2013) and previous authors (*e.g.* Lumbsch 1989), distinguishing *D. muscorum* by its 4-spored asci and growth as a parasite of *Cladonia* spp. at early stages of development. Specimens here assigned to *D. muscorum* were found growing at least in part upon *Cladonia* spp. on horizontal schist surfaces or earth-filled crevices of varying orientations, although they were always also spreading upon the soil beneath these lichens. The fact that *D. muscorum* is barely distinguishable from other members of the *D. scruposus* aggregate once it becomes independent from the host, and considering the low clade support values in the most recent phenotypical and molecular studies (Fernández-Brime *et al.* 2013) raises many doubts regarding the full separation of these species.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on wide crevices of schist outcrops, 11.05.2009, J. Marques (PO9242-L). **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on horizontal schist surfaces, 01.04.2010, J. Marques (PO9243-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9244-L); Vale Cabrões, 29TPF5750, 301 m, on wide crevices of schist outcrops, 15.10. 2010, J. Marques (PO9245-L); Vale de José Esteves, 29TPF5949, 161 m, on narrow crevices of schist outcrops, 08.05.2009, J. Marques (PO9109-L).

Diploschistes scruposus (Schreb.) Norman

Specimens here assigned to this species in fact represent an aggregate that includes forms with eight spores characteristic of *Diploschistes interpediens* (Nyl.) Zahlbr. following the synonymization of both species by Lumbsch (1989) and the latest phylogenetic studies by Fernández-Brime *et al.* (2013). Frequent, on vertical north-west facing schist surfaces as well as fully-exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on horizontal schist surfaces, 13.05.2009, J. Marques (PO9250-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2010, J. Marques (PO9258-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9252-L). **Murça**, Candedo, Caldas do Carlão, 29TPF3676, 200 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9251-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9253-L); Penascosa, 29TPF5941, 161 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9254-L); Muxagata, Fariseu, 29TPF5844, 127 m, on horizontal schist surfaces, 01.04.2010, J. Marques (PO9255-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 295 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9256-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9257-L).

Endocarpon Hedw.

Endocarpon adscendens (Anzi) Müll. Arg.

Very rare, overgrowing mosses on vertical schist surfaces along the riverbanks of the main watercourses. Further notes on Chapter 6.3. **New for Portugal**.

Endocarpon loscosii Müll. Arg.

Rare, overgrowing mosses on periodically submerged granite boulders associated with temporary riverbeds. Further notes on Chapter 6.3. **New for Portugal**.

Endocarpon pusillum Hedw.

Very frequent, on narrow and wide earth-filled crevices and earth banks between schist outcrops, as well as compacted soil in horizontal schist surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on narrow crevices of schist outcrops, 13.05.2009, J. Marques (PO9415-L). **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on schist walls, 13.10.2011, J. Marques (PO9416-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9316-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on earth banks between schist outcrops, 12.05.2009, J. Marques (PO9093-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on earth banks between schist outcrops, 17.09.2011, J. Marques (PO9417-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on wide crevices of schist outcrops, 15.05.2007, J. Marques (PO9421-L); Penascosa, 29TPF5941, 163 m, on narrow crevices of schist outcrops, 31.03.2010, J. Marques, PO9578-L; Muxagata, Fariseu, 29TPF5844, 127 m, on wide crevices of schist outcrops, 01.04.2010, J. Marques (PO9058-L); Ribeira de Piscos, 29TPF5843, 150 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9422-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9423-L); Foz do Côa, 29TPF5849, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9584-L); Tudão, 29TPF5650, 378 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9424-L) ; Vale de José Esteves, 29TPF5949, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9414-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9425-L).

Endocarpon simplicatum (Nyl.) Nyl.

Sharing the microhabitat with the similar *E. pusillum* but much rarer. Further notes on Chapter 6.2. **New for the Iberian Peninsula**.

Epiphloea Trevis

Epiphloea terrena (Nyl.) Trevis.

Relatively frequent, on horizontal schist surfaces where soil accumulates and earth banks between schist outcrops. Further notes on Chapter 6.3.

Fuscopannaria P. M. Jørg.

Fuscopannaria mediterranea (Tav.) P. M. Jørg.

Rare, overgrowing mosses on vertical north-west facing schist surfaces sheltered from direct sunlight due to the presence of tall vegetation.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Quinta das Tulhas, 29TPF5949, 140 m, overgrowing mosses on vertical schist surfaces, 03.05.2011, J. Marques (PO9410-L).

Glyphopeltis Brusse

Glyphopeltis ligustica (B. de Lesd.) Timdal

Very frequent, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3.

Koerberia A. Massal.

Koerberia sonomensis (Tuck.) Henssen

Rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3.

Lasallia Mérat

Lasallia pustulata (L.) Mérat

Relatively frequent, on vertical to inclined granite and schist surfaces, of varying orientations.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 13.10.2011, J. Marques (PO9604-L). **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on vertical granite surfaces, 08.03.2012, J. Marques (PO9605-L). **Vila Flor**, Freixiel, Ponte de Abreiro, 29TPF4478, 203 m, on vertical schist surfaces, 13.10.2011, J. Marques (PO9606-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9238-L); Penascosa, 29TPF5941, 160 m, on narrow crevices of schist outcrops, 15.05.2007, J. Marques (PO9239-L).

Lecanora Ach.

Lecanora albescens (Hoffm.) Branth & Rostr.

Relatively rare on shaded to fully-exposed and eutrophic vertical schist surfaces near watercourses together with *Caloplaca flavescens*, *Verrucaria nigrescens* and others.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Chãs, Quinta da Barca, 29TPF5941, 150 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9466-L).

Lecanora bolcana (Pollich) Poelt

Frequent, on vertical south-east facing schist surfaces as well as fully-exposed horizontal surfaces along the riverbanks of the main watercourses.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9549-L). **Mogadouro**, Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9601-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9499-L); Penascosa, 29TPF5941, 163 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9387-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9539-L).

Lecanora dispersa (Pers.) Röhl.

Rare, on inclined south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9539-L).

Lecanora muralis (Schreb.) Rabenh.

Frequent, on vertical south-east facing and fully-exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on horizontal schist surfaces, 13.05.2009, J. Marques (PO9559-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on horizontal schist surfaces, 15.10.2011, J. Marques (PO9601-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9550-L); Penascosa, 29TPF5941, 166 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9471-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on vertical schist surfaces, 11.10.2010, J. Marques (PO9553-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9551-L); Vale do Forno, 29TPF5748, 269 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9472-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9552-L); Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9602-L).

Lecanora orosthea (Ach.) Ach.

Relatively rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9556-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 348 m, on vertical schist surfaces, 13.09.2012, J. Marques (PO9555-L).

Lecanora polytropa (Ehrh.) Rabenh.

Frequent, on inclined and vertical, both north-west and south-east facing, schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9537-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 127 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9607-L); Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9490-L).

Lecanora pseudistera Nyl.

TLC: atranorin, 2’-O-methylperlatolic acid

Very frequent on north-west and south-east facing vertical schist surfaces. Further notes on Chapter 6.3.

Lecanora subcarnea (Lilj.) Ach.

TLC: atranorin, protocetraric acid

Relatively frequent, on vertical south facing schist and granite surfaces. Distinguished from other members of *Lecanora rupicola* group, namely *L. swartzii* (Ach.) Ach. (Edwards *et al.* 2009) by the K^+ yellow, P^+ orange and C^- thallus.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 13.10.2011, J. Marques

(PO9468-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 08.03.2012, J. Marques (PO9556-L); Vale de Moinho, 29TPF3477, 200 m, on vertical granite surfaces, 18.09.2011, J. Marques (PO9469-L).

Lecanora sulphurea (Hoffm.) Ach.

Rare, on exposed inclined schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9586-L).

Lecanora swartzii (Ach.) Ach.

TLC: atranorin

Rare, on vertical south facing schist surfaces together with *Acarospora hilaris* and *A*. aff *bullata*. Distinguished from other members of *Lecanora rupicola* group, namely *L*. *subcarnea*, by the K^+ yellow, C^+ orange-red and P^- thallus.

SPECIMENS EXAMINED – **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9470-L).

Lecidea Ach.

Lecidea fuscoatra (L.) Ach.

Frequent, on horizontal to inclined schist surfaces facing north.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9502-L); Penascosa, 29TPF5941, 161 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9495-L); Vila Nova de Foz Côa, Vermelhosa, 29TPF5950, 147 m, on horizontal schist surfaces, 14.10.2010, J. Marques (PO9507-L).

Lecidea grisella Flörke

Relatively rare, on horizontal to inclined schist surfaces facing north.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9359-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 127 m, on horizontal schist surfaces, 08.02.2006, J. Marques (PO9608-L).

Lecidea sarcogynoides Körb.

Rare, on inclined north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9486-L).

Lecidella Körb.

Lecidella carpathica Körb.

Relatively rare, on horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on horizontal schist surfaces, 08.02.2006, J. Marques (PO9218-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9356-L).

Lepraria Ach.

Lepraria caesioalba (B. de Lesd.) J. R. Laundon

TLC: atranorin, fumarprotocetraric acid

Very frequent, on vertical south-east facing schist surfaces and extending towards the wide crevices of those surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 156 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9562-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9563-L).

Lepraria lobificans Nyl.

TLC: atranorin, stictic acid complex

Very frequent, on fully exposed horizontal to vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – Vila Nova de Foz Côa, Muxagata, Fariseu, 29TPF5844, 127 m, on horizontal schist surfaces, 01.04.2010, J. Marques (PO9560-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on horizontal schist surfaces, 07.05.2009, J. Marques (PO9561-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9558-L); Vale de José Esteves, 29TPF5849, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9557-L).

Leprocaulon Nyl.

Leprocaulon microscopicum (Vill.) Gams

Frequent, on vertical both north-west and south-east facing schist surfaces, as well as horizontal schist surfaces among terricolous lichens such as *Endocarpon pusillum*, *Lepraria* spp, *Solenopsora vulturiensis*, *Trapeliopsis gymnidiata*, and others.

SPECIMENS EXAMINED – **Mogadouro**, Brunhoso, Ribeiro de Juncaínhos, 29TPF6880, 474 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9364-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 9.05.2009, J. Marques (PO9609-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on horizontal surfaces, 07.05.2009, J. Marques (PO9564-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9558-L); Vale do Forno, 29TPF5748, 297 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9524-L); Vale de José Esteves, 29TPF5949, 146 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9438-L).

Leproloma Nyl. ex Cromb.

Leproloma membranaceum (Dicks.) Vain.

Rare, on vertical schist and granite surfaces facing north.

SPECIMENS EXAMINED – **Murça**, Candedo, Vale de Moinho, 29TPF3477, 200 m, musciculous on vertical schist surfaces, 18.09.2011, J. Marques (PO9565-L). Vila Nova de Foz Côa, Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 248 m, on narrow crevices of schist outcrops, 18.03.2012, J. Marques (PO9405-L).

Leptochidium M. Choisy

Leptochidium albociliatum (Desm.) M. Choisy

Relatively frequent in the study area, muscicolous on horizontal and vertical schist surfaces in the floodplains of small watercourses, possibly compensating for the low relative humidity and high temperatures that characterize the main valleys.

SPECIMENS EXAMINED – **Alijó**, Carlão, Louzedo, 29TPF3576, 230 m, on vertical schist surfaces, 07.03.2012, J. Marques (PO9315-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9309-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9310-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on earth banks between schist outcrops, 11.05.2009, J. Marques (PO9311-L); Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9312-L). **Murça**, Candedo, Vale de Moinho, 29TPF3477, 200 m, musciculous on vertical schist surfaces, 18.09.2011, J. Marques (PO9313-L). Vila Nova de Foz Côa, Castelo Melhor, Canada do Amendoal, 29TPF5946, 315 m, on vertical schist surfaces, 30.09.2011, J. Marques, PO9152-L; Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9314-L).

Leptogium (Ach.) Gray

Leptogium cochleatum (Dicks.) P. M. Jørg. & P. James

Very rare, muscicolous on vertical but moist and shaded north-west facing schist surfaces. Further notes in López de Silanes *et al.* 2012. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Quinta das Tulhas, 29TPF5949, 140 m, muscicolous on vertical schist surfaces, 03.05.2011, J. Marques (PO9317-L; PO9395-L).

Leptogium cyanescens (Rabenh.) Körb.

Relatively frequent, overgrowing mosses on vertical schist surfaces of varying orientations. Further notes in López de Silanes *et al.* 2012. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9327-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9326-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9418-L); Vale Figueira, 29TPF5845, 200 m, on vertical schist surfaces, 13.03.2012, J. Marques (PO9325-L).

Leptogium gelatinosum (With.) J. R. Laundon

Relatively rare, overgrowing mosses on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9328-L).

Leptogium lichenoides (L.) Zahlbr.

Frequent, overgrowing terricolous mosses on wide crevices and inclined schist surfaces facing north.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on inclined schist surfaces, 9.05.2009, J. Marques (PO9484-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9419-L); Vale Cabrões, 29TPF5750, 301 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9558-L).

Leptogium magnussonii Degel & P. M. Jørg.

Rare, on vertical north-west facing schist and granite surfaces. Further notes in López de Silanes *et al.* 2012. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Alijó**, Carlão, Vale do Moinho, 29TPF3477, 203 m, on vertical granite surfaces, 07.03.2012, J. Marques (PO9329-L). **Carrazeda de ansiães**, Pombal, São Lourenço, 29TPF3572, 156 m, on vertical granite surfaces, 14.10.2011, J. Marques (PO9330-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 251 m, on vertical schist surfaces, 4.05.2011, J. Marques (PO9420-L).

Leptogium palmatum (Huds.) Mont.

Relatively frequent, muscicolous on vertical to inclined schist surfaces and wide crevices of schist outcrops of varying orientations.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, S. Lourenço, 29TPF3672, 188 m, muscicolous on earth banks between granite outcrops, 14.10.2011, J. Marques (PO9330-L). **Murça**, Candedo, Vale do Moinho, 29TPF3477, 200 m, on vertical granite surfaces, 18.09.2011, J. Marques (PO9331-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, 29TPF5846, 130 m, Canada do Inferno, muscicolous on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9322-L); Tudão, 29TPF5650, 378 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9332-L).

Leptogium plicatile (Ach.) Leight.

Frequent, on vertical south-east facing schist surfaces. Further notes in López de Silanes *et al.* 2012. **New for Portugal**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9326-L); Vale de José Esteves, 29TPF5849, 196 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9333-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9334-L).

Leptogium pulvinatum (Hoffm.) Otálora

Very rare, on earth banks between ultrabasic rock outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9335-L).

Leptogium schraderi (Bernh.) Nyl.

Specimens here assigned to this species are anatomically consistent with *L. schraderi*, including the presence of a distinct cortical layer of isodiametric cells, and a lax medullar zone that disappears towards the center of the thallus, but are formed mainly by small, turgid and very distinctly wrinkled squamules, with few visible lobules, thus falling under the range of *L. turgidum* (Ach.) Nyl. (Jørgensen 1994). However, *L. turgidum* has been regarded by Jorgensen (1994) as a possible growth form of *L. schraderi*, lacking the characteristic fruticose structures of this species. The status of some of the examined specimens is therefore considered doubtfull until further studies on the *L. schraderi* complex.

Frequent, on south-east facing crevices and earth banks in the vicinity of schist outcrops, often with *Epiphloea terrena* and *Moelleropsis nebulosa* (Hoffm.) Gyeln. Further notes in López de Silanes *et al.* 2012. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9336-L); Vale Cabrões, 29TPF5750, 301 m, on narrow crevices of schist outcrops, 15.10.2010, J. Marques (PO9337-L); Vale de José Esteves, 29TPF5849, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9414-L); Vale do Forno, 29TPF5748, 258 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9338-L).

Leptogium teretiusculum (Flörke) Arnold

Rare, among terricolous lichens on wide south-east facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9610-L); Penascosa, 29TPF5941, 164 m, on wide crevices of schist outcrops, 15.05.2007, J. Marques (PO9340-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9339-L).

Lichinella Nyl.

Lichinella cribellifera (Nyl.) P. P. Moreno & Egea

Frequent, on vertical south-east facing schist surfaces as a member of rain-track communities described by Llimona & Egea 1985. Further notes on Chapter 6.3. **New for the province of Trás-os-Montes e Alto Douro.**

Lichinella nigritella (Lettau) P. P. Moreno & Egea

Frequent, on vertical south-east facing schist surfaces as a member of rain-track communities described by Llimona & Egea 1985. Further notes on Chapter 6.3. **New for Portugal**.

Lichinella stipatula Nyl.

Frequent, on vertical south-east facing schist surfaces and narrow crevices of schist outcrops. **New for the province of Trás-os-Montes e Alto Douro**. Further notes on Chapter 6.3.

Lobothallia (Clauzade & Cl. Roux) Hafellner

Lobothallia recedens (Taylor) A. Nordin, S. Savić & Tibell

Relatively rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9489-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9490-L).

Massalongia Körb.

Massalongia carnosa (Dicks.) Körb.

Relatively frequent, forming minute squamulose thalli among other terricolous lichens on vertical south-east facing schist surfaces and earth banks between schist outcrops. Fully developed, fertile specimens however occur in more humid situations, usually overgrowing mosses on granite boulders along the riverbanks of the main rivers.

SPECIMENS EXAMINED – **Alijó**, Carlão, Louzedo, 29TPF3576, 230 m, on vertical schist surfaces, 07.03.2012, J. Marques (PO9317-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2011, J. Marques (PO9318-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO9309-L; PO9316-L). **Murça**, Candedo, Vale do Moinho, 29TPF3477, 200 m, musciculous on vertical granite surfaces, 18.09.2011, J. Marques (PO9319-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9541-L); Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 302 m, on wide crevices of schist outcrops, 30.09.2011, J. Marques (PO9130-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9154-L); Vale de José Esteves, 29TPF5849, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9320-L); Vermelhosa, 29TPF5950, 147 m, on horizontal schist surfaces, 14.10.2010, J. Marques (PO9540-L).

Melanelixia O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch

Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch

Relatively rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 295 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9574-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9572-L).

Miriquidica Hertel & Rambold

Miriquidica deusta (Stenh.) Hertel & Rambold

Rare, on horizontal to less frequently vertical schist and granite surfaces facing south.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9083-L). **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on horizontal granite surfaces, 13.10.2011, J. Marques (PO9611-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on horizontal schist surfaces, 12.05.2009, J. Marques (PO9612-L).

Moelleropsis Gyeln.

Moelleropsis nebulosa (Hoffm.) Gyeln.

Relatively frequent but easily overlooked due to its small size and ephemeral nature on earth banks between schist outcrops and earth-filled crevices of those outcrops. Also occurring on vertical north-west facing schist surfaces, sheltered from direct sunlight.

SPECIMENS EXAMINED – **Alijó**, Carlão, Louzedo, 29TPF3576, 230 m, on earth banks between schist outcrops, 07.03.2012, J. Marques (PO9412-L). **Murça**, Candedo, Alto do Vale de Moinho, 29TPF3477, 324 m, on earth banks between schist outcrops, 18.09.2011, J. Marques (PO9413-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 302 m, on wide crevices of schist outcrops, 30.09.2011, J. Marques (PO9130-L); Vale do Forno, 29TPF5748, 258 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9411-L).

Nephroma Ach.

Nephroma tangeriense (Maheu & A. Gillet) Zahlbr.

Rare, muscicolous on vertical granite surfaces sheltered from direct sunlight.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Castanheiro do Norte, 29TPF3466, 378 m, on vertical granite surfaces, 12.10.2011, J. Marques (PO9613-L). **Murça**, Candedo, Vale de Moinho, 29TPF3477, 200 m, on vertical granite surfaces, 18.09.2011, J. Marques (PO9614-L).

Ochrolechia A. Massal.

Ochrolechia parella (L.) A. Massal.

Frequent, on vertical north-east facing schist surfaces.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on earth banks between ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9447-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 295m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9448-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9449-L).

Parmelia Ach.

Parmelia omphalodes (L.) Ach.

Relatively rare, on fully exposed horizontal granite surfaces.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on horizontal granite surfaces, 13.10.2011, J. Marques (PO9615-L).

Parmelia saxatilis (L.) Ach.

Relatively frequent, on vertical north-east facing schist and granite surfaces.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 13.10.2011, J. Marques (PO9616-L); Vila Nova de Foz Côa, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9617-L).

Parmelina Hale

Parmelina tiliacea (Hoffm.) Ach.

Frequent, on vertical north-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9617-L); Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 302 m, on vertical schist surfaces, 30.09.2011, J. Marques (PO9618-L); Vale de José Esteves, 29TPF5849, 208 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9619-L).

Peltigera Willd.

Peltigera canina (L.) Willd.

Relatively frequent, on earth banks between granite outcrops.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on earth banks between granite outcrops, 13.10.2011, J. Marques (PO9450-L). **Carrazeda de Ansiães**, Pombal, S. Lourenço, 29TPF3672, 188 m, on earth banks between granite outcrops, 14.10.2011, J. Marques (PO9451-L). Murça, Candedo, Vale de Moinho, 29TPF3477, 200 m, on earth banks between granite outcrops, 18.09.2011, J. Marques (PO9452-L).

Peltigera neckeri Hepp. ex Müll. Arg.

Relatively frequent, on earth banks between granite outcrops.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pereiros, Codeçais, 29TPF4177, 296 m, on earth banks between granite outcrops, 16.10.2011, J. Marques (PO9453-L); Pombal, S. Lourenço, 29TPF3672, 188 m, on earth banks between granite outcrops, 14.10.2011, J. Marques (PO9454-L).

Peltigera praetextata (Flörke ex Sommerf.) Zopf

Relatively frequent, on mossy granite surfaces and earth banks between schist and granite outcrops.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Castanheiro do Norte, 29TPF3466, 378 m, on vertical granite outcrops, 12.10.2011, J. Marques (PO9455-L). **Murça**, Candedo, Vale de Moinho, 29TPF3477, 200 m, on earth banks between granite outcrops, 18.09.2011, J. Marques (PO9456-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on earth banks between schist outcrops, 07.05.2009, J. Marques (PO9457-L).

Peltigera rufescens (Weiss) Humb.

Relatively rare, on earth banks between granite outcrops.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on earth banks between granite outcrops, 13.10.2011, J. Marques (PO9458-L).

Peltula Nyl.

Peltula bolanderi (Tuck.) Wetmore

Relatively frequent, colonizing the shaded areas of vertical south-east facing schist surfaces. Further notes on Chapter 6.1. **New for mainland Europe**.

Peltula euploca (Ach.) Poelt ex Ozenda & Clauzade

One of the most frequent species in the study area, colonizing virtually all sheltered to fully exposed vertical schist surfaces facing south, as a dominant species of the rain-track communities described by Llimona & Egea 1985. Further notes on Chapter 6.1.

Peltula farinosa Büdel

Rare, growing on sheltered to fully exposed vertical south-east facing schist surfaces. Further notes on Chapter 6.1. **New for mainland Europe**.

Peltula lobata J. Marques, M. Schultz & Paz-Berm.

Frequent, on narrow crevices of schist outcrops along the riverbanks of main rivers and small watercourses. Full details on Chapter 6.1. **New species**.

Peltula obscurans (Nyl.) Gyeln.

Relatively frequent, on crevices and compacted soil between schist outcrops. Further notes on Chapter 6.1. **New for Portugal**.

Peltula patellata (Bagl.) Swinscow & Krog

Frequent, on wide crevices or compacted calcareous soil between schist outcrops, and less often spreading towards the neighboring schist surfaces. Further notes on Chapter 6.1. **New for Portugal**.

Peltula placodizans (Zahlbr.) Wetmore

Relatively rare, on vertical south facing schist surfaces. Further notes on Chapter 6.1. **New for mainland Portugal**.

Peltula zahlbruckneri (Hasse) Wetmore

One of the rarest species in the study area, on inclined south facing schist surfaces. Further notes on Chapter 6.1. **New for Europe**.

Pertusaria DC.

Specimens revised by: Dr. Samantha Fernandez-Brime (University of Barcelona)

Pertusaria aspergilla (Ach.) J.R. Laundon

TLC: fumarprotocetraric, protocetraric and stictic acids

Characterized by the presence of fumarprotocetratic acid (Boqueras & Llimona 2003). Frequent, on vertical north-west facing schist surfaces. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9572-L); Vale do Forno, 29TPF5748, 295 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9574-L).

Pertusaria flavicans Lamy

Rare, on vertical north-west facing schist surfaces. **New for Portugal.**

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9579-L).

Pertusaria leucosora Nyl.

TLC: protocetratic and (traces of) norstictic acids

Distinguished from the similar *P. aspergilla* by the different chemistry, lacking picrolichenic acid and having protocetratic acid as a major constituent, as well as traces of norstictic acid

(Boqueras & Llimona 2003). Frequent, on vertical north-west facing schist surfaces. **New for Portugal.**

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9575-L).

Pertusaria melanochlora (DC.) Nyl.

TLC: protocetratic, stictic and picrolichenic acids

Characterized by the presence of picrolichenic acid (Boqueras & Llimona 2003). Frequent, on vertical north-west facing schist surfaces. **New for the province of Trás-os-Montes e Alto Douro.**

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9571-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9570-L).

Pertusaria pertusa (L.) Tuck.

TLC: stictic acid complex, (traces of) norstictic acid and coronatone

Relatively frequent, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9576-L).

Pertusaria pseudocorallina (Lilj.) Arnold

Distinguished by the isidiate thallus and K+ red reaction. Very rare, on inclined schist surfaces. **New for the province of Trás-os-Montes e Alto Douro**.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9536-L).

Phaeophyscia Moberg

Phaeophyscia cernohorskyi (Nádv.) Essl.

Very rare in the study area, muscicolous on shaded vertical schist surfaces facing south.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 190 m, on vertical schist surfaces, 14.03.2012, J. Marques (PO9178-L).

Phaeophyscia orbicularis (Neck.) Moberg

Relatively frequent, on vertical north-west facing to less frequently south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9342-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9343-L); Vale do Forno, 29TPF5748, 295 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9344-L).

Physcia (Schreb.) Michx.

Physcia caesia (Hoffm.) Hampe ex Fürnr.

Relatively frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 164 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9345-L).

Physcia tribacia (Ach.) Nyl.

Frequent, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9346-L); Penascosa, 29TPF5941, 161 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9347-L); Muxagata. Ribeira de Piscos, 29TPF5843, 130 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9348-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9349-L).

Physconia Poelt

Physconia enteroxantha (Nyl.) Poelt

Relatively frequent, on vertical schist surfaces at varying orientations.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9350-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on vertical schist surfaces, 11.10.2010, J. Marques (PO9351-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9352-L).

Physconia grisea (Lam.) Poelt

Rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5846, 190 m, on vertical schist surfaces, 14.03.2012, J. Marques (PO9178-L).

Placidium A. Massal.

Placidium imbecillum (Breuss) Breuss

Relatively frequent, on crevices of vertical south-east facing schist surfaces and earth banks between schist outcrops. Further notes on Chapter 6.3. **New for the province of Trás-os-Montes e Alto Douro.**

Placidium rufescens (Ach.) A. Massal.

Previously cited by Paz-Bermúdez *et al.* (2009) for the province of Trás-os-Montes e Alto Douro growing on amphibolite and pteridotite, it is one of the few species of *Placidium* known to grow directly on rock (Prieto *et al.* 2010). In the study area it was frequently found growing directly on vertical south-east facing schist surfaces together with *Peltula euploca* and *Glyphopeltis ligustica*.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on vertical surfaces of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9382-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 310 m, on vertical schist surfaces, 07.04.2011, J. Marques (PO9383-L); Muxagata, Fariseu, 29TPF5844, 127 m, on wide crevices of schist outcrops, 01.04.2010, J. Marques (PO9384-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9385-L); Vale Figueira, 29TPF5845, 223 m, on vertical schist surfaces, 13.03.2012, J. Marques (PO9386-L).

Placynthium (Ach.) Gray

Placynthium tremniacum (A. Massal.) Jatta

This species is morphologically identical to *Placynthium nigrum* (Huds.) Gray and difficult to separate from the latest when sterile (Burgaz 2011). Distinction from *Placynthium nigrum* is based on spore size and septation as suggested by this author. The studied specimen presents uniseptate spores reaching 15 µm in width and 6 µm in length. Very rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9321-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9341-L).

Polychidium (Ach.) Gray

Polychidium muscicola (Sw.) Gray

Relatively frequent, muscicolous on periodically submerged schist and granite outcrops in temporary riverbeds.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Praia fluvial, 29TPF3466, 136 m, on horizontal granite surfaces, 12.10.2011, J. Marques (PO9324-L). **Mogadouro**, Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9312-L; PO9323-L). **Murça**, Candedo, Rio Tinhela, 29TPF3576, 225 m, on vertical schist surfaces, 18.09.2011, J. Marques (PO9582-L).

Polysporina Vězda

Polysporina simplex (Davies) Vězda

Relatively rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 164 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9471-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 127 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9620-L).

Porocyphus Körb.

Porocyphus coccodes Flot. ex Körb.

Very rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3. **New for the province of Trás-os-Montes e Alto Douro**.

Psora Hoffm.

Psora decipiens (Hedw.) Hoffm.

Relatively frequent, on fully exposed horizontal schist surfaces and wide south-east facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9484-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 297 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9492-L); Vermelhosa, 29TPF5950, 147 m, on horizontal schist surfaces, 14.10.2010, J. Marques (PO9540-L).

Psora gresinonis B. de Lesd.

Frequent, on horizontal schist surfaces and earth banks between schist outcrops. Further notes on Chapter 6.3. **New for the province of Trás-os-Montes e Alto Douro.**

Psorotichia A. Massal.

Psorotichia schaereri (A. Massal.) Arnold

Rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3. **New for Portugal.**

Pterygiopsis Vain.

Pterygiopsis affinis (A. Massal.) Henssen

Frequent, on vertical south-east facing schist and granite surfaces. Further notes on Chapter 6.3. **New for Portugal**.

Pyrenopsis (Nyl.) Nyl

Pyrenopsis triptococca Nyl.

Very frequent, on vertical south-east facing schist surfaces associated with the rain-track communities described by Llimona & Egea (1985).

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO8993-L). **Carrazeda de Ansiães**, Castanheiro, Fiolhal, 29TPF3264, 203 m, on vertical schist surfaces, 23.03.2012, J. Marques (PO9459-L). **Mirandela**, Valverde, Serra de Valverde, 29TPF5183, 219 m, on vertical quartzite surfaces, 15.10.2011, J. Marques (PO8994-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9460-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9461-L); Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9467-L); Penascosa, 29TPF5941, 163 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9464-L); Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9008-L); Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO8991-L); Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9211-L); Vale do Forno, 29TPF5748, 301 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9462-L).

Ramalina Ach.

Ramalina capitata (Ach.) Nyl.

Rare, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9493-L).

Rhizocarpon Ramond ex DC.

Rhizocarpon geographicum (L.) DC.

TLC: rhizocarpic and psoromic acids

Frequent, on inclined schist surfaces with various orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9592-L); Penascosa, 29TPF5941, 161 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9593-L).

Rhizocarpon lecanorinum Anders

TLC: rhizocarpic, stictic acid complex

Rare, on vertical schist surfaces facing south.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9636-L).

Rhizocarpon richardii (Nyl.) Zahlbr.

TLC: gyrophoric and stictic acids

Frequent, on inclined schist surfaces with various orientations.

SPECIMENS EXAMINED – **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on inclined schist surfaces, 15.10.2011, J. Marques (PO9591-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9590-L).

Rhizocarpon tetrasporum Runemark

TLC: rhizocarpic, stictic, constictic, menegazziaic and (traces of) norstictic acids

The most frequent species of *Rhizocarpon* in the study area, on vertical to slightly inclined south-east facing schist surfaces. Also found on granite in similar situations.

SPECIMENS EXAMINED – **Alijó**, Amieiro, Amieiro, 29TPF3471, 153 m, on vertical granite surfaces, 13.10.2011, J. Marques (PO9637-L); **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9638-L); **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9579-L); Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9639-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9641-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9640-L).

Rhizoplaca Zopf

Rhizoplaca maheui (Hue) Gómez-Bolea & M. Barbero

TLC: usnic, fumarprotocetraric, protocetratic and psoromic acids

Relatively rare, on vertical schist and granite surfaces facing south. Further notes on Chapter 6.3. **New for Portugal.**

Rinodina (Ach.) Gray

Rinodina atrocinerea (Fr.) Körb.

Relatively rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 156 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9276-L).

Rinodina beccariana Bagl.

Frequent, on vertical to slightly overhanging, mostly north-west facing schist surfaces. Also found on ledges and narrow crevices of schist outcrops with the same orientation.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Castanheiro, Praia fluvial, 29TPF3466, 136 m, on vertical granite surfaces, 12.10.2011, J. Marques (PO9277-L). **Mogadouro**, Brunhoso, Ribeiro de Juncaínhos, 29TPF6880, 474 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9364-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9581-L); Penascosa, 29TPF5941, 164 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9284-L); Chãs, Quinta da Barca, 29TPF5941, 150 m, on overhanging schist surfaces, 14.10.2010, J. Marques (PO9281-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on overhanging schist surfaces, 09.05.2009, J. Marques (PO9278-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on vertical schist surfaces, 07.05.2009, J. Marques (PO9280-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9279-L); Vale Cabrões, 29TPF5850, 248 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9282-L).

Rinodina intermedia Bagl.

Very frequent, on wide south-east facing crevices of schist outcrops and horizontal schist surfaces among other terricolous lichens.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on wide crevices of schist outcrops, 13.05.2009, J. Marques (PO9011-L; PO9186-L). **Alijó**, Carlão, Alto do Vale de Moinho, 29TPF3477, 324 m, on wide crevices of schist outcrops, 07.03.2012, J. Marques (PO9287-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2011, J. Marques (PO9285-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on wide crevices of schist outcrops, 12.05.2009, J. Marques (PO9288-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 160 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9585-L); Muxagata, Fariseu, 29TPF5844, 127 m, on horizontal schist surfaces, 01.04.2010, J. Marques (PO9621-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9289-L); Vale de José Esteves, 29TPF5849, 190 m, on wide crevices of schist outcrops, 02.05.2011, J. Marques (PO9290-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9291-L).

Rinodina oxydata (A. Massal.) A. Massal.

Very frequent, on vertical south-east facing surfaces and respective crevices. Doubtfully separated from the morphological and chemically identical *R*. *vezdae* H. Mayrhofer by spore size, which is only slightly smaller in *R*. *oxydata* (Giralt 2001).

SPECIMENS EXAMINED – **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9053-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9431-L); Penascosa, 29TPF5941, 160 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9292-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9293-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on wide crevices of schist outcrops, 07.05.2009, J. Marques (PO9294-L); Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9195-L); Vale do Forno, 29TPF5748, 320 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9538-L).

Rinodina teichophila (Nyl.) Arnold

Very frequent, on vertical south-east facing surfaces and respective crevices.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 164 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9340-L); Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9298-L); Monte do Fariseu, 29TPF5844, 263 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9041-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9180-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9299-L); Vale de José Esteves, 29TPF5849, 190 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9296-L); Vale do Forno, 29TPF5748, 269m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9297-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9148-L).

Rinodina trachytica (A. Massal.) Bagl. & Carestia

Very frequent, on vertical to inclined south-east facing surfaces and respective crevices.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on vertical schist surfaces, 13.05.2009, J. Marques (PO9083-L). **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9300-L); Penascosa, 29TPF5941, 158 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9301-L); Muxagata, Ribeira de Piscos, 29TPF5843, 150 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO9302-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9539-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9303-L); Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9304-L).

Rinodina vezdae H. Mayrhofer

Frequent, on vertical south-east facing schist surfaces and respective crevices. Further notes on Chapter 6.3. **New for Portugal.**

Romjularia Timdal

Romjularia lurida (Ach.) Timdal

Very rare, on wide south-east facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Brunhoso, Ribeiro de Juncaínhos, 29TPF6880, 474 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9369-L).

Sarcogyne Flot.

Sarcogyne privigna (Ach.) A. Massal.

Relatively frequent, on fully exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Monte do Fariseu, 29TPF5844, 263 m, on horizontal schist surfaces, 01.04.2010, J. Marques (PO9487-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9488-L).

Sarcogyne regularis var. *intermedia* (Körb.) N. S. Golubk.

Rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9098-L).

Solenopsora A. Massal.

Solenopsora holophaea (Mont.) Samp.

Rare, on vertical north-west facing schist surfaces, overgrowing mosses. Also on ultrabasic rocks.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on wide crevices of ultrabasic rock outcrops, 11.05.2009, J. Marques (PO9406-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 330 m, on vertical schist surfaces, 29.09.2010, J. Marques (PO9407-L); Quinta das Tulhas, 29TPF5949, 140 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9409-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 320 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9408-L).

Solenopsora vulturiensis A. Massal.

Very frequent, on horizontal to vertical schist surfaces of varying orientations although morphology varied significantly from a totally pulverulent thallus at south-east facing surfaces to a squamulose sorediate thallus at north-west facing surfaces. Found fertile in both situations.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on wide crevices of schist outcrops, 13.05.2009, J. Marques (PO9402-L). **Carrazeda de Ansiães**, Castanheiro, Quinta da Ribeira, 29TPF3265, 132 m, on schist walls, 12.10.2011, J. Marques (PO9401-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9206-L). **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 248 m, on narrow crevices of schist outcrops, 18.03.2012, J. Marques (PO9405-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9403-L); Vale do Forno, 29TPF5748, 258 m, on wide crevices of schist outcrops, 04.05.2011, J. Marques (PO9404-L).

Squamarina Poelt

Squamarina cartilaginea (With.) P. James

Rare, on vertical schist surfaces of varying orientations and respective crevices.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on wide crevices of schist outcrops, 13.05.2009, J. Marques (PO9365-L). **Mogadouro**, Brunhoso, Ribeiro de Juncaínhos, 29TPF6880, 474 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9364-L); Soutelo, Alto da Fraga, 29TPF8886, 580 m, on wide crevices of schist outcrops, 11.05.2009, J. Marques (PO9485-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques, PO9366-L. **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9367-L; PO9368-L).

Squamarina concrescens (Müll. Arg.) Poelt

Very frequent, on wide south-east facing crevices of schist outcrops and fully-exposed horizontal schist surfaces. Further notes on Chapter 6.3.

Squamarina gypsacea (Sm.) Poelt

Very rare, on wide south-east facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Brunhoso, Brunhoso, 29TPF6880, 474 m, on wide crevices of schist outcrops, 17.02.2011, J. Marques (PO9369-L).

Tephromela M. Choisy

Tephromela atra (Huds.) Hafellner

Relatively rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 177 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9566-L); Vale do Forno, 29TPF5748, 295 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9473-L).

Toninia A. Massal.

Toninia aromatica (Turner) A. Massal.

Relatively frequent, on vertical south-east facing schist surfaces and respective crevices.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9434-L). **Murça**, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on narrow crevices of schist outcrops, 17.09.2011, J. Marques (PO9435-L). **Vila Nova de Foz Côa**, Muxagata, Ribeira de Piscos, 29TPF5843, 136 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9436-L); Vila Nova de Foz Côa, Vale Cabrões, 29TPF5750, 301 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9295-L; PO9437-L); Vale de José Esteves, 29TPF5949, 146 m, on wide crevices of schist outcrops, 08.05.2009, J. Marques (PO9438-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9400-L).

Toninia cinereovirens (Schaer.) A. Massal.

The most frequent species of the genus in the study area, on vertical south-east facing schist surfaces, as a characteristic of species of the rain-track communities described by Llimona & Egea (1985). Also terricolous on narrow and wide crevices along with *Endocarpon pusillum*, *Peltula patellata* and other ecologically similar lichens. Further notes on Chapter 6.3.

Toninia opuntioides (Vill.) Timdal

Relatively rare, on vertical south-east facing schist surfaces. Further notes on Chapter 6.3.

Toninia sedifolia (Scop.) Timdal

Relatively frequent, on wide south-east facing crevices of schist outcrops.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9439-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9440-L); Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9441-L); Vermelhosa, 29TPF5950, 147 m, on wide crevices of schist outcrops, 14.10.2010, J. Marques (PO9442-L).

Toninia squalida (Ach.) A. Massal.

Frequent, on north-west facing schist surfaces. Further notes on Chapter 6.3.

Toninia cf. *taurica* (Szatala) Ochsner

According to Timdal (1991) this species is distinguished from *Toninia sedifolia*, with which it is easily confused, by the presence of pruina exclusively in apothecia while the thallus is completely deprived of it. The examined specimens do have most of the pruina concentrated on apothecia, differing from *Toninia sedifolia*, but a few pruinose areas are also found, after a close look, on the lower surface of some squamules. Rare, on wide crevices of schist outcrops.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on narrow crevices of schist outcrops, 25.04.2009, J. Marques (PO9443-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on narrow crevices of schist outcrops, 30.03.2010, J. Marques (PO9444-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 130 m, on horizontal schist surfaces, 07.05.2009, J. Marques (PO9445-L); Vale Cabrões, 29TPF5750, 301 m, on horizontal schist surfaces, 15.10.2010, J. Marques (PO9446-L).

Toninia toepfferi (Stein) Navàs

Relatively frequent in the study area, on wide crevices filled with compacted soil. Further notes on Chapter 6.3. **New for mainland Portugal.**

Toninia tristis (Th. Fr.) Th. Fr. subsp. *pseudotabacina* Timdal

Very rare, on wide south-east facing horizontal crevices. Further notes on Chapter 6.3. **New for Trás-os-Montes e Alto Douro.**

Trapelia M. Choisy

Trapelia coarctata (Turner) M. Choisy

Rare, on horizontal schist surfaces and wide crevices of schist outcrops of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9359-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9360-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9361-L).

Trapelia involuta (Taylor) Hertel

Rare, on horizontal schist surfaces and wide crevices of schist outcrops of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 161 m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9359-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 126 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9362-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9357-L).

Trapeliopsis Hertel & Gotth. Schneid.

Trapeliopsis flexuosa (Fr.) Coppins & P. James

Rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Vale do Forno, 29TPF5748, 251 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9363-L).

Trapeliopsis gymnidiata Aptroot & Schumm

Very frequent, on wide south-east facing crevices of schist outrcrops. Further notes on Chapter 6.2. **New for mainland Europe**.

SPECIMENS EXAMINED – **Carrazeda de Ansiães**, Pombal, Amieiro, 29TPF3570, 186 m, muscicolous on vertical granite surfaces, 14.10.2011, J. Marques (PO9358-L). **Mogadouro**, Soutelo, Alto da Fraga, 29TPF8886, 580 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9577-L). **Torre de Moncorvo**, Cardanha, Ribeira de Relvas, 29TPF6669, 259 m, on earth banks between schist outcrops, 18.02.2011, J. Marques (PO9353-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on wide crevices of schist outcrops, 15.10.2010, J. Marques (PO9354-L); Canada do Amendoal, 29TPF5946, 344 m, on horizontal schist surfaces, 30.03.2010, J. Marques (PO9355-L); Penascosa, 29TPF5941, 160 m, on wide crevices of schist outcrops, 15.05.2007, J. Marques (PO9147-L; PO9356-L); Muxagata, Ribeira de Piscos, 29TPF5843, 130 m, on horizontal schist surfaces, 11.10.2010, J. Marques (PO9567-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 131 m, on inclined schist surfaces, 07.05.2009, J. Marques (PO9476-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9356-L); Vale do Forno, 29TPF5748, 297 m, on wide crevices of schist outcrops, 30.03.2010, J. Marques (PO9357-L).

Umbilicaria Hoffm.

Umbilicaria grisea Hoffm.

Rare, on vertical north-west facing schist surfaces.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9426-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada do Amendoal, 29TPF5946, 344 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9427-L).

Vahliella P. M. Jørg.

Vahliella leucophaea (Vahl) P. M. Jørg.

Rare, on vertical schist surfaces facing north. Further notes on Chapter 6.3. **New for the province of Trás-os-Montes e Alto Douro**.

Verrucaria Schrad.

Verrucaria geophila Zahlbr.

Rare, on wide south-east facing crevices of schist outcrops. Further notes on Chapter 6.3. **New for Portugal**.

Verrucaria nigrescens Pers.

Frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9428-L); Vale de José Esteves, 29TPF5849, 178 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9429-L); Vale do Forno, 29TPF5748, 269 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9430-L).

Xanthoparmelia (Vain.) Hale

Xanthoparmelia conspersa (Ehrh. ex Ach.) Hale

TLC: usnic and norstictic acids, stictic acid complex

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9496-L). **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9622-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9623-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 156 m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9498-L); Quinta das Tulhas, 29TPF5949, 140 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9624-L); Muxagata. Ribeira de Piscos, 29TPF5843, 136 m, on vertical schist surfaces, 09.05.2009, J. Marques (PO8991-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9497-L); Vale de José Esteves, 29TPF5949, 146 m, on horizontal schist surfaces, 08.05.2009, J. Marques (PO9109-L); Vale do Forno, 29TPF5748, 251 m, on vertical schist surfaces, 04.05.2011, J. Marques (PO9209-L).

Xanthoparmelia loxodes (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

TLC: glomelliferic, glomellic, perlatolic and gyrophoric acids

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Murça**, Candedo, Alto das Eirinhas, 29TPF3978, 260 m, on vertical schist surfaces, 16.10.2011, J. Marques (PO9625-L). **Vila Nova de Foz Côa**, Muxagata. Ribeira de Piscos, 29TPF5843, 130 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9501-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 146 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9626-L).

Xanthoparmelia pokornyi (Körb.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

Rare, on fully exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Murça**, Candedo, Foz do Tinhela, 29TPF3676, 181 m, on horizontal schist surfaces, 17.09.2011, J. Marques (PO9627-L).

Xanthoparmelia protomatrae (Gyeln.) Hale

TLC: usnic and fumarprotrocetraric acids

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Mirandela**, Barcel, Longra, 29TPF4680, 219 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9628-L). **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9513-L). Murça, Candedo, Ponte do Tinhela, 29TPF3676, 204 m, on vertical schist surfaces, 17.09.2011, J. Marques (PO9512-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9629-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316 m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9499-L; PO9504-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9497-L); Foz do Côa, 29TPF5849, 187 m, on vertical schist surfaces, 03.05.2011, J. Marques (PO9299-L).

Xanthoparmelia pulla (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

TLC: stenosporic and gyrophoric acids

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Alfândega da Fé**, Parada, Santo Antão da Barca, 29TPF7669, 175 m, on horizontal schist surfaces, 13.05.2009, J. Marques (PO9519-L). **Alijó**, Carlão, Alto do Vale de Moinho, 29TPF3477, 324 m, on wide crevices of schist outcrops, 07.03.2012, J. Marques (PO9630-L). **Mogadouro**, Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9516-L). **Vila Flor**, Vilarinho das Azenhas, Vilarinho das Azenhas, 29TPF4982, 206 m, on vertical schist surfaces, 15.10.2011, J. Marques (PO9631-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 158 m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9500-L); Muxagata, Fariseu, 29TPF5844, 127 m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9520-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110 m, on wide crevices of schist outcrops, 08.02.2006, J. Marques (PO9515-L); Tudão, 29TPF5650, 378 m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9527-L); Vale de José Esteves, 29TPF5949, 146 m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9517-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques

(PO9506-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9503-L; PO9507-L).

Xanthoparmelia somloensis (Gyeln.) Hale

TLC: usnic, salazinic and norstictic acids

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Mogadouro**, Bemposta, Faia da Água Alta, 29TQF0775, 514 m, on vertical schist surfaces, 12.05.2009, J. Marques (PO9632-L); Soutelo, Ponte de Remondes, 29TPF8385, 200 m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9521-L). **Vila Nova de Foz Côa**, Castelo Melhor, Penascosa, 29TPF5941, 158 m, on vertical schist surfaces, 31.03.2010, J. Marques (PO9508-L); Muxagata. Ribeira de Piscos, 29TPF5843, 130 m, on wide crevices of schist outcrops, 09.05.2009, J. Marques (PO9633-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5849, 188 m, on vertical schist surfaces, 02.05.2011, J. Marques (PO9634-L); Vale do Forno, 29TPF5748, 297 m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9524-L); Vermelhosa, 29TPF5950, 147 m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9523-L).

Xanthoparmelia subverrucigera O. Blanco, A. Crespo & Elix

TLC: usnic and lusitanic acids, stictic acid complex

Chemically identical to *X*. *verrucigera* (containing usnic acid, the stictic acid series and lusitanic acid) but distinguished by the mid-brown to dark brown lower surface and branched coralloid isidia. Relatively frequent on fully exposed horizontal schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 110m, on inclined schist surfaces, 08.02.2006, J. Marques (PO9525-L); Vale de José Esteves, 29TPF5949, 146m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9526-L).

Xanthoparmelia tinctina (Maheu & A. Gillet) Hale

TLC: usnic, salazinic and norstictic acids

Very frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Mogadouro**, Soutelo, Ponte de Remondes, 29TPF8385, 200m, on vertical schist surfaces, 11.05.2009, J. Marques (PO9521-L). **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9499-L); Vila Nova de Foz Côa, Tudão, 29TPF5650, 378m, on inclined schist surfaces, 08.05.2009, J. Marques (PO9527-L); Vale do Forno, 29TPF5748, 297m, on vertical schist surfaces, 30.03.2010, J. Marques (PO9506-L; PO9524-L); Vermelhosa, 29TPF5950, 147m, on vertical schist surfaces, 14.10.2010, J. Marques (PO9503-L; PO9507-L).

Xanthoparmelia verrucigera (Nyl.) Hale

TLC: usnic and lusitanic acids, stictic acid complex

The analysed specimens contain usnic acid, the stictic acid series and lusitanic acid. The lack of norstictic acid is the prime difference from *X. conspersa,* also found in the study area. Frequent, on vertical schist surfaces of varying orientations.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Canada da Moreira, 29TPF6047, 316m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9504-L); Penascosa, 29TPF5941, 164m, on inclined schist surfaces, 15.05.2007, J. Marques (PO9510-L); Muxagata, Fariseu, 29TPF5844, 127m, on vertical schist surfaces, 01.04.2010, J. Marques (PO9505-L); Vila Nova de Foz Côa, Canada do Inferno, 29TPF5846, 127m, on vertical schist surfaces, 08.02.2006, J. Marques (PO9635-L); Vale Cabrões, 29TPF5850, 248m, on horizontal schist surfaces, 15.10.2010, J. Marques (PO9529-L); Vale do Forno, 29TPF5748, 320m, on vertical schist surfaces, 15.10.2010, J. Marques (PO9528-L).

Xanthoparmelia verruculifera (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

TLC: divaricatic and gyrophoric acids

Rare, on vertical south-east facing schist surfaces.

SPECIMENS EXAMINED – **Vila Nova de Foz Côa**, Castelo Melhor, Alto da Penascosa, 29TPF5846, 270m, on horizontal schist surfaces, 15.05.2007, J. Marques (PO9502-L); Penascosa, 29TPF5941, 161m, on vertical schist surfaces, 15.05.2007, J. Marques (PO9530-L); Vila Nova de Foz Côa, Vale de José Esteves, 29TPF5949, 146m, on vertical schist surfaces, 08.05.2009, J. Marques (PO9356-L).

6.4.2 Discussion

Lichens are by far the dominant component of vegetation growing on exposed schist outcrops in the Upper Douro region. From a physiognomic point of view, these lichen assemblages are mainly constituted by crustose epilithic species, possibly influenced by both the xeric climatic conditions as well as the rock surface resistance, although chasmolithic lichens of various growth forms are also quite abundant on earth-filled crevices and earth-banks between outcrops, certainly owing to the stability of relatively undisturbed hills after several years of land abandonment. From the ecological point of view, it is well known that schist creates warmer and dryer microclimates which, enhanced by a harsh climate and topography, favours the occurrence of a high number of thermophilic and xerophilic species.
The study area is therefore especially rich in Mediterranean species such as *Acarospora hilaris*, *A. epithallina*, *Caloplaca pellodella*, *Glyphopeltis ligustica*, *Lichinella cribellifera*, *Physcia tribacia*, among many others. Recent discoveries or range extensions of species usually found in arid regions of both hemispheres such as *Endocarpon simplicatum*, *Peltula euploca*, *P. obscurans*, *P. patellata*, *P. placodizans* and *P. zahlbruckneri* clearly demonstrate a close relationship at species or genus level to other dry areas of the Earth. A high number of Ibero-Macaronesian elements such as *Pyrenopsis triptococca*, *Toninia toepferii* or *Trapeliopsis gymnidiata*, and Ibero-African elements, including *Acarospora charidema*, were also detected. A few sites constitute a refuge for the so far Iberian endemics *Caloplaca cancarixiticola* and *Rhizoplaca maheui*. Ocurrence of carbonate intercalations is quite obvious by the exceptionally large amount of calciphilous species including *Caloplaca arnoldii*, *Caloplaca flavescens*, *Pterygiopsis affinis*, *Psora decipiens* and *Squamarina*

concrescens, among others. The studied lichen flora is nevertheless composed of numerous cosmopolitan to subcosmopolitan elements, including some oceanic species such as *Diploicia canescens*, *Koerberia sonomensis*, *Moelleropsis nebulosa* and *Ochrolechia parella*, just to name a few. The richness of lichen species in the Upper Douro region is increased by the presence of some shady and moist microhabitats produced by narrow ravines or other protected places in the proximity of major rivers. Several taxa more commonly found as epiphytes are sporadically encountered on north-west facing surfaces sheltered from the summer drought by the denser vegetation, including the usually epiphytes *Fuscopannaria mediterranea* and *Leptogium cochleatum*.

6.4.3 References

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7 Ecological tools for rock-art condition assessment in the Upper Douro region

7.1 Choosing between sides in the battle for pioneer colonization of schist in the Côa Valley Archaeological Park: a community ecology perspective

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Abstract

Lichens and bryophytes are the dominant colonizers of rock-art on schist in the Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal). They should play an important role in the process of biologically induced deterioration of the engraved surfaces, yet little is known about the composition and distribution of these communities and how they respond to local environmental constraints. This study provides a first detailed survey of the lichen and bryophyte species assemblages on the Côa Valley's rock surfaces and assesses the effect of slope aspect on the composition of those assemblages with the aim of providing a selection of the most appropriate species for future approaches of biologically induced rock-art deterioration. Ninety lichens and forty one bryophytes were identified on a selection of twenty four rock surfaces, with significant variation in the composition of their assemblages dependent on slope aspect, here analysed in detail. The potential implications of such variation for differential rock-art deterioration are also discussed. An emphasis is given on the need to directly quantify the impacts of individual species and species assemblages before assuming the effective role of biological activity on (differential) rock surface weathering.

7.1.1 Introduction

The Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal) is a UNESCO World Heritage site holding the most remarkable collection of open-air Prehistoric rock-art in the Iberian Peninsula (Baptista 2009) and the longest rock-art cycle known in western Europe (Baptista & Fernandes 2007), ranging from the Upper Palaeolithic (Zilhão 1995) to the early modern era, with important representations from the Neolithic and the Iron Age as well as Historical and Contemporary (Baptista 2009). These are preserved on approximately 1000 vertical schist surfaces located along the steep slopes of the Côa River Valley, and extending into a portion of the Douro riverbanks (Aubry *et al.* 2012, Fernandes 2012).

Archaeologists have sought to understand the currently observed pattern of clustering and orientation of the engraved surfaces in the Côa valley, mainly exposed toward the south-south-east (Baptista & García Diez 2002) and spawned a whole series of papers on its likely origin, with contradictory opinions regarding the influence of either human choice or natural phenomena (*e.g.* Aubry *et al.* 2012, Baptista & García Diez 2002, Fernandes 2010). The consensus viewpoint is that a suite of highly complex and interacting physical, chemical and biological processes operate on these surfaces, whose knowledge is crucial for open-air rock-art conservation (Fernandes 2004). Lichens and bryophytes (mosses and liverworts) are known to have a wide range of direct and indirect impacts on rock surfaces (Adamo & Violante 2000, Altieri & Ricci 1997, Chen *et al.* 2000, de los Rios *et al.* 2004, Shirzadian & Uniyal 2008, Warscheid & Braams 2000), and are likely to be the main agents of biologically induced weathering in the Côa valley, given their prevalence over other colonizing organisms. Based on the well-known effects of aspect in the occurrence and distribution of lichens and bryophytes on rock surfaces (*e.g.* John & Dale 1991, Hespanhol *et al.* 2010, 2011, Pentecost 1979) and considering the recent demonstrations of the influence of aspect in the weathering ability of individual species (Adamson *et al.* 2013, Hall *et al.* 2005), one might be led to overestimate the role of bryophyte- and lichen-induced processes in differential weathering, unless changes brought about by a number of factors are also accounted for, including: 1) the local- and micro-scale patterns of community structure, composition and distribution (Kuntz & Larson 2006); 2) the internal readjustments of species assemblages as a result of ecological succession (Woolhouse *et al.* 1985); 3) the impact of several other physical and chemical agents (Hall *et al.* 2012), superimposed upon the effect of lichen and bryophyte growth on rock surfaces - and certainly interacting with it - that are simultaneously affected by aspect, namely water and temperature regimes (Caneva *et al.* 2008, Viles 1995). The nature and extent of the impact of lichen and bryophyte assemblages upon rock surfaces in the Côa valley, although still poorly understood, is thus expected to depend on the weathering ability of individual species (Favero-Longo *et al.* 2005, Prieto *et al.* 1997) and surrounding environment, as well as on the intrinsic properties of the substrate (Guillitte 1995, Prieto & Silva 2005).

The ecological determinants of lichen and bryophyte distribution on monuments have been studied by Prieto *et al.* (1995, 1999) and Sparrius *et al.* (2007) on granite and limestone churches in Spain and The Netherlands, respectively; by Steinbauer *et al.* (2013) and Weber & Büdel (2001) on medieval castles in Germany; and by Nascimbene *et al.* (2009) and Nascimbene & Salvadori (2008), on calcareous statues in Italy. Prevailing microclimatic conditions of light, water and temperature regimes, rock pH and nutrient availability are as much influential in cultural environments (Adamson *et al.* 2013, Carter & Viles 2004, Hall *et al.* 2012, Prieto *et al.* 1995, 1999, Steinbauer *et al.* 2013) as they are on natural contexts (Prieto *et al.* 1994).

The use of community ecology to address interactions within and between species assemblages on monuments has been pioneered by the works of Monte (1991) and Ramette (2007), but followed by few researchers (Barberousse *et al.* 2006, Sparrius *et al.* 2007, Steinbauer *et al.* 2013, Ortega-Morales *et al.* 2013), probably because such an approach is usually dependent on field recognition of all colonizing organisms up to the species level, which for many groups such as bacteria, fungi or algae is highly demanding, and results in large, complex data sets. Data interpretation in community ecology has long been relying on multivariate techniques such as ordination (Legendre & Legendre 1998) that allows extracting the underlying structure out of such complexity and relating this structure to environmental variables, displayed in the reduced space of a diagram (ter Braak & Verdonschot 1995). It is therefore considered an excellent tool to evaluate differences in species composition between samples and to identify the environmental variables responsible for those differences, in a single analysis (van den Brink *et al.* 2003).

Despite the already mentioned existing studies about the influence of macro and micro-scale factors on the structure of lichen and bryophyte communities, relatively little research has been carried out to untangle the contributions of aspect to observed changes in the composition and development of lichen and bryophyte assemblages on vertical schist surfaces. Studies concerning the lichen and bryophyte flora in the Côa Valley Archaeological Park have focused mostly on biodiversity assessments (Caldas *et al.* 2002, Romão 1999, Vänskä 2001), aiming at identifying some of the most frequent species on the engraved surfaces, but do not consider the processes that structure their occurrence and distribution at the local-scale. The purpose of this study is to specifically address the occurrence patterns of lichen and bryophyte species assemblages on vertical schist surfaces under the influence of opposite slope aspect in order to (1) assess the effect of slope aspect on lichen and bryophyte species richness and composition in the Côa Valley Archaeological Park and (2) provide a selection of species representing the local-scale changes in these communities that can be related to aspect, and to their respective role in the deterioration of the Côa valley's open-air rock-art.

An important outcome of the community ecology approach is that knowledge about species-environment relationships allows for species colonizing rock surfaces to be used as early indicators of the effects of environmental change, by relating species assemblages to environmental drivers that are relevant for rock-art conservation (Prieto *et al.* 1999). Such knowledge is also critical for the choice of the most appropriate preventive or cleaning methods (Nascimbene *et al.* 2009), if that is to be considered in the framework of the Côa valley's rock-art conservation plan.

7.1.2 Material and methods

The study area

Fig. 7.1.1. The typical landscape of the Côa Valley (left) and one example of the thousands of schist outcrops that can be found throughout the valley (right).

The study area is situated in the valley of River Côa close to its confluence with River Douro, approximately 200 km upstream of the mouth of the Douro, in the city of Oporto. The lithology of the Côa River Valley is dominated by meta-sedimentary rocks of the schist-greywacke complex said by Ribeiro (2001) to be ranging in age from the Precambrian to the Ordovician. The Côa and its tributary streams have cut deeply through the schist and metagreywacke basement, taking advantage of pre-existing major faults roughly oriented from NE to SW (Aubry *et al.* 2012) and forming numerous steep-walled valleys that play a major influence on regional landscape. A special feature resulting from the down-cutting of the Côa Valley is the occurrence of massive vertical schist surfaces arranged in layers along the valley's slopes (Fig. 7.1.1), which have been gradually exposed by rock toppling, *i.e.* a sequence of gravity-induced block displacement after splitting of vertically orientated joints, along the schistosity plane (Fernandes 2006, Fernandes & Delgado-Rodrigues 2008).

Climate is predominantly dry meso-Mediterranean (Costa *et al.* 1998), sheltered from the Atlantic influence by mountains to the north and the west, but thermo-Mediterranean microclimates are usually produced in the bottom of the valleys, where temperature is frequently above 40°C in late spring and summer (June-August), daily thermal amplitudes may reach 10º to 15º C and mean annual precipitation is often bellow 400 mm (Fernandes 2012).

Sampling design

The study was conducted at three sites: Vale de José Esteves, Vale do Forno and Foz do Côa, following Aubry *et al.* (2012) in their assessment of differential weathering in the Côa valley. At each site, data was collected from four randomly selected vertical schist surfaces, independently of the occurrence of rock-art, within each of the two opposing slopes (facing NW and SE, respectively), leading to a total of 24 sampled surfaces (12 sampled surfaces per slope aspect and 8 sampled surfaces per site). Among these were six rock-art surfaces. Sampling was therefore based on a split-plot design with four replications, where the main variable of interest, slope aspect, defines the so-called whole-plots (the between plots factor) which are split according to site (the within plots factor) (Quinn & Keough 2002).

Data collection and analysis

Data collection started by first recording the predominant orientation of the selected schist surface with a compass, in order to latter verify its conformity with corresponding slope aspect. Then, lichen and bryophyte species percentage cover was estimated through visual inspection. Total percentage cover of both groups was also visually estimated. The entire surface was considered, instead of sub-sampling, since: 1) it was physically possible (the area was small enough); 2) sub-sampling would introduce an additional, and unnecessary, level of variability in the data set and 3) there was not enough previous knowledge on the composition of the target communities to allow for the recognition of representative assemblages (sub-samples) of the whole surface. Surface area varied between 2 m^2 and 60 m^2 but explained only 3.67% of the total variability in the data set (analysis not shown) and its effect on species composition was not statistically significant (p-value= 0.690), proving that this decision was correct. Data collection took place in the spring (late March) of 2011. Bryophyte nomenclature follows Hill *et al.* (2006) and Ros *et al.* (2007) for mosses and liverworts, respectively. Lichen nomenclature is according to the *Index Fungorum* (2013). The nomenclature for *Peltula lobata*, a recently described lichen species from the Iberian Peninsula is according to Marques *et al.* (2013).

For the purpose of the statistical analysis, the 24 samples were grouped according to location (Vale de José Esteves, Vale do Forno and Foz do Côa) and classified as belonging to either north-west or south-east facing slopes, providing two explanatory variables (site and aspect). The primary focus was on the effects of slope aspect, but the fact that samples were taken at different sites could introduce an undesirable effect of location, resulting in a biased analysis. If the effect of location is removed, a much clearer picture is provided of the influence of slope aspect on the composition of target lichen and bryophyte communities.

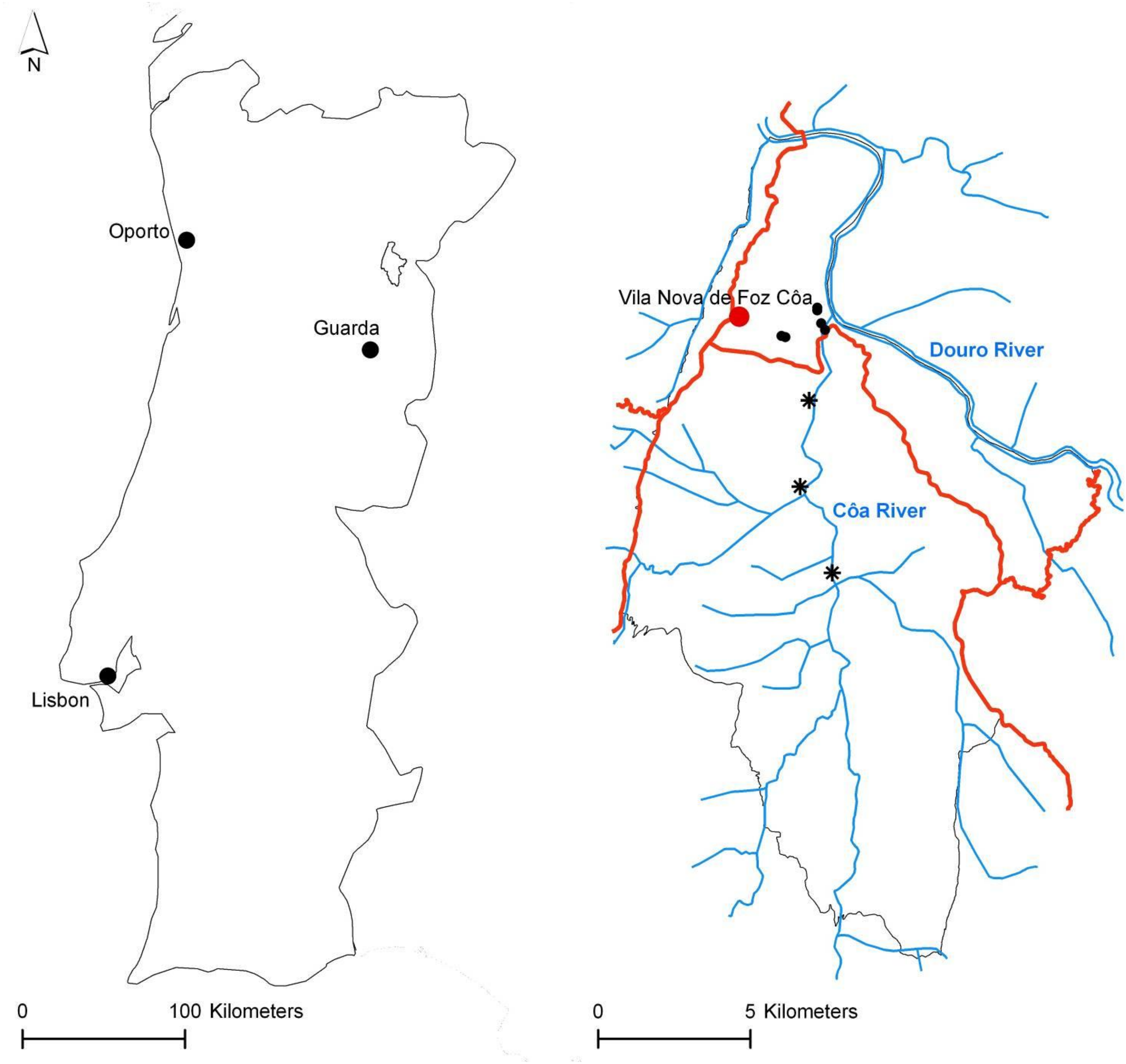

Fig. 7.1.2. Sampling location. Sampling sites are represented by black dots (●). Rock-art sites open to the public are represented by asterisks (*).

Split-plot analysis of variance (ANOVA) performed in IBM SPSS Statistics 20 was used to detect significant differences in total, bryophyte and lichen species richness between opposite slopes, while accounting for the effect of location. ANOVA's assumption of homogeneity of variances was tested with Levene's test, and that of normality of the residual distribution checked graphically by normal probability plots. The multivariate counterpart of split-plot ANOVA was achieved by carrying out a series of constrained analyses, summarized in Table 7.1.2, that allowed to quantify the different components of variation in the data set and determine their statistical significance by Monte Carlo permutation tests (see Lepš & Šmilauer (2003) and Legendre & Legendre (1998) for details on the procedure of variance partition and special permutation schemes in split-plot designs). Detrended Correspondence Analysis (DCA) with detrending by segments (Hill's scaling) was carried out initially to provide an estimate of the length of gradient in the data set and afterwards to clarify the relationships among sampling sites based on their floristic composition. The length of the first axis in Hill's scaling for the analysed data was 3.647, which is in the so-called 'grey zone' regarding the decision about the most appropriate method of multivariate analysis, where both linear and unimodal methods perform reasonably well (Lepš & Šmilauer 2003). Since sample composition was quite heterogeneous, Constrained Correspondence Analysis (CCA), which is based on relative rather than absolute species abundances, was considered the best option (ter Braak & Šmilauer 2002). Both partial and complete CCA were done with Hill's scaling focused on inter-sample distances, and downweighting of rare species after exclusion of single occurrences. All multivariate analyses were performed in CANOCO ver. 4.5 (ter Braak & Šmilauer 2002).

7.1.3 Results and discussion

General patterns of bryophyte and lichen species richness and composition on the studied rock surfaces

A total of 131 different taxa (consisting of 90 lichens and 41 bryophytes), were found on the studied schist surfaces, though only 10 of these taxa were present in more than half of them: *Aspicilia contorta* subsp. *hoffmanniana* (*Aspicilia hoffmanniana* hereafter), *Caloplaca subsoluta*, *Collema ryssoleum*, *Lecanora pseudistera*, *Lepraria* sp., *Peltula euploca* and *Xanthoparmelia tinctina* (lichens); *Bartramia stricta* and *Fissidens crispus* (bryophytes). The complete list of species found in the studied rock surfaces is given in Table 7.1.3 with respective codes used in Figs. 7.1.3 and 7.1.4. Average species richness, of 27 taxa in total, was higher for lichens (17 species) than for bryophytes (10 species). Average bryophyte cover was also lower (25%) than average lichen cover (60%).

Lichens and bryophytes share ecological roles and may have similar environmental requirements, being sometimes regarded as competitors for space and resources. However lichens, in general, are thought to have a strongest desiccation resistance, and to be best colonizers of dry exposed southerly aspects, while bryophytes are generally more abundant at northerly aspects, where they receive higher levels of moisture and less light supply (Hespanhol *et al.* 2010). In fact, at south-east facing slopes, average cover for bryophytes is much lower than average cover for lichens (10% and 60%, respectively). At north-west facing slopes both groups are equally abundant (50% for bryophytes and 60% for lichens), probably because shade and moisture at north-west facing slopes, although higher than at heavily exposed south-east facing slopes, is not high enough for many bryophyte species, and is only available for a short period of time throughout the year (Fernandes 2012), hampering bryophyte competition against lichens. The analysis of lichen and bryophyte species richness in opposite slopes (Table 7.1.1), revealed statistically significant changes in the number of bryophytes (p-value= 0.006) in relation to slope aspect but not in the number of lichens (p-value= 0.112). While bryophyte species richness and cover are closely related to slope aspect (much higher at north-west than at south-east facing slopes), lichen species richness and cover are not. Total species richness in opposite slopes is strongly influenced by such differences in bryophyte species richness, being higher at north-west facing slopes with an average of 30 species (12 bryophytes and 19 lichens), than at south-east facing slopes with an average of 23 species (8 bryophytes and 15 lichens).

Table 7.1.1. Results of split-plot ANOVA for bryophyte, lichen and total species richness, using slope aspect as the between-plots effect and site as the within-plots effect.

	Bryophytes		**Lichens**		**Total**	
	F-ratio	*p-value*	*F-ratio*	*p-value*	*F-ratio*	*p-value*
Aspect	17.523	0.006	3.472	0.112	9.786	0.020
Site	5.205	0.034	0.017	0.935	0.395	0.603
Aspect x Site	4.200	0.055	0.184	0.732	0.244	0.698

The DCA biplot in Fig. 7.1.3, where aspect and site are passively projected to help interpretation, shows evident sample clustering, with samples from north-west facing slopes placed on the left side of the diagram, and samples from south-east facing slopes on the right side, suggesting that it is the effect of aspect that is underlying the first axis.

In fact, aspect and site together explain 31% of the total variation in the studied bryophyte and lichen species assemblages (Table 7.1.2: F-ratio= 3.000; p-value= 0.002), which is a large amount considering the high degree of variability that is usually inherent to ecological data, but not so surprising since aspect and site are actually proxies of several environmental variables known to influence lichen and bryophyte species occurrence and distribution on rock surfaces.

The selection of sampling sites was based on the assumption of similarity between the three sites with respect to climatic, topographic and edaphic conditions (Aubry *et al.* 2012).

However, differences in lichen and bryophyte species composition between sites, after adjusting for aspect, explain 16.5% of the total variation in these assemblages, and this variation is significantly different from that resulting from chance (Table 7.1.2: F-ratio= 1.974; p-value= 0.002).

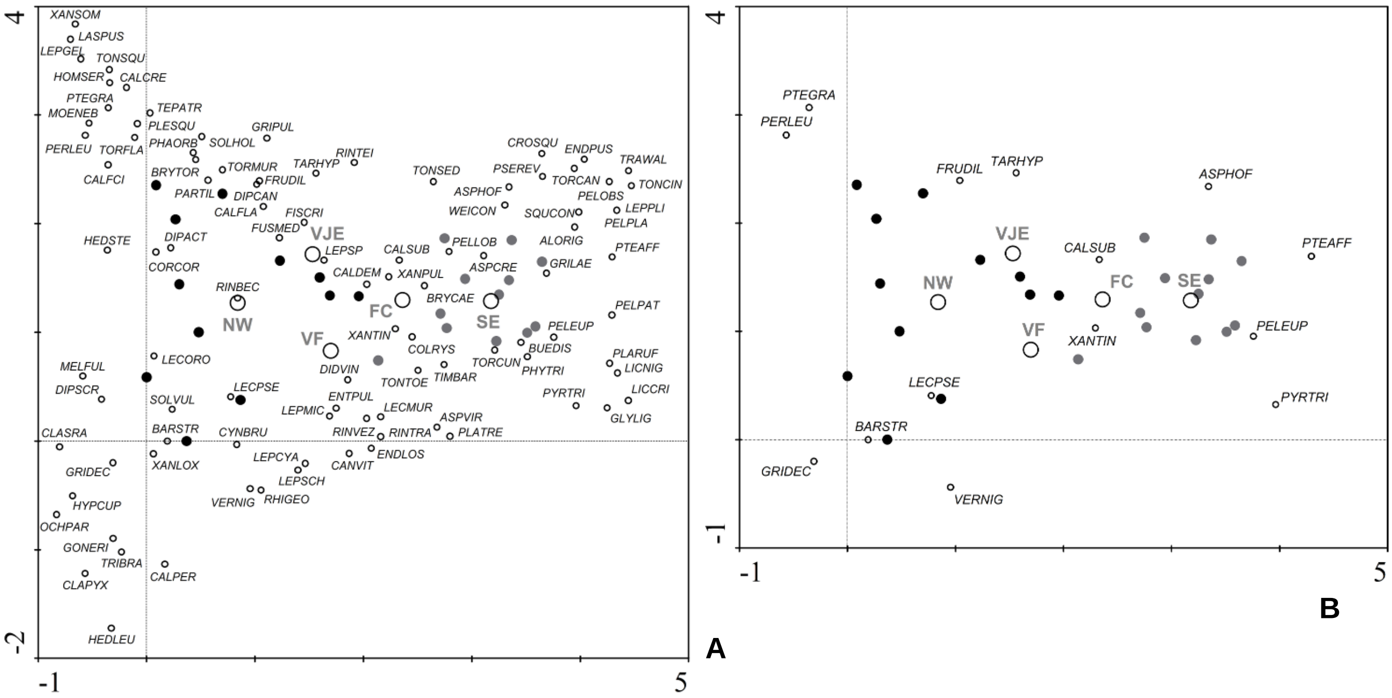

Fig. 7.1.3. Ordination diagram of DCA with detrending by segments. Sampled surfaces at north-west facing slopes are represented by black dots (●). Sampled surfaces at south-east facing slopes are represented by grey dots (●). A: All species used in the analysis are displayed; B: Only the species with highest weight (the most frequent ones) are selected for display. Species codes are according to Table 7.1.3.

Nevertheless, there are unquestionable differences in the composition of the studied species assemblages that can be related to aspect solely (third row in Table 7.1.2: F-ratio= 4.981; p-value= 0.002), explaining the largest amount of variation in lichen and bryophyte composition (19.9%). Fig. 7.1.4 displays the first two axes of partial CCA using slope aspect as the only explanatory variable and site as covariable, in order to quantify the effects of aspect upon lichen and bryophyte species assemblages on vertical schist surfaces after controlling for the variability associated with site (*i.e.* to remove the variability which can be explained by sample location and perform the analysis of interest on the remaining variation). The first ordination axis in CCA is very well correlated with aspect (r= 0.965 in Table 7.1.2). Only the most influential species - whose weights in the analysis where over 20% - are depicted in the ordination diagram of Fig. 7.1.4 B. In this way, not only does it facilitate the reading but it also evidentiates the species that better represent lichen and bryophyte assemblages on the studied surfaces, of interest for rock-art conservation. Given that aspect has proved to be a major differentiating factor of lichen and bryophyte occurrence in opposite slopes, the resulting CCA diagram is not so different from previous DCA (Fig. 7.1.3 B).

Table 7.1.2. Results of the analysis made to partition the variance in the data set into the contributions of Site (S), Aspect (A) and both variables simultaneously, in the lichen and bryophyte data. Columns on the right side contain the results of the same analyses restricted to the lichen data set. FC: Foz do Côa; VJE: Vale de José Esteves; VF: Vale do Forno; NW: north-west facing slopes; SE: south-east facing slopes; EV: Explanatory variables; CV: Covariables; Var (%): percentage of total variation in the data set; r: species-environmental correlations of first axis in CCA. VJE, VF, FC, NW and SE are all coded as dummy variables. Species codes are according to Table 7.1.3.

Analysis	**EV**	**Lichens and bryophytes**					**Lichens only**			
		CV	*Var (%)*	*R*	*F-ratio*	*p-value*	*Var (%)*	*r*	*F-ratio*	*p-value*
A + S	VJE VF FC NW SE	None	31.0	0.962	3.000	0.0020				
A x S	VJE*NW VJE*SE VF*NW VF*SE FC*NW FC*SE	VJE VF FC NW SE	11.0	0.960	1.860	0.0460				
A (S)	NW SE	VJE VF FC	19.9	0.965	4.981	0.002	17.6	0.947	4.259	0.002
S (A)	VJE VF FC	NW SE	16.5	0.878	1.974	0.002	13.6	0.815	1.575	0.002

So which side do bryophyte and lichen species choose? Some aspect-related differences in lichen and bryophyte species assemblages on the studied rock surfaces

Some thin early-colonizing lichens such as the dark crustose *Verrucaria nigrescens*, occurring at north-west facing schist surfaces, are well adapted to shade and moisture, and by absorbing water they increase the water holding capacity at the lichen-rock interface. By doing so, they favour the occurrence (Fig. 7.1.4 A, on the left) of thick crustose higro- to mesophytic lichens that tend to avoid extreme solar radiation as well, such as *Diploicia canescens*, *Lecanora orosthea* and *Rinodina beccariana*, and long-lived shuttle bryophytes such as *Bartramia stricta*, *Grimmia decipiens*, *Pterogonium gracile* and *Frullania dilatata*, with a few foliose and fruticose lichens growing on top of them, including *Fuscopannaria mediterranea* or *Cladonia pyxidata*, just to quote some examples. These are all perennial species that require a high degree of substrate stability in order to proliferate on rock surfaces. Foliose lichens and bryophytes are true epiliths attached by rhizines and rhizoids, respectively. Their contact with the surface and presumed weathering effect should be reduced to the vicinity of these structures and limited to a certain degree of mineral dissolution (Viles 1995). Water retention not only keeps moisture available but also creates niches, by opening crevices and holes where earth accumulates (Adamson *et al.* 2013, Caneva *et al.* 2008) and favouring the occurrence of terricolous bryophytes that increase the number of bryophyte species at north-east facing slopes, as already shown. The white crustose *Lecanora pseudistera* and *Pertusaria leucosora* are some of the lichens able to cope with bryophyte competition and dominate on north-west facing surfaces (Fig. 7.1.4 B, on the left). The latter is very abundant on north-west facing surfaces in Vale de José Esteves, but rare in Vale do Forno and completely absent from the similarly oriented slopes in Foz do Côa. As a consequence of such variation among sites, it assumes a very extreme position in the CCA diagram (Fig. 7.1.4 B, on the lower left) and is probably one of the species responsible for the already mentioned variation associated with sampling location. The orange crustose lichen *Caloplaca subsoluta* seems to be more or less evenly distributed between north-west and south-east facing surfaces, meaning that *Caloplaca subsoluta* is equally dominant on both slopes (Fig. 7.1.4 B, in the middle). Lichens are clearly dominant on south-east facing surfaces. The most frequent and abundant species among these lichen-rich assemblages is the green *Aspicilia hoffmanniana* (Fig. 7.1.4 B, on the right) together with *Pyrenopsis triptococca* and *Pterygiopsis affinis*, both characterized by a black thin gelatinous thallus that closely resembles a biofilm. The term biofilm is commonly used to refer to epilithic assemblages of bacteria, algae and fungi and respective mucilage (Viles 1995) but usually excludes lichens. However, *Pyrenopsis triptococca*, *Pterygiopsis affinis* and suchlike species are usually intermingled with those microorganisms, especially cyanobacteria, have a thin black gelatinous habit, do not produce any lichen substances and are ecologically (and presumably functionally) similar to these biofilms, thus expected to play a very similar role in rock surface weathering. Also closely associated with the studied south-east facing surfaces are bryophyte species with a colonist life strategy, such as *Weissia controversa*, *Aloina rigida* and *Pseudocrossidium revolutum* (Fig. 7.1.4 A), which are well-adapted to recently exposed or recently disturbed environments, unsuitable for the occurrence of perennial species. This indicates a certain degree of substrate instability in rock surfaces located at south-east facing slopes that is not verified at north-west facing slopes. *Peltula euploca* is an early colonizer characteristic of the rain-track communities described by Llimona & Egea (1985) that had been known to predominate on the south-east facing schist surfaces of the region (Marques *et al.* 2013) and one of the first species to re-colonize the engraved surfaces after cleaning (data not shown). It has a squamulose to small-foliose habit, attached to the rock by a single holdfast, but nothing is known about its weathering ability. Also frequent and abundant is the chasmolith *Squamarina concrescens*, that thrives on the numerous pre-formed crevices and cavities filled with compacted soil found on south-east facing surfaces. The same ecological niche is occupied mainly by bryophytes (*e.g. Targionia hypophylla*) and numerous fruticose lichens on north-east facing surfaces.

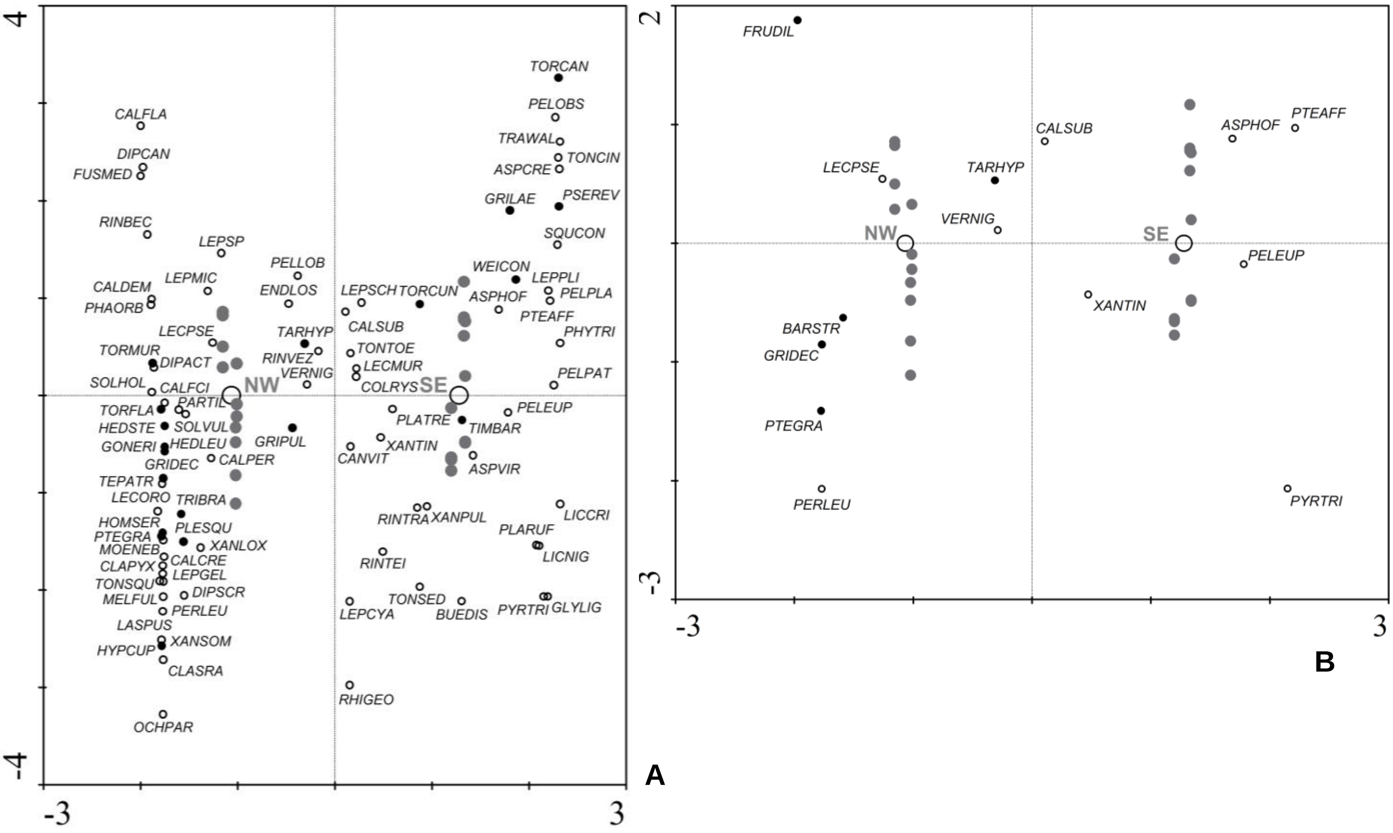

Fig. 7.1.4. Ordination diagram of partial CCA of lichen (clear dots) and bryophyte (black dots) species assemblages on vertical schist surfaces focusing on inter-sample distances. In this constrained analysis only aspect is used as explanatory variable after partialing out the variation explained by location (sites used as covariables). A: All species used in the analysis are displayed; B: Only the species with highest weight in the analysis are selected for display. Species codes are according to Table 7.1.3.

Based on their frequency and relative abundance on vertical schist surfaces at opposite slopes (Fig. 7.1.4) the species that better represent lichen and bryophyte species assemblages on open-air rock-art in the Côa Valley are *Aspicilia hoffmanniana* (at south-east facing surfaces), *Lecanora pseudistera* (at north-east facing surfaces) and *Caloplaca subsoluta* (on both surfaces). Bryophytes, foliose and fruticose lichens should also be capable of surface weathering but since they appear later in ecological succession after some degree of surface weathering and are almost absent in south-east facing surfaces, where rock-art predominates, priority should be given to the former species.

Inferences from a community ecology perspective on the studied rock surfaces

The relationship between aspect-related patterns of biological colonization and presumably resulting differential weathering in the Côa Valley was approached briefly by Aubry *et al.* (2012). Based on the patterns of lichen and bryophyte cover at the same sites studied here, these authors suggest that biological colonization has been playing a major differentiating role in rock surface weathering since the end of the Pleistocene. However, careful must be taken in order to avoid a biased measure of association between changes in lichen and bryophyte abundance and differences in the current weathering degree of the studied surfaces, confounded by the environmental variables influencing both phenomena, thus overestimating the role played by lichen and bryophyte colonization in the weathering process.

The amount of variation in lichen and bryophyte assemblages among slopes (19.9%) is surprisingly low compared to variation among sites (16.5%), suggesting that the relationship between bryophyte and lichen communities and differential surface weathering associated with slope, although plausible, is not straightforward. Of great interest are also the interactions of slope aspect with sampling site depicted in Table 7.1.1 and Table 7.1.2. Differences between sites that are observed at north-west and not at south-east, as well as differences between south-east and north-west that are produced at one site, and not on the other site, are both reflections of interaction between these variables. The effect of interaction between site and slope aspect on species richness is reflected only by changes in the number of bryophyte species (Table 7.1.1: F-ratio= 4.200; p-value= 0.055) and such interaction explains 11.0% of the total variability in lichen and bryophyte assemblages in the study area (second row in Table 7.1.2). But when subtracting the partial effects of slope aspect (19.9%) and site (16.5%) from the total amount of variability explained by the two variables (31.0%) the resulting value for the effect of both variables is negative. A negative value for a shared variance indicates that the effects of the two variables are competitive in the sense that the effect of one of the variables somehow blocks the contribution of the other (Legendre & Legendre 1998). This can be readily explained by field observations: in some cases, especially at north-west facing slopes, individual outcrops and respective surfaces were slightly deviated from overall slope aspect, towards the west-southwest (for example, surface orientation of FCN3, corresponding to an engraved surface coded as Quintas das Tulhas nº 5, was 210º according to field measurements). Such micro-site differences (Kuntz & Larson 2006) produce micro-scale variations in light, temperature, water and nutrient availability regimes, with consequent effects on lichen and bryophyte assemblages, closer in terms of species richness and composition to the assemblages occurring at the opposite slope. This is evidenced by the strong association of some xerophytic to very xerophytic lichens, including *Caloplaca demissa* and *Xanthoparmelia somloensis* (Fig. 7.1.4 A) to north-west facing slopes.

An additional source of bias may arise from considering total cover rather than the cover of individual species since every species (and species assemblage) is expected to act differently on the same substrate and make a unique contribution to rock weathering (Favero-Longo *et al.* 2005, Prieto *et al.* 1997). In fact, although not reflected on lichen species richness or total cover (as seen in Table 7.1.1), aspect has a significant influence on lichen composition (Table 7.1.2: F-ratio= 4.259, p-value= 0.002), explaining 17.6% of variation in the studied lichen assemblages, meaning that equally rich, but different, lichen species assemblages occur on both slopes, as described above.

Moreover, biological colonization on rock surfaces is not only derived by climate but also by the properties of the rock itself. Substrate related differences were supposedly controlled by the fact that the studied surfaces are homogenous in terms of the properties that affect its biorreceptivity (Prieto & Silva 2005). This was not necessarily true. The weathering degree of an individual surface will depend on the same micro- and local-scale factors (Hall *et al.* 2012, Pope 2000) that act on bryophyte and lichen community composition, and should also vary with the within site deviations from overall orientation. In many cases, on both north-west and south-east facing slopes, toppling causes water runoffs and seepage paths to deviate from their natural course along the rock surface, thereby resulting in smoother and dryer areas underneath the displaced blocks, which few or none of these organisms are able to colonize.

Time is also an important factor in biologically induced weathering. The ecological timescale is usually in the order of tens to few hundred years. There are some reported cases of lichen thalli surviving hundreds of years but these do still constitute exceptions in very peculiar environments. Adamson *et al.* (2013) in Northern Ireland proved that greening (*i.e.* biological growth mainly attributed to green algae and some species of cyanobacteria), which usually precedes lichen and bryophyte colonization (Caneva *et al.* 2008) appeared on newly exposed sandstone and limestone after six months of exposure and that, after approximately one year, some lichens were already present, showing that biological colonization is a surprisingly rapid process.

Interestingly enough for the subject of this article, lichen establishment on the freshly exposed sandstone blocks used by Adamson *et al.* (2013) was faster at north facing surfaces than at south facing surfaces and so was the associated rock weathering, but some authors alert to the danger of extrapolating results from other climates and environments (Stretch & Viles 2002). Species that colonize north-west facing surfaces are not necessarily more active in biologically induced weathering and may require the aid of other weathering agents. Previous works by Prieto *et al.* (1997) described a similarly fast process of biological colonization on almost permanently damp granite but these authors concluded that biological weathering had been favoured by the use of a severely weathered granite type. Viles (1995) studies even suggest that biological contributions to weathering should be greatest in arid environments where climatic stress promotes endolithic growth forms that interact very differently with rock surfaces (Carter & Viles 2004) when compared to epilithic growth forms. The absence of endolithic lichens on the south-east facing surfaces in the Côa valley might be explained by the fact that these surfaces are not arid enough together with some properties of the substrate that hamper endolithic growth, namely rock hardness and low porosity (Fernandes 2012). *Verrucaria nigrescens*, a cosmopolitan pioneer lichen occurring at both north-west and south-east facing slopes in the Côa Valley (see Fig. 7.1.4), has been used to demonstrate how in moist hot environments, such as the ones occurring in the north-west facing slopes at the Côa valley (Fernandes 2012), the lichen thallus is able to reduce the amplitude of surface and internal temperature fluctuations, thereby protecting the rock surface from thermal stress (Carter & Viles 2003) while at dry hot environments, comparable to the ones occurring in the south-east facing slopes at the Côa valley (Fernandes 2012), the same species is associated with significant increase in surface temperature and thermal gradient beneath the lichen thallus therefore increasing the rock susceptibility to breakdown (Carter & Viles 2004).

Theoretically, opposing slopes in the Côa Valley Archaeological Park are being subjected to different biological-induced weathering processes, given the differences in the composition of both species assemblages. However evidence is still insufficient to conclude whether north-east facing slopes are more affected by the activity of these organisms than southwest facing slopes. The link between the observed differential weathering in north-west and south-east facing rock surfaces and differential weathering activity of bryophyte and lichen species will necessarily come from controlled experiments with individual species tested against bare rock controls (Aghamiri & Schwartzman 2002, Carter & Viles 2003, 2004, Jackson & Keller 1970, Prieto *et al.* 1997, Scarciglia *et al.* 2012, Zambell *el al*. 2012) a task that will be conducted in the near future.

Table 7.1.3. List of lichen and bryophyte taxa found in the study area, with respective code used in diagrams. Taxa are assigned to either north-west (NW) or south-east (SE) facing surfaces based on their scores in CCA or field observations.

Code	Lichens	NW	SE
	Acarospora hilaris (Dufour) Arnold		X
	Acarospora umbilicata Bagl.		X
	Agonimia opuntiella (Buschart & Poelt) Vězda		X
ASPHOF	*Aspicilia contorta* (Hoffm.) Kremp. subsp. *hoffmanniana* S. Ekman & Fröberg		X
ASPCRE	*Aspicilia crespiana* V. J. Rico		X
BUEDIS	*Buellia dispersa* A. Massal.		X
	Caloplaca aff. *aractina* (Fr.) Häyrén		X
CALCRE	*Caloplaca crenularia* (With.) J. R. Laundon	X	

Code	Lichens	NW	SE
CALDEM	*Caloplaca demissa* (Flot.) Arup & Grobe	X	X
CALFLA	*Caloplaca flavescens* (Huds.) J. R. Laundon	X	
CALFCI	*Caloplaca flavocitrina* (Nyl.) H. Olivier	X	
CALFUS	*Caloplaca fuscoatroides* J. Steiner	X	
CALSUB	*Caloplaca subsoluta* (Nyl.) Zahlbr.	X	X
CANVIT	*Candelariella vitellina* (Ehrh.) Müll. Arg.	X	X
	Cladonia foliacea (Huds.) Willd.	X	X
CLAPYX	*Cladonia pyxidata* (L.) Hoffm.	X	
CLARAN	*Cladonia rangiformis* Hoffm.	X	
CLASQU	*Cladonia squamosa* (Scop.) Hoffm.	X	
COLRYS	*Collema ryssoleum* (Tuck.) A. Schneider	X	X
	Collema subflaccidum Degel.	X	X
	Dermatocarpon miniatum (L.) W. Mann	X	
DIPCAN	*Diploicia canescens* (Dicks.) A. Massal.	X	
DIPACT	*Diploschistes actinostomus* (Ach.) Zahlbr.	X	
DIPSCR	*Diploschistes scruposus* (Schreb.) Norman	X	
ENDLOS	*Endocarpon loscosii* Müll. Arg.	X	
ENDPUS	*Endocarpon pusillum* Hedw.		X
FUSMED	*Fuscopannaria mediterranea* (Tav.) P. M. Jørg.	X	
GLYLIG	*Glyphopeltis ligustica* (B. de Lesd.) Timdal		X
LASPUS	*Lasallia pustulata* (L.) Mérat	X	
	Lecanora albescens (Hoffm.) Branth & Rostr.	X	
LECMUR	*Lecanora muralis* (Schreb.) Rabenh.	X	X
LECORO	*Lecanora orosthea* (Ach.) Ach.	X	
LECPSE	*Lecanora pseudistera* Nyl.	X	
	Lepraria sp.	X	
LEPMIC	*Leprocaulon microscopicum* (Vill.) Gams	X	
	Leptogium cochleatum (Dicks.) P. M. Jørg. & P. James	X	
LEPCYA	*Leptogium cyanescens* (Rabenh.) Körb.	X	X
LEPGEL	*Leptogium gelatinosum* (With.) J. R. Laundon	X	
	Leptogium magnussonii Degel & P. M. Jørg.	X	
LEPPLI	*Leptogium plicatile* (Ach.) Leight.		X
LEPSCH	*Leptogium schraderi* (Bernh.) Nyl.	X	X
LICCRI	*Lichinella cribellifera* (Nyl.) P. P. Moreno & Egea		X
LICNIG	*Lichinella nigritella* (Lettau) P. P. Moreno & Egea		X
	Massalongia carnosa (Dicks.) Körb	X	X
MELFUL	*Melanelixia fuliginosa* (Fr. Ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	X	
MOENEB	*Moelleropsis nebulosa* (Hoffm.) Gyeln.	X	
OCHPAR	*Ochrolechia parella* (L.) A. Massal.	X	
	Parmelia saxatilis (L.) Ach.	X	
PARTIL	*Parmelina tiliacea* (Hoffm.) Ach.	X	
	Peltula bolanderi (Tuck.) Wetmore		X
PELEUP	*Peltula euploca* (Ach.) Poelt ex Ozenda & Clauzade		X
PELLOB	*Peltula lobata* J. Marques, M. Schultz & Paz-Berm.		X
PELOBS	*Peltula obscurans* (Nyl.) Gyeln.	X	X
PELPAT	*Peltula patellata* (Bagl.) Swinscow & Krog		X

PELPLA	*Peltula placodizans* (Zahlbr.) Wetmore		X
	Peltula zahlbruckneri (Hasse) Wetmore		X
PERLEU	*Pertusaria leucosora* Nyl.	X	
PHAORB	*Phaeophyscia orbicularis* (Neck.) Moberg	X	
PHYTRI	*Physcia tribacia* (Ach.) Nyl.		X
	Physconia enteroxantha (Nyl.) Poelt	X	X
PLARUF	*Placidium rufescens* (Ach.) A. Massal.		X
PLATRE	*Placynthium tremniacum* (A. Massal.) Jatta	X	X
	Psora decipiens (Hedw.) Hoffm.		X
	Pterygiopsis affinis (A. Massal.) Hessen		X
PYRTRI	*Pyrenopsis triptococca* Nyl.		X
RHIGEO	*Rhizocarpon geographicum* (L.) DC.	X	X
	Rhizocarpon richardii (Nyl.) Zahlbr.	X	X
RINBEC	*Rinodina beccariana* Bagl.	X	
RINTEI	*Rinodina teichophila* (Nyl.) Arnold	X	X
RINTRA	*Rinodina trachytica* (A. Massal.) Bagl. & Carestia	X	X
RINVEZ	*Rinodina vezdae* H. Mayrhofer	X	X
SOLHOL	*Solenopsora holophaea* (Mont.) Samp.	X	
SOLVUL	*Solenopsora vulturiensis* A. Massal.	X	
	Squamarina cartilaginea (With.) P. James	X	
SQUCON	*Squamarina concrescens* (Müll. Arg.) Poelt		X
TEPATR	*Tephromela atra* (Huds.) Hafellner	X	
TONCIN	*Toninia cinereovirens* (Schaer.) A. Massal.		X
	Toninia opuntioides (Vill.) Timdal	X	
TONSED	*Toninia sedifolia* (Scop.) Timdal	X	X
TONSQU	*Toninia squalida* (Ach.) A. Massal.	X	
TONTOE	*Toninia toepfferi* (Stein) Navàs	X	X
	Toninia tristis (Th. Fr.) Th. Fr. subsp. *pseudotabacina* Timdal		X
	Trapeliopsis flexuosa (Fr.) Coppins & P. James	X	
TRAGYM	*Trapeliopsis gymnidiata* Aptroot & Schumm		X
VERNIG	*Verrucaria nigrescens* Pers.	X	X
XANLOX	*Xanthoparmelia loxodes* (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch	X	X
XANPUL	*Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch	X	X
	Xanthoparmelia somloensis (Gyeln.) Hale	X	
XANTIN	*Xanthoparmelia tinctina* (Maheu & A. Gillet) Hale	X	X
Code	**Bryophytes**	**NW**	**SE**
ALORIG	*Aloina rigida* (Hedw.) Limpr.		X
BARSTR	*Bartramia stricta* Brid.	X	
BRYCAE	*Bryum caespiticium* Hedw.		X
BRYTOR	*Bryum torquescens* Bruch & Schimp.	X	
	Cephaloziella stellulifera (Taylor ex Spruce) Schiffn.		X
CORCOR	*Corsinia coriandrina* (Spreng.) Lindb.	X	
CROSQU	*Crossidium squamiferum* (Viv.) Jur.		X
CYNBRU	*Cynodontium bruntonii* (Sm.) Bruch & Schimp.	X	
	Didymodon insulanus (De Not.) M. O. Hill	X	
DIDVIN	*Didymodon vinealis* (Brid.) R. H. Zander	X	X
ENTPUL	*Entosthodon pulchellus* (H. Philib.) Brugués	X	X

FISCRI	*Fissidens crispus* Mont.	X	X
	Fossombronia angulosa (Dicks.) Raddi	X	
FRUDIL	*Frullania dilatata* (L.) Dumort.	X	
GONERI	*Gongylanthus ericetorum* (Raddi) Nees	X	
GRIDEC	*Grimmia decipiens* (Schultz) Lindb.	X	
GRILAE	*Grimmia laevigata* (Brid.) Brid.		X
GRIPUL	*Grimmia pulvinata* (Hedw.) Sm.	X	X
	Hedwigia ciliata (Hedw.) P. Beauv.	X	
HEDLEU	*Hedwigia ciliata* (Hedw.) P. Beauv. var. *leucophaea* Bruch & Schimp.	X	
HEDSTE	*Hedwigia stellata* Hedenäs	X	
HOMSER	*Homalothecium sericeum* (Hedw.) Schimp.	X	
HYPCUP	*Hypnum cupressiforme* Hedw.	X	
	Lunularia cruciata (L.) Lindb.	X	
	Pleuridium acuminatum Lindb.		X
PLESQU	*Pleurochaete squarrosa* (Brid.) Lindb.	X	
	Pottiopsis caespitosa (Brid.) Blockeel & A. J. E. Sm.		X
PSEREV	*Pseudocrossidium revolutum* (Brid.) R. H. Zander		X
PTEGRA	*Pterogonium gracile* (Hedw.) Sm.	X	
	Scleropodium touretii (Brid.) L. F. Koch	X	
	Syntrichia montana Nees	X	
TARHYP	*Targionia hypophylla* L.	X	X
TIMBAR	*Timmiella barbuloides* (Brid.) Mönk.		X
TORFLA	*Tortella flavovirens* (Bruch) Broth.	X	
TORCAN	*Tortula canescens* Mont.		X
TORCUN	*Tortula cuneifolia* (Dicks.) Turner	X	X
	Tortula lanceolata R. H. Zander	X	
TORMUR	*Tortula muralis* Hedw.	X	
TRIBRA	*Trichostomum brachydontium* Bruch	X	
	Triquetrella arapilensis Luisier	X	
WEICON	*Weissia controversa* Hedw.		X

7.1.4 Conclusions

Standard sampling and analysing procedures in the framework of community ecology allows examining how interactions among species and their environment affect community composition while controlling for confounding variables, *i.e.* to isolate the effect of the interaction between species and the environment from the single effect of the environmental variables.

Slope aspect is a major constraint for lichen and bryophyte species occurrence in the Côa Valley Archaeological Park but so are some other unknown sources of variation among the studied species assemblages that depend on location, probably related to micro-scale factors acting on individual surfaces. Assumptions about aspect related effects of lichen and bryophyte colonization must therefore be addressed carefully in the framework of rock-art conservation.

Aspect dependant variation in lichen and bryophyte species assemblages in the Côa Valley Archaeological Park is significant enough, though, to justify separate consideration in assessments of bryophyte- and lichen-induced rock surface weathering. Lichen and bryophyte communities in advanced stages of ecological sucession are not easy to study or simulate under controlled environments but a selection of the most representative species should provide a close picture of what happens in the natural context. Based on their frequency and abundance on differently oriented vertical surfaces of the study area at least three crustose lichens (*Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Lecanora pseudistera*) should be selected to proceed with the evaluation of the susceptibility of rock-art on schist to biological deterioration in the Côa Valley Archaeological Park.

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7.2 Searching for surrogates of lichen diversity for rock-art condition assessment and monitoring

Submitted to the Journal of Cultural Heritage with the following co-authors: Beatriz Prieto and Graciela Paz-Bermúdez

Abstract

One of the basic tools to quantify biodiversity is based on the use of surrogates or proxies. However, the studies conducted so far have been in the framework of disciplines such as Conservation Biology aiming at finding indicators of threatened species in a given region, site or habitat. An important research task in rock-art conservation is therefore to find equally fast and cost-effective tools for measuring and monitoring lichen colonization of rock surfaces. The use of indicator species or lichen classifications into easily recognizable groups can be developed on the basis of simple traits such as thallus colour and growth form. This study is situated in the Upper Douro region (north-east Portugal) and based on the inventory of lichen assemblages on 64 vertical schist surfaces, comprising 109 different lichens. Three lichen traits were selected because of their significance for lichen-induced rock weathering. Such traits were then used to characterize lichen functional groups that are relevant for rock-art condition assessment and monitoring. Overall lichen species diversity was accurately reproduced by species diversity of either the resultant functional groups or a strongly reduced subset made out of group representatives, although not by individual species. The proposed functional groups have distinct ecophysiological characteristics, with the potential to respond differently to environmental factors, which is of great importance for rock-art condition assessment.

7.2.1 Introduction

Lichens have long been demonstrated to be useful indicators of the health status of many different ecosystems (Eldridge & Tozer 1996, McCune 2000, Rogers & Ryel 2008) and of the effects of disturbance on ecosystem structure and functioning, including air pollution (*e.g.* Branquinho *et al.* 1999, Hawksworth & Rose 1970, Giordani 2007, Lackovicová *et al.* 2013), fire regimes (Wolseley & Aguirre-Hudson 1997), land-use and land-use management (Nascimbene *et al.* 2013, Stofer *et al.* 2006, Wolseley *et al.* 2006) or climate change (Aptroot 2009, Sancho *et al.* 2007). The indicator value of lichens has also been acknowledged by archaeologists and conservators ever since the first use of lichenometry in dating of monuments (Follmann 1961, Müller 2005, Trudgill *et al.* 1979). Yet a different kind of demand for lichen indicators may arise in the context of open-air rock-art conservation as evidences of the close relationship between lichen activity and rock surface weathering continue to accumulate in the literature (Cann 2012, de la Rosa *et al.* 2012, Nugari *et al.* 2009, Scarciglia *et al.* 2012, Strech & Viles 2002).

Ideally, evaluation of lichen-induced weathering would be based on detailed surveys, including a thorough knowledge of the effect of each species, its distribution patterns, species interactions within lichen communities and between these and the surrounding environment in each local context (Armstrong 2013, Edwards *et al.* 1995, Lawrey 1991, Prieto *et al.* 1999, Viles 1995). However, such detailed surveys, especially of those small inconspicuous taxa, are dependent on time-consuming expert knowledge and adequate funding, since lichens on rocks are rather laborious to sample and identify. Lichen surveys of rock-art surfaces are additionally hampered by sampling restrictions imposed by conservation issues. Lichen diversity is therefore often neglected despite its generally accepted contribution to rock-art deterioration (Adamo & Violante 2000, Chen *et al.* 2000). The same difficulties have been experienced over the years by ecologists while attempting to capture information on the overall species diversity within communities and ecosystems. A popular approach in ecological studies to overcome the inconvenience of surveying total species richness is to restrict the survey to subsets of easily recognized species diversity surrogates (Caro & O'Doherty 1999, Favreau 2006). Several methods have been suggested for finding such surrogates based on assumed relationships between: (1) species diversity of an habitat subset and that of the whole set (Caro & O'Doherty 1999, Will-Wolf *et al.* 2000) (2) species diversity of unrelated taxonomic groups such as birds and plants or invertebrates, *i.e.* cross-taxa surrogacy (*e.g.* Djupström *et al.* 2010, McMullan-Fisher *et al.* 2010, Negi & Gadgil 2002, Pharo *et al.* 1999, Saetersdal *et al.* 2003), (3) species diversity and the diversity of supra-specific taxa such as genera or families *i.e.* higher-taxa approach (*e.g.* Aragón *et al.* 2013, Bergamini *et al.* 2005, 2007, Gaston 2000, Gaston & Williams 1993), (4) single species abundance and species diversity (*e.g.* Campbell & Fredeen 2004) and (5) single species abundance and that of other species within certain groups (Cushman *et al.* 2010). Another common strategy for reducing the complexity of surveying highly diverse communities and ecosystems is to search for surrogates of species diversity among the relationships between the patterns of overall species diversity and that of some well-known and relatively easy-to-measure species aggregate. This might be accomplished by finding sets of strongly related species that share similar abundance patterns (i.e co-occurring species) or some kind of structural or physiological attributes (functional groups) (Box 1996), and exhibit similar responses to environmental conditions (Garcia-Mora *et al.* 1999). The use of species functional groups as surrogates of overall species diversity has the advantage of simultaneously directing diversity surrogacy towards some ecological function of interest, as it might be the case of species weathering ability.

A promising approach for non-expert assessment of lichen colonization of rock surfaces in the framework of rock-art conservation may be thus to similarly focus on lichen diversity surrogates rather than on overall lichen diversity. These groups would be much easier to quantify than the occurrences and abundances of all lichen taxa, and their respective effect on rock surfaces. Furthermore, lichen species grouping might allow ecological comparisons among lichen communities at a much larger scale than would be possible through traditional taxonomic approaches, linking different spatial and temporal scale-dependent changes of lichen activity on rock surfaces. Therefore the main goal of this study is to test thoroughly the possibility of evaluating and monitoring the extent of lichen colonization on rock surfaces (and degree of lichen-induced rock weathering) by means of surrogate species and species functional groups. The effectiveness of the compositional pattern of different sets of lichen functional groups as predictors of overall lichen species richness and lichen community composition is specifically addressed.

7.2.2 Conceptual framework

The functional groups approach

Perhaps the most comprehensive framework of the trait-based approaches in Ecology is the one by Violle *et al.* (2007), acknowledging general confusion around the definitions in previous literature and proposing an integrative terminology. According to these authors a species trait is any morphological, physiological or phenological feature, either continuous or categorical, that is measured at the individual level, without considering species-environment or inter-species relationships. When these traits influence the performance of the species in terms of growth, reproduction and survival rates these are referred to as species functional traits. Regarding species-environment interactions, any trait whose attributes vary in response to changes in environmental conditions is a response trait. Finally, any trait that reflects the effects of a species on environmental conditions or community properties is an effect trait. The term functional group is used to refer to a group of organisms that share a certain amount of functional traits and whose functional roles in the community or ecosystem are similar (Will-Wolf *et al.* 2000). The relevance of the functional classification depends on the domain of application, as traits that are relevant for rock-art conservation may be quite different from those that are relevant for conservation ecology (Stofer *et al.* 2006).

When it comes to functional trait screening and functional classifications of species, less attention has been paid to lichens than to better known groups such as vascular plants (Diaz *et al.* 2004) despite the paramount importance of lichens for biogeochemical cycling in ecosystems worlwide and the considerable number of traits available (Cornelissen *et al.* 2007). The building of lichen functional groups therefore typically relies on three easily discernible traits: growth form, reproductive strategy, and photobiont type (Bowker 2011, Canullo *et al.* 2013, Koch *et al.* 2013, Stofer *et al.* 2006) that have been of low information value for rock-art condition assessment.

The effect of lichens on rocks apparently results from a direct involvement in physical and chemical weathering processes by means of thallus adhesion and hyphal penetration in the first case, and production of secondary metabolites in the second (Adamo & Violante 2000, Chen *et al.* 2000), which may therefore constitute key lichen functions for rock-art condition assessment (Table 7.2.1). The ability to adhere to rock surfaces and penetrate into the rock is dependent on the growth-rate and biomass as well as growth form (St. Clair *et al.* 2002), whereas the production of many lichen substances is reflected in thallus colour (Edwards *et al.* 2005). Traits that are shared by a large number of species are most likely to act as surrogates of lichen diversity patterns than those found in only a few taxa. Therefore, in the context of rock-art conservation, thallus colour, growth rate, biomass and growth form are lichen traits for which a functional relationship to rock weathering and rock-art deterioration can be hypothesized. Growth rate can be considered a ‘hard’ trait (Violle *et al.* 2007), while biomass and growth form are ‘soft’ traits already familiar to the fields of geomorphology and heritage conservation (*e.g.* Viles 1995).

Thallus colour

Thallus colour is a reflection of secondary metabolite production inside lichen thallus and thus widely used as a reliable taxonomic character in lichen identification (Culberson 1970). Secondary metabolites in lichens are commonly referred to as lichen acids. The production of lichen acids is affected by (and therefore reflects) environmental changes (Deduke *et al.* 2012) even if there are no changes in thallus dimensions. Therefore colour can be used as an alternative early indicator of changes in chemical activity when there are no changes in physical activity on rock surfaces.

In the context of rock-art conservation, chemical deterioration is the lichen function of interest, production of lichen acids the proposed functional trait, while thallus colour is a proxy of such function, easier to obtain and readily available. The following categories (attributes sensu Violle *et al.* 2007) of thallus colour are here considered: black, grey, green, brown, red, orange, yellow, white (Table 7.2.1).

Growth forms

Growth form is one of those integrative traits that do not have a single physiological basis, but is possibly the most relevant proxy of species performance in nature (Duarte 2007). Rock-inhabitant organisms, also known as lithobionts, are commonly divided into epiliths (which live entirely on the rock surface), endoliths (which colonize the interior of rocks) and chasmoliths (which occupy pre-formed spaces within the rock) (Golubic *et al.* 1981, Viles 1995). Golubic *et al.* (1981) clarified previous concepts and further divided endolithic microorganisms into several growth form categories based on a combination of topical and functional criteria. Lichens, however, are capable of a much more variety of growth forms (Viles 1995). A revised terminology is proposed here describing lichen growth forms on rock surfaces, that in addition to the terminology used by previous authors (Friedmann 1967, Viles 1995, Golubic *et al.* 1981), also considers two new or rarely used terms - “Hemiendoliths” and “Hemiepiliths” - applied to those species that are partly endolithic and partly epilithic, respectively (Barreno 1989, Viles 1995). Epilithic lichens are only partially attached to the external surface of the rock (Golubic *et al.* 1981) by means of rhizines, stipes or umbilicus, and lack a hyphal penetration component. These are usually of the foliose (including squamulose and umbilicate), fruticose and compound types, when considering the more traditional morphological groups. Their weathering action is probably restricted to the most superficial layers of the rock, in the vicinity of their attachment structures (de los Rios *et al.* 2002, Viles 1995). Hemiepilithic lichens are those whose thallus develops mostly external and partly internal, penetrating the rock by means of rhizomorphs that cause mineral disintegration, being also capable of incorporating substrate particles (*e.g.* Sanders *et al.* 1994). Thallus of hemiendolithic lichens develops partly external and partly internal, penetrating the rock through medullary hyphae and in much closer contact with the rock surface than hemiepiliths. Effects on rock include mineral disintegration, mineral dissolution, detachment of mineral fragments and incorporation of mineral particles into the lichen thallus (Adamo & Violante 2000, Chen *et al.* 2000). Endolithic lichens are a peculiar group of lichens whose thallus develops inside the rock and is almost invisible at the rock surface, except for the sexual structures (apothecia and perithecia). These are more common on calciferous rocks (*e.g.* Bungartz *et al.* 2004, de la Rosa *et al.* 2012) but can also be found on siliceous ones (*e.g.* de los Rios *et al.* 2005). All endolithic lichens are euendoliths (true endoliths) sensu Golubic *et al.* (1981). Lichens who occupy pre-formed cracks, crevices or holes in rocks that are open to the rock surface are referred to as chasmolithic (Golubic *et al.* 1981). Another argument in favour of the use of growth form in functional group surrogacy lies in the possibility that a species is able to slightly change its growth form in response to changes in environmental factors (Barreno 1989, Scarciglia 2012).

Fig. 7.2.1. The typical species assemblage of south-east facing schist surfaces in the Upper Douro region showing the orange *Caloplaca subsoluta* and brown *Peltula euploca* on a black biofilm dominated by *Pyrenopsis triptococca*.

Biological coating

The morphological and ecological resemblance of some lichen species with biofilms has already been hypothesized in another paper (Marques *et al.* in review). The species in question are characterized by redish-brown to black thin gelatinous thalli lacking secondary metabolites and are therefore expected to play a similar role in rock surface weathering as biofilms (Table 7.2.1). Viles (1995) additionally coined the terms ‘biorind’ to define up to 5 mm thick lithobiontic communities presumably involved in both weathering and precipitation of minerals and ‘biocrust’ to the thickest of the lithobiontic communities where the ‘bioconstructive’ role dominates.

The single species approach

Key attributes of a successful surrogate species are its ability to indicate the abundance patterns of many other species (Cushman *et al.* 2010); its similarity in terms of environmental requirements; and its ability to show the same effects of environmental constraints as the group which they indicate (Favreau 2006). Parallel studies on the composition of lichen assemblages in the study area (Marques *et al.* in review) revealed considerable changes related to aspect and identified the most frequent and abundant species on vertical schist surfaces of opposing valley slopes. The effectiveness of using the isolated and combined abundance of these and other species as indicators of overall lichen diversity on vertical schists in the study area is to be tested in this study.

7.2.3 Material and methods

The study area

The study was conducted in the Upper Douro region (north-east Portugal). Located in one of the most complex areas of the variscan Central Iberian Zone (CIZ), the Upper Douro region has great geological diversity, dominated by a sequence of autochthonous metasedimentary units from the Neoproterozoic to the Cenozoic, and a sequence of units belonging to the allochthonous terranes of the Galicia-Trás-os-Montes Zone (GTMZ) (Moreira *et al.* 2010), as well as a wide variety of variscan granitic rocks. Climate is tipically mediterranean and bioclimate is predominantly meso-Mediterranean although a so-called topographic thermo-mediterranean belt (Costa *et al.* 1998) is produced on the floodplains of the steeper river valleys. Data collection took place between May 2011 and September 2012, from 64 randomly selected vertical schist surfaces, independently of the occurrence of rock-art.

Data collection

Field data

Species percentage cover was estimated through visual inspection as in Marques *et al.* (in review). Lichen specimens were identified in the field based on previous knowledge on the lichen flora of the region (Marques *et al.* 2013) except for dubious cases, which were later confirmed in the laboratory. A total of 108 species and one subspecies (treated as ‘species’ hereafter) were registered in all sampled surfaces. A ‘species x samples’ matrix was finally built, composed of the abundance values of individual species in the 64 sampled surfaces. Lichen nomenclature is according to the *Index Fungorum* (2013). The nomenclature for *Peltula lobata*, recently described from the Iberian Peninsula, is according to Marques *et al.* (2013).

Functional trait data

Traits were chosen to reflect recognizable aspects of lichen morphology related with colonization ability and presumed effect on rock surfaces (see Table 7.2.1 for detailed information on the functional significance of the traits). They were all categorical but further expanded into as many binary variables as trait attributes (Table 7.2.1). This procedure resulted in 16 attributes stemming from the original three recorded traits. Each species was classified with respect to these 16 trait attributes (presence/absence of each attribute). Data was finally compiled into a matrix of 16 attributes (variables) x 109 species (cases). Differences between samples in the abundance of each attribute were taken into account by means of species abundances, emphasizing the most represented trait attribute.

Table 7.2.1. Lichen traits and respective attributes selected for TWINSPAN analysis with information on their functional significance. TC: Thallus colour; GF: Growth form; BC: Biological coating. Information based on Edwards *et al.* 1995, 2003, Mottershead & Lucas 2000, Muchen *et al.* 1988, Šoun *et al.* 2011.

Traits	Attributes	Rationale	Presumed function
TC	Black	A proxy of secondary metabolite production which cause mineral dissolution and neoformation of biogenic minerals.	Reduced chemical weathering (no secondary metabolites)
	Grey		Sedifolia-grey, atranorin or no lichen substances
	Green		Chemical weathering (*e.g.* usnic acid)
	Brown		Chemical weathering (*e.g.* gyrophoric acid) or absence of chemical weathering (no lichen substances)
	Red		Absence of chemical weathering (no lichen substances)
	Orange		Chemical weathering (anthraquinones)
	Yellow		Chemical weathering (*e.g.* rhizocarpic acid)
	White		Chemical weathering (*e.g.* lecanoric acid, atranorine); Oxalate production.
GF	Endolithic	An integrative trait for several features that determine lichen ability to physical and chemically weather rock surfaces, including surface adhesion, hyphal and rhizomorph penetration and chemical interaction with rock.	Physical weathering (hyphae)
	Hemiendolithic		Physical weathering (hyphae).
	Hemiepilithic		Physical weathering (rhizomorphs).
	Epilithic		Reduced physical weathering (rhizines).
	Chasmolithic		Agregation of mineral particles and substrate stabilization.
BC	Biofilm	A proxy of the mechanisms associated with ecological succession where biologically-induced weathering progressively replaces inorganic processes and weathering processes reinforce one another to produce a complex weathering environment.	Modification of the water holding properties of the rock; Dissolution and precipitation of minerals.
	Biorind		Physical and chemical weathering.
	Biocrust		Bind sediments.

Data analysis

The ‘species x traits’ matrix was analysed by the polythetic divisive classification method TWINSPAN (Hill 1979) in order to identify species groups with similar trait attributes. Species groups retrieved by this method were assumed to represent lichen functional groups (García-Mora *et al.* 1999).

Lichen functional groups

Determination of relationships between groups is commonly and most simply done by examining correlations between species richness. The relationship between overall species richness and that of the various functional groups in the 64 samples surfaces was tested by means of Spearman rank correlations. Yet, even if there are close relationships between the richness of the surrogate and that of the indicated group, one will rarely know anything about how such a relationship may change with changes in the surrounding environment and must still determine whether the relationship between the surrogate and the indicated group remains the same across space and time. A good surrogate should respond to the same underlying main gradients and be at least as sensitive to ecological and environmental constraints as the group it is supposed to represent (Pharo *et al.* 1999). Therefore analyzing the relationship between environmental variables and studied species is vital to determine if the surrogate was appropriately chosen. Covariation in species composition of the entire species ensemble and that of each functional group along underlying gradients was quantified by means of Spearman rank correlations between the respective samples scores in a Principal Components Analysis (PCA). Length of compositional turnover, as revealed by Detrended Correspondence Analysis (length of gradient through DCA 1.429) had indicated the use of ordination based on the linear model (Lepš & Šmilauer 2003). Because second and higher order axes are often less explanatory (Legendre & Legendre 1998), all correlations and comparisons were based only on the sample scores along the first two PCA axes.

Single species

The usefulness of each species as surrogate, *i.e.* its ability to explain species abundances either within its functional group or across the entire species assemblage was quantified by means of Redundancy Analysis (Legendre & Legendre 1998). The analysis was performed as suggested by Cushman *et al.* (2010), using each species as the only independent variable, therefore obtaining the proportion of variance in the observed data that can be explained by the species that is removed. The response was either restricted to the members of the corresponding functional group or considering the entire species assemblage. This analysis was applied only to a few putative surrogate species (Table 7.2.3) based on the following criteria: 1) Abundance – the putative surrogate species should cover a significant part of the vertical schist surfaces in the study area thus being the most influential as possible in terms of lichen-induced weathering; 2) Frequency – preferably, a surrogate should be frequent enough (occurring at least in half of the sampling surfaces) in order to allow for comparisons between rock-art sites; 3) Functional group representation – each functional group should have at least one representative (the most frequent and abundant among group members).

The significance of the relationships between the abundance of individual species and the compositional pattern of species assemblages was tested by Monte Carlo permutation tests (Lepš & Šmilauer 2003). The potential use of the compositional pattern of each lichen functional group (Table 7.2.2) as a surrogate of that of all other lichen species was similarly tested. All ordinations were performed in CANOCO 4.5 (ter Braak & Smilauer 2002) and TWINSPAN analysis in Community Analysis Package 4.0 (Seaby & Henderson 2007) softwares. Other statistical analyses were done through the vegan package in R statistical software.

7.2.4 Results and discussion

Building lichen functional groups

The TWINSPAN analysis based on similarity of trait attributes (Fig. 7.2.2) separated, at the first level of division (eigenvalue 0.6048), a small cluster of 29 species that form biocrusts from a wider cluster of 80 species either associated with biorinds or biofilms. At the second level of division the later was further split (eigenvalue 0.5013) into light coloured hemiendoliths and endoliths (groups 1 and 2), although endoliths are rare in the studied surfaces; and dark coloured epiliths and hemiepiliths (groups 3 and 4). Group 2 is mainly composed of brightly coloured species, with pure white to deep orange thallus. It is simultaneously the most species rich (28 species) and presumably the most biodeteriogenic, since its members grow in close contact with the substrate, developing penetrating hyphae, and most of them producing secondary metabolites. The species previously recognized as the most adequate for an assessment of lichen-induced weathering of vertical schist surfaces in the study area (Marques *et al.* in review) are distributed between groups 1, 2 and 3 which is indicative of the functional diversity behind that selection. Group 4 includes the previously referred black gelatinous lichens that form biofilms. The cluster of the remaining 29 chasmolithic and epilithic species resulting from the first level of division was further separated into group 5 (the poorer in terms of species richness and defined solely by the green species of *Toninia*), group 6 (also species poor and mainly consisting of brown chasmolithic species of genus *Endocarpon*, *Toninia* and *Peltula*), group 7 (grey epiliths) and group 8 (green epiliths). Members of groups 5 and 6 could be joined to form a unit of chasmoliths that do not produce secondary metabolites, and are therefore functionally similar from the point of view of rock-art conservation.

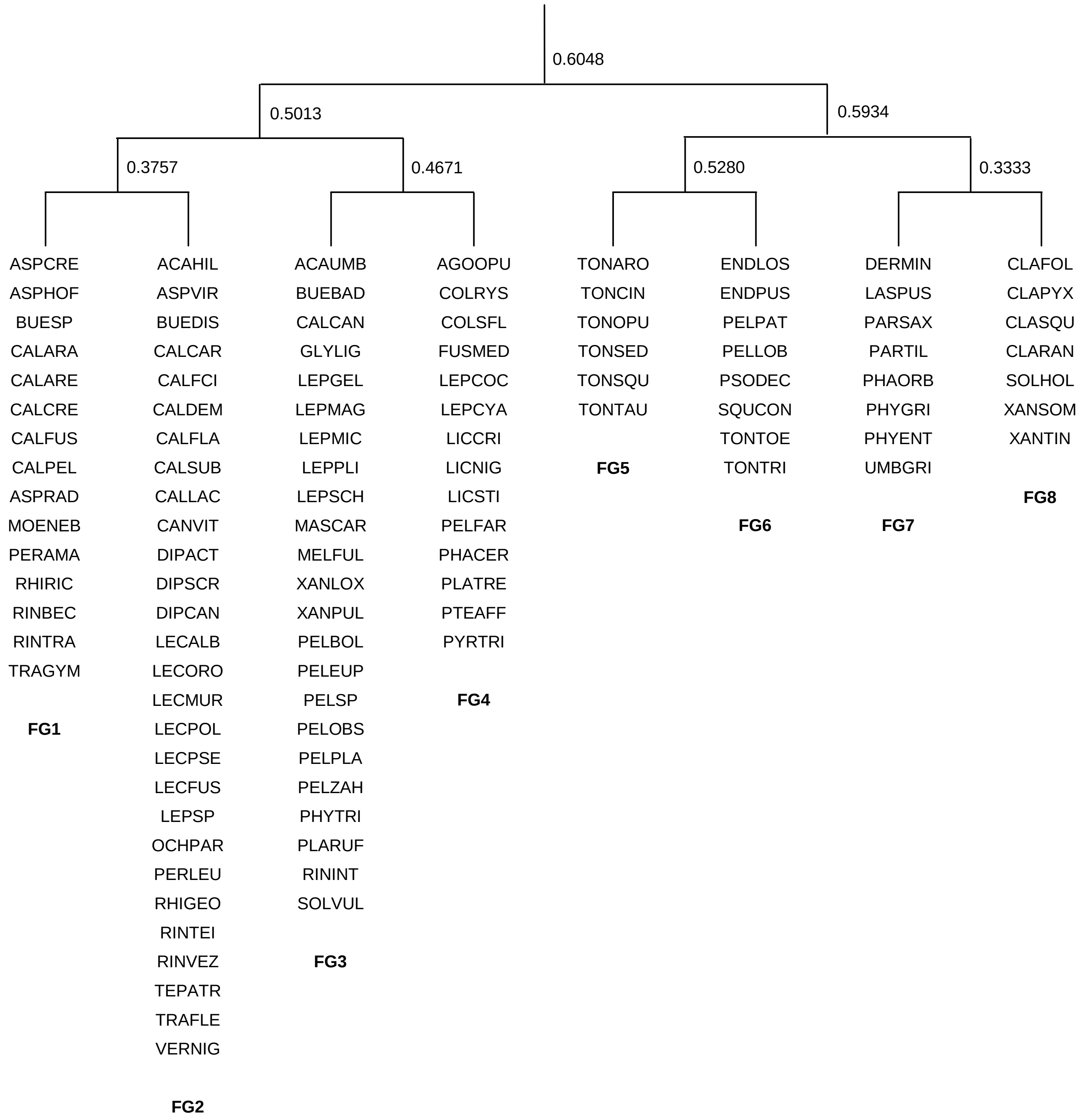

Fig. 7.2.2. Grouping of species by TWINSPAN based on similarity of selected trait attributes**.** FG1-8: Functional groups obtained by TWINSPAN. Species codes are according to Table 7.2.4.

Testing surrogate species

All values of species richness within functional groups are correlated with the values of species richness in the full assemblage (Table 7.2.2). This means that an increase in overall species richness is associated with an increase in species richness of all functional groups. Therefore if, for example, an increase in species richness of black biofilms (FG4) is detected, one would expect that an increase had also been produced in overall species richness.

The ability of the selected species (putative surrogates) to explain the variance in the abundance of other group members varied markedly among functional groups (Table 7.2.3) but generally accounted for very little variance in species abundance. Variance explained by species within the functional groups is generally higher than variance explained by the species when the full species pool is considered. The opposite clearly occurs with both representatives of FG4, which were able to explain a significant amount of variation in the full species pool but not in the respective functional group. Across groups, species explained at best an average of 6.1% of the variance in abundance of the other group members. *Lecanora pseudistera* was one of the best surrogate species because it explained the most in terms of variance of any species both within its functional group (9.9%) and across the entire species assemblage (4.0%).

Table 7.2.2. Relationships between lichen species richness and composition of putative surrogate groups and those of the full lichen assemblage by means of Spearman rank correlations of the number of species of each group and the sample scores on the first and second axis of a Principal Componentes Analysis (PCA). Variance explained by the species subset and each functional group is based on Redundancy Analysis (RDA). Asterisks (*) indicate the significance of correlation: *p-value< 0.05; **p-value< 0.01.

	All Species			
	Species richness	*Samples scores*		*Explained variance (%)*
		PCA axis 1	PCA axis 2	
Subset (18)	0.689**	0.978**	0.992**	81.1**
FG1 (15)	0.531**	0.526*	0.487**	50.3**
FG2 (28)	0.468**	-0.380**	0.123	66.2**
FG3 (23)	0.625**	0.903**	0.160	63.3**
FG4 (14)	0.440**	0.675**	-0.237	52.7**
FG5 (6)	0.593**	0.316*	0.165	17.3*
FG6 (8)	0.361**	0.362**	-0.004	21.6*
FG7 (8)	0.373**	-0.263*	0.015	18.3
FG8 (7)	0.513**	0.097	-0.052	22.8*

Testing surrogate functional groups

Significant correlations indicate parallel variation in the species composition among compared groups. Significant correlations between sample scores along the PCA axes indicate that lichen assemblages originating them respond to the same underlying main gradients and can therefore be used as surrogates, not only at optimal conditions (Bergamini *et al.* 2007) but also within the framework of environmental changing scenarios.

The probability of related groups to react similarly to environmental variation was expected to be high because of their similar ecological requirements. In fact, spliting species into the previously described functional groups on the basis of thallus colour, growth form and type of rock surface coating produced a considerable amount of statistically significant surrogate relationships (Table 7.2.2) with the best being the species subset defined by the representatives of each group. The group 'coloured hemiendoliths and endoliths' (FG2) has an order of magnitude in terms of species than any of the other groups, therefore, it is not surprising that it covers well the variation of the other groups. Even the group with fewer members (FG8), thus holding little information about overall species diversity, captured significant variation in the species composition of the full assemblage. Among species poor groups (FC5 to FG8), species richness was found to be more efective at capturing overall lichen diversity than species composition.

Table 7.2.3. Variance explained by the selected lichen species representing each functional group (FG) within its group (*Group*) and across the entire species pool (*All*) based on Redundancy Analysis (RDA). Aspect preference is based on species scores on axis 1 from partial Canonical Correspondence Analysis (Marques *et al.* in review). FG1-8: Functional groups obtained by TWINSPAN (Fig. 7.2.2). Asterisks (*) indicate the significance of correlation: *P<0.05, **P<0.01.

Species subset	**Aspect preference**	**Group membership**	**Variance explained (%)**	
			Group	*All*
Aspicilia contorta subsp. *hoffmanniana*	South-east	FG1	5.5*	1.8
Rinodina beccariana	North-west	FG1	3.5	4.5*
Caloplaca subsoluta	Indiferent	FG2	6.2**	4.1*
Diploschistes actinostomus	North-west	FG2	5.9*	4.8*
Lecanora pseudistera	North-west	FG2	9.9**	4*
Verrucaria nigrescens	South-east	FG2	5.4*	2.4
Leprocaulon microscopicum	North-west	FG3	0.2	3.2
Peltula euploca	South-east	FG3	3.3	8.1**
Solenopsora vulturiensis	North-west	FG3	3.4	3.2
Collema rysoleum	Indiferent	FG4	1.3	5.8**
Pyrenopsis triptococca	South-east	FG4	6.4	9.6**
Toninia cinereovirens	South-east	FG5	4.6	3.8*
Endocarpon loscosii	Indiferent	FG6	7	2.1
Endocarpon pusillum	South-east	FG6	3.7	3.4
Dermatocarpon miniatum	South-east	FG7	1.8	2.4
Phaeophyscia orbicularis	North-west	FG7	6.5*	4.1*
Solenopsora holophaea	North-west	FG8	4.7	2.4
Xanthoparmelia tinctina	Indiferent	FG8	5.5	4.3**

Monitoring efficiency is often judge on the basis of the power to detect change (Cushman *et al.* 2010). Manley *et al.* (2004) suggested that being able to detect a 20% change in abundance is a reasonable expectation for change-detection monitoring. In this analysis the average variance in species abundance explained by the group assignment was 39.1%, with the lowest explanation being the one from functional group 5 (Table 7.2.2), meaning that at least 17.3% of the variability of the species assemblage is dependent of the abundance of this group. Any of the functional groups between FG1 and FG4 explains more than 50% of total variation and should be considered a good surrogate of overall lichen diversity on the vertical schist surfaces of the study area. The surrogate with the highest power to detect change in overall species diversity is the species subset formed by 18 representatives of the functional groups, explaining 81.1% of the variation in the species assemblage. These results indicate that functional groups and species subsets respond to the same main environmental gradients as complete species assemblages, and will respond in a similar way to habitat alterations.

Table 7.2.4. List of lichen taxa found in the study area, with respective code used in diagrams. Taxa are organized by functional group (FG) according to Fig. 7.2.2.

FG	Code	Taxa
1	ASPHOF	*Aspicilia contorta* (Hoffm.) Kremp. subsp. *hoffmanniana* S. Ekman & Fröberg
1	ASPCRE	*Aspicilia crespiana* V. J. Rico
1	ASPRAD	*Aspicilia radiosa* (Hoffm.) Poelt & Leuckert
1	BUESP	*Buellia* sp.
1	CALARA	*Caloplaca* aff. *aractina*(Fr.) Häyrén
1	CALARE	*Caloplaca arenaria* (Pers.) Müll. Arg. *s. lat.*
1	CALCRE	*Caloplaca crenularia* (With.) J. R. Laundon
1	CALFUS	*Caloplaca fuscoatroides* J. Steiner
1	CALPEL	*Caloplaca pellodella* (Nyl.) Hasse
1	MOENEB	*Moelleropsis nebulosa* (Hoffm.) Gyeln.
1	PERAMA	*Pertusaria amara* (Ach.) Nyl.
1	RHIRIC	*Rhizocarpon richardii* (Nyl.) Zahlbr.
1	RINBEC	*Rinodina beccariana* Bagl.
1	RINTRA	*Rinodina trachytica* (A. Massal.) Bagl. & Carestia
1	TRAGYM	*Trapeliopsis gymnidiata* Aptroot & Schumm
2	ACAHIL	*Acarospora hilaris* (Dufour) Arnold
2	ASPVIR	*Aspicilia viridescens* (A. Massal.) Hue
2	BUEDIS	*Buellia dispersa* A. Massal.
2	CALCAR	*Caloplaca carphinea* (Fr.) Jatta
2	CALDEM	*Caloplaca demissa* (Flot.) Arup & Grobe
2	CALFLA	*Caloplaca flavescens* (Huds.) J. R. Laundon
2	CALFCI	*Caloplaca flavocitrina* (Nyl.) H. Olivier
2	CALLAC	*Caloplaca lactea* (A. Massal.) Zahlbr.
2	CALSUB	*Caloplaca subsoluta* (Nyl.) Zahlbr.
2	CANVIT	*Candelariella vitellina* (Ehrh.) Müll. Arg.
2	DIPCAN	*Diploicia canescens* (Dicks.) A. Massal.
2	DIPACT	*Diploschistes actinostomus* (Ach.) Zahlbr.
2	DIPSCR	*Diploschistes scruposus* (Schreb.) Norman

FG	Code	Taxa
2	LECALB	*Lecanora albescens* (Hoffm.) Branth. & Rostr.
2	LECMUR	*Lecanora muralis* (Schreb.) Rabenh.
2	LECORO	*Lecanora orosthea* (Ach.) Ach.
2	LECPOL	*Lecanora polytropa* (Ehrh.) Rabenh.
2	LECPSE	*Lecanora pseudistera* Nyl.
2	LECFUS	*Lecidea fuscoatra* (L.) Ach.
2	LEPSP	*Lepraria* sp.
2	OCHPAR	*Ochrolechia parella* (L.) A. Massal.
2	PERLEU	*Pertusaria leucosora* Nyl.
2	RHIGEO	*Rhizocarpon geographicum* (L.) DC.
2	RINTEI	*Rinodina teichophila* (Nyl.) Arnold
2	RINVEZ	*Rinodina vezdae* H. Mayrhofer
2	TEPATR	*Tephromela atra* (Huds.) Hafellner
2	TRAFLE	*Trapeliopsis flexuosa* (Fr.) Coppins & P. James
2	VERNIG	*Verrucaria nigrescens* Pers.
3	ACAUMB	*Acarospora umbilicata* Bagl.
3	BUEBAD	*Buellia badia* (Fr.) A. Massal.
3	CALCAN	*Caloplaca cancarixiticola* Nav.-Ros., Egea & Llimona
3	GLYLIG	*Glyphopeltis ligustica* (B. de Lesd.) Timdal
3	LEPMIC	*Leprocaulon microscopicum* (Vill.) Gams
3	LEPGEL	*Leptogium gelatinosum* (With.) J. R. Laundon
3	LEPMAG	*Leptogium magnussonii* Degel & P. M. Jørg.
3	LEPPLI	*Leptogium plicatile* (Ach.) Leight.
3	LEPSCH	*Leptogium schraderi* (Bernh.) Nyl.
3	MASCAR	*Massalongia carnosa* (Dicks.) Körb.
3	MELFUL	*Melanelixia fuliginosa* (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch
3	PELBOL	*Peltula bolanderi* (Tuck.) Wetmore
3	PELEUP	*Peltula euploca* (Ach.) Poelt ex Ozenda & Clauzade
3	PELOBS	*Peltula obscurans* (Nyl.) Gyeln.
3	PELPLA	*Peltula placodizans* (Zahlbr.) Wetmore
3	PELSP	*Peltula* sp.
3	PELZAH	*Peltula zahlbruckneri* (Hasse) Wetmore
3	PHYTRI	*Physcia tribacia* (Ach.) Nyl.
3	PLARUF	*Placidium rufescens* (Ach.) A. Massal.
3	RININT	*Rinodina intermedia* Bagl.
3	SOLVUL	*Solenopsora vulturiensis* A. Massal.
3	XANLOX	*Xanthoparmelia loxodes* (Nyl.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch
3	XANPUL	*Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch
4	AGOOPU	*Agonimia opuntiella* (Buschart & Poelt) Vezda
4	COLRYS	*Collema ryssoleum* (Tuck.) A. Schneider
4	COLSUB	*Collema subflaccidum* Degel.
4	FUSMED	*Fuscopannaria mediterranea* (Tav.) P. M. Jørg.
4	LEPCOC	*Leptogium cochleatum* (Dicks.) P. M. Jørg. & P. James
4	LEPCYA	*Leptogium cyanescens* (Rabenh.) Körb.
4	LICCRI	*Lichinella cribellifera* (Nyl.) P. P. Moreno & Egea

FG	Code	Taxa
4	LICNIG	*Lichinella nigritella* (Lettau) P. P. Moreno & Egea
4	LICSTI	*Lichinella stipatula* Nyl.
4	PELFAR	*Peltula farinosa* Büdel
4	PHACER	*Phaeophyscia cernohorskyi* (Nádv.) Essl.
4	PLATRE	*Placynthium tremniacum* (A. Massal.) Jatta
4	PTEAFF	*Pterygiopsis affinis* (A. Massal.) Hessen
4	PYRTRI	*Pyrenopsis triptococca* Nyl.
5	TONARO	*Toninia aromatica* (Turner) A. Massal.
5	TONTAU	*Toninia* cf. *taurica* (Szatala) Ochsner
5	TONCIN	*Toninia cinereovirens* (Schaer.) A. Massal.
5	TONOPU	*Toninia opuntioides* (Vill.) Timdal
5	TONSED	*Toninia sedifolia* (Scop.) Timdal
5	TONSQU	*Toninia squalida* (Ach.) A. Massal.
6	ENDLOS	*Endocarpon loscosii* Müll. Arg.
6	ENDPUS	*Endocarpon pusillum* Hedw.
6	PELLOB	*Peltula lobata* J. Marques, M. Schultz & Paz-Berm
6	PELPAT	*Peltula patellata* (Bagl.) Swinscow & Krog
6	PSODEC	*Psora decipiens* (Hedw.) Hoffm.
6	SQUCON	*Squamarina concrescens* (Müll. Arg.) Poelt
6	TONTOE	*Toninia toepfferi* (Stein) Navàs
6	TONTRI	*Toninia tristis* (Th. Fr.) Th. Fr. subsp. *pseudotabacina* Timdal
7	DERMIN	*Dermatocarpon miniatum* (L.) W. Mann
7	LASPUS	*Lasallia pustulata* (L.) Mérat
7	PARSAX	*Parmelia saxatilis* (L.) Ach.
7	PARTIL	*Parmelina tiliacea* (Hoffm.) Ach
7	PHAORB	*Phaeophyscia orbicularis* (Neck.) Moberg
7	PHYENT	*Physconia enteroxantha* (Nyl.) Poelt
7	PHYGRI	*Physconia grisea* (Lam.) Poelt
7	UMBGRI	*Umbilicaria grisea* Hoffm.
8	CLAFOL	*Cladonia foliacea* (Huds.) Willd.
8	CLAPYX	*Cladonia pyxidata* (L.) Hoffm.
8	CLARAN	*Cladonia rangiformis* Sandst.
8	CLASQU	*Cladonia squamosa* (Scop.) Hoffm.
8	SOLHOL	*Solenopsora holophaea* (Mont.) Samp.
8	XANSOM	*Xanthoparmelia somloensis* (Gyeln.) Hale
8	XANTIN	*Xanthoparmelia tinctina* (Maheu et A. Gillet) Hale

7.2.5 Conclusions

There appears to be good evidence for a general coincidence in the patterns of species diversity on the vertical schist surfaces of the Upper Douro region. Lichen functional groups based on few morphological features but specifically targeted at presumed lichen activity on rock surfaces were shown to be excelent surrogates for overall lichen species richness and composition and should constitute a good alternative to exhaustive species surveys in the study area. The groups most correlated with the entire species pool are those formed by the most ubiquitous species. On the contrary, variation in the cover of overall lichen species on the studied rock surfaces is not really depicted by individual lichen abundances, given that these explain only a small amount of total variation in full assemblages, unless one considers a subset of species built by the most common representatives of the proposed functional groups. A strongly reduced subset of the original data composed of such species has been proposed here, to facilitate cost-effective monitoring of lichen-induced weathering. This subset should also guide the zonation of rock surface vulnerability to lichen-induced weathering, for rock-art condition assessment purposes, if combined with local environmental and geochemical data. Habitat requirements must still be determined, but only for a handful of species.

Proven the possibility of defining surrogates of overall lichen species diversity on vertical schist surfaces, the question remains whether these can be used as *proxies* of lichen-induced weathering in the study area. Assessing intra- vs inter-specific variability of thallus colour is clearly of interest in this context in order to precise if there are changes in species colour along environmental gradients or through time that can be detected through colorimetric techniques. Additionally, further investigation on the importance of suitable substrate caracteristics in determining micro-scale patterns of lichen species distributions on rock surfaces may point to some of the diferences in preventive conservation strategies required for protection of rock-art from lichen-induced weathering. The extent to which these results are transferable to other rock-art sites and other substrate types will depend on what the controlling factors are for each functional group. Earlier work showed that slope and aspect are important explanatory variables of lichen species diversity and distribution on rock surfaces (Dale 1995). If the provision of microhabitat were to explain an additional and substantial portion of the observed variance, then it may be important to firstly compare the general character of these rock-art sites and secondly, the diversity and types of substrate available.

7.2.6 References

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8 Towards a comprehensive model of the mechanism of lichen-induced schist weathering

8.1 The susceptibility of weathered versus unweathered schist to biological colonization in the Côa Valley Archaeological Park (north-east Portugal)

Submitted to the Science of the Total Environment with the following co-authors: Daniel Vazquez-Nion, Graciela Paz-Bermúdez and Beatriz Prieto

Abstract

Concern about all aspects of schist weathering has increased with the discovery of one of the world's largest collection of open-air rock-art of Upper Palaeolithic age in the Côa Valley (Vila Nova de Foz Côa, north-east Portugal), today classified by UNESCO as World Heritage. However the consequences of schist weathering for open-air rock-art preservation are poorly understood because of a dearth of data from schistose environments. This study addresses the bioreceptivity of unweathered and weathered schist from the Côa Valley Archaeological Park in order to determine primary and secondary bioreceptivity of schist used as a support for rock-art in the Côa Valley and provide parameters that can be related to the risk of biologically induced schist weathering. Samples of freshly quarried and naturally weathered schist were characterized in terms of their intrinsic properties and let grow in controlled environmental conditions after inoculation with biofilm-forming cyanobacteria. The physical properties of the studied schist, as well as its abrasion pH, all varied according to the origin of the samples and so did its susceptibility to colonization by biofilm-forming cyanobacteria. Complete separation between weathered and unweathered schist samples in terms of photosynthetic biomass was obtained by measuring total colour change in the CIE L*a*b* colour space. Weathered schist was more bioreceptive than unweathered schist, associated with increased open porosity, water saturation, capillary water and capilarity coefficient, and decreased pH, suggesting that rock-art preservation might be affected adversely by the removal of biological colonization. In the future it might be possible to determine the susceptibility of schist surfaces to biological colonization through evaluation of

colour differences associated with the different weathering degrees presented by those surfaces, prior to colonization.

8.1.1 Introduction

The Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal) holds one of the world's largest collections of open-air rock-art from the Upper Palaeolithic, classified in 1998 by UNESCO as world heritage. Most rock-art is in the form of petroglyphs carved in thousands of flat, vertical schist surfaces, found among the countless blocks of rock that crop out along the steep slopes the Côa River Valley.

Open-air rock-art surfaces in the Côa Valley, as many other exposed vertical rock surfaces, often present an almost continuous layer of dark brown to black crusts marking the track of water along the rock surface. The composition of these specific crusts was not fully analyzed but found so far are the extensive patches of dark gelatinous cyanolichens of genus *Peltula*, *Pyrenopsis* and *Pterygiopsis* (Marques *et al.* 2013) spreading upon a black biofilm of non-symbiotic cyanobacteria. In fact, biofilm-forming cyanobacteria are known to constitute a large part of the biomass developing on exposed natural rocks of both tropical and desert environments (*e.g.* Büdel 1999, Warren-Rhodes *et al.* 2007) and thought to play a crucial role in the weathering of stone monuments worldwide (Crispim & Gaylard 2005). Although there is limited experimental evidence, both colonial and free-living cyanobacteria in these biofilms are assumed not only to contribute to water and nutrient retention at the surface level (Llimona & Egea 1985) but also to constitute important sources of photobionts for the lichen symbiosis, thus favouring the process of ecological succession on exposed rock surfaces leading to lichen colonization. Other early colonizers found on these surfaces include numerous bryophytes, some ferns and to a lesser extent vascular plants. All of these organisms contribute to the series of geophysical and geochemical changes, inherent to ecological succession, which lead to rock surface weathering and open-air rock-art deterioration.

Biological colonization on rock surfaces is itself under the influence of two main groups of contributing factors: those related with the properties of the rock, such as chemical composition, porosity and surface roughness, strongly determining the colonization potential of the substrate, *i.e.*, its bioreceptivity (Guillite 1995); and those related with the colonization potential of the surrounding environment, including accessibility (Heimans 1954 *apud* Guillite 1995). The latter can be further divided into large-scale environmental constraints, including those arising out of climate change; and small-scale microclimatic factors due to variations in surface orientation, slope aspect, and presence of vegetation, among others (Miller 2009). The effect of small-scale factors on the composition and morphology of species assemblages on exposed rock surfaces has been the focus of previous works (Adamson *et al.* 2013, Steinbauer *et al.* 2013), with large-scale environmental constraints and bioreceptivity factors controlled to a certain extent by the use of relatively homogenous study areas and lithotypes.

The state of current research in the field of bioreceptivity has been described in detail elsewhere (Miller *et al.* 2012), but a few concepts will be briefly recovered because of their importance for the subject of this paper. Guillite (1995) defined three types of bioreceptivity termed primary, secondary and tertiary bioreceptivity on the basis of the extent of changes in the intrinsic properties of the material in consideration: no or very limited change, change by the action of natural phenomena and change by human action, respectively. Perhaps more important for open-air rock-art conservation and for the focus of this study, however, are the intermediary stages between these main types, also contemplated in Guillite's approach, that consider the external factors adding to intrinsic bioreceptivity a certain degree more of susceptibility to biological colonization, either independently (extrinsic biorreceptivity) or combined with the intrinsic properties of rock (semi-extrinsic).

A rock taken from a quarry is obviously not totally isolated from such exogenous contributions but represents the most similar to what can be considered the initial state of the rock, when susceptibility to biological colonization is dependent mostly of its intrinsic properties. Once extracted from the quarry, the rock is subjected to irreversible changes brought about by the synergic action of external and internal physical and chemical factors, therefore presenting variation in the weathering degree. Differences in the weathering degree are expected to be reflected in the expression of bioreceptivity. In a natural context, the condition of the rock allowing the very first organism to establish on its surface can be regarded as semi-extrinsic primary bioreceptivity, and is followed by a series of semi-extrinsic secondary bioreceptivity states driving ecological succession. Within the realm of what can be considered secondary bioreceptivity, the degree varies tremendously, depending on the stage of ecological succession, but no single definition may adequately cover the full range of factors influencing biological colonization as a hole.

A great body of research has been carried out into the links between the bioreceptivity of natural and artificial stones and their intrinsic properties, reflecting the geographical origin of the target lithotype and the importance of the corresponding stone in terms of its commercial or cultural use. Limestone and marble are the most commonly employed lithotypes followed by artificial stone materials, sandstone and granite (Miller *et al.* 2012). Most experiments have been designed with the purpose of improving the accuracy of bioreceptivity assessment (Escadeillas *et al.* 2007, 2009, Fernández-Silva *et al.* 2001, Guillite & Dreesen 1995, Prieto *et al.* 2004, 2006, Prieto & Silva, 2005, Shirakawa *et al.* 2003) or of finding proxies of rock bioreceptivity that are easier or faster to measure (Prieto & Silva 2005). Some have been focusing on comparing the primary bioreceptivity of different lithotypes (Guillite & Dreesen

1995, Miller *et al.* 2006, 2010, Prieto & Silva 2005, Tiano *et al.* 1995), fewer on the characterization of a single lithotype (Adamson *et al.* 2013, Miller *et al.* 2009), while simultaneously addressing other questions such as those related with the ecological preferences of the colonizing organisms (Adamson *et al.* 2013, Guillite & Dreesen 1995, Tiano *et al.* 1995). Only a few studies have aimed at testing the efficiency of eradication treatments (Tomaselli *et al.* 2002, Urzí & De Leo 2007) or of techniques for preventing biological growth (De Muynck *et al.* 2009).

Attempts to follow the causes of secondary bioreceptivity in the same lithotype are very rare. Silva *et al.* (1997) observed that weathered granite used in the construction of a museum was more prone to colonization than sound granite of the same type, and related this difference with the occurrence of naturally weathered alkali feldspars from glacially-scoured granite surfaces. Prieto & Silva (2005) briefly addressed the differences between weathered and unweathered granite (Baleante) in terms of their intrinsic properties and consequent bioreceptivity, concluding that in the controlled environment of a growth chamber, bioreceptivity of weathered granite was higher to that of sound granite due to increased water absorption and water holding capacity. Cámara *et al.* (2008) took a different approach to the relationship between bioreceptivity and the intrinsic properties of rock, basing their study on samples taken from the field with different stages of colonization. This approach has the advantage of being more realistic in terms of the structural and functionally complex species assemblages that constitute biological colonization, but is also prone to some subjectivity, since the ‘stage of colonization’ is a condition that is difficult to define and recognize, and homogeneity of the substrate samples is more difficult to control.

The schistose rocks outcropping throughout the Côa Valley Archaeological Park have been the subject of some mineralogical and geochemical characterizations (Aires *et al.* 2011, Noronha *et al.* 2012) given the recent increased commercial interest of schist as a building stone. Additional concern about all aspects of schist weathering has arisen from the discovery of the Côa Valley’s rock-art, but there has never been an attempt to characterize schistose rocks in terms of their bioreceptivity. Their geological structure, the intensity of deformation, the degree of metamorphism and the state of fracturing of the rock mass are important to determine their susceptibility to weathering especially that brought about by biological colonization. The purpose of this study is: i) to address the bioreceptivity of schist; ii) to compare the bioreceptivity and physical properties of unweathered and weathered schist from the Côa Valley Archaeological Park.

8.1.2 Material and methods

The studied schist is the substrate upon which local rock-art has been produced, of the so-called schist-greywacke complex (Sousa 1982). Unweathered schist samples were obtained from a local quarry known as Poio, whereas presumably weathered schist samples were gathered from recently exposed outcrops at Vale de José Esteves, a rock-art site located in the vicinity of the quarry but well away from traffic or quarrying activities. Source outcrops at Vale de José Esteves were facing south-east as most of the engraved surfaces in the Côa Valley.

Poio rock has been traditionally called schist (Noronha *et al.* 2012) or slate (Aires *et al.* 2012) but is in fact a relatively low-grade metamorphic (greenshist facies) phyllite consisting of thin alternating layers of whitish psammitic and dark pelitic components (Aires *et al.* 2011). The psammitic component is sometimes more abundant and the rock is then classified as a metaquartzwacke instead of a phyllite. For a matter of simplicity, this phyllite-metaquartzwacke sequence will be addressed under the broad term schist. This schist is mainly composed of quartz, sericite and/or muscovite, chlorite and biotite minerals, as well as plagioclase feldspars (mostly albite) in variable amounts depending of the psammitic contribution. Calcite is usually present in the matrix of these rocks in sufficient amounts to produce effervescence when treated with dilute hydrochloric acid. Magnetite and, more sporadically, pyrite crystals are present in both the psamitic and pelitic levels as accessory constituents (Sousa 1982). Both calcite and pyrite are potentially highly alterable (Prieto *et al.* 2011). Additional accessory minerals are illite, kaolinite, montmorillonite, graphite, turmaline, zircon, apatite, epidote, hematite, leucoxene and some alkali feldspars, such as microcline and orthoclase (Aires *et al.* 2011, Gomes & Almeida 2003, Sousa 1982).

Table 8.1.1. Mineral composition of the rocks used in this study based on Prieto & Silva (2005) and Solicel (2003).

Lithotype	**Percentage in volume**						
	Quartz	*K feldspar*	*Plagioclase*	*Chlorite*	*Muscovite*	*Biotite*	*Accessory minerals*
Blanco Cristal	39	35	20	-	-	5	1
Poio	19	16	2	5	58		1

Blocks of each type of schist were cut into six cubes of 5x5x5 cm and twelve smaller square blocks of 4x4x1 cm that were used as samples for physical properties testing and bioreceptivity assessment, respectively. Blocks of the Spanish granite Blanco Cristal were cut into 4x4x1cm square blocks and were also included in this study to test for the effect of lithotype, provided that information about the mineralogy (Table 8.1.1), physical properties and bioreceptivity of this type of granite was already available from previous studies (Prieto & Silva 2005). Surface of all samples were sawn smooth in order to avoid any influence of surface roughness in the final results. All blocks were sterilized, oven dried at 65ºC for 24 hours and let cool down to room temperature in a plastic container with silica gel prior to their use in the experiments.

Characterization of the studied lithotypes

Replicate samples of each weathering grade were polished with sandpaper attached to an Ecomet 3000 (Buehler, Düsseldorf, Germany) polisher machine and mineralogically and geochemically characterized by X-ray microdiffraction (XRmD) and X-ray fluorescence (XRF), respectively. XRmD measurements were performed on an Empyrean (PANalytical, Almelo, The Netherlands) diffractometer, equiped with a five-axis Chi-Phi-x-y-z stage goniometer, a copper sealed anticathode X-ray tube (Empyrean Tube Cu Lff Hr) and a PIXcel^{3D} (PANalytical, Almelo, The Netherlands) X-ray detector. Bragg angles were scanned between 4 and 47º, with steps of 0.02° (12 minutes time length). XRF measurements were performed with a Tracer IV handheld XRF analyser (Bruker, Billerica (MA), USA).

The bigger samples (5x5x5 cm) were characterized in terms of their physical properties according to European standards (EN). These included bulk density (EN 1936:1999), open porosity (EN 1936:1999), water saturation (adapted from EN 13755:1999 as in Sanmartín 2007) and capillarity coefficient (EN 1925:1999) following RILEM's recommendations (RILEM TC 25-PEM 1980). Capillarity was determined in the orientation corresponding to that of the engraved surfaces, *i.e.* perpendicularly to the schistosity plane.

Determination of abrasion pH followed the protocol developed by Grant (1969) where 20 g of each rock type is grinded in distilled water for 2.25 min and allowed to settle for another 2.00 min before taking the measurements. Physical properties and abrasion pH were tested on freshly-quarried and naturally weathered schist samples not only to estimate their potential bioreceptivity (Prieto & Silva 2005) but also in order to ascertain whether the samples were in fact different in terms of their weathering degree.

Bioreceptivity experiments

The upper surface of each 4x4x1 cm sample of weathered and unweathered schist was inoculated with 10 ml of a cyanobacterial mixture of known chlorophyll-a concentration (21.67 µg/ml) containing the strains *Nostoc* sp. PCC 9025, *Nostoc* sp. PCC9104 and *Scytonema* sp. CCC9801 grown in BG-11_0 liquid medium (Rippka *et al.* 1979) and allowed to reach the exponential growth phase in aerified Erlenmeyer flasks under the controlled environment of a plant growth chamber with temperature kept at 22ºC, relative humidity of 95% and a 12/12h day/night cycle (light intensity of 1,600 lx). The choice of cyanobacteria as the test organism relates to the fact that these are among the first colonizers of the target rock surfaces, are quite abundant on those surfaces and seem to be important for the establishment of other pioneer species during ecological succession leading to surface weathering (and rock-art deterioration). The use of cyanobacteria will also allow objective comparison with results from other studies if the common goal is to produce a bioreceptivity index (Guillite 1995, Miller *et al.* 2006, Prieto *et al.* 2006).

The experiment ran for 120 days for full-term samples, whilst a set of nine samples, three from each type, were removed after 30, 60 and 90 days for estimation of photosynthetic biomass. This was done by extraction of chlorophyll-a in dimethyl-sulfoxide (DMSO) and determination of total colour change following Prieto & Silva (2005) and Prieto *et al.* (2004) as they both have yield reliable results.

Quantification of chlorophyll-a content in rock samples after inoculation

Chlorophyll-a was extracted by direct sonication of the intact rock in a 0.43 ml DMSO/cm^2 sample bath followed by incubation of this bath at 63°C for 40 min according to the protocol optimized by Fernández-Silva *et al.* (2011). The absorbance of the extract was then measured at 665 and 649 nm as required by Wellburn (1994) in his proposed equation for the determination of chlorophyll-a concentration.

Determination of total colour difference (ΔE*)

Colour measurements were performed with a CM-700d handheld spectrophotometer (Konica Minolta, Tokyo, Japan) coupled with a glass protected 8 mm target mask, for equivalent diameter of the measuring area. Measurement results were obtained under the CIE standard daylight illuminant D65 with specular components included (SCI). A total of 14 measurements were done in a semi-systematic unaligned manner across the surface of each block according to the protocol optimized by Prieto *et al.* (2010). Although previous analysis of the measurement conditions showed that the minimum number of measurements required to determine the colour of schist surfaces is substantially lower than the number required to determine the colour of granitic rocks due to the greater homogeneity of the colour and texture of schist when compared to that of granite (Sanmartín *et al.* 2010), a conservative approach was taken and the same number of measurements suggested for granite were used for schist. Data stored in the spectrophotometer was loaded with the CM-S100w (SpectraMagicTM NX) software. Analysis of the colour measurements was based on the CIE L*a*b* colour space (CIE 1976), a three-dimensional model of colour representation defined by three parameters (or Cartesian coordinates) plotted along the three orthogonal axes of a Cartesian coordinate system: L* is for lightness or luminosity of colour, which varies from 0 (absolute black) to 100 (absolute white); and a* and b* are chromaticity coordinates associated with changes in redness/greenness and in yellowness/blueness, respectively: a* ranges from green (negative) to red (positive) and b* from blue (negative) to yellow (positive). Measurements were always taken on water sprayed blocks, unless indicated otherwise. Results are expressed as the difference between the colour of the inoculated block at the previously set time and that presented by the exact same block before inoculation (ΔE*):

$$\Delta E_i^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

Where:

$$\Delta L^* = L^*_i - L^*_0$$
$$\Delta a^* = a^*_i - a^*_0$$
$$\Delta b^* = b^*_i - b^*_0$$

L^*_0, a^*_0 and b^*_0 are the parameters of the colour in the block before inoculation and L^*_i, a^*_i and b^*_i are the same parameters of the inoculated block at a time i (i= 0, 30, 60, 90 or 120 days).

The same technique was used for colour characterization of the schist samples prior to inoculation. Differences between the colour of weathered and unweathered samples were followed throughout the bioreceptivity experiment in order to test if colour differences due to weathering prior to colonization were able to explain the differences between weathered and unweathered schist in terms of partial and/or total colour change brought about by biological colonization, *i.e.* in order to test if an evaluation of the weathering degree of non-colonized schist could be used to estimate the extent of biological colonization after a certain time of exposure. Results in this case are expressed as the difference between the colour of the unweathered schist samples and that of the weathered schist samples at a given moment in time, and calculated as follows:

$$\Delta L^* = L^*_u - L^*_w$$
$$\Delta a^* = a^*_u - a^*_w$$
$$\Delta b^* = b^*_u - b^*_w$$

Where:

L^*_u, a^*_u and b^*_u are the colour parameters of unweathered samples (freshly-quarried). L^*_w, a^*_w and b^*_w are the colour parameters of weathered samples (outcropped).

Data analysis

Estimation of potential bioreceptivity of the studied lithotypes from their intrinsic properties was based on the model developed by Prieto & Silva (2005):

$$\mu g\ \text{chlorophyll-a/cm}^2 = -206.397 - 1.708\ \text{pH} - 6.965\ \text{OP} + 84.977\ \text{BD} + 21.362\ \text{Wc}$$

Where OP is for open porosity, BD is for bulk density and Wc is for capillary water.

Data on chlorophyll-a content and total colour change over time, as well as total colour difference between weathered and unweathered schist was analysed by means of linear mixed models with ‘nlme’ (Pinheiro *et al.* 2013) and ‘multcomp’ (Hothorn *et al.* 2013) packages in R. The remaining tests were based on univariate and multivariate analysis of variance (ANOVA and MANOVA, respectively), also performed in R.

8.1.3 Resuls and discussion

Quarry samples differed significantly from outcrop samples in all physical properties. The most obvious difference was visible to the naked eye and related with their colour. Weathered schist samples varied between a light-coloured grey or brownish-grey to greyish-brown, depending on which sample was considered. Unweathered schist samples were much more identical in terms of colour, which is consistent with previous descriptions of the slates of north-west Iberian Peninsula by Prieto *et al.* (2011). Brownish colourations in Poio schist have been attributed to the occurrence of iron oxides (Aires *et al.* 2011). However, although the two types of schist differed significantly in the content of some major elements, namely Al, K, Ca and Mg, the amount of Fe detected by XRF was roughly the same (Table 8.1.2).

Table 8.1.2. Major elements (%) detected by X-ray fluorescence in weathered and unweathered schist. Different letters in brackets indicate statistically significant differences (p-value < 0.05) between samples of different weathering degree.

	Unweathered schist (mean ± sd)	Weathered schist (mean ± sd)
SiO2	72.02 ± 1.63 (a)	71,43 ± 5.92 (a)
AlO3	21.31 ± 0.90 (a)	23,51 ± 3.11 (b)
FeO3	8.39 ± 0.57 (a)	9,62 ± 1.49 (a)
K2O	4.17 ± 0.22 (a)	4,51 ± 0.46 (b)
CaO	1.56 ± 0.07 (a)	1,68 ± 0.13 (b)
MgO	1,78 ± 0.54 (a)	2,21 ± 0.72 (b)
TiO2	1,07 ± 0.04 (a)	1,09 ± 0.09 (a)

Regarding the detection of such differences by spectrophotometric-assisted colour measurements depicted in Table 8.1.3, the predominance of a brownish colouration in weathered samples is clearly reflected by the shift in the values of parameter b* towards the yellow (higher values) in both wet and dry samples while the values for parameter a* are roughly the same as in the unweathered samples. The values of L* parameter also differed significantly between weathered and unweathered schist samples, indicating a lighter colour (higher values) in weathered samples that also corresponds to what is apparent from direct observation. The effect of weathering in the colour of schist samples was detected in both dry and moist samples by significant change in all components of the CIE L*a*b* colour space except for a* in dry samples. However, differences between the colour of weathered and unweathered schist samples are clearly more pronounced in wet samples (Table 8.1.3), which is apparently due to the higher water-holding capacity of weathered samples as will be discussed bellow (Table 8.1.4). Measures taken in moist samples will therefore provide the most robust predictions of total colour differences resulting from weathering among non-colonized schist samples.

Table 8.1.3. Mean values of the CIE L*a*b* parameters measured in wet and dry samples of different weathering degree. Different letters in brackets indicate statistically significant differences (p-value< 0.05) between samples of different weathering degree. Pu= unweathered Poio; Pw= weathered Poio.

Code	**Wet**			**Dry**		
	L (mean)	a (mean)	b (mean)	L (mean)	a (mean)	b (mean)
Pu	28.8 (a)	-0.757 (a)	0.67 (a)	55.2 (a)	-1.200 (a)	0.62 (a)
Pw	60.5 (b)	-1.401 (b)	6.70 (b)	52.6 (b)	-1.417 (a)	6.75 (b)

Differences between weathered and unweathered schist in terms of fissuration are not apparent in the small blocks submitted to the bioreceptivity experiment but are quite evident in the larger samples that were tested for the physical properties, being extensive in the weathered and almost null in the unweathered schist samples.

The values of open porosity, water saturation, capillary water and capillarity coefficient were significantly higher, and those of bulk density and abrasion pH significantly lower, in outcrop samples than in quarry samples (Table 8.1.4), indicating that sampling options were adequate to the purpose of this study.

Decrease of abrasion pH in weathered samples with respect to that of unweathered samples is a well known phenomenon, previously observed in other lithotypes (Prieto & Silva 2005, Taboada *et al.* 1996) that is related with the leaching of basic cations from the rock. Such relationship is useful to the point of abrasion pH being considered a good indicator of the weathering degree of a rock (Grant 1969). Open porosity of unweathered schist was not as low as that of some marbles and granites (Miller *et al.* 2006) but similar to the open porosity of Blanco Cristal. Open porosity was greatly increased by weathering, probably due to the high fissuration of the samples. This increase was not enough to reach the highest porosity of limestones (Miller *et al.* 2006) neither does it equal the high porosity of some weathered granites (Prieto & Silva 2005). Open porosity of weathered schist is comparable to that of the unweathered Baleante granite (Prieto & Silva 2005). Since open porosity is a measure of the volume of interconnected pores and cracks linked to the external surface of the rock, it is of paramount importance for the movement and storage of water in a rock, and consequently to its bioreceptivity.

Table 8.1.4. Physical properties and abrasion pH for Poio schist and the granite Blanco Cristal. Information on Blanco Cristal derived from Prieto & Silva (2005). BD= bulk density (g/cm^3); OP= open porosity (%); Ws= water saturation (%); Wc= capillary water (%); CC= capillarity coefficient (g/cm^2). Pu= unweathered Poio; Pw= weathered Poio; BC= Blanco Cristal. Different letters in brackets indicate statistically significant differences (p-value< 0.05) between samples of different weathering degree.

Code	pH	BD	OP	WS	WC	CC
Pu	8.97 ± 0.01 (a)	2.73 ± 0.01 (a)	0.81 ± 0.10 (a)	0.30 ± 0.04 (a)	0.23 ± 0.11 (a)	0.001 ± 0.0001 (a)
Pw	7.24 ± 0.02 (b)	2.64 ± 0.04 (b)	4.33 ± 0.82 (b)	1.64 ± 0.34 (b)	1.69 ± 0.38 (b)	0.014 ± 0.0002 (b)
BC	9.56	2.59	0.9	0.6	0.45	0.02

The importance of capillary water for bioreceptivity is that it determines the time that the rock remains wet, given that capillary water is more strongly retained. From a practical point of view and taking into consideration the position of the Côa Valley's engravings on vertical surfaces, capillary water content is very important, since, although water easily infiltrates through the joints affecting the hole outcrop, the engravings are more directly and sooner affected by what happens at the surface exposed to the exterior and therefore to the water that enters and is retained by the strength of the capillary suction. The water retained by capillarity in the unweathered schist is much lower than that of most granites studied by Prieto & Silva (2005) whose Wc was higher than 1%. However, weathered schist is significantly different from unweathered schist and comparable to those granites in terms of the water retained by capillarity. Both water saturation and capillary water followed the same pattern in terms of differences between unweathered and weathered schist and respective differences against Blanco Cristal. The maximum amount of water that the schist samples were able to absorb (water saturation) are obviously related to the open porosity of each rock, so that weathered schist, with higher open porosity, showed higher ability to absorb water than unweathered schist, with lower open porosity. The weathered schist had the highest capillary coefficient, *i.e.* it took up water most rapidly, absorbing more water than the unweathered schist. The fact that the value for capillary water is practically equal to that of water saturation indicates that most of the pores present in the rocks are of capillary size (Prieto & Silva, 2005).

Applying the predictive equation for the bioreceptivity of granite proposed by Prieto & Silva (2005), the bioreceptivity of the studied schist, expressed in µg chlorophyll-a/cm^2 would be estimated at 10.868 for the weathered schist and at 8.734 for the unweathered schist. The bioreceptivity of Blanco Cristal is close to zero (Table 8.1.7). This equation needs to be calibrated with data from a wider range of lithotypes to be used with more confidence. In the meantime these values are merely indicative and should be used only for comparison purposes, as will become clear from the following results.

The progress of biological colonization expressed by chlorophyll-a content and total colour difference after inoculation is depicted in Fig. 8.1.1.

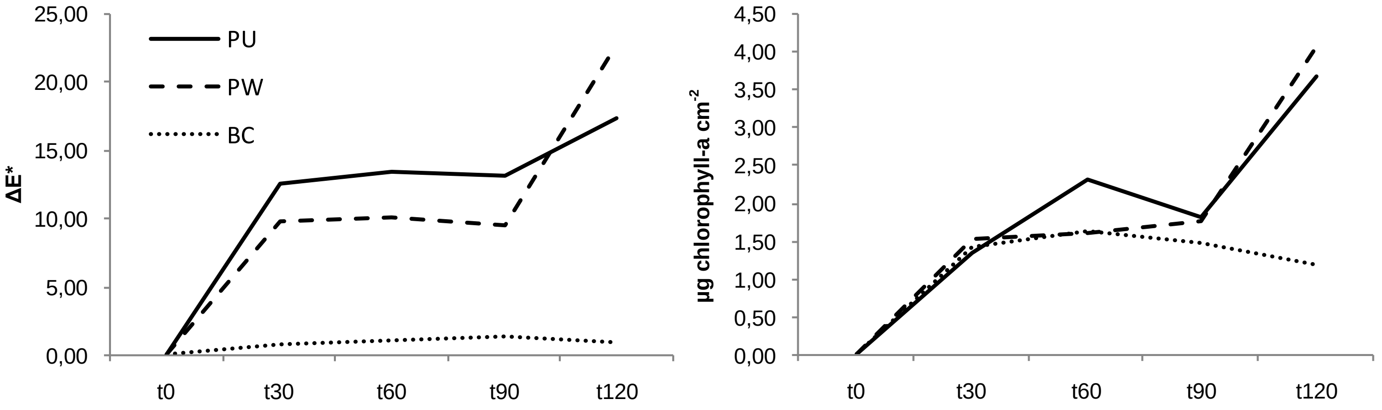

Fig. 8.1.1. Total colour difference (ΔE*) (left) and mean chlorophyll-a content (right) in samples over time after inoculation. Pu= unweathered Poio; Pw= weathered Poio; BC= Blanco Cristal.

The cross-temporal comparison shows that there was great loss of photosynthetic biomass soon after inoculation (Table 8.1.5) which was never recovered. Granite samples did not experience any statistically significant differences in chlorophyll-a content or total colour difference throughout the experiment, whereas schist samples did: during the first 90 days, biological growth was quite slow or inexistent, as shown by the almost flat lines representing chlorophyll-a content and total colour difference (Fig. 8.1.1) but a sharp increase in the speed of colonization happened between 90 and 120 days after inoculation, leading to significant biological growth after 120 days (Table 8.1.5). Only a small portion of the inoculated cyanobacteria was able to establish on the samples of Blanco Cristal but not to develop considerably. On the contrary, biological colonization in these samples started to decline somewhere between 60 and 90 days after inoculation, depending on the used method for photosynthetic biomass quantification, something that is in conformity with the extremely low bioreceptivity of this type of granite (Prieto & Silva 2005).

Table 8.1.5. Biomass results (mean ± sd) over time as expressed by chlorophyll-a content (µg cm^{-2}), total colour difference (ΔE*) and difference in CIE L*a*b* parameters (ΔL*, Δa*, Δb*). Pu= unweathered Poio; Pw= weathered Poio; BC= Blanco Cristal. Different letters in brackets indicate statistically significant differences (p-value< 0.05).

Code	**Time after inoculation (days)**				
	T0	*T30*	*T60*	*T90*	*T120*
Chlorophyll-a content					
Pw	0.00	1.51 ± 0.21 (a)	1.60 ± 0.72 (a)	1.75 ± 0.56 (a)	4.06 ± 0.33 (b)
Pu	0.00	1.35 ± 0.10 (a)	2.31 ± 1.42 (a)	1.82 ± 0.30 (a)	3.68 ± 1.08 (b)
BC	0.00	1.44 ± 0.02 (c)	1.65 ± 0.57 (c)	1.49 ± 0.15 (c)	1.19 ± 0.11 (c)
Total colour difference					
Pw	0.00	9.87 ± 0.18 (a)	10.08 ± 1.01 (a)	9.45 ± 0.43 (a)	22.83 ± 5.1 (b)
Pu	0.00	12.50 ± 1.34 (a)	13.39 ± 0.75 (a)	13.17 ± 1.91 (a)	17.35 ± 1.92 (c)
BC	0.00	0.78 ± 0.20 (d)	1.16 ± 0.42 (d)	1.43 ± 1.22 (d)	0.97 ± 0.17 (d)
ΔL*					
Pw	0.00	9.79 ± 0.18 (a)	13.15 ± 0.71 (a)	9.35 ± 0.44 (a)	22.69 ± 5.23 (c)
Pu	0.00	12.35 ± 1.31 (a)	10.03 ± 1.04 (b)	12.92 ± 1.80 (b)	16.70 ± 2.00 (d)
Δa*					
Pw	0.00	1.05 ± 0.23 (a)	0.58 ± 0.13 (a)	0.94 ± 0.22 (a)	1.12 ± 0.31
Pu	0.00	0.69 ± 0.38 (a)	0.30 ± 0.24 (a)	0.55 ± 0.29 (a)	0.19 ± 0.64
Δb*					
Pw	0.00	-0.36 ± 0.82 (a)	-0.21 ± 0.79 (a)	-0.73 ± 0.75 (a)	-1.74 ± 1.51 (a)
Pu	0.00	1.80 ± 0.48 (b)	2.49 ± 0.37 (b)	2.34 ± 1.12 (b)	4.63 ± 0.62 (b)

Colour differences between weathered and unweathered schist during the first 90 days after inoculation (Fig. 8.1.1) probably resulted from a differential survival success at the very beginning of the experiment which was finally overcome after 120 days, when colonization was much higher in weathered schist than that of unweathered schist, as expected.

The effect of lithotype on total colour difference over time is statistically significant (Table 8.1.6: F-value= 188.299; p-value< 0.001), consequently on the left side of Fig. 8.1.1 the lines for the three lithotypes are rather far apart. The within samples test indicates that there is also a statistically significant effect of time (Table 8.1.6: F-value= 22.607; p-value< 0.001). In other words, colour does change over time in weathered and unweathered schist samples. However, it was not until 120 days were passed since inoculation that these differences were statistically significant, both in weathered and unweathered schist samples (Table 8.1.5). In fact, 90 days after inoculation, there was no statistically significant distinction in total colour difference between weathered (Table 8.1.5: 9.45 ± 0.43) and unweathered (Table 8.1.5: 13.17 ± 1.91) samples. Moreover, the interaction of time and lithotype, depicted by the non parallel lines on the right side of Fig. 8.1.1, is statistically significant (Table 8.1.6: F-value= 10.300; p-value< 0.001), which means that the colour in both lithotypes is changing over time but is changing in different ways: the rate of colour change was higher in weathered schist samples than in unweathered schist samples.

The pattern of change in chlorophyll-a content with time and lithotype was somewhat similar (Fig. 8.1.1): both weathered and unweathered schist samples expressed statistically significant differences in chlorophyll-a content after 120 days of experiment (Table 8.1.5), and the interaction between lithotype and time was statistically significant (Table 8.1.6: F-value= 4.488; p-value= 0.006). The significant interaction confirms that the pattern of change in chlorophyll-a content over time differs depending on the lithotype. However, the content of chlorophyll-a was found to be very similar in weathered and unweathered schist samples throughout the experiment (Table 8.1.5: 4.06 and 3.68 after 120 days, respectively). Given that determination of total colour difference is able to distinguish between weathered and unweathered schist samples in terms of their photosynthetic biomass after 120 days, it is possible that quantification of chlorophyll-a content is not as accurate and requires larger differences in the amount of photosynthetic biomass to accomplish the same.

Table 8.1.6. Mixed model ANOVA considering chlorophyll-a content (µg.cm^{-2}) and total colour difference (ΔE*) as the response variable, Lithotype and Time (and their interaction) as the explanatory variables and Sample as the blocking variable. Pu= unweathered Poio; Pw= weathered Poio; BC= Blanco Cristal.

	Chlorophyll-a content		**Total colour difference**	
	F-value	*p-value*	*F-value*	*p-value*
Lithotype	6.956	0.027	188.299	<0.001
Time	10.902	<0.001	22.607	<0.001
Lithotype*Time	4.488	0.006	10.300	<0.001

Similarly to what was previously observed by Sanmartín *et al.* (2012), among the analysed CIE L*a*b* parameters, Δb* provided earlier indication of colour change, and presumed change in the amount of photosynthetic biomass, than ΔE*: 30 days after the inoculation, the partial means for Δb* of weathered schist was much lower than that of unweathered schist and the p-value for the effect of the weathering degree on Δb* was 0.001, whereas the p-value for the same effect on total colour change was 0.834 and the partial means for ΔE* identical (see Table 8.1.5). However, Δb* did not significantly change over the considered time period despite the obvious importance of time for the overall increase in photosynthetic biomass, indicating that differences in Δb* are mostly reflecting the effect of sample heterogeneity. In ΔE* this effect is compensated by the other two parameters resulting in a more robust measure of colour change due to biological colonization, able to simultaneously detect differences over time and across lithotypes, while controlling for sample heterogeneity.

Considering the different ways to express the bioreceptivity of a lithotype (Table 8.1.7) and previous assessments on the bioreceptivity of other lithotypes (Câmara *et al.* 2007, Miller *et al.* 2006, Prieto & Silva 2005), unweathered schist quarried in the Côa Valley can be viewed as low bioreceptive while weathered schist, such as the one used as a support for rock-art in the Côa Valley, is moderately bioreceptive. Blanco Cristal was however the least bioreceptive of the three studied lithotypes.

The ratio of the bioreceptivity of weathered to that of unweathered Poio schist, as defined by their intrinsic properties, was 1.24, meaning that weathering was responsible for an increase of approximately 25% in the bioreceptivity of outcropping schist relative to the freshly quarried (Table 8.1.7). As can be seen from the same table, the three available methods for determining the bioreceptivity of a lithotype, arrive to very similar ratios, indicating that for comparison purposes, any of these methods is useful. The method based on the physical properties is faster and avoids the disadvantages of working with living organisms (*e.g.* maintainance, time constraints), whereas the determination of total colour difference is the most accurate and practical method among the other two.

Table 8.1.7. Bioreceptivity of the studied lithotypes and those used for comparison from Prieto & Silva (2005). Pu= unweathered Poio; Pw= weathered Poio; BC= Blanco Cristal.

Code	**Bioreceptivity**				
	Lithotype	*Origin*	*DMSO (120 days)*	*ΔE (120 days)*	*Intrinsic properties*
PW	Schist	exposed	4.06	22.83	10.868
PU	Schist	quarry	3.68	17.35	8.734
BC	Granite	quarry	1.19	0.97	0.000
		PW/PU	1.10	1.31	1.24

Given that the extent of biological colonization accumulated through time on a rock surface depends on the initial properties of the lithotype (including those reflecting its weathering degree) and that total colour difference is able to accurately detect those differences, it is reasonable to assume that an evaluation of the initial weathering degree of the lithotype based on colour can be used to predict the extent of biological colonization after a certain period of time.

In fact, total colour difference between schist samples with different weathering degrees, before inoculation, explains a significant amount of the variation in the difference between the colour of weathered and unweathered schist samples through time after inoculation, *i.e.* colour difference prior to colonization seems to be a good predictor of colour difference after colonization, thus reflecting differences in terms of bioreceptivity.

The best model (r^2= 0.7759), after testing on dry/wet samples and SCE/SCI geometry, is the one that considers the measures of total colour difference between wet samples and based on SCI geometry:

$$\Delta Ed^*(T_i) = -7.2279 + 0.6877 \times \Delta Ed^*(T_0) + 8.9393 \times T_i$$

Such relationship would allow predicting the extent of the effect of different weathering degrees across a rock surface in its bioreceptivity prior to colonization by total colour difference between weathered and unweathered parts of that rock surface. This model is merely indicative, as there was no statistically significant difference in biological colonization in the first three months of the experiment and statistically significant differences in biological colonization were detected only after four months since inoculation; and requires validation through more extensive test time periods, further lithotypes, as well as more structural and functionally complex rock colonizers.

The results reported in laboratory-based colonisation experiments can only be considered pertinent for the used lithotypes, the tested organisms and the applied incubation conditions (Miller *et al.* 2009). If the intention is to reach to an Index of Bioreceptivity (Miller *et al.* 2012, Prieto *et al.* 2006) as Guillite (1995) predicted, an important point is that there must be a fixed and agreed methodology. Tests carried out with a single type or limited set of organisms may therefore be somewhat atypical (Miller *et al.* 2012) but so are the controlled laboratory conditions under which they are carried on, based on the assumption that “the bioreceptivity of a material will be best expressed under maximum accessibility and optimal environmental conditions for the development of organisms” and related with the necessity of carrying studies “under experimental conditions that are as standard as possible to remove any subjectivity attached to the concept”. However, as there is no standardized protocol for measuring bioreceptivity, aspects such as the number of measurements and the area of measurement vary across studies, hampering the comparison of results obtained by different authors and instruments.

8.1.4 Conclusions

This study demonstrates, like others beforehand, that the bioreceptivity of a rock is a reflection of its intrinsic properties. All parameters of physical properties as well as abrasion pH differentiated significantly between weathered and unweathered schist. Besides the differences in the weathering degree, differences between rock types were also well represented by the physical properties and abrasion pH. Schist samples expressed the highest bulk density and the lowest open porosity, water saturation, capillary water and capilarity coefficient, among the unweathered. The predictive model proposed by Prieto & Silva (2005), although developed for granites, is also relevant for schist and can probably be applied with confidence to a range of other lithotypes, at least when it comes to compare the bioreceptivity associated with different weathering degrees of the same lithotype.

After four months since inoculation, there was clear biofilm development in both weathered and unweathered schist samples. Even if there were differences in the final extent of colonization, the time pattern of cyanobacterial growth was similar in both weathered and unweathered schist: low colonization success rate, slow recovery until 90 days and fast growth after 90 days.

The time required for the cyanobacterial biofilm to settle and expand on both weathered and unweathered schist samples was much higher than previously thought, provided that data on the bioreceptivity of other lithotypes (Miller *et al.* 2006, Prieto & Silva 2005) indicated that after 90 days microbial growth would be perfectly settled. In fact, these biofilms remained relatively stable in terms of photosynthetic biomass until three months had passed since inoculation, and only after did they start growing rapidly. This has practical implications for future experiments focusing on low to moderately bioreceptive lithotypes, and should also be taken in consideration when calibrating the parameters of a putative bioreceptivity index.

The effect of the weathering degree on bioreceptivity was well reflected by total colour change but not by chlorophyll-a content. Both methods were equally capable of detecting differences in photosynthetic biomass at the end of the bioreceptivity experiment but total colour change is probably more sensitive to the small differences in photosynthetic biomass found between differently weathered schist. CIE L*a*b* colour space had already proved to be efficient in characterizing and differentiating between other homogenously coloured schistose substrata. Here it is proved that it can also be used to characterize differently weathered schist. Determination of total colour change (ΔE^*) is the best alternative of those tested, because, as was shown, it is statistically robust, and has some important additional advantages associated with it: it is quick, easy, non-destructive and can be used *in situ* and on site, *i.e.* directly on the rock surface in the field, which is very important in bioreceptivity assessments.

It appears that bioreceptivity as estimated from the intrinsic properties of the rock is consistent with bioreceptivity estimated by photosynthetic biomass. The initial weathering degree had a statistically significant effect on the amount of cyanobacterial biomass accumulated on the surface of the schist samples at the end of the experiment. Bioreceptivity of weathered schist was significantly higher than that of unweathered schist. Bioreceptivity of schist surfaces in the Côa valley is therefore increased by outcrop exposure prior to colonization and should also be affected by small-scale variations in the weathering degree at the surface level.

Preliminary results suggest that total colour difference between differently weathered rocks prior to colonization could be used to predict secondary bioreceptivity and thus constitute an instrument for preventive conservation of open-air rock-art.

8.1.5 References

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8.2 Evaluation of lichen-induced weathering of exposed schist surfaces under contrasting microclimates

Submitted to International Biodeterioration and Biodegradation with the following co-authors: João Gonçalves, Cláudia Oliveira, Rubim Almeida, Beatriz Prieto

Abstract

The present study was undertaken in the Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal), where there is some debate as to the extent of lichen contribution to the differential weathering of vertical schist surfaces and resulting pattern of rock-art distribution. The study aimed at evaluating the effect of rock surface orientation in the weathering ability of three crustose (*Aspicilia hoffmanniana*, *Caloplaca subsoluta*, *Lecanora pseudistera*) and one squamulose (*Peltula euploca*) lichens, occurring on schist surfaces exposed to the north-west and the south-east.

Periodic-Acid Schiff (PAS) stained cross-sections of colonized schist samples revealed a unidirectional pattern of hyphal penetration, up to 37 mm deep, following lichen produced and/or expanded intermineral voids, and appearing to be related with the lamellar nature of schist minerals. Image analysis of the same cross-sections allowed to accurately measure the hyphal spread of each species into the rock interior and test the effect of surface orientation in the ability of each lichen to spread into the rock. Hyphal spread was higher in *Lecanora pseudistera*, one of the dominant species on north-west facing schist surfaces. Lichen-induced physical weathering is thus likely to be more severe at north-west facing surfaces by means of an indirect effect of surface orientation on the individual pattern of species abundance. Surface orientation also seems to relate with the form of calcium oxalate accumulated inside the lichen thallus, as depicted by FT-Raman spectroscopy, since whewellite was found exclusively in specimens taken from north-west facing surfaces whereas weddellite was found in specimens taken from both orientations. An apparently opposite effect of surface orientation was additionally noticed at the lichen-rock interface based on the results of X-ray microdiffraction, since weddellite accumulated at the lichen-rock interface of south-east but not at north-west facing surfaces.

These results suggest that variation in microclimatic factors related to surface orientation produce different effects depending on the nature of lichen-induced weathering. While hyphal spread is likely to be more aggressive on north-west facing surfaces and dependent on the species, calcium oxalate formation is apparently higher on south-east facing surfaces and dependent on microclimatic conditions, giving only weak support to the theory that lichens

are responsible for the differential weathering of vertical schist surfaces in the Côa Valley and respective rock-art. These findings should be considered preliminary as information on quantitative variation of some of the analysed parameters is still lacking, as well as of other species and their assemblages.

8.2.1 Introduction

The last three decades have been extremely rich in contributions to the knowledge of the various aspects of lichen-induced rock weathering, as seen by the good number of reviews available (Adamo & Violante 2000, Chen *et al.* 2000, Jones 1988, Jones & Wilson 1985, 1986, Saiz-Jimenez 1999, Seaward 1997, Viles & Pentecost 1994). Common approaches to this subject have been focusing on (adapted from Scarciglia *et al.* 2012): i) identifying individual species and species assemblages colonizing rock surfaces and making assumptions on their impact based on previous knowledge about their ecological requirements (*e.g.* Carballal *et al.* 2001, Nimis & Monte 1988); ii) determining the climatic constraints and habitat preferences of colonizing species based on field observations (*e.g.* de los Rios *et al.* 2005, Camuffo 1993, Cicek *et al.* 2009, Prieto *et al.* 1995, 1999a, Steinbauer *et al.* 2013, Weber & Büdel 2001) or controlled experiments (Adamson *et al.* 2013, Carter & Viles 2003, Kidron & Termina 2010) thus contributing to the knowledge of environmental factors that are also important for rock conservation; iii) detecting geophysical and geochemical changes at the lichen-rock interface associated with the growth of individual species (*e.g.* Adamo *et al*. 1993, Ariño *et al.* 1997, Ascaso & Wierzchos 1995, Edwards & Seaward 1993, Hall *et al.* 2005, Fry 1927, Prieto *et al.* 1999a, 1999b, Souza-Egipsy *et al.* 2002, McCarroll & Viles 1995) including the occurrence of organic and mineral by-products of lichen activity (*e.g.* Arocena *et al.* 2007, Edwards *et al.* 1997, Jorge-Villar & Edwards 2010, Favero-Longo *et al.* 2005, Giordani *et al.* 2003, Prieto *et al.* 1997, Wierzchos & Ascaso 1996) and iv) developing methods to quantify the weathering rates induced by individual species or by a limited set of the most representative ones on the surface of interest (Aghamiri & Schwartzman 2002, Brady *et al.* 1999, Gazzano *et al.* 2009a, 2009b, McCarroll & Viles 1995, Stretch & Viles 2002).

The majority of work has been applied at characterizing the biodeterioration of monuments, statues or any other kind of stonework in Europe and only sporadically in other regions of the globe. Few papers have dealt with the relationship between lichen growth and the weathering of schist (Aghamiri *et al.* 2002, Cann *et al.* 2012, Galvan *et al.* 1981, Fry 1924, 1927, Sanders *et al.* 1994) despite the increasing demand of schist as a building stone (Noronha *et al.* 2012) and the fact that schist outcrops constitute in many countries an important support for ancient rock-art that is worth preserving.

The processes of schist weathering, including those induced by lichen activity, are a primary concern in the Côa Valley Archaeological Park (Vila Nova de Foz Côa, north-east Portugal) where one of the most important sets of European Prehistoric rock-art is located, dating back to the Upper Palaeolithic. Efforts are being made there to understand the weathering dynamics acting on the schist outcrops that support the rock-art, integrating biological, geophysico-chemical and environmental data in order to prevent major damages to the engraved surfaces (Fernandes 2004). There is general consensus about the combination of physical (mechanical) and chemical changes brought about by lichens to rock surfaces but the extent and relative contribution of their weathering action is a central question – still unanswered – in the Côa Valley as in the field of rock-art conservation in general. Recently, Aubry *et al.* (2012) suggested that aspect-related differences in the extent of lichen (and bryophyte) colonization of vertical schist surfaces in the Côa Valley could be partly responsible for the differential weathering of those surfaces and resulting pattern of rock-art distribution in the Côa Valley, currently higher at south-east facing slopes.

A key uncertainty in the assumptions on the relationship between lichen colonization and rock weathering is precisely in the way that lichens act under different weathering environments. The extreme environments of cold and hot deserts have been particularly interesting for research (Ascaso *et al.* 1990, Bungartz *et al.* 2004, Chen & Blume 1999, McCarroll & Viles 1995) since every species is expected to act differently in distinct weathering contexts and change its performance under the influence of environmental change. However, although a useful first approximation, these studies commonly assume species neutrality, with substrate and climate as the primary controls. As a result, knowledge about lichen-induced rock weathering is still based on static views of the influence of environmental climatic parameters such as temperature, solar radiation, exposure or humidity. Studies aimed at evaluating the response of individual species to changes in environmental conditions have typically demonstrated a shift in oxalate production (thus in the biodeteriorative action) in response to environmental variation (Caneva 1993, Edwards *et al.* 1995, Prieto *et al*. 2000). The use of bare-rock controls is a necessary condition for isolating the lichen-induced effects from the ones induced by other weathering agents (either biotic or abiotic) (Scarciglia *et al.* 2012).

The variation of the diversity and composition of lichen assemblages with rock surface orientation is a well-known phenomenon in both natural and cultural contexts (Adamson *et al.* 2013) as orientation acts as a proxy of those environmental variables that affect the structure and dynamics of saxicolous lichen communities. Some components of that variation in the Côa Valley have been previously addressed from an ecological perspective (Marques *et al.* in review). This study will directly 1) investigate the lichen-induced physical and chemical weathering of schist surfaces, and 2) assess the effects of surface orientation on both processes; as well as 3) the influence of small scale microclimatic parameters, including temperature and relative humidity, on the weathering ability of these species; in order to determine the contribution of lichen action to differential rock weathering in the Côa Valley.

8.2.2 Material and methods

Mineralogical characterization of the studied lithotype

Previous studies had already demonstrated the mineralogical and geochemical composition of the target lithotype (*e.g*. Búrcio 2004, Sousa 1982). It is a relatively low-grade metamorphic (greenshist facies) phyllite consisting of thin alternating layers of whitish psammitic and dark pelitic components (Aires *et al.* 2011). The psammitic component is sometimes more abundant and the rock is then classified as a metaquartzwacke instead of a phyllite. For a matter of simplicity, this phyllite-metaquartzwacke sequence will be addressed under the broad term schist. This schist is mainly composed of quartz, sericite and/or muscovite, chlorite and biotite minerals, as well as plagioclase feldspars (mostly albite) in variable amounts depending of the psammitic contribution. Calcite is usually present in the matrix of these rocks in sufficient amounts to produce effervescence when treated with dilute hydrochloric acid. Magnetite and, more sporadically, pyrite crystals are present in both the psamitic and pelitic levels as accessory constituents (Sousa 1982). Both calcite and pyrite are potentially highly alterable (Prieto *et al.* 2011). Additional accessory minerals including illite, kaolinite, montmorillonite, graphite, turmaline, zircon, apatite, epidote, hematite, leucoxene and some alkali feldspars, such as microcline and orthoclase were detected by polarized light (petrographic) microscopy and X-ray diffraction (Aires *et al.* 2011, Búrcio 2004, Gomes & Almeida 2003, Solicel 2003, Sousa 1982).

Microclimatic characterization of vertical schist surfaces in the Côa Valley

To characterize the thermal and hydric contrasts of opposite slopes in the Côa Valley, 12 Hygrochron iButton dataloggers (Maxim Integrated Products Inc., Sunnyvale (CA), USA) were placed on key vertical schist surfaces of varying orientations. The results were then grouped in the two aspect classes of interest for this study: north-west and south-east. Dataloggers were synchronized and set to record both Temperature (ºC) and Relative Humidity (%), at hourly or half-hourly intervals during a 3-year period from late September 2010 to late September 2013.

Species and sample strategy

Physical and chemical weathering was assessed by means of four locally-common lichens: *Aspicilia hoffmanniana* hereafter, *Caloplaca subsoluta*, *Lecanora pseudistera* and *Peltula* *euploca*. Taxa selection was based on their frequency and abundance on the vertical schist surfaces of the Côa Valley Archaeological Park (Marques *et al.* in review). *Aspicilia hoffmanniana* is a crustose lichen varying in colour from greenish-grey in shaded north-west facing surfaces to light brown in exposed south-east facing surfaces, where it is more abundant. *Caloplaca subsoluta* is a deep orange coloured crustose species that is common on siliceous rocks throughout the Mediterranean. In the Côa Valley it was found equally abundant on the vertical schist surfaces of the two opposing slopes. *Lecanora pseudistera* is a white crustose species proliferating on north-west facing surfaces, although it can also be found less abundantly on south-east facing schist surfaces. *Peltula euploca* is a widespread squamulose epilith characteristic of the rain-track communities of vertical schist surfaces (Marques *et al.* 2013) and exclusive of south-facing exposures. Each squamule is attached to the substrate by a central umbilicus and its lower surface is pale to dark brown.

The major secondary metabolites produced by *Lecanora pseudistera* are atranorin and 2'-O-methylperlatolic acid (Brodo 1984). Secondary compounds in *Aspicilia hoffmanniana* are either lacking or include only aspicilin (Rico 1999). *Caloplaca subsoluta* produces anthraquinones of chemosyndrome A3 (Søchting 1997). *Peltula euploca* lacks secondary metabolites. The three crustose lichens are able to reproduce sexually, although *Aspicilia hoffmanniana* is most frequently sterile. *Peltula euploca* is often fertile in the study area but its characteristic mode of dispersal is through vegetative propagules (soredia). The algal partner is *Trebouxia* spp. in the three crustose lichens and unicellular cyanobacteria (*Chroococcidiopsis* spp.) in *Peltula euploca* (Büdel 1987).

Colonized rock samples were taken at random from non-engraved north-west and south-east facing schist surfaces, located at representative rock-art sites, namely Canada do Amendoal, Foz do Côa, Quinta das Tulhas, Vale do Forno and Vale de José Esteves, and are therefore very similar to those holding rock-art surfaces in terms of their macro- and micro-environmental constraints. Colonized rock was therefore studied *ex situ* but the lichen thallus kept intact. Uncolonized rock samples were also taken from the source outcrops to be used as controls. Since *Peltula euploca* is virtually exclusive of south-east facing surfaces, appropriate samples of rock colonized by this particular species could only be found and collected at south-east.

The collected samples were cut perpendicular to the colonized or previously exposed surface, up to a depth of 3 to 4 cm, with an Isomet 1000 Precision Saw (Buehler, Düsseldorf, Germany) and the surfaces of the resulting cross-sections were polished with sandpaper attached to an Ecomet 3000 (Buehler, Düsseldorf, Germany) polisher machine. Nine replicates were prepared for each combination of species vs orientation, and respective control (uncolonized) in order to evaluate if weathering associated with lichen colonization differs from the weathering produced on identical but lichen-free surfaces. Three subsets of three replicates each were taken from the initial sample set, and processed accordingly for Periodic Acid-Schiff (PAS) staining, X-ray microdiffraction and FT-Raman spectroscopy. Almost all samples had been included in polyester resin (Recapoli 2196 styrene and phthalic anhydride, Methyl Ethyl Ketone peroxide as catalyst) before cutting, to avoid excessive loss of material due to the fragile nature of the schist samples, except for those used in Raman analysis, since resin inclusion would preclude from taking measures directly on the lichen thallus.

Periodic Acid-Schiff staining

This procedure aimed at highlighting the Hyphal Penetration Component (HPC) in the cross-sections following Favero-Longo *et al.* (2005). Microphotographs of the stained cross-sections were then acquired at a ×10 magnification using a Nikon SMZ1000 stereomicroscope equipped with a Nikon DS Fi1 digital camera, at three random locations of each cross-section. Data on the depth of hyphal penetration was obtained by visual inspection of stained samples under the same stereomicroscope.

In order to quantify the length and extent of hyphal penetration, as well as the size of other important weathering-related features (*e.g.* weathering rind), the acquired images were submitted to a protocol of pixel-based supervised classification using colour and texture features, which can be described briefly as follows: 1) Image pre-processing in ImageJ (Abramoff *et al.* 2004), including resampling of 40% of the original images (to increase computation speed and efficiency) and contrast and sharpening enhancement to allow a better discrimination of the HPC and weathering rind (whenever present), from the rock core; 2) Feature extraction, including a total of 162 texture features based on run-length, co-occurrence, image histogram and gradient matrices in MaZda (Szczypiński *et al.* 2009) and colour features based on several colour spaces namely RGB, HIS, YUV, YIQ and XYZ using ‘adimpro’ package (Polzehl & Tabelow 2007) in R; 3) Generation of model input data (classification) in ImageJ, by manually assigning points (XY coordinates) in the original images to the corresponding structures of interest, namely ‘lichen thallus’, ‘hyphae’, ‘weathering rind’ and ‘rock core’; 4) Random Forest model calibration (Breiman 2001, Liaw & Wiener 2002) in R with ‘ntree’= 200, ‘mtry’= 6, ‘nodesize’= 5 and the remaining parameters kept as default, based on the relationship between the classified points and a reduced subset of 20 features (colour and texture) after ranking all 162 extracted features according to their relative predictive importance (Boulesteix *et al*. 2012, Oppel *et al.* 2009); 7) Model selection through Monte-Carlo cross-validation (Xu *et al*. 2004) among 100 generated classifiers. The dimensions of the HPC and weathering rind in each sample are among the descriptive statistics retrieved by the model.

FT-Raman spectroscopy

The spectroscopic study was concerned with 1) the upper layer (cortex) of the lichen thalli as in Edwards *et al*. (2003a, 2003b, 2005) 2) the surface of the rock in contact with the lichen thallus, named lichen-rock interface (Ascaso *et al*. 1976), where mineral neoformation, if taking place at all, is most likely to be due to lichen-rock interactions, and 3) the rock interior (at least 2 cm away from the surface) which is used as a specific control for each sample since lichen-induced weathering is supposed not to reach such deeper areas. Control FT-Raman spectra were also recorded on non-colonized schist samples collected from the same schist outcrops as the colonized ones, including 1) the exposed surface (taken from the top as in colonized samples), 2) a 5 mm deep virtual interface and 3) the rock interior. A Bruker RFS 100/S FT-Raman spectrometer was used with a Nd:YAG laser operating at 1064 nm as the excitation light source and a resolution of 4 cm^{-1}. Spectral data were acquired after 1024 laser scans of 20 mW in lichen thallus, to minimize lichen damage, and 64 laser scans of 250 mW in rock and lichen-rock interface.

X-ray microdiffraction (XRmD)

XRmD measurements were performed on an Empyrean (PANalytical, Almelo, The Netherlands) diffractometer, equiped with a five-axis Chi-Phi-x-y-z stage goniometer, a copper sealed anticathode X-ray tube (Empyrean Tube Cu Lff Hr) and a PIXcel^{3D} (PANalytical, Almelo, The Netherlands) X-ray detector. Each sample was first submitted to a set of five random line scan readings along the polished cross-section in order to obtain a preliminary depth profile of the mineralogical composition of the studied schist. Yet no significant differences were detected between the surface of the sample and its interior so priority was given to the lichen-rock interface as in FT-Raman spectroscopy. The final measurements are based on ten random readings along the lichen-rock interface. Bragg angles were scanned between 4 and 47º, with steps of 0.02º (12 minutes time length) for exploratory measurements and between 3.5 and 60º, with steps of 0.02º (2 hours time length) for the final measurements, with a laser diameter of 0.6 mm.

Statistical analysis

The effects of species and orientation on the depth and extent of the Hyphal Penetration Component (HPC) was tested by means of two-way analysis of variance (ANOVA) and post-hoc Tukey HSD for pair-wise comparisons with ‘agricolae’ (de Mendiburu 2013) package in R. ANOVA’s assumption of normality of residuals was checked graphically using qq-plots and that of homocedasticity tested by means of the Levene test, also in R.

8.2.3 Results and discussion

The pattern of hyphal penetration into the rock substrate is highly influenced by the characteristics of the rock (Sanders *et al.* 1994) since hyphae tend to follow paths of least resistance. Length of hyphal penetration of the four lichens analysed was on average 3.4 mm in samples coming from north-west facing surfaces, 4.6 mm in samples coming from south-east facing surfaces, and varied between 0.1 to 37 mm when considering all samples. Maximum depth of hyphal penetration is apparently higher at south-east than at north-west facing surfaces (Table 8.2.1). However, variation within samples, as depicted by the standard deviations, was extremely high and there was no statistically significant effect of orientation or species in the depth of hyphal penetration. Additionally, although maximum depth of hyphal penetration detected among the analysed samples was 37 mm (in *Aspicilia hoffmanniana* from south-east facing surfaces), hyphal bundles frequently reached the lower extremity of the samples, which were roughly between 30 and 40 mm long, and measure of real maximum depth of penetration could not be accurately estimated. As suggested by previous works (Favero-Longo *et al.* 2005, Prieto *et al.* 1995, Wierzchos & Ascaso 1996, 1998) schistose rocks may be susceptible to greater lichen-induced weathering than igneous rocks due to their higher predisposition to break and the easier progression of growing hyphae along the planes of weakness, parallel to the schistosity (Fig. 8.2.1). Additional planes of weakness come from the intense tectonically-related fracturing that characterizes the studied rock. One feature observed in almost every sample, and also commonly referred in literature (Fry 1927), is the extremely penetrative type of hyphal bundles originating from apothecia, that reach much deeper into the schist than adjacent hyphae (Fig. 8.2.1).

Analysis of the effects of penetrating hyphae have long been relying on the use of scanning electron microscopy (SEM) and other high-resolution laboratory techniques (*e.g.* Adamo *et al.* 1993, Hallbauer & Jahns 1977, Strech & Viles 2002, Jones *et al*. 1981). Although extremely useful for examining the very specific changes occurring in rock minerals by direct contact with individual hyphae, the scale of these approaches is often too small to answer questions related with the performance of the entire lichen or lichen community at the scale of the whole surface (micro-scale) or site where these surfaces are located (meso-scale). Additionally, these techniques may only be considering worst (or best) case scenarios resulting from chance unless they are based on large sample sizes and use full random sampling as in Strech & Viles (2002), so important for the statistical robustness of the findings.

Many studies have determined the maximum or average depth of hyphal penetration for a wide range of lithotypes (Pinna *et al.* 1998, Favero-Longo *et al.* 2005), but usually ignore hyphal spread, with few exceptions (Gazzano *et al.* 2009a). Image analysis of colonized cross-sections after PAS staining retrieved accurate values for the hyphal spread of each species inside the rock (Table 8.2.1; Fig. 8.2.2). These values could then be used to determine if surface orientation had any effect on species ability to spread into the rock interior and induce mineral breakdown.

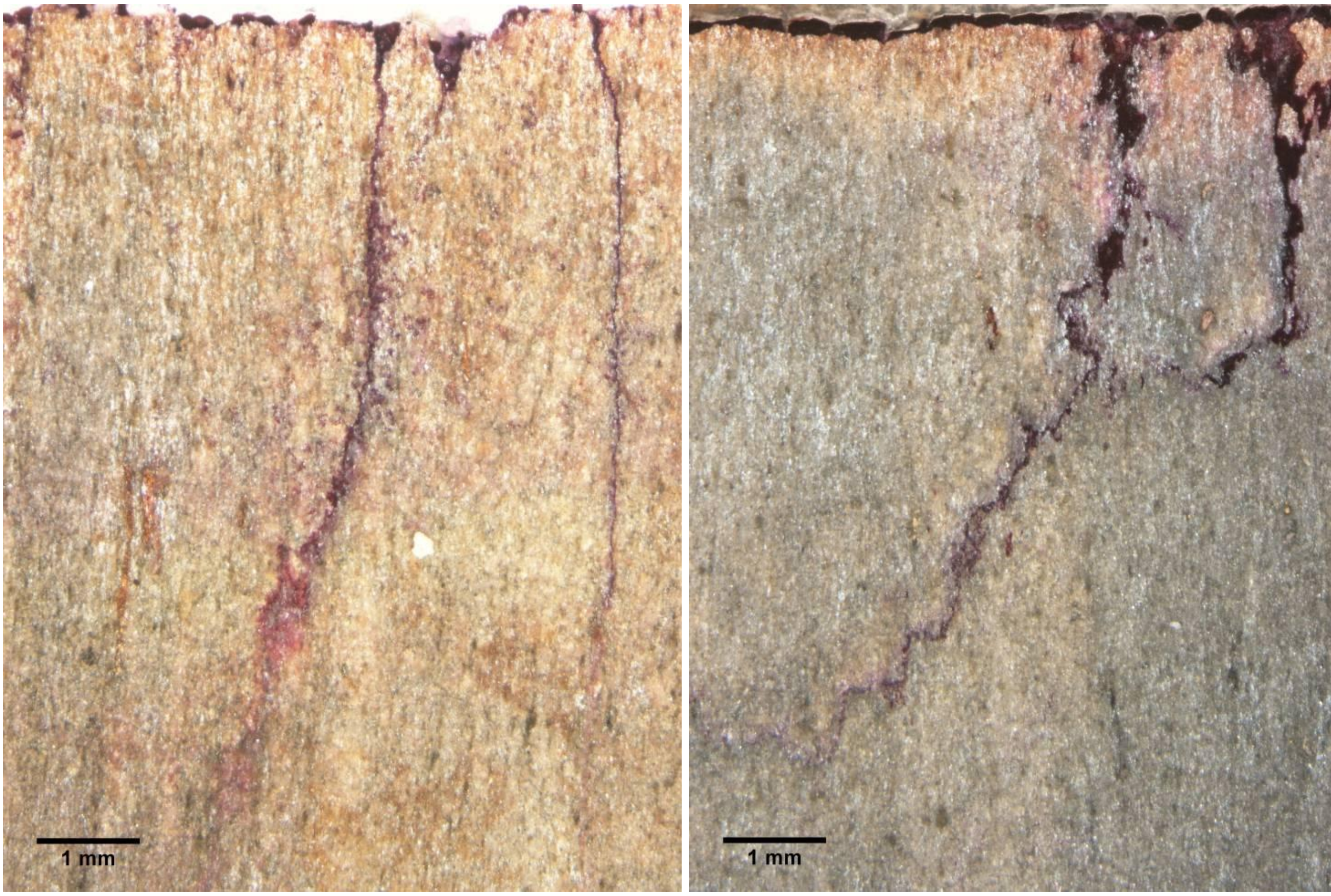

Fig. 8.2.1. Cross-sections of samples colonized by *Lecanora pseudistera* from a north-west facing surface (left) and by *Aspicilia hoffmanniana* from a south-east facing surface (right).

The studied lichens differed significantly in the spread of the HPC irrespective of surface orientation (Table 8.2.2: F-value= 10.974, p-value< 0.001). Post-hoc pair-wise comparisons (Tukey HSD) more specifically showed that these differences were caused by a significantly higher hyphal spread in *Lecanora pseudistera* than in any other of the lichens analysed, whereas *Aspicilia hoffmanniana*, *Caloplaca subsoluta* and *Peltula euploca* were very similar among each other (Table 8.2.1). The effect of interaction between species and surface orientation was not statistically significant neither was the effect of orientation itself, which means that even if there were differences between species in terms of their ability to spread inside the rock, there are no statistically significant differences between north-west and south-east oriented surfaces in any of the studied species.

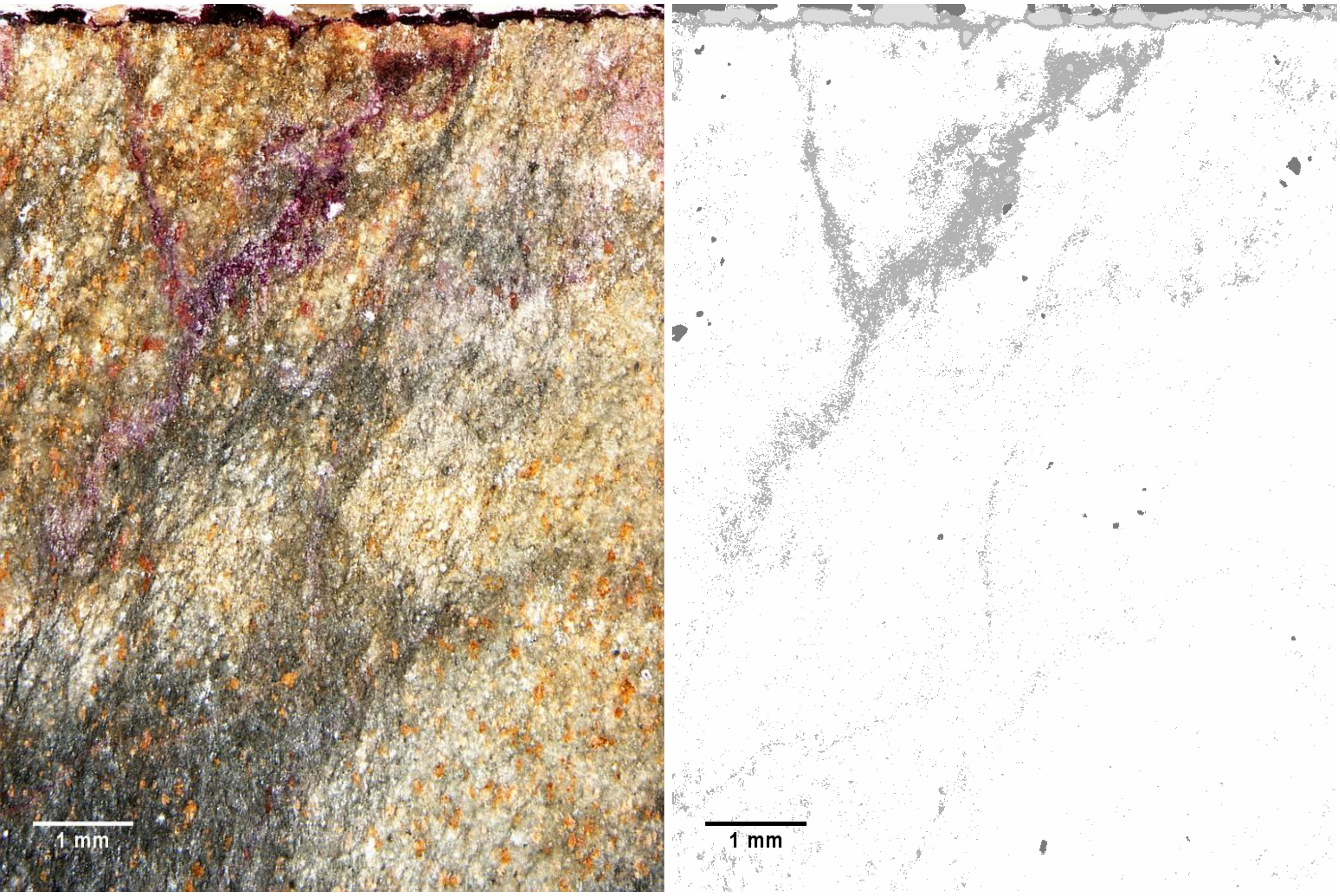

Fig. 8.2.2. PAS stained cross-section of a schist sample colonized by *Aspicilia hoffmannianna* after PAS staining (on the left) and respective classification (on the right) with medium grey areas corresponding well with the area occupied by the hyphal penetration component (in purple) in the original image.

A weathering rind was shown by all samples from north-west facing surfaces, against only 13% of the samples from south-east facing surfaces. The dimension of the weathering rind was unrelated with the extent of the HPC (Table 8.2.1: Spearman= – 0.012) but there might be some species affinity at north-west facing surfaces to such kind of weathering feature.

Although the extent of the HPC is unrelated to surface orientation, as species differ in their ability to spread inside the rock, an indirect effect of orientation on lichen-induced physical weathering will be produced by means of the abundance of individual species. Since *Lecanora pseudistera* is the most effective in terms of hyphal spread among the four lichens analysed and having been the most frequent and abundant on north-west facing slopes (Table 8.2.1, also Marques *et al*. in review) one might assume a more intensive lichen-induced physical weathering happening at north-west than at south-east facing surfaces in the Côa Valley.

Table 8.2.1. Results of measures taken out of colonized cross-sections after PAS staining. Aspect preference is according to Marques *et al.* (in review). Depth: estimated depth of hyphal penetration in mm; HPC: area occupied by the hyphal penetration component in mm^2; WR: area occupied by the weathering rind in mm^2. Total area of the each cross-section was 57.5 mm^2. Different letters in brackets indicate statistically significant pair-wise comparisons (p-value< 0.05)

Species	Aspect preference	Depth (mean ± sd)	Depth (max)	HPC (mean ± sd)	WR
Aspicilia hoffmanianna	south-east	5.7 ± 7.6	37.0	2.96 ± 2.03 (a)	0.00
	north-west	3.5 ± 3.5	12.0	2.37 ± 0.81 (a)	0.00
Caloplaca subsoluta	south-east	4.1 ± 5.3	30.0	2.09 ± 1.71 (a)	4.07
	north-west	1.8 ± 1.9	6.8	1.74 ± 0.71 (a)	7.56
Lecanora pseudistera	south-east	5.1 ± 6.6	31.0	8.01 ± 2.02 (b)	0.00
	north-west	4.1 ± 3.8	13.0	5.79 ± 2.97 (b)	30.36
Peltula euploca	south-east	2.4 ± 1.0	3.9	0.69 ± 0.60 (a)	6.72
				Spearman: – 0.012	

Table 8.2.2. ANOVA test results for the comparison between species and orientation of the original surface in terms of the extent of the Hyphal Penetration Component (HPC).

	MS	F-value	p-value
Species	38.227	10.974	<0.001
Orientation	4.663	1.339	0.262
Species x Orientation	3.019	0.867	0.364

Among the four lichens analysed, *Peltula euploca* was probably the most surprising in terms of its physical performance since, contrary to previous belief, the attachment of this peltate (shield-like) growth form is far from being limited to superficial layers, penetrating up to 4 mm into the rock.

Examples of FT-Raman spectra of the wavenumber region 100-1700 cm^{-1} for the three sample positions of each species are shown in Figs. 8.2.3-09. The complementary results of X-ray microdiffraction and FT-Raman spectroscopy are listed in Tables 8.2.3-10 and summarized in Tables 8.2.13-14. The results of the same analyses applied to bare-rock control samples are listed in Tables 8.2.11-12 and depicted in Figs. 8.2.10-11. X-ray microdiffraction analysis of bare-rock controls and internal layers of all samples confirms the presence of quartz, chlorite, muscovite and albite as the major minerals in the presumably unweathered schist.

The region 100-1700 cm^{-1} contains useful spectroscopic information about metabolic by-products of the lichen-induced weathering process (Edwards *et al.* 1995, Jorge-Villar *et al.* 2004). Quartz and phyllosilicates are the strongest features in the rock interior of all samples, corresponding well with the presumably unweathered parent rock type. Phyllosilicates have complex structures and highly variable compositions (Wang *et al.* 2002) reflected in complex FT-Raman spectra, but for the purpose of this paper the occurrence of phyllosilicates can be usefully discussed in terms of the 100-600cm^{-1} spectral region, since the other regions do not present any visible peak that cannot be related to other substances (see bellow). According to Wang *et al.* (2002) di-octahedral phyllosilicates such as muscovite produce a strong FT-Raman peak at 260 cm^{-1}, which is depicted in the FT-Raman spectra of the rock interior and lichen-rock interface of almost all studied schist samples.

Table 8.2.3. Wavenumbers (WN) for the Raman spectra of *Peltula euploca* from the Côa Valley Archaeological Park. Wavenumber assignments are based on Freeman *et al.* (2008), Frost (1997), Wang *et al*. (2002), Jorge-Villar *et al.* 2004, Edwards *et al.* (1997, 2000, 2003a). R: rock; I: lichen-rock interface; T: thallus.

		South-east		
	WN cm^{-1}	*R*	*I*	*T*
Kaolinite minerals (halloysite)	127	X		
?	162	X	X	
Chlorite/Biotite	200	X	X	
Muscovite	260	X	X	
Albite	291	X		
Quartz/Phyllosilicate	432			X
Quartz	464	X	X	
Albite	507	X		
?	582			X
Carotene	1004			X
Carotene	1157			X
Chlorophyll	1350			X
?	1554			X
Scytonemin	1593			X
Weddellite	1634			X

Table 8.2.4. Main results of X-ray microdiffraction of samples colonized by *Peltula euploca* from the Côa Valley Archaeological Park.

	Å	**South-east**
Albite	3.19	X
Chlorite	4.72, 14	X
Kaolinite	2.56, 4.47, 7.06	X
Muscovite	5, 5.96, 10	X
Quartz	3.33	X
Weddellite	2.12, 4.39, 6.10	X

Mg-bearing phyllosilicates, such as chlorite, peak strongly at approximately 350 cm^{-1}. The peak at 356 cm^{-1} visible in the FT-Raman spectra of the rock and lichen-rock interface of schist samples colonized by *Aspicilia hoffmanniana* (Fig. 8.2.5) and of the rock-lichen interface of those colonized by *Caloplaca subsoluta* (Fig. 8.2.7), can therefore be assigned to chlorite. Peaks at 200 cm^{-1} are characteristic of tri-octahedral phyllosilicates (Wang *et al.* 2002) and most probably indicate the presence of either chlorite or biotite.

Table 8.2.5. Wavenumbers (WN) for the Raman spectra of *Aspicilia hoffmanniana* from the Côa Valley Archaeological Park. Wavenumber assignments are based on Freeman *et al.* (2008), Frost (1997), Wang *et al*. (2002), Jorge-Villar *et al.* 2004, Edwards *et al.* (1997, 2003a). R: rock; I: lichen-rock interface; T: thallus.

		North-west			**South-east**		
	WN cm^{-1}	*R*	*I*	*T*	*R*	*I*	*T*
Kaolinite minerals (halloysite)	127	X			X	X	
Chlorite/Biotite	200	X	X		X	X	
Muscovite/Kaolinite	260	X	X		X	X	X
Albite	291					X	
Chlorite	356				X	X	
Quartz	464	X	X		X	X	X
Weddellite	503			X			X
Albite	507	X			X	X	
Whewellite	897			X			
Weddellite	912						X
Carotene	1003						X
Carotene	1157						X
Carotene	1190						X
?	1286						X
?	1440						X
Whewellite	1463			X			
Weddellite	1476						X
Carotene	1525			X			X
?	1554			X			
?	1607						X
?	1670						X

Table 8.2.6. Main results of X-ray microdiffraction of samples colonized by *Aspicilia hoffmanniana* from the Côa Valley Archaeological Park.

	Å	**North-west**	**South-east**
Albite	3.19		X
Chlorite	4.72, 14		X
Kaolinite	2.56, 4.47, 7.2, 7.06		X
Muscovite	5.10, 5.96, 10.5		X
Quartz	3.33		X
?	9.09		X
?	2.15	X	
?	2.32		X

Quartz is very resistant to weathering and persists even at the surface of bare rock (Figs. 8.2.10-11) and at the lichen-rock interface of all samples. It was detected in every sample by a sharp band at 464 cm^{-1} in FT-Raman spectra and at 3.33 Ǻ in X-ray microdiffraction. The presence of the same band in the FT-Raman spectra of *Aspicilia hoffmanniana* (Fig. 8.2.5) and *Caloplaca irrubescens* (Fig. 8.2.6) indicates an incorporation of quartz particles by the thallus of these lichens.

Evidence for the ability of *Aspicilia hoffmanniana* to incorporate phyllosilicate particles is also seen in its FT-Raman spectra, with characteristic bands at 200 and 260 cm^{-1} (Wang *et al*. 2002).

Table 8.2.7. Wavenumbers (WN) for the Raman spectra of *Caloplaca irrubescens* from the Côa Valley Archaeological Park. Wavenumber assignments are based on Freeman *et al.* (2008), Frost (1997), Wang *et al*. (2002), Jorge-Villar *et al.* 2004, Edwards *et al.* (1997, 2003a). R: rock; I: lichen-rock interface; T: thallus.

		North			**South**		
	WN cm^{-1}	*R*	*I*	*T*	*R*	*I*	*T*
Kaolinite minerals (halloysite)	127				X	X	
Chlorite/Biotite	200				X	X	
Muscovite	260				X	X	
Albite	291					X	
?	332					X	
Chlorite	356					X	
Quartz	464	X	X	X	X	X	
Parietin	927			X			X
Carotene	1005						X
Carotene	1157						X
Parietin	1280			X			X
Chlorophyll	1340						X
Chlorophyll	1352						X
Carotene	1525			X			X
?	1557			X			X
?	1612			X			X
?	1672			X			X

Table 8.2.8. Main results of X-ray microdiffraction of samples colonized by *Caloplaca subsoluta* from the Côa Valley Archaeological Park.

	Å	**North-west**	**South-east**
Albite	3.19	X	X
Chlorite	2.46, 4.72, 14, 14.14	X	X
Kaolinite	2.56, 4.47, 7.2		X
Muscovite	1.99, 5, 5.96, 9.98, 10, 10.13	X	X
Quartz	3.33, 4.24	X	X
Weddellite	2.12, 2.78, 4.39		X
?	11.25		X
?	2.32		X

However, the occurrence of quartz and phyllosilicates in lichen thalli and lichen-rock interface can also have an exogenous origin from airborne dust, as suggested by Vingiani *et al.* (2013) after detecting the same kind of mineral incorporation in lichens growing on quartz- and phyllosilicate-free vulcanic rocks. Incorporation of quartz and phyllosilicate minerals by lichens is not exclusive of crustose lichens as can be inferred by the FT-Raman spectra of

Peltula euploca showing a band at 432 cm^{-1}, which is assignable to either of these silicate minerals. This would require further confirmation through higher resolution techniques such as scanning electron microscopy (SEM).

Besides those minerals that are known to characterize the unweathered rock, X-ray microdiffraction and FT-Raman spectroscopy confirmed the occurrence of kaolinite and halloysite at both the virtual interface of bare rock samples and the lichen-rock interfaces, irrespective of the species and the orientation of the parent outcrop (Tables 8.2.11 and 8.2.12). Kaolinite and halloysite are common products of schist weathering, resulting from the transformation of chlorite, biotite, muscovite and feldspars (Banfield & Eggleton 1990, Kretzschmar *et al.* 1997, Parham 1969). In fact, the peak at 260 cm^{-1} in FT-Raman spectra could correspond to kaolinite minerals instead of muscovite (Wang *et al.* 2002). Differentiation of kaolinite minerals from muscovite, and the latter from vermiculite, can be problematic because peaks shared by the three minerals are not fully differentiable by X-ray microdiffraction or FT-Raman spectroscopy. Weathering is quite obvious to the naked eye in most samples, where the black to more usually dark-grey rock core presumably corresponding to unweathered parent rock gradually changes into the light brown to greyish-brown coloured weathering rind that was measured through image analysis, as seen before (Fig. 8.2.1, on the right). No differences were observed in terms of the occurrence of kaolinite and halloysite between north-west and south-east facing surfaces.

Within this low wavenumber region, FT-Raman bands are observed for calcium oxalate dihydrate, known as weddellite, at 191 and 503 cm^{-1} in the thallus of *Aspicilia hoffmanniana* and *Lecanora pseudistera* on schist samples of both orientations in the former, or exclusively of south-east facing surfaces in the latter species. Weddellite is known to produce other characteristic peaks between 505 and 507 cm^{-1} in FT-Raman spectra (Jorge-Villar *et al.* 2004). A band at 507 cm^{-1} is clearly visible in the rock interior, and to a lesser extent the lichen-rock interface, of *Peltula euploca* (Fig. 8.2.3) and *Aspicilia hoffmanniana* (of both south-east and north-west facing surfaces). Relating this band to calcium oxalate is somewhat problematic because it also falls within the narrow region of 505 to 515 cm^{-1} attributed to feldspars (Freeman *et al.* 2008). In fact, albite is known to peak precisely at 507 and 291 cm^{-1}. The occurrence of both bands simultaneously is here attributed to albite.

An additional band assignable to calcium oxalate in *Lecanora pseudistera* peaks at 910 cm^{-1} but key molecular signatures for oxalates occur in the 1400-1500 cm^{-1} region (Edwards *et al.* 2003b) where 1476 cm^{-1} is considered distinctive for weddellite. Other distinguishing bands for weddellite occur at 912 and 1634 cm^{-1}. The signature of weddellite was found either completely or partially in the thalli of *Aspicilia hoffmanniana*, *Lecanora pseudistera* and *Peltula euploca* from south-east facing surfaces, but not in the thalli of *Caloplaca subsoluta* neither in any of the specimens taken from north-west facing surfaces. In fact, previous experimental works had indicated that microclimatic factors could be important in determining the state of hydration of calcium oxalate in lichens (Edwards *et al.* 1995) and have associated the occurrence of the dyhidrate form with lichen's strategy for maintaining its water balance in dry exposed surfaces (Prieto & Silva 2003).

Table 8.2.9. Wavenumbers (WN) for the Raman spectra of *Lecanora pseudistera* from the Côa Valley Archaeological Park. Wavenumber assignments are based on Freeman *et al.* (2008), Frost (1997), Wang *et al*. (2002), Jorge-Villar *et al.* 2004, Edwards *et al.* (1997, 2003a). R: rock; I: lichen-rock interface; T: thallus.

		North-west			**South-east**		
	WN cm^{-1}	*R*	*I*	*T*	*R*	*I*	*T*
Kaolinite minerals (halloysite)	127	X	X				
Chlorite/Biotite	200	X	X			X	X
Muscovite	260	X	X			X	
Quartz	464	X	X		X	X	
Weddellite, Whewellite	503						X
Weddellite	910						X
Carotene	1006			X			
?	1134						X
Carotene	1155						X
Carotene	1157			X			
Carotene?	1186			X			
?	1302						X
?	1306			X			
?	1440			X			
Weddellite	1476						X
?	1512						X
Carotene	1525			X			
?	1601			X			X
Whewellite	1631			X			
?	1655			X			

Table 8.2.10. Main results of X-ray microdiffraction of samples colonized by *Lecanora pseudistera* from the Côa Valley Archaeological Park.

	Å	**North-west**	**South-east**
Albite	3.19	X	X
Chlorite	3.53, 4.72, 14	X	X
Kaolinite	2.56, 4.47, 7.2, 7.06		X
Muscovite	5, 10	X	X
Quartz	3.33, 4.24	X	X
Weddellite	2.12, 2.78, 4.39, 6.10		X
?	11.25		X
?	9.09		X
?	2.32		X

Calcium oxalate monohydrate, known as whewellite, has been detected in the thalli of *Aspicilia hoffmanniana* (Fig. 8.2.4) and *Lecanora pseudistera* (Fig. 8.2.8) on samples from north-west facing surfaces, peaking in FT-Raman spectra at 1463 and 1631 cm^{-1}, respectively. In the particular case depicted in Fig. 8.2.4, corresponding to *Aspicilia hoffmanniana* from north-west facing surfaces, a mixture of both forms of calcium oxalate has been detected.

Table 8.2.11. Wavenumbers (WN) for the Raman spectra of bare rock controls from the Côa Valley Archaeological Park. Mineral assignments to wavenumbers are based on Freeman *et al.* (2008), Frost (1997), Wang *et al*. (2002). R: rock; I: virtual interface; S: surface.

		North-west			**South-east**		
	WN cm^{-1}	*R*	*I*	*S*	*R*	*I*	*S*
Kaolinite minerals (halloysite)	127	X	X	X			
Chlorite/Biotite	200	X	X	X		X	X
Muscovite	260	X	X	X		X	
Quartz	464	X	X	X	X	X	
Albite	507	X	X				

Table 8.2.12. Main results of X-ray microdiffraction of bare rock controls from the Côa Valley Archaeological Park.

	Á	**North-west**	**South-east**
Albite	3.19	X	X
Chlorite	3.53, 4.72, 14	X	X
Quartz	3.33, 4.24	X	X
Muscovite	5, 10, 10.5	X	X
Kaolinite	7.20	X	X

Variation in measured temperature and relative humidity between north-west and south-east facing surfaces is summarized in Table 8.2.15. There are similarities in the general pattern of annual rock surface temperature and relative humidity regimes. Both north-west and south-east facing surfaces exhibited a seasonal pattern of high temperature and low relative humidity values from around June to September followed by a much colder and moist period between November and February. Variation in temperature was huge at both orientations (Table 8.2.15), but nevertheless higher at south-east than at north-west facing surfaces. The same happened with relative humidity, although relative humidity was always higher at north-west facing surfaces than at south-east facing surfaces.

The occurrence of oxalates in the lichen thallus of *Aspicilia hoffmanniana* and *Lecanora pseudistera* is not completely unexpected since the fruiting bodies of the former are well known for being pruinose (*i.e.* covered by oxalate crystals) and the later belongs to a group of lichens that are characterized precisely by the presence of 'large amphithecial crystals'

(Brodo 1984). The occurrence of the monohydrate form in specimens that grow under less contrasting humidity and temperature regimes is consistent with the physiological role attributed to calcium oxalate.

The origin of Ca ions for calcium oxalate production in lichens, however, is still an unsolved matter. Calcite ($CaCO_3$) is usually present in the matrix of these rocks in sufficient amounts to produce effervescence when treated with dilute hydrochloric acid. Calcite is highly alterable and a potential source of Ca ions for calcium oxalate formation promoted by biological colonization. However, calcite, with characteristic features in FT-Raman spectra being a strong, sharp band at 1086 and weaker bands at 712 and 286 cm^{-1} (Edwards *et al.* 1995, 2003b) is missing in all schist samples.

Although it has been proved that lichens are able to uptake Ca ions from calcareous rocks such as limestones and marbles for calcium oxalate production (*e.g.* Seaward & Edwards 1995), the rock is definitely not the only source of this element as the occurrence of both forms of calcium oxalate has been reported in lichens colonizing non-calcareous substrates such as granites (Prieto *et al.* 1995, Prieto & Silva) or even tree-bark (Edwards *et al*. 2005) and tree leafs (de Oliveira *et al*. 2002). The presence of calcium oxalates inside the lichen thallus is therefore not necessarily indicative of its biodeteriogenic activity. Occurrence of calcium oxalates in lichens growing on iron-rich siliceous rocks instead of the most expected ferrous oxalate dehydrate (humboldtine) has also been reported before (Prieto *et al.* 1997, 2000, Prieto & Silva 2003, Arocena *et al.* 2003). This phenomenon can, according to Prieto & Silva (2003), be explained by the higher water solubility of humboldtine as well as higher susceptibility to oxidation.

The detection of calcium oxalates at the lichen-rock interface by X-ray microdiffraction (Tables 8.2.4, 8.2.6, 8.2.8 and 8.2.10), however, is not as easily assignable to external sources of Ca. Only weddellite has been detected and that was exclusively on samples taken from south-east facing surfaces, colonized by *Caloplaca subsoluta*, *Lecanora pseudistera* and *Peltula euploca* (Table 8.2.14). Weddellite was apparently absent from the lichen-rock interface of *Aspicilia hoffmanniana* whose thallus presented this form of oxalate. The opposite occurred with *Caloplaca subsoluta*, which showed no detectable form of calcium oxalate inside the lichen thallus. These results suggest that calcium oxalates at the lichen-rock interface and inside the lichen thallus may have different origins and/or functions. Except for the occurrence of weddellite, FT-Raman spectroscopy and X-ray microdiffraction of the lichen-rock interface retrieved quite similar results to that of the virtual interface in bare rock controls.

The relevance of these results in the search for the causes of differential weathering of schist surfaces in the Côa Valley is opposite to those already mentioned for lichen-induced physical weathering. Assuming that calcium oxalate at the lichen-rock interface is being produced from Ca taken from the rock, lichen-induced chemical weathering should be more intense on south-east facing surfaces.

Table 8.2.13. Summary of the minerals detected in lichen thalli (FT-Raman spectroscopy)

	Aspicilia hoffmanniana		***Caloplaca subsoluta***		***Lecanora pseudistera***		***Peltula euploca***
	north-west	south-east	north-west	south-east	north-west	south-east	south-east
Whewellite	X				X		
Weddellite	X	X		X		X	X
Quartz		X	X				X
Phyllosilicates		X				X	

Table 8.2.14. Summary of the neoformed calcium minerals detected in the lichen-rock interface (FT-Raman spectroscopy and X-ray microdiffraction).

	Aspicilia hoffmanniana		***Caloplaca subsoluta***		***Lecanora pseudistera***		***Peltula euploca***
	north-west	south-east	north-west	south-east	north-west	south-east	south-east
Whewellite							
Weddellite				X		X	X

Other bands present in FT-Raman spectra include 1158, 1552 and 1612 cm^{-1} are characteristic of parietin and therefore present in the FT-Raman spectra of *Caloplaca irrubescens*. The resemblance of the FT-Raman spectra of *Caloplaca irrubescens* and that of other members of the lichen family Teloschistales (Jorge-Villar *et al.* 2004) is quite obvious mainly due to the profile of parietin.

The FT-Raman spectra of the thallus of *Aspicilia hoffmanniana* and *Lecanora pseudistera* on samples taken from north-west and south-east facing surfaces, respectively, contain a series of bands that could not be assigned to any substance for the time being, but are probably related with the lichen acids produced by these lichens. Also unknown were the peaks at 5.96 an 9.09 Ǻ in X-ray microdiffraction of samples colonized by *Aspicilia hoffmanniana* and 2.32 Ǻ in samples colonized by *Aspicilia hoffmanniana* and *Caloplaca subsoluta*. The very broad bands at 1332 and 1595 cm^{-1} are due to amorphous carbon.

Table 8.2.15. Data on rock surface microclimate at opposite orientations in the Côa Valley.

		North-west	**South-east**
Temperature	Average	17 ºC	20 ºC
	Minimum	13 ºC	15 ºC
	Maximum	23 ºC	30 ºC
Relative humidity	Average	70 %	63 %
	Minimum	55 %	45 %
	Maximum	81 %	77 %

South-east facing schist surfaces

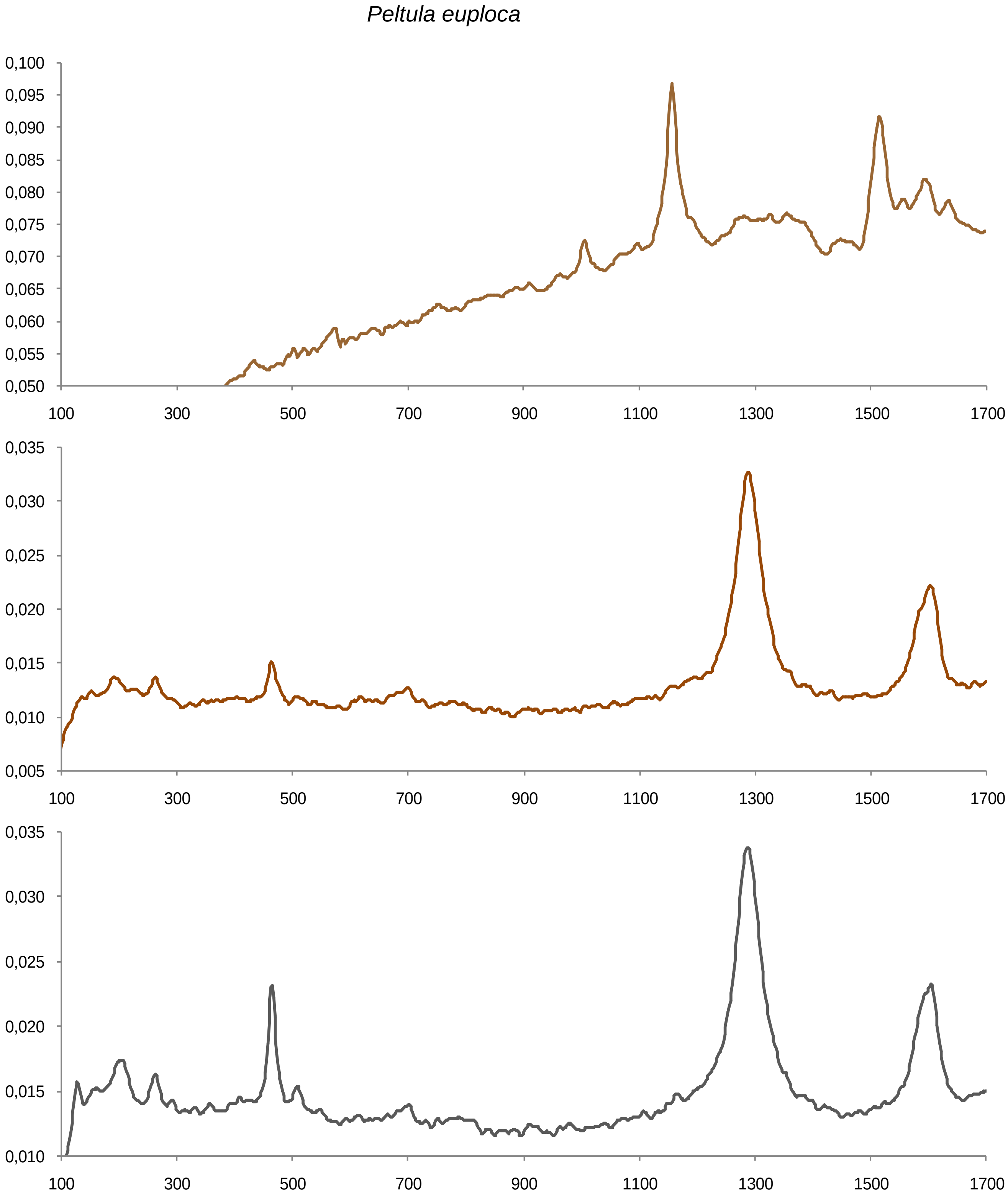

Fig. 8.2.3. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Peltula euploca* from south-east facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

North-west facing schist surfaces

Aspicilia hoffmanniana

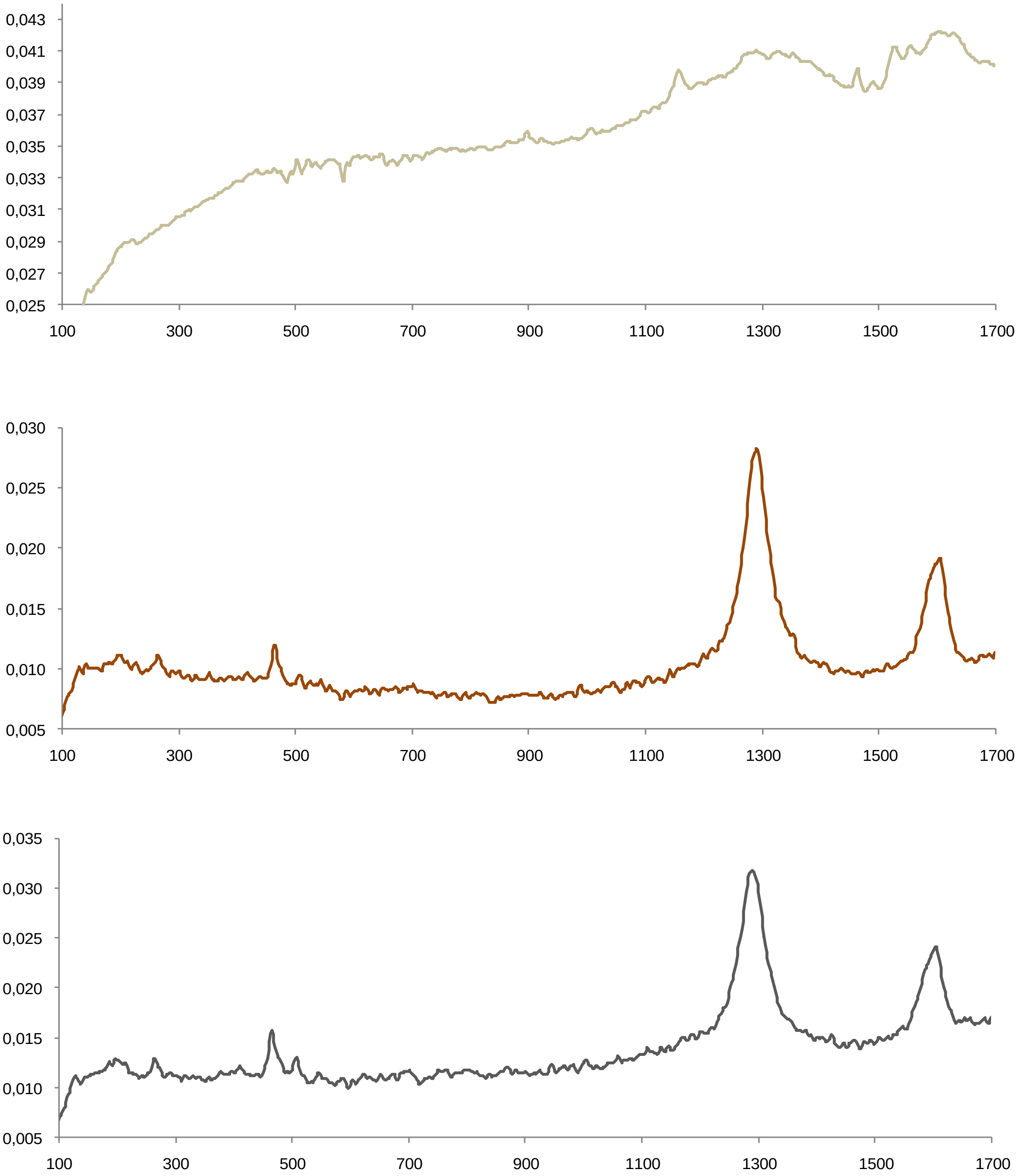

Fig. 8.2.4. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Aspicilia hoffmanniana* from north-west facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

South-east facing schist surfaces

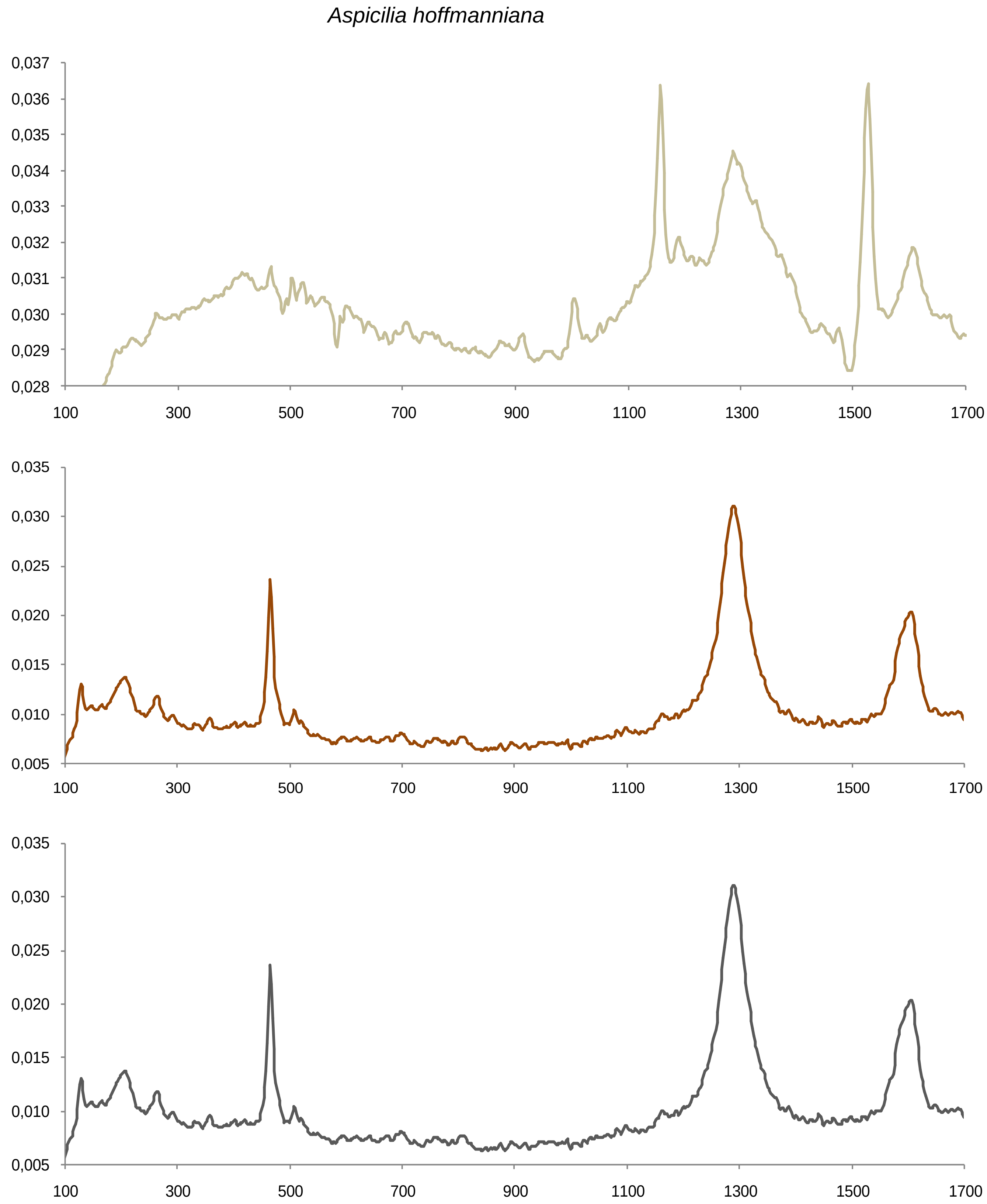

Fig. 8.2.5. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Aspicilia hoffmanniana* from south-east facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

North-west facing schist surfaces

Caloplaca subsoluta

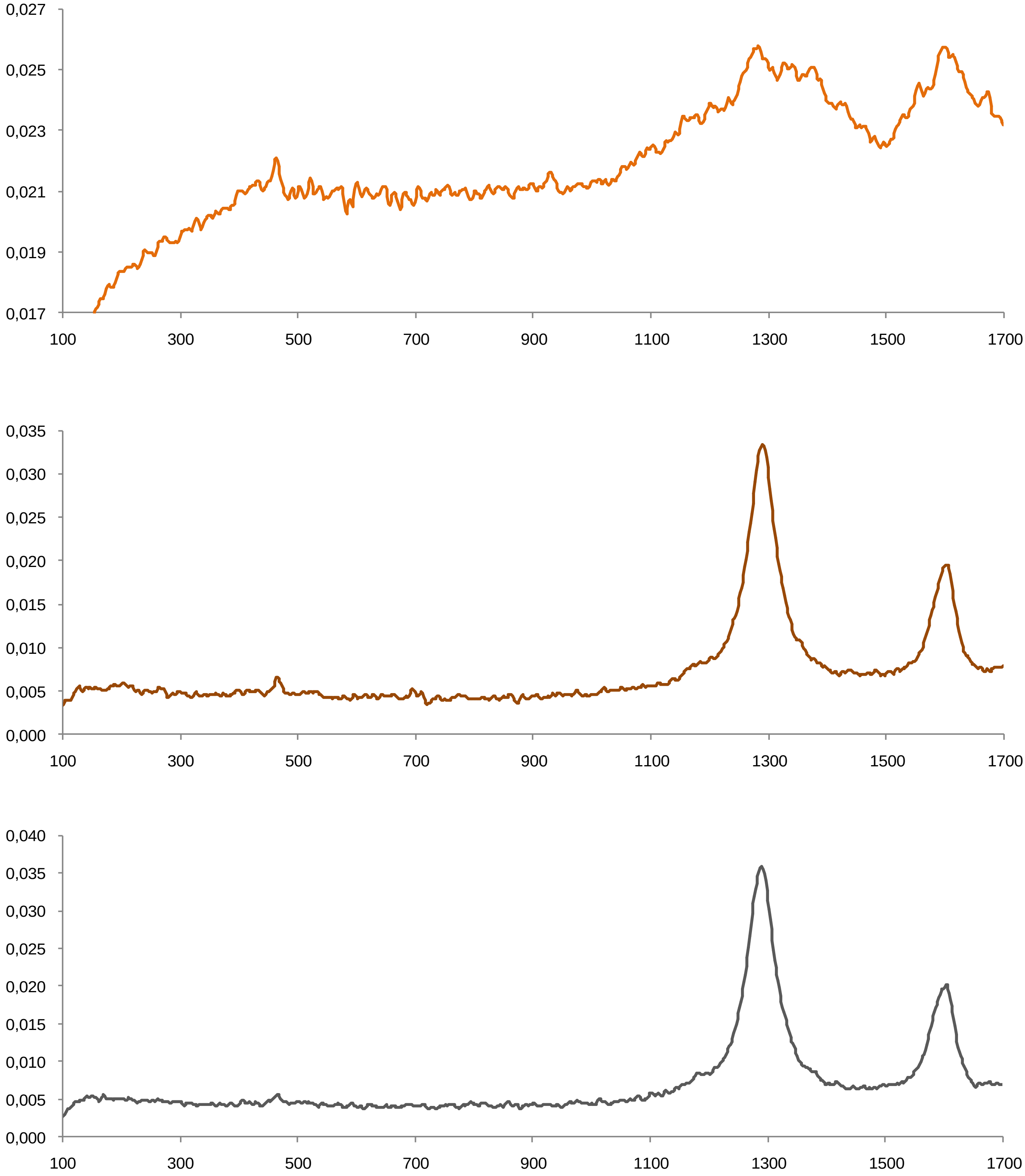

Fig. 8.2.6. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Caloplaca subsoluta* from north-west facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

South-east facing schist surfaces

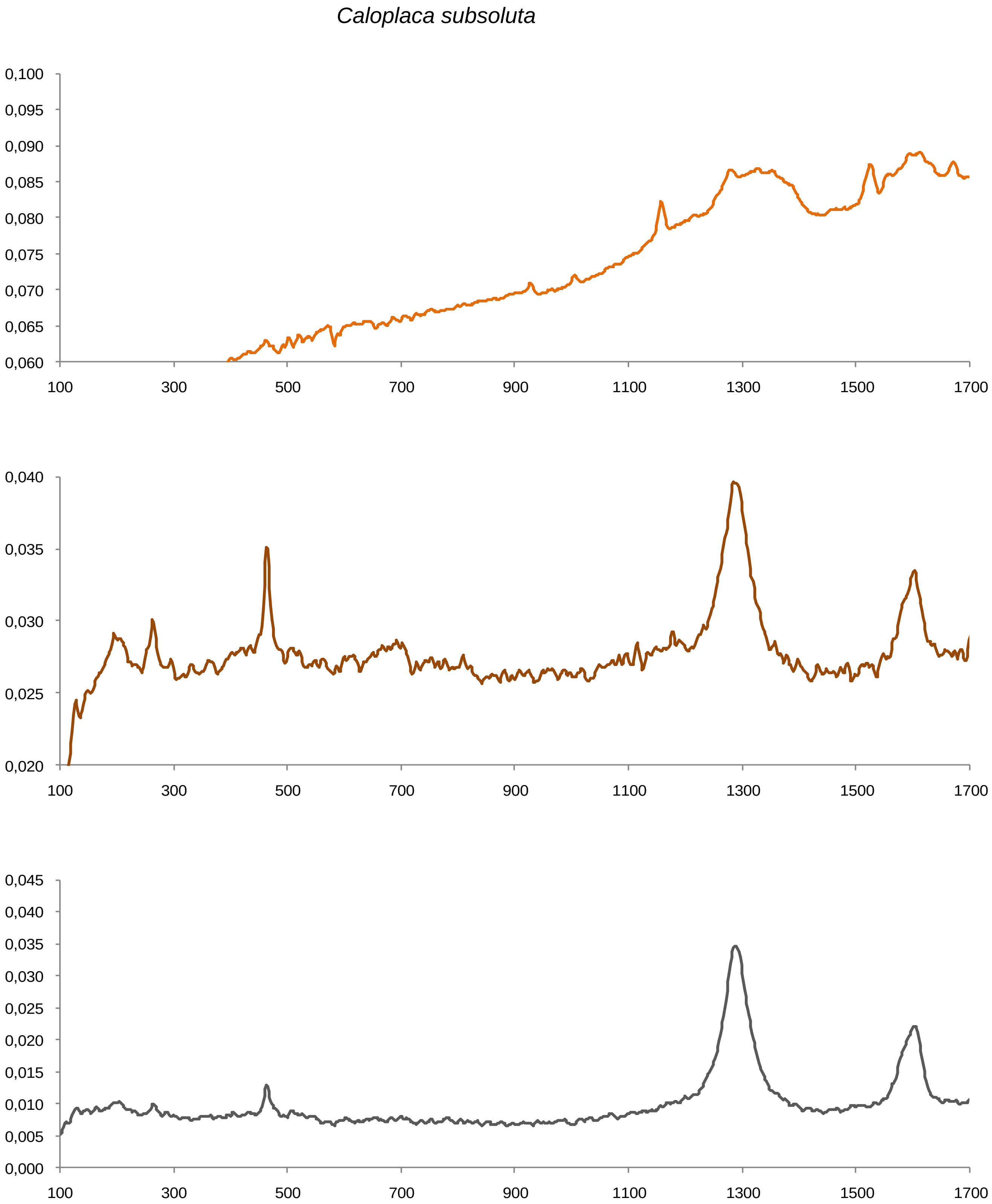

Fig. 8.2.7. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Caloplaca subsoluta* from south-east facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

North-west facing schist surfaces

Lecanora pseudistera

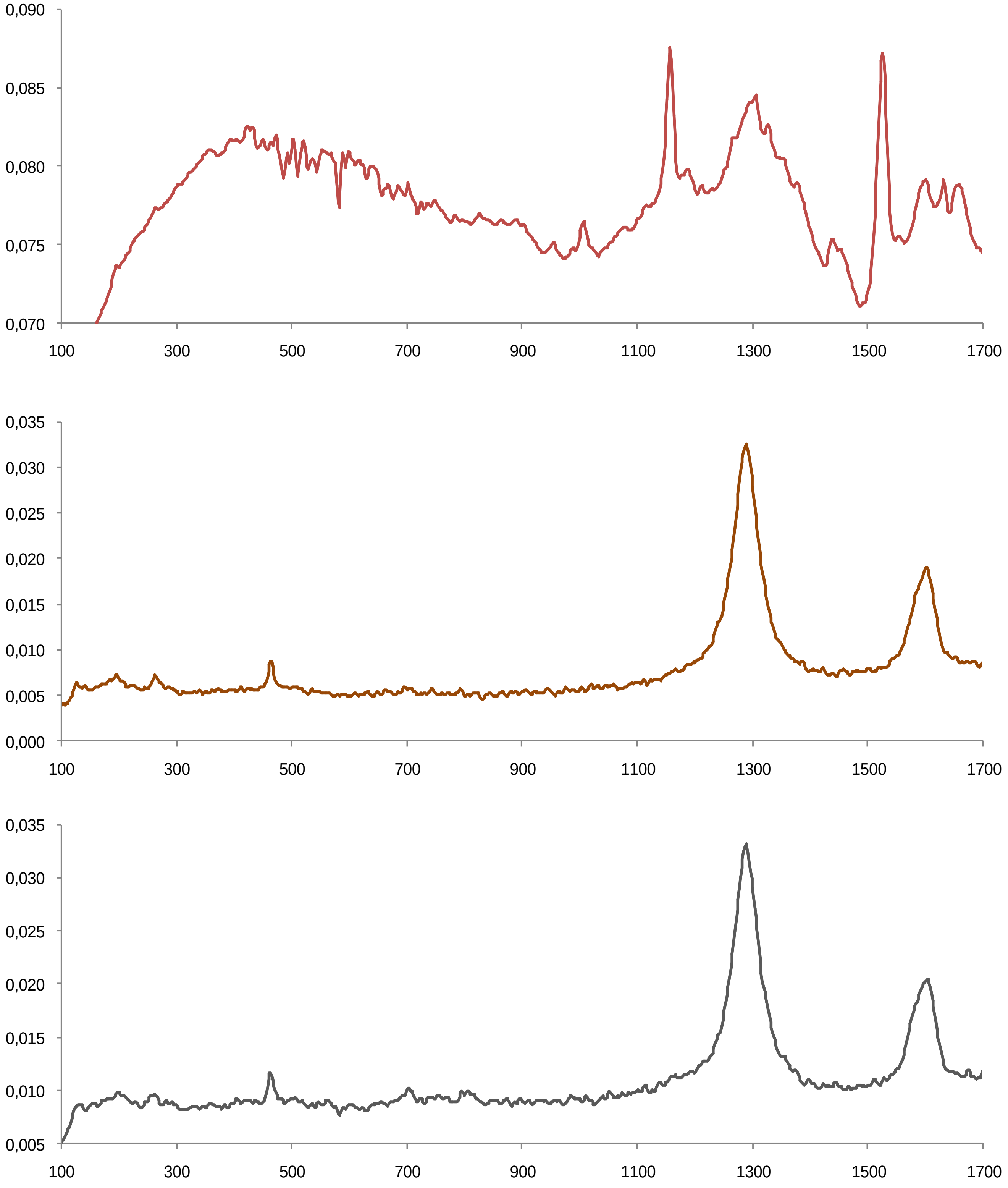

Fig. 8.2.8. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Lecanora pseudistera* from north-west facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

South-east facing schist surfaces

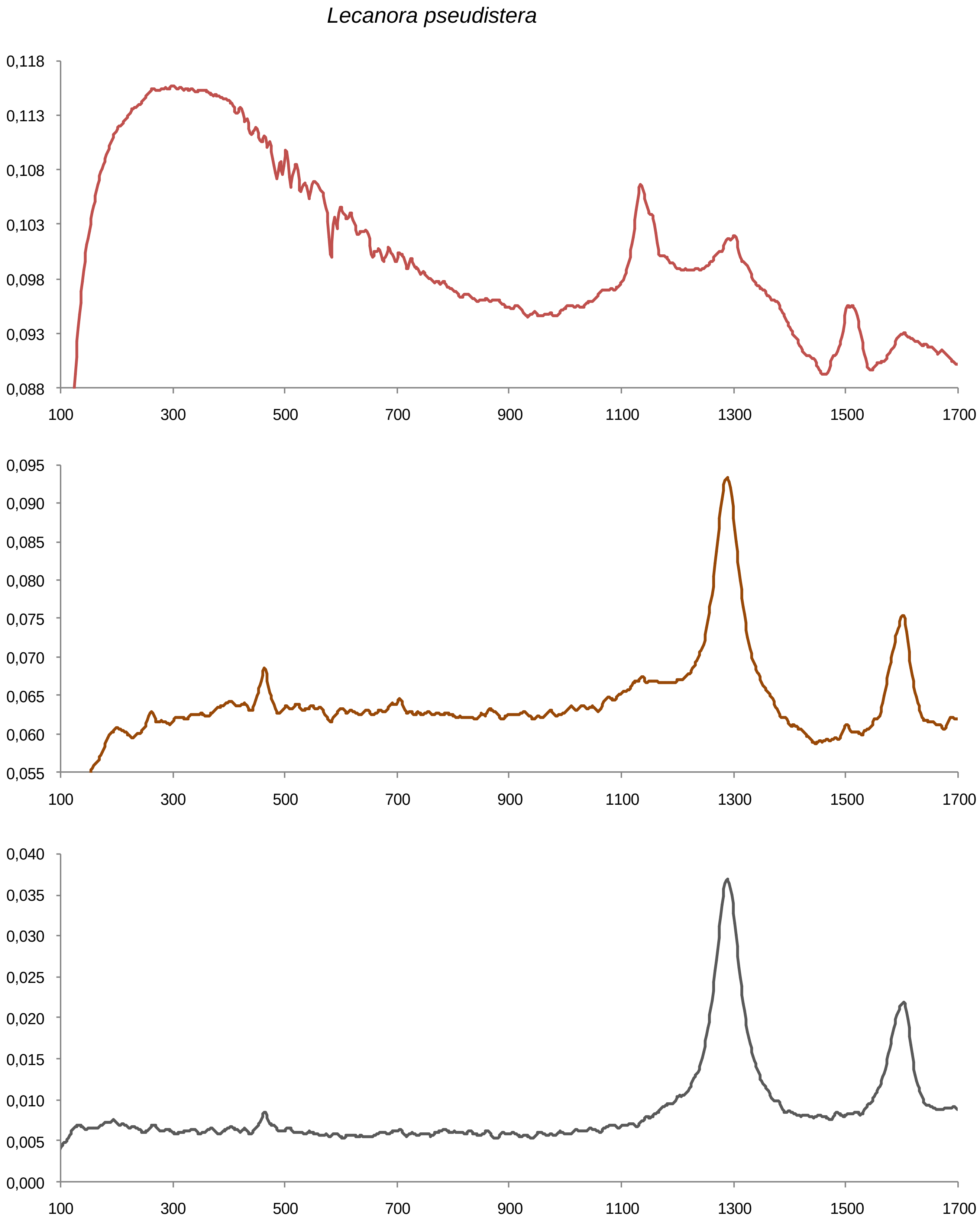

Fig. 8.2.9. FT-Raman spectra of thallus surface (top), rock-lichen interface (middle) and rock core (bottom) of *Lecanora pseudistera* from south-east facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

North-west facing schist surfaces

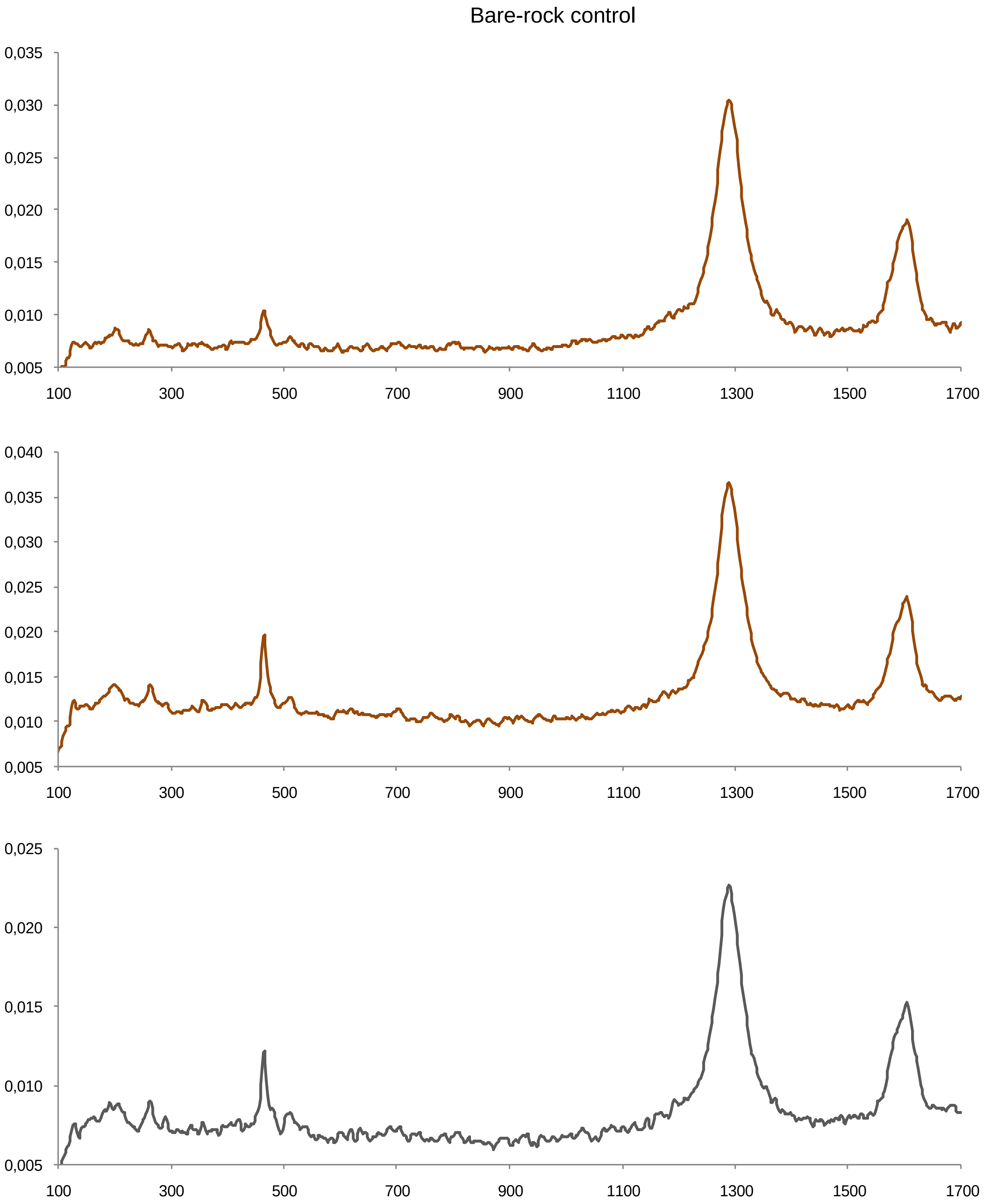

Fig. 8.2.10. FT-Raman spectra of external surface (top), internal surface (middle) and core (bottom) of the bare rock control from north-west facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

South-east facing schist surfaces

Bare-rock control

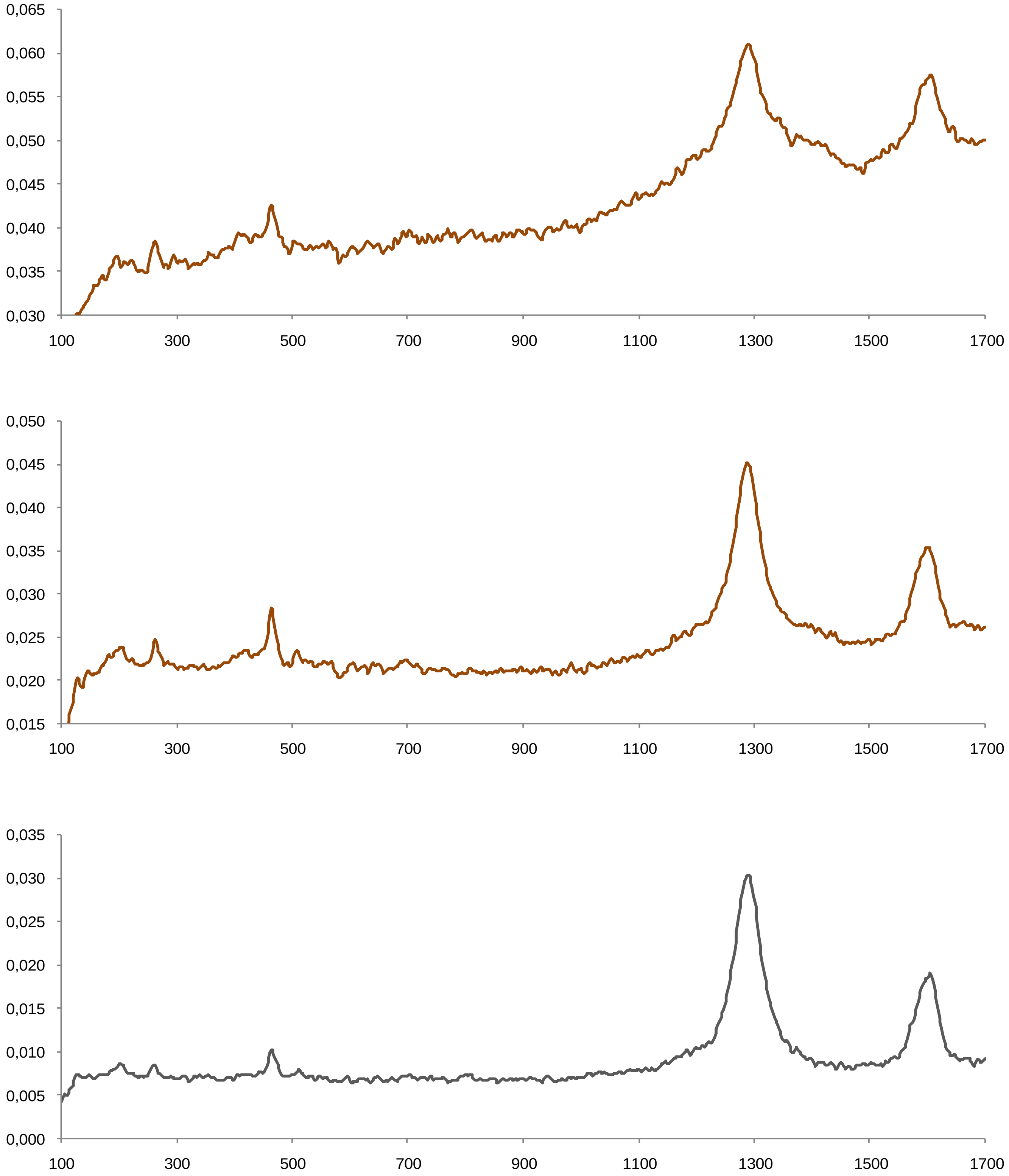

Fig. 8.2.11. FT-Raman spectra of external surface (top), internal surface (middle) and core (bottom) of the bare rock control from south-east facing vertical schist surfaces. Conditions as explained in material and methods. Wavenumber region: 100-1700 cm^{-1}.

8.2.4 Conclusions

Analysis of stained polished cross-sections of schist samples colonized by the crustose *Aspicilia hoffmanniana*, *Caloplaca subsoluta*, *Lecanora pseudistera* and the squamulose *Peltula euploca* showed that hyphae originating from the medulla of these lichens penetrate more than 30 mm and follow a unidirectional pattern along the depth rock profile. The lamellar nature of schist minerals offering pathways of least resistance along intermineral voids, probably favours this deep penetration. Therefore the effects of hyphal penetration on schist should go far beyond the surface and also involve the minerals in the deeper layers. The spread of the hyphal penetration component in the analysed species was similar on north-west and south-east facing surfaces, but may turn out to be more severe at north-west facing surfaces, due to the higher frequency and abundance of species with higher penetrative ability, such as *Lecanora pseudistera*. Orientation is thus likely to have an indirect effect on lichen-induced physical weathering by means of the abundance patterns of individual species, highlighting the importance of accurate estimates of the relative abundances of colonizing species, stemming from community ecology approaches, for rock-art condition assessments.

Other evidences of lichen-induced weathering produced in these rocks are related with the incorporation of quartz and phyllosilicate particles by the thalli of all the lichens studied, including the squamulose *Peltula euploca*. The external origin of these particles cannot be ruled out, however, while the mechanisms of airborne mineral incorporation by the lichen thallus are not fully understood. Also of interest for the purpose of this study is the presence of kaolinite and halloysite, two common products of schist weathering, at the lichen-rock interface and on the surface of bare rock controls, irrespective of surface orientation. Variations in the amount of these minerals depending on the colonizing species and microclimatic factors remain to be tested.

Evidence for the occurrence of metabolic by-products of lichen activity in the analysed samples is limited to calcium oxalates. Specimens of *Aspicilia hoffmanniana* from dry south-east facing surfaces produced weddellite exclusively, while those from moist north-west facing surfaces produced a mixture of weddellite and whewellite. Specimens of *Lecanora pseudistera* produced weddellite on south-east facing surfaces and whewellite on north-west facing surfaces. Weddellite was also detected inside the thalli of squamulose *Peltula euploca*, occurring only on south-east facing surfaces. None of these forms of calcium oxalate were detected inside the thalli of *Caloplaca subsoluta*. These results indicate, as others beforehand, that there is some preference among the studied lichens for the production of the dehydrate form of calcium oxalate under the highly variable microclimate conditions provided by south-east facing surfaces, while on the slightly less variable north-west facing surfaces, a mixture of both monohydrate and dehydrate forms can occur. Given the possibility of calcium uptake from airborne particles, it is impossible to state unequivocally whether calcium ions used in the formation of calcium oxalates were acquired from the substrate, but the probability that this might have happened is higher in those cases were calcium oxalates were also detected at the lichen-rock interface. Weddellite was detected at the interface of all species except *Aspicilia hoffmanniana* with south-east facing surfaces.

This study therefore suggests that lichen-induced physical weathering in the Côa Valley is species-specific and stronger on north-east facing surfaces, whereas lichen-induced chemical action is microclimatically controlled and more severe on south-east facing surfaces. There is probably some variation in the relative abundance of alteration minerals and calcium oxalates at different portions of the samples but according to present evidence, the lichens currently dominant on the vertical schist surfaces in the Côa Valley are unlikely to be responsible for the differential weathering, and distribution pattern of engraved schist surfaces.

Calcium oxalate production by lichens not attributable to any kind of biodeteriogenic activity, as it happens with *Aspicilia hoffmanniana*, adds to the doubts concerning its importance in lichen-induced weathering, especially since this seems to be limited to a few species, and, as demonstrated by this study, change with microclimatic conditions.

8.2.5 References

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9 General conclusions

Little was known about the lichen diversity of schist surfaces in the Upper Douro region before this study. Some of the findings are truly unexpected, mainly in what concerns the numerous chorological novelties for Europe and the Iberian Peninsula as well as the new species to science adding to the floristic, geological and landscaping interest of the region, a lichenological interest worth preserving both at national and European level.

Some notes on the main threats to conservation of the studied lichen communities in the Upper Douro region include fire and grazing as well as the expansion of vineyards, since vineyard plantation involves breaking up the schist outcrops where these species proliferate. The significance of the Côa Valley Archaeological Park in particular, goes far beyond its archaeological value, given that it hosts some of the most significant populations of the rarest species, thus particularly important for their conservation.

If little was known about the lichen diversity of schist surfaces in the Upper Douro region even less was known about the composition of lichen assemblages upon those surfaces. Significant differences in species distribution were observed which are regional and climatic, but also local and probably surface controlled. Temperature, irradiation and water availability are presumed to be the main constraining factors.

Structural and compositional variation in these assemblages associated with surface orientation was significant enough to require separate consideration in the analysis of lichen-induced weathering. A strongly reduced subset of surrogate species and species functional groups based on selected vegetative traits, accurately reproduced overall lichen species richness and may thus constitute a good alternative to exhaustive species surveys in the study area. Based on their frequency and abundance at opposite slopes in the Côa Valley, one squamulose (*Peltula euploca*) and three crustose lichens (*Aspicilia contorta* subsps. *hoffmanniana*, *Caloplaca subsoluta* and *Lecanora pseudistera*) were selected to address the phenomenon of lichen-induced weathering under the effect of major microclimatic constraints.

The few observations that existed in the literature as to how schist surfaces are affected by lichen activity in the Côa Valley were mostly speculative. The link between the observed differential weathering in north-west versus south-east facing schist surfaces and differential weathering activity of lichens came from controlled experiments with individual species tested against bare rock controls. The most obvious mode of action by these lichens seems to be the mechanical, through hyphal penetration along the schistosity plane.

Surface orientation produced no detectable effect on the extent of hyphal penetration of individual species. However, the studied species differed in their ability to penetrate the rock, and the extent of hyphal penetration was significantly lower in species dominant on south-east facing surfaces. Such intrinsic differences among species, irrespective of microclimatic or environmental variation, highlights the importance of obtaining accurate estimates of the relative abundances of individual species, stemming from community ecology approaches, in rock-art condition assessments.

The amount and composition of acids produced by lichens is also species-specific and is usually accounted for their species-specific rock weathering action. The narrow range of metal-organic by-products of lichen action is nevertheless somewhat below expected and limited to calcium oxalates at the lichen-rock interface of three of the four studied species. Unlike other products of schist weathering detected on both colonized and bare rock surfaces, however, calcium oxalate seems to be produced more frequently on south-east than on north-west facing surfaces, contradicting certain assumptions of a causal relationship between lichen-induced weathering and rock-art deterioration in the Côa Valley.

Different conclusions therefore arise regarding the impact of the weathering promoted by lichens, depending on surface orientation, and probably due to the relative contribution of other weathering agents. Weathering previous to lichen colonization was shown to increase schist bioreceptivity and there are certainly cases where water and temperature regimes act synergistically with lichen activity to produce damage. It is possible that lichen-induced physical weathering turns out to be higher at north-west facing surfaces than at south-east facing surfaces due to the abundance of more physically aggressive species, whereas the contrary happens with lichen-induced chemical weathering, seeming to be higher at south-east facing surfaces and environmentally controlled.

10 Implications for future research

The study area possesses undeniable lichenological values which are not exhausted by the content of this thesis. During the course of this study, further taxonomic questions arose and are already the subject of ongoing research:

- The morphological and molecular variation within *Peltula euploca* and *P. bolanderi* with the collaboration of Dr. Matthias Schultz (University of Hamburg);
- The taxonomy and systematic position of *Caloplaca pellodella* and allied species in collaboration with Dr. Ester Gaya (Royal Botanical Gardens, Kew) and Dr. Ulrik Sochting (University of Copenhagen);
- The taxonomy of Lichinaceae in the Iberian Peninsula including the revision of a few holotypes by Gonçalo Sampaio (*e.g. Lemmopsis affinis* Samp., *Psorotichia macrospora* Samp.) currently deposited in PO Herbarium;

Using this framework as a starting point, a predictive model of the lichen-induced rock-art deterioration in the Upper Douro region can be developed by relating information about:

- The occurrence and distribution of potentially biodeteriogenic lichen species and their environmental constraints (including rock bioreceptivity);
- The degree of geochemical and geophysical alteration caused by those species.

The uncertainties behind these preliminary estimates and controversies which persist regarding the role played by lichens in rock weathering in the study area highlight the need for a variety of new and continued research efforts. Based on the findings of this study, the highest priority research needs are related with:

- Increased basic and targeted research on the effects of individual species as well as the variation within and across species, in schist weathering;
- The spatial and temporal patterns of lichen species distribution on schist surfaces;
- The effects of small scale variation of environmental factors on the bioreceptivity of those surfaces;
- The evaluation of early indicators of lichen activity on schist, including intraspecific trait variability and environmentally driven shifts in species weathering ability;

- A more geographically comprehensive assessment of lichen activity on schist surfaces, particularly for the most ubiquous species.

Finally, given that lichens are fast responsive to changes in their environmental surroundings, changes in the responses of individual species and species assemblages to the contrasting weathering microclimates provided by the opposing slopes in the Côa Valley are expected to mirror broader-scale patterns along current latitudinal and altitudinal climatic gradients. Some processes of lichen-induced weathering may have been accelerated or worsened by climate change since the act of engraving, whilst others may have been retarded. Currently detected differences in these species related with surface orientation could thus be used to infer past scenarios of rock-art biodeterioration in the Côa Valley Archaeological Park but this remains a challenge while data on past regional climate is unavailable or uncertain.