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# Effect of old-growth forest attributes on lichen species abundances: a study performed within Ceahlău National Park (Romania)

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**Abstract** – This study is based on finding a representative pattern of interactions between old-growth forest attributes and lichen species abundances. The field activities were performed within Ceahlău National Park, Neamț County, North-Eastern Romania. In total 38 sampling units of 10 by 10 m were randomly selected, within these all phorophytes with circumferences that exceed 70 cm were selected. On each phorophyte, a frame of 20 by 20 cm was randomly fixed and all lichen specimens included were sampled. Relationships between macrovariables, microvariables and response variables were tested using the Kendall rank order correlation coefficient. To explain the influence of macrovariables and microvariables on response variables, polynomial regression was used.The results indicated that lichen species abundances are significantly influenced by macrovariables such as herb coverage, altitude, slope, multilayered canopies and host phorophyte diversity.With regard to the microvariables, the aspect, rhytidome crevice depth and circumferences of host phorophytes have a significant influence on lichen species abundances. The output of the regression analysis highlighted significant relationships among macrovariables and lichen species abundances. The multilayered canopies and host phorophytes diversity are of interest as predictors which explain lichen species diversity. Of all microvariables, only the rhytidome crevice depth plays an important role as a predictor of lichen species abundances. The main conclusion is based on the identification of the old-growth forest attributes which significantly affect the lichen species abundances and uses them to predict the conservation level of forests of Ceahlău National Park.

**forest conservation / macrovariables / microvariables / multi-layered canopies / phorophyte diversity / red list**

## INTRODUCTION

The conservation value of an ecosystem can be indicated by lichen species abundances due to their importance as indicators of the environmental conditions (Das *et al*., 2013). Factors which have an important role on the distribution of lichen species include the historical and contemporary density of the habitats, habitat connectivity (Ranius *et al*., 2008) geographical factors (aspect), physical factors, e.g. light, temperature and humidity (Çobanoğlu & Sevgi, 2009) and biotic factors such as phorophyte diversity (Vicol, 2015).

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Previous work on the ecology of lichen species abundance and environmental drivers has highlighted increased abundance as a function of factors related to the trees such as higher circumferences (Rolstad & Rolstad, 1999; Boudreault *et al*., 2009) and trunk morphology (Johansson *et al*., 2013; Perhans *et al*., 2014). At stand level the complexity of the forest structure is an important driver which significantly affects lichen species (Rheault *et al*., 2003). Large phorophytes and a complex forest structure are relevant old-growth forest attributes (Calamini *et al*., 2011). In addition, forest age, multilayered canopies, bark characteristics, humidity, light, and competition affect the forest lichen community (Will-Wolf *et al*., 2002; Armstrong & Welch, 2007; Seaward, 2008). On large phorophytes, humidity, light and rhytidome morphology greatly affect lichen colonisation (Kantvilas & Jarman, 2004).

The purpose of the present project was to improve knowledge of the ecological pattern of lichen synusiae as an important contribution to the conservation of lichen species and their phorophytes. The hypothesis tested in this study is that in natural conditions lichen species abundances are significantly influenced by the attributes of the investigated old-growth forests. Thus, the following questions are of interest: (a) are there any old-growth attributes with significant effects on lichen species abundances? and (b) could lichen species abundances be used to predict the importance of phorophyte species diversity in a biodiversity conservation context? The main objective of this research is based within a context of integrated forest conservation with a particular interest in how old-growth forest attributes affect lichen species abundances.

## MATERIALS AND METHODS

*Studied area.* – The Ceahlău Massif is located in the central part of the Oriental Carpathians (Fig. 1) and covers an area of 290 km$^2$. The field researches were performed within Ceahlău National Park, which represents 26.5% (7742.5 ha) of the overall area of the Ceahlău Massif (Comănescu & Dobre, 2009). Geologically, Ceahlău Mountain comprises internal flysch that belongs to the Moldavian Carpathians. The flysch zone is composed of detrital rock, basalts and granites (Manoliu *et al*., 1998; Comănescu & Dobre, 2009). The highest peak is Ocolașul Mare (1907 m), followed by Toaca Peak which attains 1900 m (Manoliu *et al.*, 1998).

The climate is temperate-moderate-continental, the annual mean temperature ranges between 0.7°C and 7.2°C, the precipitation exceeds 700 mm and the prevailing winds are from the west, blowing up to 6-8 m/s in summer and 12-14 m/s in winter (Manoliu *et al*., 1998).

*Field survey.* – Field activities were performed during May-September 2014. Within the studied area, random sampling was performed based on a 10 × 10 m unit in which all phorophytes with a circumference more than 70 cm (as an important old-growth attribute) were tested (Prigodina-Lukošienė & Naujalis, 2006). Thus, a total of 38 sampling units of 10 × 10 m were randomly selected. A frame of 20 × 20 cm was hung on selected phorophytes at 1 m above the ground. Within each sampling unit of 20 by 20 cm all individuals which belong to identified lichen species were counted. A total of 50 sampling units of 20 × 20 cm were investigated.

The crevice depth of the rhytidome was measured (cm) using a ruler for the tested phorophytes. In total 50 phorophytes were sampled *i.e*. *Picea abies* (L.)

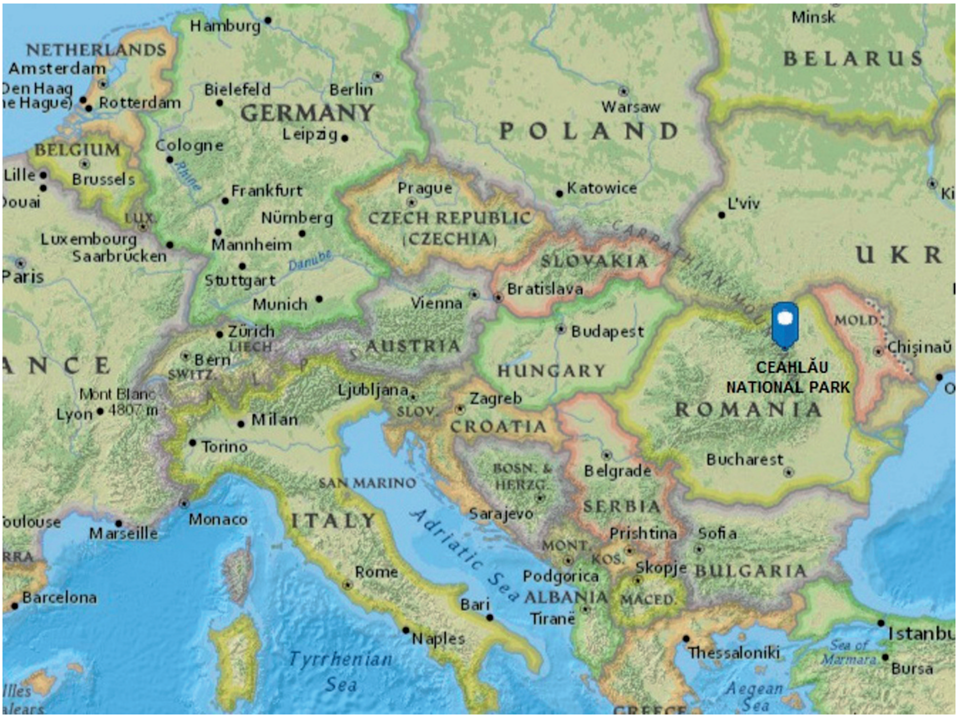


Fig. 1. Geographical position of Ceahlău National Park.

H. Karst (31 trees), *Abies alba* Mill. (7 trees), *Larix decidua* Mill. (1 tree), *Fagus sylvatica* L. (9 trees) and *Acer pseudoplatanus* L. (2 trees).

Within each 10 × 10 m sampling unit, the following macrovariables were examined: altitude, coverage of herbs, subarbuscles and arbuscles, canopy openness, host phorophytes and slope. The percentage cover of herbs, subarbuscles and arbuscles was estimated according to the Braun-Blanquet cover abundance scale (Wikum & Shanholtzer, 1978). Canopy openness was assessed according to Mistry & Berardi (2005) based on the following scale: 1 = low canopy interleaved, 2 = moderate canopy interleaved and 3 = strongly canopy interleaved. As regard microvariables (phorophyte level variables), were considered following: phorophyte circumferences, aspect and crevice depth.

Lichen species abundances were evaluated as absolute abundances *i.e*. the total number of specimens counted within the 20 by 20 cm frame.

*Laboratory study.* – All identified lichen species were determined using a stereomicroscope (Zeizz Stereo CL 1500 ECO), and an optical microscope (Zeizz Scope A1). The determination of lichen species which had not been identified in the field was performed according to Ciurchea (2004). The following chemical reagents were used to identification lichen species: IIK (iodine-potassium iodide), $Cl_2$ (chlorine), KOH (potassium hydroxide). The nomenclature for lichen species is according to www.indexfungorum.org and for phorophytes is according to Ciocârlan (2009).

The map was constructed using National Geographic Map (Basemap Gallery) powered by **Environmental Systems Research Institute, Inc.** (www.arcgis.com/home/webmap/viewer.html).

*Statistical analysis.* – Firstly, relative abundance of each lichen species was calculated according to the following formulae (Botnariuc & Vădineanu, 1982): $A = n/N$ x 100, in which **A** is relative abundance; n is the number of individuals counted for each species and **N** is the total number of individuals recorded for all species.

The statistical analyses were performed using PAST software (Hammer *et al.*, 2001). The collected data were firstly tested for normal distribution using the Shapiro-Wilk test (Mărușteri, 2006). This test indicated a non-normal distribution of the data set ($p < 0.05$). To test the relationships among response variables (abundance of the lichen species) and environmental variables (altitude, coverage of herbs, subarbuscular and arbuscular plants, canopy openness and slope) the Kendall rank order correlation coefficient ($\tau$) was used (Ditham, 2011).

Coverage by herbs was used to indicate the degree of illumination, such that a higher percentage of herb coverage indicates an optimum illumination degree.

To predict the influence of macrovariables and microvariables on response variables, polynomial regression of the second order was used. Low values of both chi-squared test and Akaike Information Criterion were used as indicators of the best fit among explanatory (environmental) and response variables (Hammer *et al.*, 2001).

## RESULTS

A total of 21 lichen species were sampled within studied area (Table 1). *Hypogymnia physodes* (L.) Nyl. is the most abundant lichen species, whilst the relative abundances of all other species does not exceed 10%.

We report in the next paragraphs the observed relationships between three macro- and three microvariables and lichen species abundance:

– ***degree of illumination:*** moderate positive correlations were observed between species with foliose thalli (*Platismatia glauca* (L.) W. Culb. & C. F. Culb. and *H. physodes*) and the coverage of herbs ($\tau = 0.33$; $p < 0.01$ and $\tau = 0.40$; $p < 0.001$, respectively). In contrast, lichen species with crustose thalli (*Opegrapha vulgata* Ach. and *Pyrenula macrospora* (Degel.) Coppins & P. James) grew on substrata that were strongly shaded ($\tau = 0.34$; $p < 0.01$ and $\tau = 0.50$; $p < 0.00001$). On better illuminated substrata, the abundance of *Pyrenula macrospora* was significantly lower ($\tau = -0.36$; $p < 0.01$). Higher coverage of arbuscles is related to a greater abundance of *Ramalina pollinaria* (Westr.) Ach. ($\tau = 0.28$; $p < 0.05$).

– ***topography:*** the abundance of *O. vulgata*, *Pyrenula macrospora* and *Lepraria* ssp. decreased as a function of altitude ($\tau = -0.23$; $p < 0.05$, $\tau = -0.33$; $p < 0.05$, and $\tau = -0.29$; $p < 0.01$) and these species all occurred under 950 m. *H. physodes* was present both at lower and higher altitude, though with greater abundance at higher altitude ($\tau = 0.25$; $p < 0.05$). The abundance of *R. pollinaria* is negatively correlated to steep mountain slopes ($\tau = -0.38$; $p < 0.001$).

– ***host phorophytes:*** *Picea abies* and *Fagus sylvatica* are the best represented phorophytes in the studied area. Greater abundance of *P. glauca*, *H. physodes* and *Lepraria* ssp. were observed on *P. abies* ($\tau = 0.30$; $p < 0.01$, $\tau = 0.46$; $p < 0.0001$, and $\tau = 0.22$; $p < 0.05$). The abundances of lichen species with crustose thalli (such as *O. viridis*, *O. vulgata* and *Pyrenula macrospora*) were greater on *F. sylvatica* ($\tau = 0.42$; $p < 0.001$, $\tau = 0.51$; $p < 0.00001$, and $\tau = 0.75$; $p < 0.000001$). The abundance of *O. viridis* was greatest on *Abies alba* ($\tau = 0.49$; $p < 0.0001$).

– ***orientation:*** of all the lichen species recorded, two (*P. glauca* and *Evernia prunastri* (L.) Ach.) appeared preferential for the north-facing part of phorophyte trunks while *H. physodes* was negatively correlated to the south-east facing part of sampled trunks ($\tau = -0.28$; $p < 0.05$) and *Lepraria* ssp. was positively correlated with the east-facing part of tested trunks ($\tau = 0.32$; $p < 0.01$).

– ***rhytidome crevice depth:*** abundances recorded for *E. prunastri* and *O. vulgata* increased with greater rhytidome crevice depth ($\tau = 0.22$; $p < 0.05$ and $\tau = 0.22$; $p < 0.05$).

– ***phorophyte circumferences:*** the abundances of the *P. macrospora* and *O. vulgata* were positively correlated to trunk circumferences ($\tau = 0.26$; $p < 0.05$ and $\tau = 0.24$; $p < 0.05$).

Regression analysis indicated certain significant predictors which affect lichen species abundances. Amongst the macrovariables these predictors included: the herb coverage, arbuscle coverage, slope, canopy strongly interleaved, altitude,

Table 1. List of identified lichen species within Ceahlău National Park

| *Lichen species* | *Host phorophytes* | *Relative abundances (%)* |
|---|---|---|
| *Bryoria implexa* (Hoffm.) Brodo & D. Hawksw. (1977) | *Picea abies* | 0.11 |
| *Bryoria subcana* (Nyl. ex Stizenb.) Brodo & D. Hawksw. (1977) | *Picea abies* | 1.22 |
| *Buellia disciformis* (Fr.) Mudd. (1861) | *Fagus sylvatica* | 0.22 |
| *Evernia divaricata* (L.) Ach. (1810) | *Picea abies* | 0.22 |
| *Evernia prunastri* (L.) Ach. (1810) | *Picea abies*, *Acer pseudoplatanus*, *Abies alba* | 4.21 |
| *Graphina anguina* (Mont.) Müll. Arg. (1882). | *Fagus sylvatica* | 1.66 |
| *Hypogymnia physodes* (L.) Nyl. (1896) | *Picea abies*, *Abies alba*, *Larix decidua* | 66.14 |
| *Lecanora subintricata* (Nyl.) Th. Fr. (1871) | *Abies alba* | 2.88 |
| *Lecidea turgidula* Fr. (1824) | *Abies alba* | 1.55 |
| *Lecidella elaeochroma* (Ach.) M. Choisy (1950) | *Abies alba* | 0.33 |
| *Lepraria* ssp. | *Abies alba*, *Picea abies* | 6.99 |
| *Opegrapha niveoatra* (Borrer) J. R. Laundon (1963) | *Abies alba* | 0.77 |
| *Pseudoschismatomma rufescens* (Pers.) Ertz. & Tehler (2014) | *Acer pseudoplatanus* | 0.88 |
| *Opegrapha viridis* Pers. (1803) | *Abies alba* | 0.33 |
| *Opegrapha vulgata* (Ach.) Ach. (1803) | *Abies alba*, *Fagus sylvatica* | 2.21 |
| *Pertusaria dacica* Erichsen (1934) | *Acer pseudoplatanus* | 0.22 |
| *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb. (1968) | *Picea abies* | 2.44 |
| *Pyrenula macrospora* (Degel.) Coppins & P. James (1980) | *Fagus sylvatica* | 2.88 |
| *Pyrenula chlorospila* (Nyl.) Arnold (1887) | *Fagus sylvatica* | 0.66 |
| *Ramalina farinacea* (L.) Ach. (1810) | *Picea abies* | 0.11 |
| *Ramalina pollinaria* (Westr.) Ach. (1810) | *Picea abies*, *Acer pseudoplatanus*, *Abies alba* | 3.88 |

Table 2. Significant results of the polynomial regression performed to find a best fit among environmental variables and response variables (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

| *Lichen species* | *Explanatory variables* | *Chi-squared* | *Akaike Information Criterion* | *Coefficient of determination ($R^2$)* | *F test* | *Regression coefficients* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $a_0$ | $a_1$ | $a_2$ |
| *Platismatia glauca* | Herb coverage | 174.05 | 180.05 | 0.16 | 3.54* | 0.02 | 0.12 | – 0,001 |
| *Ramalina pollinaria* | Arbuscle coverage | 559.76 | 565.76 | 0.17 | 3.59* | – 0.24 | 0.02 | 0.0007 |
| | Slope | 493.15 | 499.15 | 0.26 | 6.44** | 10.65 | – 0.50 | 0.005 |
| *Opegrapha vulgata* | Rhytidome crevice depth | 128.93 | 134.93 | 0.39 | 11.20*** | 0.45 | – 4.34 | 5.42 |
| *Pyrenula macrospora* | Strongly canopy interleaved | 80.20 | 86.20 | 0.21 | 4.80* | 0.32 | 0.06 | 0.19 |
| | *Fagus sylvatica* | 44.88 | 50.88 | 0.56 | 22.34*** | 0.00 | 0.50 | 2.38 |
| *Lepraria* ssp. | Altitude | 1105.5 | 1111.5 | 0.21 | 4.66* | 7.97 | – 0.01 | 0.000003 |
| | *Picea abies* | 1143.4 | 1149.4 | 0.18 | 3.93* | – 1.07 | 4.71 | – 0.57 |
| *Hypogymnia physodes* | *Picea abies* | 6668.5 | 6674.5 | 0.82 | 84.75*** | 7.69 | – 2.96 | 5.41 |
| *Opegrapha viridis* | *Abies alba* | 3.71 | 9.71 | 0.22 | 4.94* | 0.00 | 0.04 | 0.38 |
| | *Fagus sylvatica* | 4 | 10 | 0.16 | 3.33* | 0.00 | 0.05 | 0.27 |
| *Opegrapha vulgata* | *Fagus sylvatica* | 177.56 | 183.56 | 0.16 | 3.34* | 0.00 | 0.39 | 1.83 |

and host phorophytes (*Fagus sylvatica*, *Abies alba*, and *Picea abies*). Amongst the microvariables the rhytidome crevice depth significantly affects the lichen species abundance (Table 2). The abundance of *P. glauca* and *R. pollinaria* increased as a function of better illuminated substrata. On strongly shaded substrata, the abundance of *P. macrospora* (a species with crustose thalli) is greater. Local topography (*e.g.* slope and altitude) negatively affect both the abundance of *R. pollinaria* and *Lepraria* ssp. Lichen species as *P. macrospora*, *Lepraria* ssp., *H. physodes*, *O. viridis*, and *O. vulgata* have their highest abundances on the following host phorophytes: *A. alba*, *P. abies* and *F. sylvatica* (Table 2).

## DISCUSSION

The correlative analysis indicated that the abundances of lichen species are related to attributes of old-growth forests. Thus, circumference of host phorophytes, phorophyte diversity and multi-layered canopies are determining factors of microhabitats with a significant influence on lichen species abundances. In addition, the complex interactions of microhabitat and macrohabitat drivers reveal ancient patterns within the site investigated. Greater phorophyte circumferences have a positive influence on the abundance of lichen species (Sorrell, 2006; Johansson *et al.*, 2009). The higher number of epiphytic lichen species on phorophytes with greater circumferences might be influenced by bark pH, a rough rhytidome, and humidity storage capacity (Çobanoğlu & Sevgi, 2009). Furthermore, tree species diversity determines greater complexity in the lichen community (Sorrell, 2006). Other authors have reported that the richness of lichen species was lower on large phorophytes (Çobanoğlu & Sevgi, 2009). The dependence of the lichen species on greater phorophyte circumferences is considered a measure of site-quality. In this context large phorophytes act as dispersal sources with a positive influence on propagule dispersal and colonisation of substrata (Johansson *et al*., 2009). Site-quality is an expression of microhabitat complexity, so that the diversity of the large phorophytes is related to epiphytic species diversity. Increased lichen species richness as a function of the phorophyte circumferences is due to habitat diversity (Kantvilas & Jarman, 2004). Previous studies have explained the positive relationships between lichen species and phorophyte circumferences as based on metapopulation theory (Hanski, 1999) because "large patches are likely to harbour large populations" (Johansson *et al*., 2009).

Higher abundances of crustose and leprose thalli (*e.g*. *O. viridis*, *O. vulgata* and *P. macrospora*) on *F. sylvatica* are due to the smooth rhytidome of this forest tree. On the other hand the higher abundance of foliose thalli on *P. abies* is determined by the rough rhytidome. Sorrell (2006) found that the rough rhytidome is an appropriate substrate especially for foliose and fruticose lichen species. Some authors found that lichens with crustose thalli inhabited the northern part of trunks on a shadowed smooth rhytidome (Bollinger *et al*., 2007; Ekman *et al*., 2013). The sunny microhabitats represent better environmental conditions for greater abundance of foliose lichen species. Generally, foliose lichen species are photophilous (Wirth, 2010) and these are more abundant on well-lit substrata (Moe & Botnen, 2000).

Aspect is one of the most important attributes with a strong influence on lichen species abundances and communities (Sorrell, 2006). Aspect is also related to variations in the topography and availability of light (Sorrell, 2006; Johansson *et al*., 2009). In a study performed in Antalya (Turkey), *P. glauca* was found on northern orientation of the phorophytes tested (Çobanoğlu & Sevgi, 2009), as also observed in the present study. This Turkish research also found that lichen species diversity was higher on northern orientation than on southern orientation.

Within the lower montane belt *F. sylvatica* is a dominant element of forest structure and therefore two lichen species strongly related to *F. sylvatica* decreased in their abundance as a function of altitude. Although *H. physodes* is widely distributed along a range of altitudes, its abundance is greater at higher altitude.

Within the studied area, although out of our sampling units, two red-listed lichen species, *Cetraria sepincola* (Ehrh.) Ach. and *Lobaria pulmonaria* (L.) Hoffm., were found that are of great conservative interest (Sârbu *et al.*, 2007). *Lobaria pulmonaria,* which was growing on an old beech with a circumference of 3.20 m, is at European level well-known to be associated with old-growth forests (Öckinger *et al*., 2005; Jüriado & Liira, 2009; Svoboda *et al*., 2011).

This study found that both macrovariables and microvariables affect the abundances of lichen species. Important factors in improving propagule dispersal of lichens are the retention of large phorophytes and a greater phorophyte diversity. Multi-layered canopies are also an important aspect of old-growth forests; therefore phorophytes of different ages affect lichen species abundances. Based on these findings, the host phorophyte diversity, their structural features and habitat components must be conserved.

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