

On the use of higher taxa in biodiversity assessment and conservation of bryophyte species

Cristiana de Maia Alves

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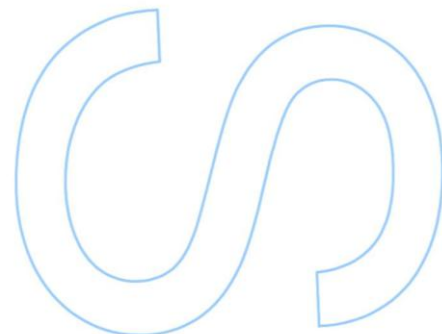
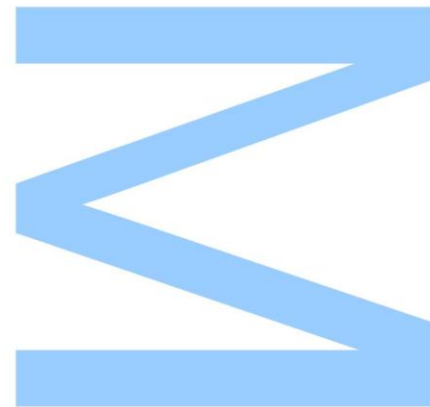
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Supervisor

Rubim Manuel Almeida da Silva, Auxiliary Professor, Faculty of Sciences, University of Porto

Co-Supervisor

Helena Canha Pinto Hespanhol, Postdoctoral Researcher, CIBIO-InBIO





Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ____/____/____

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O

Foreword

This work has already been presented in the following poster communications:

Alves C, Hespanhol H, Vieira C, Almeida R. Using higher taxa (i.e. genera and families) as surrogate for bryophytes species richness. 4^o Workshop BioPlant – Inter-university Doctoral Program in Fundamental and Applied Plant Biology. Faculty of Sciences of University of Porto.

Alves C, Hespanhol H, Vieira C, Almeida R. Using higher taxa (i.e. genera and families) as surrogate for bryophytes species richness. IX International Meeting of Phytosociology – Vegetation and landscape: a socio-ecological perspective. Parque Biológico de Gaia, Avintes, Vila Nova de Gaia.

To my mother

“It is always surprising how small a part of life is taken up by meaningful moments. Most often they are over before they start although they cast a light on the future and make the person who originated them unforgettable.”

in Anna and the King

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To my mother, my inspiration, words can't express how much important you are to me. You taught me to pursue my dreams, to never give up, and to face life always with a smile. Time was stolen from us, but thank you for our little infinity. I hope to become the most extraordinary woman, just like you.

Abstract

The distribution of biodiversity across ecosystems has proved to be difficult to describe and predict, and typically a proper characterisation of the biodiversity of multiple taxonomic groups requires much effort, expertise, and money. Therefore, biodiversity assessment and identification of priority areas for conservation nowadays relies on surrogate data. Surrogates are easier to analyse than species, and less time consuming. One approach using such surrogates is the higher taxa, which substitute information on species by that of higher taxonomic levels (i.e. genera and families). In this thesis, two studies exploring the higher taxa approach are presented.

In the first study, genera were tested as surrogates for species richness and composition of bryophytes, in two different habitats (rock exposed outcrops and watercourses), and across two scales (local- and micro-scale), in north and centre of Portugal. Moreover, we tested if environmental variables could influence the species and genera richness and composition. Our results showed strong correlations between species and genera, for both richness and composition, in both habitats and across the two scales analysed.

In the second study, we tested if genera could be used in prioritization of important areas for bryophyte conservation in Peneda-Gerês National Park, using three different approaches: Scoring, Important Plant Areas (IPA), and Complementarity-based approaches. Our results showed that all three approaches tested for prioritization of important areas ranked localities in a similar way using species or genera data .

In conclusion, genus-level information could be used as surrogates of bryophyte species richness and composition. Moreover, it could be used in the selection of important areas for bryophyte conservation. This thesis contributes for the knowledge of genera as surrogates of bryophyte richness and composition, and could be used as a tool for rapid assessment and monitoring of this taxonomic group.

Keywords

Higher taxa; Bryophytes; Rock outcrops; Watercourses; Complementarity; IPA; Scoring; Peneda-Gerês National Park

Resumo

A distribuição da biodiversidade nos ecossistemas tem-se mostrado difícil de descrever e prever, uma vez que uma caracterização exaustiva da biodiversidade requer tempo, recursos financeiros e especialistas qualificados. Assim, a avaliação e a identificação de áreas importantes para a conservação contam, atualmente, com dados recolhidos de indicadores de substituição, de análise mais fácil e menos demorada que os dados das espécies. O uso de níveis taxonómicos superiores (e.g. géneros ou famílias), para substituir a informação acerca das espécies, constitui uma das abordagens que utilizam este tipo de indicadores. Na presente tese, serão apresentados dois estudos com utilização deste tipo de indicadores de substituição.

No primeiro estudo, o nível taxonómico do género foi testado como indicador de substituição da composição e riqueza específica de briófitas, em dois habitats diferentes (saxícola e fluvial) e em duas escalas (escala local e micro), no norte e centro de Portugal. Para além disso, testou-se a influência de variáveis ambientais na composição e riqueza específica. Os resultados obtidos revelaram correlações positivas e significativas entre género e espécie, tanto para a composição como para a riqueza, em ambos os habitats estudados e nas duas escalas analisadas.

No segundo estudo, testou-se o uso de informação ao nível do género na priorização de áreas importantes para a conservação de briófitas, no Parque Nacional da Peneda-Gerês, utilizando três abordagens diferentes: “Scoring” (“Pontuação”), Áreas Importantes para Plantas (IPA) e Complementaridade. Os resultados obtidos apontam para uma priorização semelhante de locais usando espécies e géneros, independentemente da metodologia utilizada.

Este estudo permitiu concluir que a informação do género pode ser usada como substituto da informação da composição e riqueza específica de briófitas, bem como auxiliar na selecção de áreas importantes para a conservação de briófitas. A presente tese contribui para o conhecimento acerca do uso do género como indicador da riqueza e composição de briófitas, podendo ser usado como uma ferramenta para uma avaliação e monitorização expeditas da diversidade deste grupo taxonómico.

Palavras-chave

Níveis taxonômicos superiores; Briófitas; Saxícola; Fluvial; Complementaridade; IPA; Pontuação; Parque Nacional da Peneda-Gerês

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List of abbreviations

ANOSIM - Analysis of Similarities

ANOVA – Analysis of Variance

CCA – Canonical correspondence analysis

DCA – Detrended correspondence analysis

GLM – Generalized Linear Models

IPA – Important Plant Areas

PNPG – Peneda-Gerês National Park

Chapter 1. General Introduction

Biodiversity assessment

The existence of life is the most unique feature of Earth, and the most extraordinary feature of life is its diversity (Cardinale et al. 2012). Nevertheless, biodiversity, worldwide, is presently facing an extinction crisis (Kerr et al. 2000). Such loss of biological diversity will alter the functioning of ecosystems and their ability to provide society with the goods and services needed to prosper (Cardinale et al. 2012). However, the complexity of the distribution of biodiversity across ecosystems has proved to be difficult to describe and predict, and typically a proper characterisation of the biodiversity of multiple taxonomic groups requires much effort, expertise, and money (Heino 2010). In fact, the limited time and budgetary resources available for conservation force biologists to take shortcuts in biodiversity assessment (Kerr et al. 2000). There is thus a need for suitable surrogates of biodiversity (Heino 2010).

Surrogate species

Surrogates are entities that correlate strongly with species number, but more easily assessed (Olsgard et al. 2003). These surrogates may be species that represent the whole pool of species or other aspects of the environment always with the goal of conservation and monitoring of an area (Caro & Girling 2010; Wiens et al. 2008). Surrogates have some advantages, such as less time consuming, and reduced costs and data needed (Favreau et al. 2006; Noss 1990).

According to Caro and O'Doherty (1999) and Caro and Girling (2010), surrogate species include the following types: (1) flagship; (2) umbrella; (3) keystone; (4) and indicator species (Caro & O'Doherty 1999; Favreau et al. 2006).

Flagship species are charismatic single species or small collection of species, in a given area. These species are used as emblems for a given habitat and their purpose is to raise public's awareness for conservation issues. Flagship species are normally large species, like mammals, large birds, vertebrates, and also plants and invertebrates or endangered species (Caro & Girling 2010). Some examples of flagship species are: (1) giant panda (*Ailuropoda melanoleuca*) (Dietz et al. 1994); (2) Arabian oryx (*Oryx*

leucorysx) (Ostrowski et al. 1998); (3) tiger (*Panthera tigris*) (Panwar 1982); (4) mountain tapir (*Tapirus pinchaque*) (Downer 1996); (5) elephant (*Elephas maximus*) (Johnsingh & Joshua 1994) ; (6) golden lion tamarin (*Leontopithecus rosalia*) (Dietz et al. 1994).

Umbrella species are used when the goal is to protect a habitat or a species community. Moreover, umbrella species are used as a surrogate when there is a need to delineate the type of habitat or size for conservation purposes (Caro & O'Doherty 1999). Protecting umbrella species' habitats bring other species under protection (Caro & Girling 2010). Plants can be used as umbrella species of less studied taxa like bryophytes, lichens or fungi (Chiarucci et al. 2005; Nilsson et al. 1995). Other popular cases include butterflies, locally well-known and admired (Launer & Murphy 1994) and cases of umbrella species with mammals (Berger 1997) and birds (Roberge et al. 2008).

Keystone species have a very relevant impact on communities or ecosystems, despite their abundance (Caro & O'Doherty 1999). The role of one single species in functioning and integrity of an ecosystem are important and conservation efforts focus on that single species. Firstly the concept of keystone species only included top predators but presently it was extended to include keystone herbivores (Caro & Girling 2010). Examples of keystone species are: the purple sea star (*Pisaster ochraceus*) (Menge et al. 1994) and sea urchins (*Strongylocentrotus* sp.). (Estes & Palmisano 1974).

Indicator species need to have the same habitat requirements as the species, communities, or ecosystems for which they indicate. With the protection of indicator species other species are also protected (Favreau et al. 2006) and according to Caro and O'Doherty (1999), can be divided into: (1) health indicators species (e.g. diversity of invertebrates as surrogate for the accumulation of certain pollutants in an area) (Särkkä 1996); (2) population indicator species (e.g. seabird *Morus capensis* used as a surrogate of the distribution and population trends of oceanic fish) (Oatley et al. 1992); and (3) biodiversity surrogates (e.g. when the number of species of well described taxa is used as a surrogate of other poor described taxa) (Beccaloni & Gaston 1995).

Biodiversity surrogates

Biodiversity surrogates are used when the purpose is to assess and monitor biodiversity. These surrogates can be coarsely divided into: (1) taxonomic; and (2) environmental surrogates. The first is based on biological data, whilst the second is a combination of physical and biological data (Grantham et al. 2010).

Environmental surrogates are those which are expected to affect the distribution of species. In this context, environmental information is used as a proxy for species distributions, either by itself or in combination with species data (Caro & Girling 2010; Mandelik et al. 2012). The two types of environmental surrogates that are most commonly used for biodiversity assessments are: (1) coarse-resolution habitats and vegetation types that are used as a way of classification of biodiversity patterns; and (2) fine-resolution characteristics of an habitat that which include: topography, soil, micro-climate, vegetation and productivity (Mandelik et al. 2012).

Taxonomic surrogates include: (1) Morphospecies; (2) Cross taxa; and (3) Higher taxa.

According to Derraik et al. (2010), morphospecies are separated based on morphological characters that are easily observed, therefore not involving identification at species-level. Only people with minimal training in sorting morphospecies at a basic stage are required, which is less time consuming for specialists, and requires less financial resources, than identification to the species-level (Caro & Girling 2010). This approach has been tested in previous studies with arthropods (Derraik et al. 2010), ants, beetles and spiders (Caro & Girling 2010), for purposes of environmental monitoring and conservation (Derraik et al. 2010).

Cross taxa approach is the use of one taxon species richness as a surrogate of the species richness of another, less well known taxon. This approach is used based on the assumption of congruence of different taxa in patterns of species richness. Some taxa are more difficult to assess in terms of biodiversity, and cross taxa approach can be a useful tool to sample these taxonomic groups (Chiarucci et al. 2007). This approach has been tested in different groups, such as vascular plants as surrogates for bryophytes and lichens (Pharo et al. 1999), surrogacy between different groups of arthropods (Beck et al. 2012), or the dominant groups of macroinvertebrates as surrogates of pond biodiversity (Bilton et al. 2006).

Other surrogates such as functional groups, single species abundance vs abundance of other species, and single species abundance vs species diversity aren't included in the categorization abovementioned but they are recognized in the literature (Bhusal et al. 2014; Cushman et al. 2010).

Higher taxa approach

The higher taxa approach is the use of higher taxonomic levels (i.e. genera, families) as a surrogate for species (Kallimanis et al. 2012). This approach was first used in palaeontology (most of the cases families) to diminish sampling bias (Gaston &

Williams 1993). The advantages of these surrogates are essentially related to reduced requirements in terms of time, financial resources, and level of expertise (scientists) (Gaston & Williams 1993; Kallimanis et al. 2012). Specifically, because: (1) there are fewer higher taxa than species, in the same location; (2) identification of the higher taxa is not as much time-consuming as the identification of species (i.e. sampling effort is lower for the higher taxa than for species); (3) the distribution of species within the higher taxa is relatively homogenous (Mandelik et al. 2007; Vieira et al. 2012). Although higher taxa are not natural units, they tend to comprise species that are closely related. Therefore, higher taxa can be used as parcels of species richness that are easier to analyze (Gaston 2000).

In the past years the higher taxa approach has been studied as a useful tool for describing patterns of biodiversity (Gaston 2000). Since the 1990's, up until the present, many studies have been published testing this approach across both aquatic and terrestrial ecosystems (Table 1.1). Many groups have been tested, such as invertebrates (Báldi 2003), fishes (Vanderklift et al. 1998), macroalgae (Smale et al. 2010), macroinvertebrates (Heino & Soininen 2007), birds (Balmford et al. 1996a), vascular plants (Balmford et al. 1996b), lichens (Bergamini et al. 2005), amphibians (Mazaris et al. 2008), reptiles (Mazaris et al. 2008), and mammals (Grelle 2002). The majority of studies only tested the higher taxa approach by itself, but some studies tested this approach in combination with cross taxa (Báldi 2003), functional groups (Smale et al. 2010), indicator groups (Olsgard et al. 2003), and habitat surrogates (Brennan et al. 2006). The most studied biodiversity measure using higher taxa is richness, although throughout the years composition, alpha-, beta- and gama diversity, evenness, and also rarity have also been tested (Table 1.1).

The two main applications of this approach are biodiversity monitoring (Kerr et al. 2000) and conservation (Cardoso et al. 2004). For instance, Kallimanis et al. (2012) tested the ability of the higher taxa approach to detect changes in species richness, and tried to understand if this approach could be used as an efficient tool for monitoring. On the other hand, other studies have been focusing on the issue of conservation. Higher taxa approach has been tested as a tool for reserve selection and network design, using different approaches (i.e. scoring approach, complementarity-based approach, rarity-based approach) (Balmford et al. 2000; Cardoso et al. 2004; Gladstone & Alexander 2005; Mazaris et al. 2008; Vieira et al. 2012).

Table 1.1 – Synthesis of published studies on higher taxa approach.

Authors (Year)	Surrogacy approach(es)	Aims	Group	Biodiversity measures
Andersen (1995)	Higher taxa	Test higher taxa surrogacy (genera) on ants	Ants	Richness
Balmford et al. (1996a)	Higher taxa	Test higher taxa surrogacy (genera, families and orders) at regional scale	Angiosperms, birds, mammals	Richness
Balmford et al. (1996b)	Higher taxa	Test higher taxa surrogacy (genera and families) at local-scale	Woody plants	Richness
Vanderklift et al. (1998)	Higher taxa	Test higher taxa surrogacy (genera, families, orders, and classes) on marine biodiversity	Fishes, invertebrates, plants	Richness
Grelle (2002)	Higher taxa	Test higher taxa surrogacy (genera, families and orders) on Neotropical mammals	Neotropical mammals	Richness
Ferla et al. (2002)	Higher taxa	Test higher taxa surrogacy (genera, families, orders, and subclasses) on plants distributions	Angiosperms	Richness
Báldi (2003)	Higher taxa and cross taxa	Test higher taxa surrogacy (genera and families) as a good surrogate for species diversity and cross taxa surrogacy	Invertebrates	Richness
Doerries and Van Dover (2003)	Higher taxa	Test higher taxa surrogacy (genera, families, orders and classes) on chemosynthetic communities	Mussels, and other associated organisms	Richness
Olsgard et al. (2003)	Higher taxa and indicator groups	Test higher taxa surrogacy (genera, families and orders), and indicator groups on polychaetes	Invertebrates	Richness
Prinzing et al. (2003)	Higher taxa	Test higher taxa surrogacy (genera) on woody plants	Woody plants	Richness, alpha- and beta-diversity
Cardoso et al. (2004)	Higher taxa	Test higher taxa surrogacy (genera and families) on spiders	Spiders	Richness

Bergamini et al. (2005)	Higher taxa and indicator taxa	Test higher taxa surrogacy (genera) on lichens and macrolichens, and the effects of land-use intensity	Lichens, macrolichens	Richness, alpha- and beta-diversity
Gladstone and Alexander (2005)	Higher taxa	Test higher taxa surrogacy (genera, families and orders) on marine biodiversity	Intertidal molluscs, rocky reef fishes	Richness
Villaseñor et al. (2005)	Higher taxa	Test higher taxa surrogacy (genera and families) on vascular flora	Vascular plants	Richness
Bertrand et al. (2006)	Higher taxa	Test higher taxa surrogacy (genera, families)	Invertebrates	Richness, Diversity
Brennan et al. (2006)	Higher taxa and habitat surrogates	Test higher taxa surrogacy (families) on forest invertebrates	Spiders	Richness
Goldberg et al. (2006)	Higher taxa dominant taxa	Test higher taxa surrogacy (genera, families and orders) and dominant taxa on australian macroalgae	Macroalgae	Richness, Diversity
Li et al. (2006)	Higher taxa	Test higher taxa surrogacy (genera and families) on aquatic plant flora	Aquatic macrophytes	Richness, alpha-and beta-diversity
Mandelik et al. (2007)	Higher taxa	Test higher taxa surrogacy (genera and families)	Vascular plants, ground dwelling beetles, moths	Richness, rarity, composition
Heino and Soininen (2007)	Higher taxa	Test higher taxa surrogacy (genera and families) on stream macroinvertebrates and diatoms.	Macroinvertebrates and diatoms	Richness and assemblage structure
Mazaris et al. (2008)	Higher taxa	Test higher taxa surrogacy (genera) as a tool for conservation policy for four different vertebrate classes	Birds, mammals, amphibians, reptiles	Richness
Moreno et al. (2008)	Higher taxa	Test community level patterns using higher taxa surrogates in a pine-oak forest	Litter fauna	Richness, abundance, evenness, diversity

Shokri and Gladstone (2009)	Higher taxa	Test higher taxa surrogacy (genera, families, orders, classes and phyla) on marine macroinvertebrates	Marine macroinvertebrates	Richness
Terlizzi et al. (2009)	Higher taxa	Test higher taxa surrogacy (genera, families, orders and classes) on marine molluscs	Marine molluscs	Richness, beta-diversity
Gaspar et al. (2010)	Higher taxa and cross taxa	Test taxonomic (order), colonization and trophic groups of arthropods as surrogates	Arthropods	Richness, alpha- and beta-dissimilarity diversity
Smale (2010)	Higher taxa, functional groups	Test higher taxa surrogacy (genera, families, orders and classes) on subtidal macroalgae assemblages	Macroalgae	Richness
Smale et al. (2010)	Higher taxa	Test higher taxa surrogacy (genera, families, orders and classes) on subtidal macroalgae	Macroalgae	Richness
Bevilacqua et al. (2012)	Higher taxa	Test higher taxa surrogacy (genera, families, orders and classes) on molluscs	Molluscs	Richness, diversity
Rosser and Eggleton (2012)	Higher taxa	Test higher taxa surrogacy (genera) on litter/soil insects	Litter/soil insects	Richness
Vieira et al. (2012)	Higher taxa and bioindicators	Test higher taxa surrogacy (genera and tribe) and test is effectiveness in prioritization of sites for conservation	Wasps	Richness, rarity, composition
Bhusal et al. (2014)	Higher taxa	Test higher taxa surrogacy (genera) on soil microfauna	Soil nematodes	Richness, alpha and beta diversity
Heino (2014)	Higher taxa	Test higher taxa surrogacy (genera and families) on stream macroinvertebrates, and environmental relationships, across regions	Stream macroinvertebrates	Richness

Terlizzi et al. (2014)	Higher taxa	Test higher taxa surrogacy (families) on molluscs	Molluscs	Richness, beta- and gamma-diversity
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Bryophytes

The term 'bryophytes' is a general name for plants characterized by a haplodiploid life cycle with a dominant gametophyte. They are divided in three phyla: Marchantiophyta (i.e. liverworts), Bryophyta (i.e. mosses), and Anthocerotophyta (i.e. hornworts) (Vanderpoorten & Goffinet. 2009). Bryophytes are very important ecologically. They have an important role in ecosystems, such as nutrient cycling, carbon fixing, water retention, biomass production, soil maintenance and recovery (Hallingback & Tan 2014; Sérgio et al. 2006).

Recently, bryophytes have achieved more attention for their biology and conservation (Hallingback & Tan 2014). In fact, some programs have been developed, as the Important Bryophyte Areas or "IbrA" (Papp 2008). These programs offer the possibility to protect and properly manage the priority conservation sites, but one of the basic requirements is that the design of such networks must be based on sound knowledge on species' distributions. Portugal holds 40% of European bryophytes, 45% of which are liverworts and 54% are mosses occurring in the Mediterranean area. In Portugal 704 *taxa* occur, of which 28.4% are threatened bryophytes (CR, EN E VU), and 4.3% are near threatened (NT) (Sérgio et al. 2013). Similarly, there are a number of international programs which aim is to identify and protect a network of the best sites for biodiversity conservation (e.g., <http://www.cbd.int>; <http://www.natura.org>). One of them is the Important Plant Areas (IPA) Program (<http://www.plantlife.org.uk>), focused on the identification of priority areas for wild plants, fungi and their habitats around the world and to ensure their long term survival. It offers guidelines to identify and protect regions with high diversity in habitats and species based on consistent criteria (Anderson 2002).

Identification to species level is rather difficult for bryophytes and requires very much time in laboratory (Mandl et al. 2010). To the best of our knowledge, higher taxa (i.e. genus-level) have never been tested as surrogates of bryophyte species.

Aims

The principal aim of the present thesis is to test the efficiency of genera as surrogates of overall species richness and composition of bryophytes.

The specific aims of the thesis are:

- Test if higher taxa (i.e. genera) could be used as surrogates of bryophyte species richness and composition in two different habitats and across two scales.
- Investigate if higher taxa (i.e. genera) could be used to select of important areas for bryophyte conservation.

Thesis outline

This thesis is organized in four chapters: General introduction (Chapter 1), two study cases (Chapters 2 and 3), and General conclusions (Chapter 4). Furthermore, Chapters 2 and 3 follow the organization of individual scientific articles (each with: Introduction, Material and methods, Results, Discussion and Literature cited):

Chapter 2: Alves C., Vieira C., Almeida R., Hespanhol H. Genera as surrogates for bryophyte species richness and composition. Submitted to Conservation Biology.

Chapter 3: Alves C., Vieira C., Stow S., Garcia C., Sérgio C., Almeida R., Hespanhol H. Selecting important areas for bryophyte conservation: is the higher taxa approach an effective method? Submitted to Biological Conservation.

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Chapter 2. Genera as surrogates of bryophyte species richness and composition

Abstract

Surrogates offer quick and cost-efficient solutions to assess and monitor biodiversity. These can be coarsely divided into environmental and taxonomic surrogates. The higher taxa approach consists in using higher taxonomic levels (i.e. genera and families) as surrogates of species. In this study we tested the higher taxa, particularly genera, as surrogates of bryophyte species richness and composition, in two different habitats – exposed rock outcrops, and watercourses –, and across two scales – local- and micro-scale –, in centre and northern Portugal. Furthermore, we tested the influence of environmental variables on richness and composition of species and genera. Our results showed significant and positive correlations between species and genera, for both habitats, for both richness and composition. Also, for the two scales analyzed, correlations between species and genera were found to be positive and significant. Moreover, the environmental variables tested seem to influence species and genera richness and composition in the same way. In conclusion, the higher taxa approach could be an effective method for a rapid assessment and monitoring of bryophytes in the study area, for the habitats and scales studied.

Keywords

Higher taxa; Scales; Environmental variables; Rock outcrops; Watercourses;

Introduction

Nowadays assessing and monitoring biodiversity is an important task in conservation biology, but species surveys are expensive, time consuming and experts needed (Mandelik et al. 2007). Recently, surrogates have become increasingly studied as a solution for monitoring habitats or ecosystems more quickly and detect changes in biodiversity (Mazaris et al. 2008) due to time and financial constraints. According with Olsgard et al. (2003) surrogates are attributes that correlate strongly with the number of species, but easier to assess.

Biodiversity surrogates can be coarsely divided in environmental variables and taxonomic surrogates (Grantham et al. 2010). Taxonomic surrogates include: (1) cross taxa – one group is used as surrogate of another group (Chiarucci et al. 2007); (2) morphospecies – separated based on morphological characters (Derraik et al. 2010); (3) higher taxa – the use of higher taxonomic levels (e.g. genera, families or orders) rather than species (Gaston & Williams 1993). These approaches should provide stronger associations when surrogates are phylogenetically related groups, as they will more likely share climatic requirements (Bergamini et al. 2005) – which in the case of the higher taxa approach, this is easily achieved.

Surrogacy is important in the context of monitoring assessments, and biodiversity conservation and management. For these purposes, some characteristics of the higher taxa approach are particularly relevant: (1) in the same location there are fewer genera than species; (2) identification of genera is less time-consuming than the identification of species, i.e. the sampling effort is lower for genera than for species; (3) the distribution of species within genera is relatively homogenous (i.e. most genera contain very few species, and only a few contain many species justifying the retention of biological significance) (Mandelik et al. 2007; Vieira et al. 2012c). Although higher taxa are not natural units, they tend to comprise species that are closely related and, therefore, can be used as parcels of species richness that are easier to analyze (Gaston 2000).

Higher taxa approach has been tested as an effective surrogate in many different groups, such as lichens (Bergamini et al. 2005), mammals (Grelle 2002), birds (Kallimanis et al. 2012), nematodes (Bhusal et al. 2014), macroinvertebrates, diatoms (Heino & Soininen 2007), macroalgae (Smale 2010), arthropods (Gaspar et al. 2010), molluscs (Terlizzi et al. 2009), spiders (*Araneae*) (Cardoso et al. 2004) and plants (Villaseñor et al. 2005), both in terrestrial and aquatic ecosystems. Nevertheless, to the best of our knowledge, this approach has never been previously studied for bryophytes.

In order to analyze overall biodiversity, species richness is an important measure to evaluate a given area (Bergamini et al. 2005), and many studies using the higher taxa approach tested the efficacy of this approach to provide surrogates of species richness in taxonomic groups, such as ants, beetles, flies, arachnids, angiosperms, birds, mammals, and mussels (Andersen 1995; Báldi 2003; Balmford et al. 1996; Doerries & Van Dover 2003). Nevertheless, few studies have tested the usefulness of the higher taxa approach to describe patterns of species composition in taxonomic groups, such as woody plants, macroalgae, and marine molluscs (Prinzing et al. 2003; Smale et al. 2010; Terlizzi et al. 2009).

According to previous studies, spatial scale is an important factor to be considered when establishing higher taxa approach because species richness and composition are scale dependent and the surrogacy could be affected by this variable (Gaspar et al. 2010; Mac Nally et al. 2004). The majority of the studies involving higher taxa are performed at regional to continental scales (Ferla et al. 2002; Gaspar et al. 2010; Hess et al. 2006; Larsen & Rahbek 2005; Mac Nally et al. 2004).

Some research on higher taxa approach has been done focusing mainly on the influence of environmental variables in determining genera and species richness and if this relation affects genera and species taxonomic levels in the same way (Cardoso et al. 2004; Heino & Soininen 2007; Hill et al. 2001; Vieira et al. 2012c). Some testing has been done for: environmental variables like chemical variables (i.e. pH), physical variables (i.e. current velocity) (Heino & Soininen 2007), geographical location (Cardoso et al. 2004), vegetation cover (Vieira et al. 2012c); in organisms such as macroinvertebrates, spiders, and wasps.

In this study we considered the following questions: (1) Can species richness of bryophytes be predicted by richness of genera? (2) Can the higher taxa approach reflect the difference of species composition in different locations? (3) How is the effectiveness of higher taxa approach affected at different typologies of habitats and scales? (4) How are species richness and composition influenced by environmental variables?

In order to tackle the previous questions, we tested if the higher taxa approach could be effectively used as a surrogate of patterns of bryophyte species richness and composition using two datasets collected from two different habitats – exposed rock outcrops and watercourses – in northern and central Portugal. Furthermore, we tested the influence of several environmental variables at the local-scale and micro-scale.

Methods

Datasets

Data from exposed rock outcrops was collected between March 2005 and April 2007 in northern and central Portugal (Hespanhol et al. 2010). A total of 99 localities were sampled in 11 Natura 2000 Network sites (Figure 2.1A). In each selected rock outcrop with an area of 100m². Bryophyte species growing in three types of microhabitats (rock surfaces, cavities and fissures) were recorded as present/absent. In total 705 sample plots were surveyed on granite, 190 on schist and 70 on calcareous

rock. Additionally, information on exposure and slope was collected for each sample plot (Table 2.1).

Data from watercourses was collected between March 2003 and October 2008 in northwest and centre – west Portugal (Vieira et al. 2012a). A total of 187 localities, corresponding to 100 meters watercourse segments were sampled, mostly in Natura 2000 Network sites (Figure 2.1B). In each segment, bryophyte species growing in three types of microhabitats (all immersed rocks, semi-immersed rocks and rocks in splash area) were recorded as present/absent. In total 614 sample plots were surveyed on granite, 165 on schist, and 29 on calcareous rocks. Additionally, information on exposure, slope, water flow velocity, sample plot position in relation to water surface, location of the sample plot in the river bed, rock surface roughness was collected for each sample plot (Table 2.1).

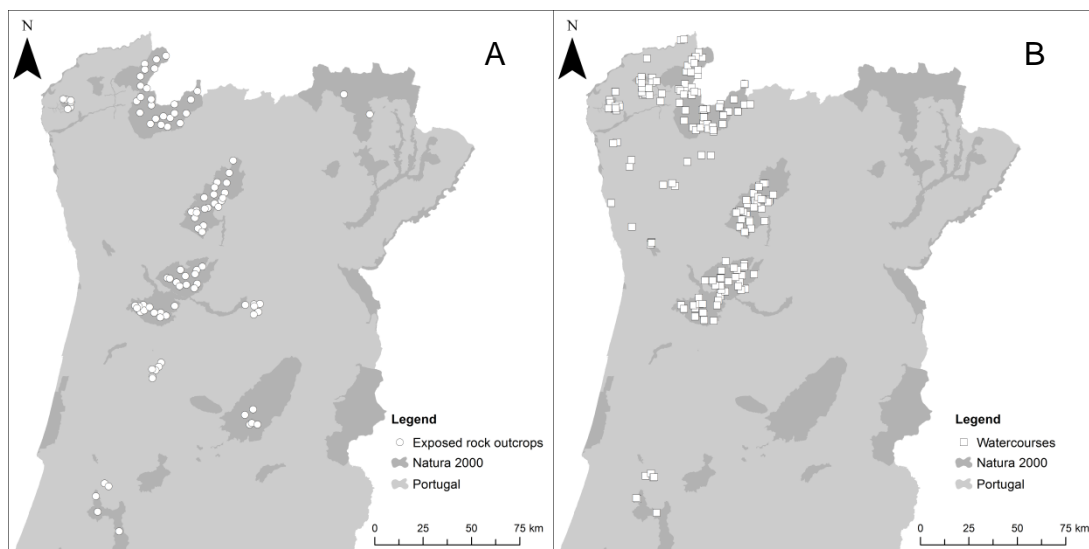


Figure 2.1 – Distribution of bryophyte survey localities for two habitats. A – exposed rock outcrops; B – watercourses.

Table 2.1 – Values assigned to the micro-environmental variables measured on exposed rock outcrops or watercourses.

Variables	Discriminated Classes
<i>For both habitats:</i>	
N-S: North-south component of exposure	(N) 1; (NE) 0.5; (E) 0; (SE) -0.5; (S) -1; (SW) -0.5; (W) 0; (NW) 0.5
E-W: East-west component of exposure	(N) 0; (NE) 0.5; (E) 1; (SE) 0.5; (S) 0; (SW) -0.5; (W) -1; (NW) -0.5
Slope	(unit = °C): (1) < 30°; (2) > 30° to < 50°; (3) > 50° to < 80°; (4) > 80° to 100°; (5) > -20° to < -60°
<i>For watercourses only:</i>	
Velocity: Water flow velocity	(unit = m/s): (1) emerged situation; (2) imperceptible or non-existent water flow; (3) splash area and unmeasurable water flow; (4) 0.01 to 0.09; (5) 0.1 to 0.3; (6) 0.4 to 0.6; (7) 0.7 to 0.9; (8) 1 to 1.5; (9) 1.6 to 2; (10) 2.1 to 2.5; (11) 2.6 to 3; (12) > 3

Position:	Sample plot	(unit = cm): (1) < -20; (2) -19 to -10; (3) -9 to 0; (4) 1 to 10; (5) 11 to 20; (6) 21 to 30; (7) 31 to 40; (8) 41 to 50; (9) 51 to 60; (10) 61 to 70; (11) > 71
Location:	Location of the sample plot in the river bed	(1) primary river bed; (2) secondary river bed; (3) margins
Roughness:	Rock surface roughness	(1) flat surface; (2) Low roughness surface with few grooves; (3) highly rough surface; (4) extremely rough surface

Data analyses

Several analyses were performed to evaluate if bryophyte genera were appropriate surrogates of patterns of bryophyte species richness and composition, using presence/absence data of species and genera from the two datasets described above.

Correlations between species richness and genera of bryophytes were tested using Spearman Correlation test in IBM SPSS v21 (IBM 2012). To test for a correlation between species and genera composition, the non-parametric Relate test, analogous to a Mantel Test was used within PRIMER v6 (Clarke & Gorley 2006). In this test Bray-Curtis similarity matrices were ranked and compared with Spearman Rank Correlation Coefficient.

At local-scale, to evaluate the influence of rock type (granite, schist and calcareous rocks) on species/genera richness, a Kruskal-Wallis analysis was used, since data did not meet the assumptions of normality and homoscedasticity. Mann-Whitney tests were performed to identify pairs of groups where these differences were significant. All these statistical analyses were done using IBM SPSS v21. To test whether species and genera composition differed among rock types we used Analysis of Similarities (ANOSIM) in PRIMER v6 (Clarke 1993). This method is analogous to a standard ANOVA for analysis of composition, and uses an *R* statistic to test if the data varies across groups. Firstly, a global test is performed to test null hypothesis that there are no differences between groups. If the null hypothesis is rejected, pairwise comparisons can give an absolute measure of how separate the groups are.

At micro-scale, to evaluate the influence of micro-environmental variables both on species and genera richness and composition, several statistical analyses were performed for each rock type (granite, schist and calcareous rocks). We tested the effects of environmental variables on species and genera richness using generalized linear models (GLM), with a Poisson distribution and logarithmic link function, since we used count data, with IBM SPSS v21. The generalized linear models expand the general linear model so that the dependent variable is linearly related to the factors and covariates via a specified link function, and allows for the dependent variable to have a

non-normal distribution. To test the influence of environmental variables on species and genera composition, a Detrended Correspondence Analysis (DCA) was used first to infer on ecological gradients from compositional data. Then, based on preliminary DCA we analyzed compositional data with Canonical Correspondence Analysis (CCA), in order to assess species and genera composition unimodal response to environmental variables. The statistical significance of the environmental variables was tested using a CCA forward selection procedure with Monte-Carlo permutation test (999 permutations). All multivariate analyses were performed using CANOCO v5 (Šmilauer & Lepš 2014).

Synthesis of all data analysis techniques used in this study are provided in Table 2.2.

Table 2.2 – Data analysis techniques used in this study.

	Species Richness	Composition
Local- and micro-scale	Spearman Correlation	Relate Test
Local-scale (variable tested: rock type)	Kruskal-wallis	Analysis of Similarities (ANOSIM)
Micro-scale (all micro- environmental variables tested for each rock type)	Generalized Linear Models (GLM)	Canonical Correspondence Analysis (CCA)

Results

Exposed rock outcrops

At local-scale, positive significant Spearman correlations were found between species and genera richness ($r = 0.92$; $n = 98$; $p < 0.05$). Relate test showed significant correlation between species and genera composition ($r = 0.861$; $n = 98$; $p < 0.05$). Kruskal-Wallis results showed significant differences ($p < 0.05$) in species and genera richness, among rock types. Mann-Whitney tests indicated very diverse pairwise differences between rock types; significant differences ($p < 0.05$) were found between granite and schist rocks, both for species and genera. No significant differences were found between schist and calcareous rocks, for neither species nor genera; and between granite and calcareous rocks, significant differences were found for species, whereas for genera no differences were found. ANOSIM analysis revealed that the differences in species composition among rock types were significant, both for species (Global $R = 0.473$, $p < 0.001$) and genera (Global $R = 0.359$, $p < 0.001$). Pairwise comparisons showed that schist and calcareous rocks, as well as granite and calcareous rocks were

different in species composition ($R > 0.75$), while granite and schist rocks were barely separable ($R < 0.25$).

At micro-scale, positive significant Spearman correlations were found between species and genera richness ($r \geq 0.933$; $p < 0.05$ for all rock types; $n_{granite} = 705$, $n_{schist} = 190$, $n_{calcareous} = 70$). Relate test showed significant correlation between species and genera composition ($r \geq 0.75$; $p < 0.05$ for all rock types; $n_{granite} = 705$, $n_{schist} = 190$, $n_{calcareous} = 70$).

GLM revealed that only microhabitat and exposure (N-S) influence both species and genera richness ($p < 0.05$), and only on granite and schist. For calcareous rocks, none of the variables seems to influence species and genera richness (Table 2.3.) In the CCA forward selection procedure, microhabitat and exposure (N-S) indicated statistically significant relationships with species and genera composition ($p < 0.05$ for all rock types). In addition, on granite and schist, slope and exposure (E-W) were also important factors for species and genera composition. In all cases congruence was found between species and genera.

Watercourses

At local-scale, the correlation between species and genera richness was positive and significant ($r = 0.848$; $n = 178$; $p < 0.05$). A significant correlation between species and genera composition was also found ($r = 0.775$; $n = 175$; $p < 0.05$). No significant differences in species and genera richness between rock types were shown by the Kruskal-Wallis test. ANOSIM analysis revealed a small but statistically significant separation between rock types, both for species (Global $R = 0.095$, $p < 0.001$) and genera (Global $R = 0.068$, $p < 0.001$). The dissimilarity in species composition is greatest between schist and calcareous rocks and granite and calcareous rocks ($R > 0.75$), whilst granite and schist were the least dissimilar in species composition ($R < 0.25$).

At micro-scale, the correlation between species and genera richness was positive and significant ($r = \geq 0.965$; $p < 0.05$ for all rock types; $n_{granite} = 614$, $n_{schist} = 165$, $n_{calcareous} = 29$). A significant correlation between species and genera composition was also found ($r \geq 0.752$; $p < 0.05$ for all rock types; $n_{granite} = 614$, $n_{schist} = 165$, $n_{calcareous} = 29$) (Table 1).

GLM revealed that, on granite, rock surface roughness influence both species and genera richness ($p < 0.05$) and exposure (N-S) influence only genera richness; on schist, only the position variable has a statistically significant influence on species and genera richness ($p < 0.05$). For calcareous rocks, none of the variables influence species and genera richness (Table 2.3). In the CCA forward selection procedure, microhabitat, slope and roughness indicated statistically significant relationships with species and

genera composition ($p < 0.05$ for all rock types). In addition, on granite, exposure (N-S), exposure (E-W), position and location were also important factors for species and genera composition; on schist, exposure (E-W), position and location influence both species and genera composition (Table 2.4). Generally, congruence was found between species and genera, with the exception of the richness in granites for the variable exposure (N-S).

Table 2.3 – Generalized linear models (GLM) for species and genera richness to test the influence of environmental variables among rock types for both datasets; (*) not applicable; Gr = granite; Sc = schist; Ca= calcareous rocks. Only significant results are shown.

Variables	Rock type	Exposed rock outcrops		Watercourses	
		Species	Genera	Species	Genera
Microhabitat (all microhabitats)	Gr	$p < 0.05$	$p < 0.05$		
	Sc	$p < 0.05$	$p < 0.05$		
	Ca				
Exposure (N-S)	Gr	$p < 0.05$	$p < 0.05$		$p < 0.05$
	Sc	$p < 0.05$	$p < 0.05$		
	Ca				
Exposure (E-W)	Gr				
	Sc				
	Ca				
Slope	Gr				
	Sc				
	Ca				
Velocity	Gr	*	*		
	Sc	*	*		
	Ca	*	*		
Position	Gr	*	*		
	Sc	*	*	$p < 0.05$	$p < 0.05$
	Ca	*	*		
Location	Gr	*	*		
	Sc	*	*		
	Ca	*	*		
Roughness	Gr	*	*	$p < 0.05$	$p < 0.05$
	Sc	*	*		
	Ca	*	*		

Table 2.4 – Canonical correspondence analysis (CCA) for species and genera composition to test the influence of environmental variables among rock types; (*) not applicable; Gr = granite; Sc = schist; Ca= calcareous rocks. Only significant results are shown.

Variables	Rock Type	Exposed rock outcrops		Watercourses	
		Species	Genera	Species	Genera
Microhabitat (all microhabitats)	Gr	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
	Sc	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
	Ca	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Exposure (N-S)	Gr	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Sc	$p<0.05$	$p<0.05$		
	Ca	$p<0.05$	$p<0.05$		
Exposure (E-W)	Gr	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Sc	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Ca				
Slope	Gr	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Sc	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$
	Ca			$p<0.05$	$p<0.05$
Velocity	Gr	*	*		
	Sc	*	*		
	Ca	*	*		
Position	Gr	*	*	$p<0.05$	$p<0.05$
	Sc	*	*	$p<0.05$	$p<0.05$
	Ca	*	*		
Location	Gr	*	*	$p<0.05$	$p<0.05$
	Sc	*	*	$p<0.05$	$p<0.05$
	Ca	*	*		
Roughness	Gr	*	*	$p<0.05$	$p<0.05$
	Sc	*	*	$p<0.05$	$p<0.05$
	Ca	*	*	$p<0.05$	$p<0.05$

Discussion

Our findings suggest that genus is a good surrogate of bryophyte species richness and composition in the studied habitats and study area. Our results showed significant correlations between species and genera richness and composition, indicating that genera can be used for that purpose in assessments at the scale of studied area. This is in congruence with other studies for different study areas, on other taxonomic groups (e.g. macrofungi, birds, macroinvertebrates and plants), for richness (Balmford et al. 2000; Gaston & Williams 1993; Heino & Soininen 2007; Villaseñor et al. 2005), as well as composition (e.g. lichens, macroinvertebrates and molluscs) (Bergamini et al. 2005; Heino & Soininen 2007; Terlizzi et al. 2009).

Our results show that habitat type does not have influence over the usefulness of higher taxa surrogacy at genus level, since significant correlations were found between species and genera richness for both habitats. Likewise, other studies – Cardoso et al. (2004), and Vieira et al. (2012c) – also tested higher taxa surrogacy in different habitat types, and no evidence of influence of habitat type was found.

Many studies have shown that, from regional to continental scales, higher taxa can be used as surrogates for species richness patterns (Ferla et al. 2002; Larsen &

Rahbek 2005; Mac Nally et al. 2004). At local-scale, this approach has been rarely studied for terrestrial ecosystems and more studies, for different taxonomic groups, are still needed, since general conclusions cannot be extrapolated from regional to local or micro-scales (Mandelik et al. 2007). In our case we studied the effectiveness of higher taxa approach at local- and micro-scales. Our results showed that higher taxa are an effective approach across local- and micro-scales. Smale (2010) reported good performance of surrogacy for subtidal macroalgae assemblages in South-west Australia, at the genus and family levels, at both small spatial scales (meters), and large spatial scales (hundreds of kilometers). By contrast, another study across different scales, (Gaspar et al. 2010), showed inconsistencies in surrogacy across the analysed scales (i.e., transect, fragment and island). As suggested in Heino (2014), within a small drainage basin, for macroinvertebrates, dispersal limitations should be negligible, leading to little to no spatial structuring of the community composition, and thus other factors should be influencing taxon diversity patterns in our case. In fact, in order to a biodiversity surrogate to be useful, it should reflect patterns of actual species turnover across multiple spatial scales, as the same scale-dependent processes should drive patterns of spatial variability for both species and the surrogate (Smale 2010).

According to Heino (2014), genus-level data should have a similar degree of community composition heterogeneity and similar environmental variation as the species-level data, if congeneric species have strictly similar response to the environmental variation, suggesting a high congruence between species and genera. At local-scale, in exposed rock outcrops, rock type influenced the patterns of bryophyte species and genera richness and composition. As expected, and already demonstrated in previous studies, rock type is an important factor influencing species richness and composition patterns in such habitat (Hespanhol et al. 2010). In watercourses, rock type only influenced species and genera composition. In general, these results showed that, at local-scale, species and genera richness and composition responded in a similar way to rock type.

For exposed rock outcrops, on granite and schist, only microhabitat and exposure (N-S) affected species and genera richness. This was in concordance with previous studies on species richness of bryophytes (Hespanhol et al. 2011). In the case of watercourses, sample plot position in relation to water surface (on schist) and rock surface roughness (on granite) were found to affect species and genera richness. The influence of microhabitat variables, particularly of the submersion level on species presence and richness was previously explored (Vieira et al. 2012a; Vieira et al. 2012b), with evidences of higher richness values in emerged levels and different patterns for

mosses and liverworts, the latter with a preference for lower depths. Only in the case of the variable N-S exposure, no concordance was shown between species and genera.

On the other hand, environmental variables influenced species and genera composition similarly. In exposed rock outcrops, microhabitat, exposure (N-S and E-W) and slope, affected species and genera composition on the different rock types. These micro-scale variables were already pointed out as important factors affecting species composition in such habitat (Hespanhol et al. 2011). Likewise, in watercourses common or correspondent environmental factors were found to be important to explain the taxon composition variation, namely, microhabitat, exposure (N-S and E-W), and slope. Although slope at the reach level has been proved to influence fluvial bryophytic communities composition (Suren 1996; Vieira et al. 2011), slope at the sample plot level has not been selected as an important variable in the referred studies, since in the referred studies, other macro and meso-scale variables superimpose in their ecological determination importance in ordination techniques or models. In addition, roughness, position and location of the sample plot in the river bed were also important factors species and genera composition. Location of the sample plot in the riverbed along a transversal gradient (margins to central zone with permanent flow) combines, in fact, the influence of several micro-scale variables (such as sample plot position, or flow turbulence and substrate stability) that are also frequently indicated as influent in communities' composition (Duncan et al. 1999; Suren & Duncan 1999).

In overview, on both habitats and at different scales, genera and species showed the same richness and composition patterns, which is in accordance with previously published works (Heino 2014; Warwick 1993), indicating that congeneric species may have strictly similar response to the environmental variation.

Our research indicates that genera could be used as a surrogate of bryophyte species. To our knowledge, this was the first study on higher taxa targeting both richness and composition of bryophytes, with the aim of understanding if this approach could be used with this group of organisms. In future, more studies are needed to attain a deeper knowledge of the use of surrogacy with bryophytes, such as using the family level, testing in other regions, habitats, and spatial scales, and comparing performance against other taxonomic groups. Nonetheless, the present work further supports the use of higher taxa approach (genera) in contexts such as conservation practice, biodiversity assessments and ecological monitoring for bryophytes.

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Chapter 3. Selecting important areas for bryophyte conservation: is the higher taxa approach an effective method?

Abstract

Surrogates have been used as support for conservation practices, since they are easier to assess and less time consuming than collecting species-level data. One of these surrogates is the higher taxa approach, i.e. the use of data with coarser taxonomic resolution than the species level, such as genus- and family-levels, as a surrogate for species richness. The aim of this work was to test if higher taxa (genera) could be used in the selection of important areas for bryophyte conservation, using three different approaches (Scoring, Important Plant Areas and Complementarity-based approaches). Our results showed that localities were ranked in a similar way using species or genera data, regardless of the methodology used. The complementarity-based approach in comparison with other methodologies protected a higher percentage of bryophyte species. In general, the three approaches selected the same areas as important areas for bryophyte conservation. Therefore, for the studied area and independently of the approach used, genera could be used in the selection of important areas for bryophyte conservation.

Keywords

Bryophytes richness; genera; complementarity; Important Plant Areas (IPA); Scoring;

Introduction

Today's challenge in conservation practice consists on the availability of complete datasets with information on species distribution that could be used for planning and management (Mandelik et al. 2007).

In recent years, surrogates (i.e. habitat, environmental, taxonomic surrogates) have been used as support of conservation practices because they are easier to assess and less time consuming (Gladstone & Alexander 2005).

Recently, the higher taxa-approach (i.e. the use of data with coarser taxonomic resolution than the species level, such as of genus- and family-levels as a surrogate for species richness) has been widely studied in terrestrial ecosystems (Balmford et al. 1996; Bergamini et al. 2005; Mandelik et al. 2007). The advantages of using these surrogates in biodiversity inventories are: (1) higher taxa (i.e. genera, families) are more easily identified than species; (2) time and cost associated with sampling and taxa identification is reduced when adopting the higher taxa approach; (3) more localities can be potentially surveyed when using higher taxa because it is less time-consuming (Gladstone & Alexander 2005).

For purposes of conservation, selection and design of reserves, surrogates have been tested in different habitats, for groups of flora and fauna and at different spatial scales (Balmford et al. 2000; Cardoso et al. 2004a; Gladstone & Alexander 2005; Guareschi et al. 2012; Larsen & Rahbek 2005; Mazaris et al. 2008; McMullan-Fisher et al. 2010). Surrogate data of finest possible geographical resolution are of the utmost importance for the selection of important areas, in order to give guidance for the identification of actual reserves on the field (Larsen & Rahbek 2005). Additionally, different underlying criteria, such as hotspots, complementarity of species or rarity (Margules et al. 1988; Vane-Wright et al. 1991) and irreplaceability (Ferrier et al. 2000) have been applied to identify a set of sites which maximize diversity conservation.

The most commonly approaches used in prioritization of areas important for conservation are scoring and complementarity-based approaches (Marignani & Blasi 2012).

Scoring procedures allow the establishment of one or several criteria (such as species richness, rarity or vulnerability) to rank sites in order of value or priority (Abellán et al. 2005). Some studies have tested this approach in terrestrial ecosystems, with spiders (Cardoso et al. 2004a), wasps (Vieira et al. 2012), and vertebrates (Mazaris et al. 2008).

Complementarity-based approaches also allow the selection of sites that represent all targeted biodiversity features altogether (Rodrigues & Brooks 2007). This approach minimizes the number of selected sites, necessary to represent the maximum number of species (Beger et al. 2003). The reason for success of this approach is the fact that sites complement one another biologically (Shokri & Gladstone 2009). Furthermore, this approach was widely studied across aquatic ecosystems (Beger et al. 2003; Shokri & Gladstone 2009), and terrestrial ecosystems (Cardoso et al. 2004a, b; Vieira et al. 2012).

Internationally, another approach commonly identified as Important Plant Areas (IPA) has been developed by *Plantlife International*, with purposes of identification and protection of a network of the best sites for plant conservation worldwide (Anderson 2002). This approach consists of three basic principles for selecting IPA: (1) the site needs to harbor significant populations of one or more species whose conservation is of global or European interest; (2) the site has an exceptionally rich flora in the European context in relation to its biogeographical zone; and (3) the site is an outstanding example of an habitat of interest for plant conservation, and of botanical interest at global or European level (Anderson 2002). This approach has been previously applied for bryophytes (Draper 2006; García-Fernández et al. 2010; Sérgio et al. 2012).

Species richness is one of many measures of diversity, and is used to evaluate the biodiversity of a site (Mazaris et al. 2008). Through species richness we can study the dynamics, spatial scale and temporal distribution of biodiversity. This biological component has been widely used for selection of important areas for conservation and for reserve networks design (Mazaris et al. 2008), but, to our knowledge, bryophyte genera richness have never been used to select areas for bryophyte conservation.

Bryophytes are usually unnoticed in conservation planning because of their small size, difficulty of identification and unrecognized levels of local diversity. However, their roles in ecosystems, contribution to overall biodiversity and potential biological resources, highlight their inclusion in conservation planning (McMullan-Fisher et al. 2010).

The aim of this study was to test if a higher taxa approach (at genus-level) could be used in the selection of areas for bryophyte conservation in the Peneda-Gêres National Park, using three different approaches for reserve selection (Scoring approach, Important Plant Areas, and Complementarity-based approach).

Methods

Study Area

The Peneda-Gêres National Park (PNPG) is the only National Park of Portugal with a total area of approximately 70,000 ha, with altitudes ranging from 50 to 1500 m (Figure 3.1). Despite the overall Atlantic climate PNPG has peculiar climatic conditions, from Rio Homem valley with thermophytic and humid conditions, to the high mountains

and interior with warm and heavy rainfall. Geologically, PNPG is dominated mainly by granites (Sérgio et al. 2012).

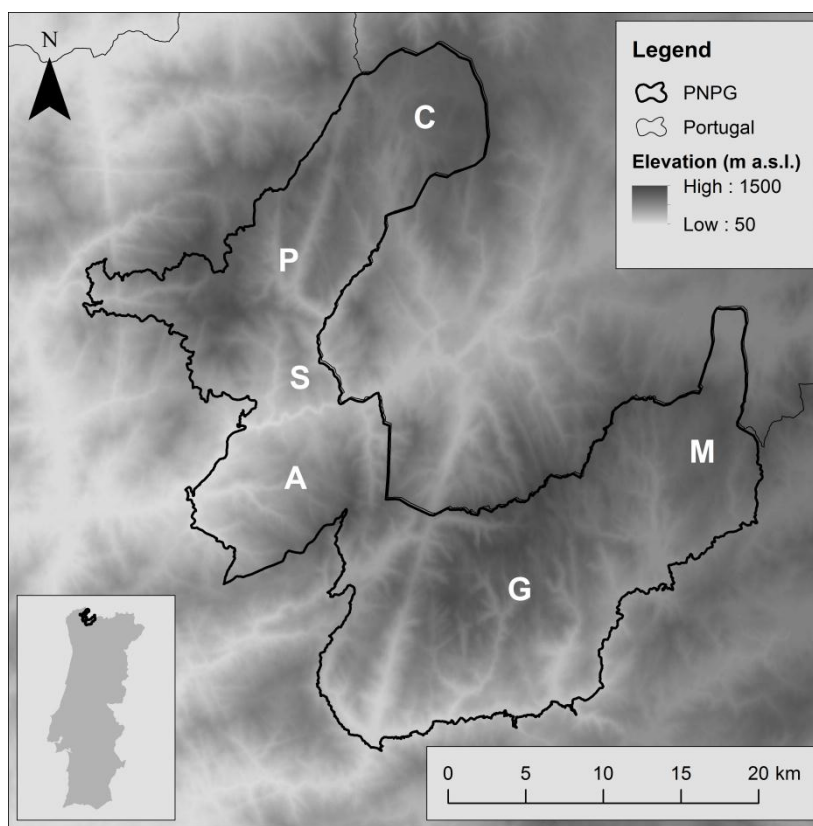


Figure 3.1 – Mountain areas comprising the Peneda-Gerês National Park (PNPG). Letters correspond to: C – Castro Laboreiro plateau; P – Peneda mountain; S – Soajo mountain; A – Amarela mountain; G – Gerês mountain; M – Mourela plateau.

Data source and recent survey

Pre-existent bryophyte data was taken from University of Lisbon (LISU) and Oporto (PO) herbaria. Additionally, a georeferenced bibliography-based dataset was used.

For each *taxon* a threat category was given, according to the Iberian Red List (Sérgio et al. 2006) and the Portuguese Red Data Book (Sérgio et al. 2013): critically endangered (CR); endangered (EN); near threatened (NT); low risk species which require special attention (LC-Att); species with insufficient data (DD and DD-n), species of low concern (LC) and vulnerable (VU).

All datasets' (herbarium, bibliographic) were georeferenced at 1×1 km scale (in MGRS UTM coordinates). For the records with insufficient information a cross-reference was made with herbarium specimens, and records without a precise indication of locality were not included (Sérgio et al. 2012).

Accumulation curves for both species and genera were performed with PRIMER v6 (Clarke & Gorley 2006) with 999 randomizations using all UTM squares, in order to summarise overall completeness of the sampling effort.

Data analysis

Scoring approach

We ranked UTM squares based on richness, from highest to lowest species and genera richness, respectively. This method corresponds to a 'scoring approach' (Cardoso et al. 2004a) or, alternatively, 'richness approach' (Vieira et al. 2012). We used IBM SPSS v.21 (IBM 2012) to calculate Spearman rank correlation in order to test the reliability of surrogacy between species and genera richness.

Important Plant Areas (IPA)

This approach was based on the methodology applied for the area of Murcia (García-Fernández et al. 2010) and in the Program of Plantlife International (Anderson 2002). Some changes were made, such as not including habitat quality, because the variations of habitat in PNPG are very high. In this study 3 criteria were used:

Criterion 1 (C1): based on the total number of species in each 1 km UTM, a richness class was attributed to each UTM: (1) 1 to 10 *taxa* - poor; (2) 11 to 50 *taxa* - moderately rich; (3) 51 to 100 *taxa* - rich; (4) more than 100 *taxa* - especially rich.

Criterion 2 (C2): based on the number of threatened bryophytes (CR, EN and VU), the value 1 is given for each of these bryophyte species present in each 1 Km UTM.

Criterion 3 (C3): based on the presence in each 1Km UTM of species of national and international importance (the value 1 is given), Habitat Directive (value 3 is given), and LC-Att or NT Red List categories (value 1 is given).

For each 1 km UTM an Area Importance Index was calculated. This was calculated by summing the values of the three criteria (C1+C2+C3). All UTM with this index equal to or greater than 9 were considered to be areas of importance for bryophytes (Sérgio et al. 2012). This sum was made for bryophyte species and, at same time, for genera. But in case of genera, the criteria 1 (C1) was based in genera richness, while the calculation of C2 and C3 used species information levels.

Complementarity-based approach

Using this more iterative approach, first we selected the UTM square with the highest species and genera richness, respectively and then in stepwise manner, the sites were selected according to the highest number of new species (i.e. the species that were not present in any of the previously selected UTM squares). This procedure was based on the algorithm described by Rebelo (1994), implemented in DIVA-GIS v7.5 software (Hijmans et al. 2012). Finally, we used species and genera accumulation curves to visualize the percentage of total bryophyte species that can be accounted for using the number of UTM squares that includes all genera.

Results

Genera and species richness

In the PNPG dataset 366 species, belonging to 155 genera, were found. Approximately 44.9% of the genera were represented by only one species in the dataset. The genera with the most species were *Bryum* and *Racomitrium*, with 13 species each (Figure 3.2).

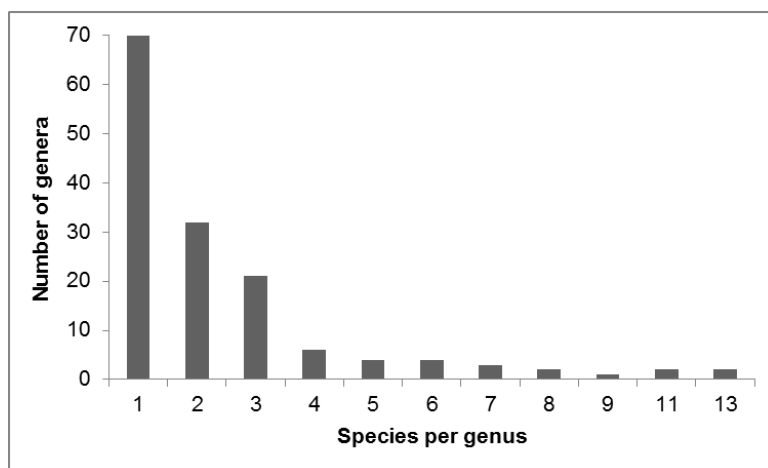


Figure 3.2 – Distribution of bryophyte species per genus.

Species and genera accumulation curves which shows the increase in the taxa observed with sampling effort, exhibited different patterns, since the genera curve reached an asymptote much earlier than the species curve (Figure 3.3).

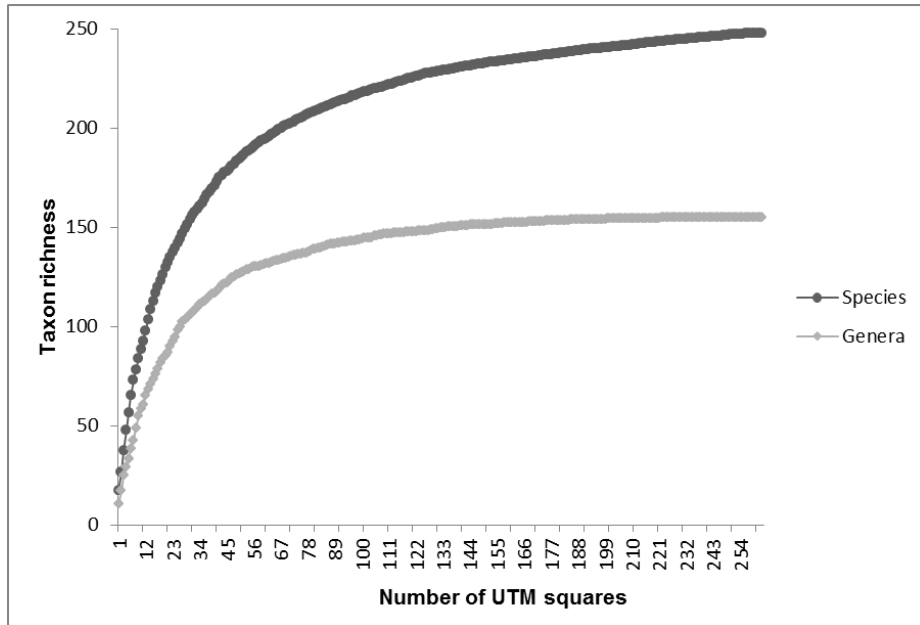


Figure 3.3 – Species and genera accumulation curves.

Reserve selection approaches

The scoring approach ranked UTM squares from highest to lowest based on richness (i.e. raw number of species). Species-level data and genus-level data were ranked in a very similar way, since a significant and positive correlation was found between species and genera richness (Figure 3.4; Appendix 1).

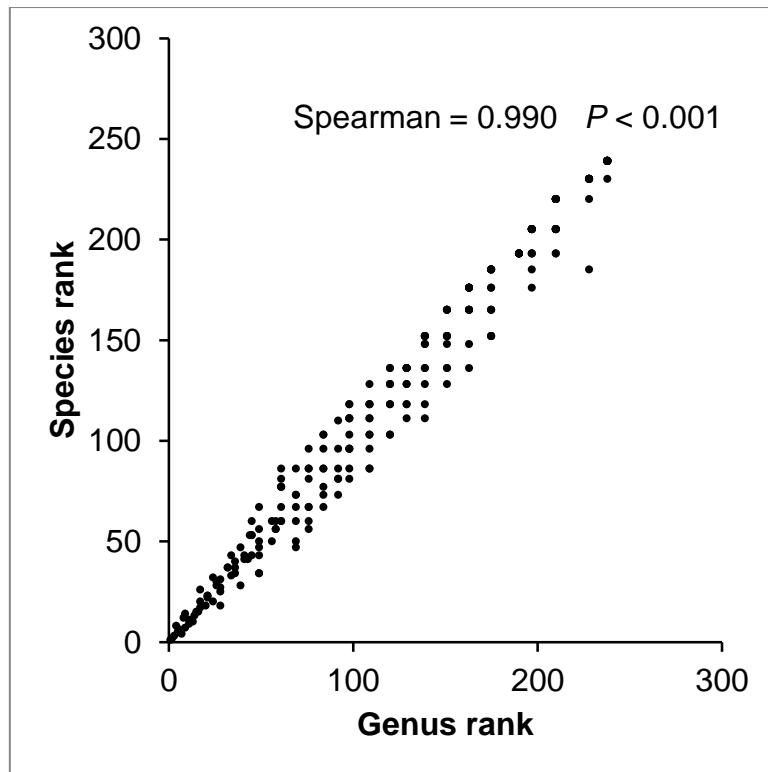


Figure 3.4 – Correlation between species and genus ranks.

Using important Plant Areas (IPA) methodology with species-level data, 24 UTM squares were selected to be important areas for bryophytes conservation, while using genus-level data 23 UTM squares were selected (Figure 3.5; Appendix 2). The important plant areas identified using genera data coincide with those areas selected using species data and are mainly located in Gerês mountain, particularly in Rio Homem valley and Caldas do Gerês, Peneda mountain, and Mourela plateau. When using genera information to select IPAs, 74.3% of the total bryophyte species would be included in those areas.

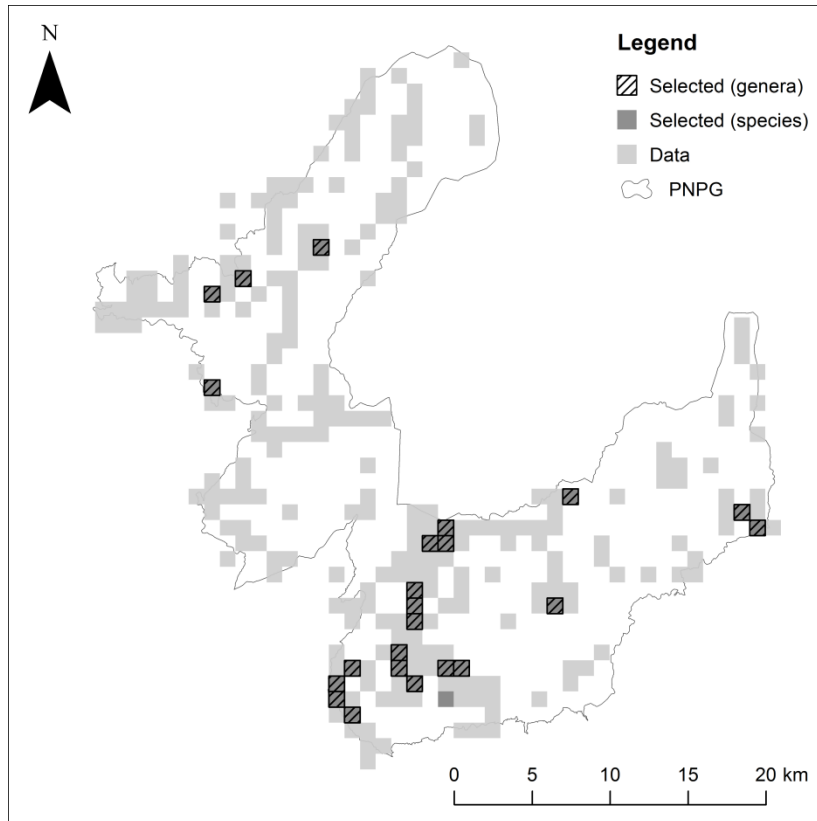


Figure 3.5 – Distribution of selected UTM squares using Important Plant Areas (IPA > 9).

The complementarity-based approach selected 42 UTM squares as important areas for bryophyte conservation using data at species-level, and 17 UTM squares at the genus-level data. Generally, the areas identified based on genera coincide with those areas selected using species data and are mainly located in Gerês mountain, particularly in Rio Homem valley and Caldas de Gerês, Peneda mountain and Mourela plateau (Figure 3.6, Appendix 2).

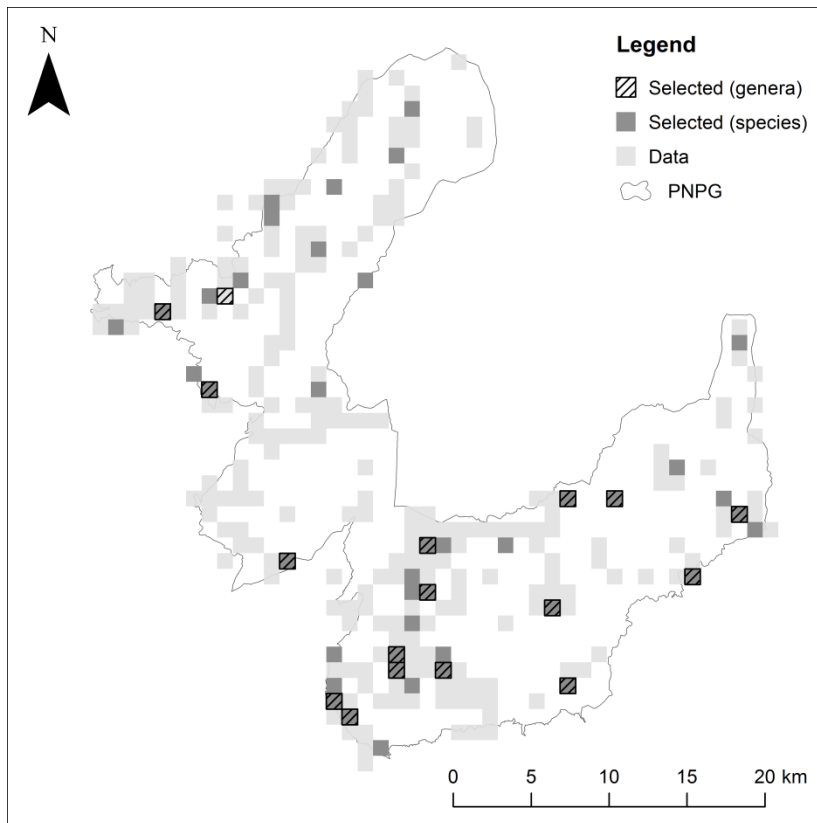


Figure 3.6 – Distribution of selected UTM squares using complementarity-based approach.

Species and genera accumulation curves revealed that the seventeen areas selected using genera data in the richness-based approach would be able to protect 91.8% of the total bryophyte species (Figure 3.7). In addition, when using the number of UTM squares that protects all genera 85.2% of threatened bryophytes would be included in those areas.

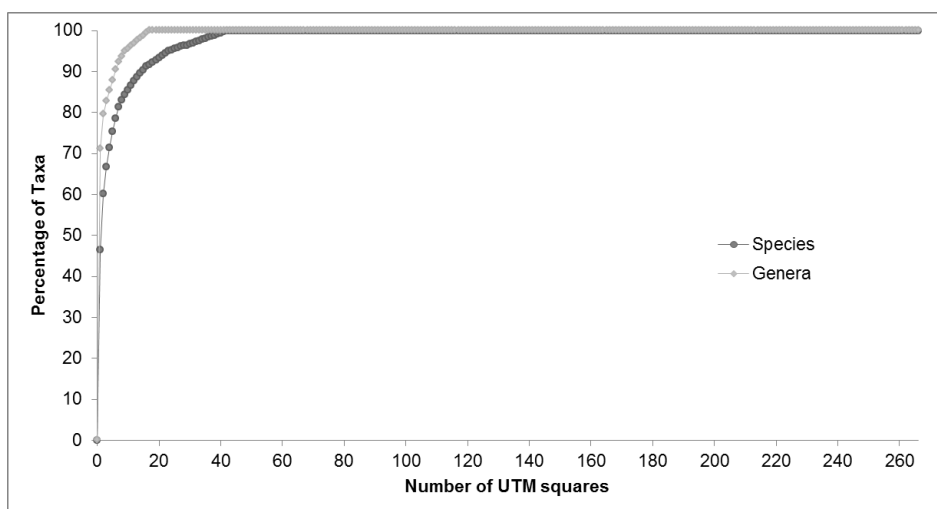


Figure 3.7 – Accumulation curves of the number of taxa, at the species- and genus-levels, represented by richness-based complementarity prioritization of UTM squares.

Discussion

Our three analyses suggest that higher taxa at genus-level could be used as a surrogate of bryophyte species richness, in the studied area and within the level of information of the dataset available, and applied in prioritization of sites for bryophyte conservation. This was in concordance with some other studies that tested this assumption in conservation biology using other taxonomic groups such as spiders, intertidal molluscs, rocky reef fishes and wasps (Cardoso et al. 2004a; Gladstone & Alexander 2005; Vieira et al. 2012). In addition, cumulative richness curves of species and genera indicated that there is a significant reduction in the sampling effort required for genus in relation to species assessments. Likewise, Bergamini et al. (2005) found the same results. Surprisingly, in other studies, sampling effort was similar for species and genera, mainly due to a high percentage of species-poor genera (Mandelik et al. 2007).

In general, regardless of the approach used, important areas selected in PNPG for bryophyte conservation are located mainly in Gerês mountain, Peneda mountain and Mourela plateau. These areas were already pointed out as vulnerable areas for bryophytes in an earlier study performed in PNPG (Sérgio et al. 2012).

In our research, the scoring approach showed that genera rank UTM squares in much the same way as species. Other studies showed similar results for other taxonomic groups such as spiders and wasps (Cardoso et al. 2004a; Vieira et al. 2012). Scoring approach has some advantages, since it is easy to perform, data needed is not complicated to obtain and does not need specific software (Abellán et al. 2005). However, this methodology has some disadvantages, such as subjectivity, lack of accountability and transparency. Also, this approach is very affected by sampling bias (Pressey & Nicholls 1989). In this study we did not use a threshold in scoring approach, as the aim was only to realize if this approach would rank all UTM squares in the same way for species and genera.

When using IPA approach, the UTM squares were ranked in a similar way using species or genera data. According to Sérgio et al. (2012), with IPA methodology, using only species data, we can protect a high number of bryophyte species, whilst also including the sensitive ones such as threatened species.

The complementarity-based approach is considered the most efficient method for finding the largest number of species that can be preserved when the number of sites allowed for protection is restricted (Abellán et al. 2005). Other studies have tested this methodology with higher taxa approach with encouraging results showing that using the

genus-level data also protects a high amount of species in other taxonomic groups such as macrofungi, fishes, invertebrates, plants and wasps (Balmford et al. 2000; Vanderklift et al. 1998; Vieira et al. 2012). On the other hand, van Jaarsveld et al. (1998) in the region of south Africa, using complementarity-based approach, found that using the higher taxonomic levels in the selection of important areas for conservation was inefficient and this approach didn't protect most of the species. Our results showed that the complementarity-based approach protects a higher amount of bryophyte species when compared to other methodologies such as IPA. Additionally, the prioritization of sites with complementarity-based approach was effective in protecting threatened bryophyte species. Therefore, it seems that the complementarity-based approach could be more efficient than other approaches when selecting areas important for conservation, as already pointed out by Abellán et al. (2005).

A study comparing IPA and complementarity-based approaches using only species and habitats richness was taken in Italy at a national scale (Marignani & Blasi 2012). Their results supported both IPA and complementarity-based approaches. In addition, it was suggested that both approaches should be combined in order to select areas important for conservation, since it would optimize the results and locate areas of highest importance for conservation. They also advocated that the focus of conservation efforts should be in several small reserves with high habitat quality, rather than in few large ones (Marignani & Blasi 2012).

Bergamini et al. (2005) debated the problem of taxonomic changes, in case of the lichen genera. This could be a problem for the applicability of genera as a surrogate of species richness, as it could hinder the effectiveness of genera as a surrogate of bryophyte species. As suggested by Bergamini et al. (2005), after major taxonomic changes, the relationship between genera and species needs to be reevaluated.

In conclusion, our results indicate that genus surrogacy could be a useful method to define a conservation priority sites network for bryophytes in PNPG, either if we apply a simple scoring approach, the IPA methodology or a much more efficient iterative approach such as the complementarity-based approach to the problem of sites ranking. Additionally, genus surrogacy can be a valuable method for conservation decision-making, especially when there are time and financial constraints. Nevertheless, more studies are needed in different regions and ecosystems and also at larger scales to test the effectiveness of genus surrogacy to select important areas for bryophyte conservation.

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Chapter 4. General conclusions

In the first study (Chapter 2), we analyzed the use of genera as surrogates of bryophyte species in two different habitats (i.e. rock exposed outcrops and watercourses), and across two scales (i.e. local-scale and micro-scale), in centre and northern Portugal. We found that genera perform well as surrogates of bryophyte species in both habitats. In addition, independently of the scale and for different rock types (schist, granite and calcareous rocks), genera could be successfully used for describing bryophyte species richness and composition. Our results showed that species and genera were influenced by the environmental variables tested in a similar way. For both rock exposed outcrops and watercourses, the main variables that influenced the distribution of species richness and composition were microhabitat, slope, and exposure (both N-S and E-W). Particularly in watercourses, other variables more specific of this habitat were found to be important factors influencing the distribution of bryophyte species richness and composition, such as sample plot position in relation to water surface, location of the sample plot in the river bed and rock surface roughness.

In the second study (Chapter 3), using genera as surrogates of bryophyte species, we compared three approaches (i.e., scoring, IPA and complementarity-based approaches) to select important areas for bryophyte conservation, in the Peneda-Gerês National Park (PNPG). We found that the use of genus-level information is an effective method to select areas for bryophyte conservation in PNPG, and that all three approaches ranked the areas selected by species and genera in a similar way. Moreover, the spatial distribution of the areas selected by the three approaches was very similar. Furthermore, we found that the complementarity-based approach protects a higher number of species with the genus-level data, while at the same time protecting threatened bryophytes, when comparing to other methodologies.

Based on the results of both studies presented here, we conclude that genera can be used as effective surrogates of bryophyte species and, therefore, this approach can be considered an important tool for the assessment and monitoring of bryophyte diversity.

In future researches, important factors should be taken into account that could influence the effectiveness of the higher taxa approach, such as taxonomic changes, which could affect the relationship between species and higher taxa. Moreover, our

results should not be extrapolated for other taxonomic groups, ecosystems, or biomes, hence the need for other studies in other contexts.

This thesis could provide a basis for future studies. Other approaches using surrogates, such as cross taxa, morphospecies, and bioindicators, could be used in comparison with higher taxa for a comprehensive understanding of surrogacy with bryophytes. On the other hand, comparison with other taxonomic groups could allow a better evaluation of the performance of each group to be used as surrogates. Moreover, guidelines could be established for the assessment and monitoring of bryophyte species using genus surrogacy. On a more methodological remark, modelling techniques could be employed in order to allow for more spatially explicit approaches, for purposes of not only studying surrogacy with bryophytes, but also selecting important areas for conservation of this taxonomic group. Finally, different approaches for the prioritization of areas for conservation, such as IPA and Complementarity-based approach, could be combined in order to allow for better optimization of both available resources and target taxa to be protected.

Appendix 1

Taxa richness of the sampled UTM squares and respective ranking according to the scoring approach.

UTM	Richness		Rank		UTM				
	Species	Genera	Species	Genus					
NG6919	170	111	1	1	NG6536	24	16	56	76
NG6920	156	99	2	2	NG9139	24	21	56	49
NG7227	119	79	3	3	NG5141	23	16	60	76
NG7127	98	65	4	7	NG6125	23	17	60	69
NG6616	93	67	5	5	NG6134	23	22	60	45
NG7022	92	66	6	6	NG6820	23	18	60	61
NG7024	91	55	7	9	NG6950	23	19	60	58
NG8030	90	68	8	4	NG6952	23	20	60	56
NG7219	89	54	9	11	NG7622	23	18	60	61
NG5743	82	52	10	13	NG5243	22	16	67	76
NG6446	81	54	11	11	NG5544	22	17	67	69
NG6517	79	58	12	8	NG6535	22	21	67	49
NG9228	69	50	13	14	NG6732	22	15	67	84
NG7023	67	55	14	9	NG7628	22	18	67	61
NG7217	66	49	15	15	NG8633	22	16	67	76
NG9129	66	45	15	16	NG6438	21	17	73	69
NG7228	59	41	17	17	NG6729	21	14	73	92
NG5944	55	32	18	28	NG6848	21	15	73	84
NG7018	55	39	18	20	NG9141	21	17	73	69
NG5737	54	41	20	17	NG6126	20	18	77	61
NG7923	54	34	20	24	NG6144	20	15	77	84
NG7021	50	37	22	21	NG8631	20	18	77	61
NG7124	49	37	23	21	NG8632	20	18	77	61
NG7319	49	37	23	21	NG6234	19	14	81	92
NG7025	48	32	25	28	NG6249	19	16	81	76
NG6518	47	41	26	17	NG6714	19	18	81	61
NG6619	44	32	27	28	NG6922	19	14	81	92
NG7020	41	33	28	26	NG7925	19	13	81	98
NG7528	41	26	28	39	NG5242	18	16	86	76
NG6635	40	33	30	26	NG5945	18	16	86	76
NG7428	39	32	31	28	NG6347	18	15	86	84
NG6519	38	34	32	24	NG6825	18	14	86	92
NG8732	36	28	33	34	NG6849	18	13	86	98
NG7518	33	21	34	49	NG6917	18	15	86	84
NG7929	33	21	34	49	NG6925	18	17	86	69
NG9328	33	27	34	36	NG7125	18	18	86	61
NG6520	32	29	37	32	NG7823	18	12	86	109
NG7122	32	29	37	32	NG8525	18	12	86	109
NG8825	32	27	37	36	NG6346	17	16	96	76
NG6146	31	27	40	36	NG6350	17	12	96	109
NG6133	30	25	41	41	NG6654	17	13	96	98
NG7627	30	24	41	43	NG6730	17	13	96	98
NG6139	29	22	43	45	NG6949	17	14	96	92
NG7129	29	21	43	49	NG7318	17	15	96	84
NG7328	29	25	43	41	NG8220	17	13	96	98
NG7417	29	28	43	34	NG5830	16	11	103	120
NG5543	28	21	47	49	NG5931	16	11	103	120
NG7323	28	17	47	69	NG6615	16	15	103	84
NG9236	28	26	47	39	NG6655	16	15	103	84
NG5638	26	21	50	49	NG6724	16	12	103	109
NG6148	26	17	50	69	NG6926	16	12	103	109
NG9229	26	20	50	56	NG7415	16	13	103	98
NG5843	25	22	53	45	NG6035	15	14	110	92
NG6447	25	22	53	45	NG6027	14	12	111	109
NG7019	25	23	53	44	NG6617	14	13	111	98
NG6034	24	19	56	58	NG6744	14	10	111	129
NG6136	24	19	56	58	NG6747	14	13	111	98
					NG6954	14	13	111	98
					NG7930	14	9	111	139
					NG9036	14	12	111	109

NG6149	13	11	118	120	NG5630	5	5	193	190
NG6242	13	12	118	109	NG6241	5	5	193	190
NG6435	13	13	118	98	NG6345	5	3	193	210
NG6550	13	12	118	109	NG6623	5	5	193	190
NG6653	13	12	118	109	NG6757	5	4	193	197
NG7027	13	13	118	98	NG7029	5	5	193	190
NG7054	13	10	118	129	NG7358	5	4	193	197
NG7515	13	11	118	120	NG7418	5	4	193	197
NG8023	13	10	118	129	NG7516	5	5	193	190
NG8731	13	9	118	139	NG7728	5	5	193	190
NG6243	12	10	128	129	NG7824	5	3	193	210
NG6814	12	10	128	129	NG9234	5	5	193	190
NG6948	12	12	128	109	NG5343	4	3	205	210
NG6953	12	11	128	120	NG5542	4	3	205	210
NG6957	12	8	128	151	NG5928	4	4	205	197
NG7128	12	11	128	120	NG6038	4	3	205	210
NG7454	12	9	128	139	NG6140	4	4	205	197
NG8024	12	11	128	120	NG6229	4	4	205	197
NG5730	11	11	136	120	NG6336	4	4	205	197
NG5930	11	11	136	120	NG6822	4	3	205	210
NG6043	11	10	136	129	NG7028	4	4	205	197
NG6437	11	8	136	151	NG7056	4	4	205	197
NG6921	11	9	136	139	NG7223	4	3	205	210
NG7053	11	8	136	151	NG7928	4	3	205	210
NG7055	11	7	136	163	NG8119	4	4	205	197
NG7226	11	10	136	129	NG8325	4	4	205	197
NG7817	11	9	136	139	NG9029	4	3	205	210
NG8018	11	10	136	129	NG5241	3	3	220	210
NG8826	11	10	136	129	NG5344	3	3	220	210
NG9140	11	10	136	129	NG5731	3	3	220	210
NG6226	10	9	148	139	NG5742	3	2	220	228
NG7317	10	8	148	151	NG5849	3	3	220	210
NG8226	10	9	148	139	NG6719	3	3	220	210
NG9238	10	7	148	163	NG6918	3	3	220	210
NG5041	9	9	152	139	NG7051	3	3	220	210
NG5729	9	8	152	151	NG7220	3	3	220	210
NG5836	9	8	152	151	NG9035	3	3	220	210
NG6030	9	8	152	151	NG5342	2	2	230	228
NG6240	9	6	152	175	NG5826	2	2	230	228
NG6434	9	8	152	151	NG5844	2	2	230	228
NG6452	9	6	152	175	NG5942	2	1	230	238
NG6646	9	9	152	139	NG6244	2	2	230	228
NG6652	9	9	152	139	NG6445	2	2	230	228
NG7453	9	6	152	175	NG6755	2	2	230	228
NG7525	9	9	152	139	NG7218	2	2	230	228
NG7924	9	6	152	175	NG7324	2	2	230	228
NG8727	9	9	152	139	NG5042	1	1	239	238
NG5244	8	7	165	163	NG5545	1	1	239	238
NG6037	8	7	165	163	NG5845	1	1	239	238
NG6516	8	8	165	151	NG5847	1	1	239	238
NG6523	8	7	165	163	NG5927	1	1	239	238
NG6718	8	6	165	175	NG5932	1	1	239	238
NG6735	8	7	165	163	NG6049	1	1	239	238
NG6817	8	8	165	151	NG6147	1	1	239	238
NG7026	8	6	165	175	NG6436	1	1	239	238
NG7126	8	8	165	151	NG6525	1	1	239	238
NG7827	8	6	165	175	NG6622	1	1	239	238
NG8330	8	8	165	151	NG6713	1	1	239	238
NG5442	7	7	176	163	NG6715	1	1	239	238
NG5828	7	4	176	197	NG7119	1	1	239	238
NG6250	7	7	176	163	NG7120	1	1	239	238
NG6334	7	7	176	163	NG7315	1	1	239	238
NG6723	7	6	176	175	NG7325	1	1	239	238
NG6835	7	7	176	163	NG7327	1	1	239	238
NG7828	7	6	176	175	NG7517	1	1	239	238
NG9030	7	7	176	163	NG7830	1	1	239	238
NG9230	7	7	176	163	NG7926	1	1	239	238
NG5142	6	6	185	175	NG8227	1	1	239	238
NG5736	6	4	185	197	NG8725	1	1	239	238
NG6150	6	6	185	175	NG8932	1	1	239	238
NG6554	6	2	185	228	NG9028	1	1	239	238
NG6629	6	6	185	175					
NG6756	6	6	185	175					
NG7017	6	6	185	175					
NG8019	6	6	185	175					

Appendix 2

Selected UTM squares for both species and genera according to IPA.

C1 – (1) 1 to 10 taxa - poor; (2) 11 to 50 taxa - moderately rich; (3) 51 to 100 taxa - rich; (4) more than 100 taxa - especially rich; **C2** – number of threatened bryophytes (CR, EN and VU) in each 1 km UTM, for each bryophyte species the value 1 is given; **C3** – species of national and international importance (the value 1 is given), Habitat Directive (value 3 is given), and LC-Att or NT Red List categories (value 1 is given) in each 1 km UTM. **IPA** – sum of the values of the three criteria (C1+C2+C3).

UTM	Species	C1	C2	C3	IPA	UTM	Genera	C1	C2	C3	IPA
NG6920	156	4	17	11	32	NG6920	99	3	17	11	31
NG6919	170	4	15	10	29	NG6919	111	4	15	10	29
NG7227	119	4	12	10	26	NG7227	79	3	12	10	25
NG6616	93	3	8	12	23	NG6616	67	3	8	12	23
NG6517	79	3	7	11	21	NG6517	58	3	7	11	21
NG7024	91	3	9	7	19	NG7024	55	3	9	7	19
NG7127	98	3	7	9	19	NG7127	65	3	7	9	19
NG7022	92	3	8	7	18	NG7022	66	3	8	7	18
NG9228	69	3	4	8	15	NG5743	52	3	3	8	14
NG5743	82	3	3	8	14	NG7023	55	3	3	8	14
NG6446	81	3	4	7	14	NG8030	68	3	7	4	14
NG7023	67	3	3	8	14	NG9228	50	2	4	8	14
NG7228	59	3	6	5	14	NG6446	54	2	4	7	13
NG8030	90	3	7	4	14	NG7219	54	3	2	8	13
NG9129	66	3	3	8	14	NG7228	41	2	6	5	13
NG7018	55	3	7	3	13	NG9129	45	2	3	8	13
NG7219	89	3	2	8	13	NG7018	39	2	7	3	12
NG5737	54	3	2	6	11	NG5737	41	2	2	6	10
NG7923	54	3	5	3	11	NG6518	41	2	3	5	10
NG5944	55	3	3	4	10	NG7923	34	2	5	3	10
NG6518	47	2	3	5	10	NG5944	32	2	3	4	9
NG6619	44	2	1	6	9	NG6619	32	2	1	6	9
NG7217	66	3	4	2	9	NG7319	37	2	3	4	9
NG7319	49	2	3	4	9						

Appendix 3

Taxa richness of the sampled UTM squares and respective ranking according to the complementary-based approach.

Sequence – indicates the order of UTM squares selected.

Classes – indicates how many different classes (species or genera) are in each selected UTM.

Additionally Classes – indicates how many new classes (species or genera) are in each UTM. These species were not present in any of the previously selected UTM squares.

UTM	Sequence (Species)	Classes (Species)	Additional Classes (Species)				
				NG6148	27	26	1
				NG6446	28	81	1
				NG6744	29	14	1
NG6919	1	170	170	NG5141	30	23	1
NG6920	2	156	50	NG9140	31	11	1
NG7219	3	89	24	NG5442	32	7	1
NG7024	4	91	17	NG6437	33	11	1
NG6517	5	79	14	NG8732	34	36	1
NG9129	6	66	12	NG7055	35	11	1
NG8030	7	90	10	NG6226	36	10	1
NG5944	8	55	6	NG7124	37	49	1
NG7627	9	30	5	NG6520	38	32	1
NG5743	10	82	4	NG7220	39	3	1
NG5737	11	54	4	NG6518	40	47	1
NG7127	12	98	4	NG7018	41	55	1
NG8018	13	11	4	NG6814	42	12	1
NG8330	14	8	3				
NG7923	15	54	3				
NG6616	16	93	3				
NG5638	17	26	2				
NG9030	18	7	2				
NG9228	19	69	2				
NG7227	20	119	2				
NG7025	21	48	2				
NG8825	22	32	2				
NG7022	23	92	2				
NG6952	24	23	1				
NG6550	25	13	1				
NG6149	26	13	1				

UTM	Sequence (Genera)	Classes (Genera)	Additional Classes (Genera)
NG6919	1	111	111
NG6920	2	99	13
NG7127	3	65	5
NG5737	4	41	4
NG8030	5	68	4
NG7923	6	34	4
NG8330	7	8	3
NG8018	8	10	2
NG6517	9	58	2
NG5442	10	7	1
NG5843	11	22	1
NG9129	12	45	1
NG6226	13	9	1
NG8825	14	27	1
NG7124	15	37	1
NG7219	16	54	1
NG6616	17	67	1