Lichenicolous fungi are more specialized than their lichen hosts in primeval forest ecosystems, Białowieża Forest, northeast Poland

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A B S T R A C T

Taxonomy and diversity of symptomatic lichenicolous fungi (visible as fruitbodies on lichen thalli, their discoloration, and/or deformation) and their specificity to lichen hosts is becoming more and more studied. However, information on their ecology is still scarce. We assess how large the specialization of these fungi towards their hosts and microhabitat is. Epiphytic, epixylic and epigeic lichens and associated lichenicolous fungi were studied on 144 permanent plots in Białowieża Forest in relation to forest communities, species of tree phorophyte and substrates. On all these three studied levels lichenicolous fungi were more specialized than their lichen hosts. Our study provides the first estimation of ecological dependences between associations of lichenicolous fungi and their hosts, microhabitats and forest communities in a primeval forest ecosystem representative of lowland Europe.

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1. Introduction

Lichenicolous fungi are specialized organisms growing on or in lichen thalli as obligate or facultative saprotrophs, commensals or parasites (Lawrey and Diederich, 2003; Diederich et al., 2018a). They inhabit various habitats of almost all climatic zones (e.g. Flakus et al., 2008, 2014; Bilovitz et al., 2011; U’Ren et al., 2012; Zhurbenko et al., 2012; Etayo et al., 2013; Gasparyan et al., 2015; Diederich et al., 2017) and are evolutionarily younger than lichens, and many evolved from typically lichenized fungal evolutionary lineages (Diederich et al., 2018a; Flakus et al., 2019). Lichenicolous fungi are an intermediate form in the evolution between fungi associated with algae in the lichen symbiosis and free growing fungi (Lutzoni et al., 2001; Hafellner, 2004; Hawkesworth, 2008; Divakar et al., 2015). By freeing themselves from lichen symbiosis they colonize lichen thalli (becoming parasites, commensals or saprobes) and indirectly or directly still use carbohydrates produced by symbiotic algae (Lutzoni et al., 2001). Numerous lichenicolous fungi show high preference for the specific genus or even for the species of lichen hosts on which they grow. Fleischhacker et al. (2015) and Lawrey and Diederich (2003) estimated that nearly 99% of the species of lichenicolous fungi are highly specialized to their hosts. However, in the case of extremely rare species of lichenicolous fungi, the main factors determining their occurrence are not only suitable hosts, but also microclimate conditions generated by a specific type of habitat (Lawrey and Diederich 2003). A similar relationship between the specificity of the plant community and the presence of parasitic fungi growing on plants was noted by Majewski (1971), who reported that the presence of a potential plant host does not determine the occurrence of its specialized parasite.

Relationships between lichens and lichenicolous fungi are still not well understood (Richardson, 1999; Lawrey, 2000, 2011; Lutzoni et al., 2001; Lawrey and Diederich, 2003; U’Ren et al., 2010; Fleischhacker et al., 2015). There are a limited number of works reporting on the distribution of lichenicolous fungi in habitats and plant communities (Lücking, 1999; U’Ren et al., 2012). Lücking (1999) studied only species of lichenicolous fungi growing on foliicolous lichens in lowland and montane rain forests of Central America, and concluded that lowland forest sites hosted more than.
twice as many fungi of species as montane forests. In North America the diversity and composition of endolichenic and endophytic fungal communities are shaped by climatic patterns, geographic separation, host type, and host lineage (U’Ren et al., 2012). Also Zhang et al. (2015) found that different fungal communities (of lichenicolous and endolichenic fungi) were adapted to different types of lichen thalli growing on different substrates, in Ny-Ålesund region of High Arctic. Up to now, research dealing with the relations between lichenicolous fungi and their lichen hosts in connection with microhabitat and forest communities have not been carried out in a temperate forest ecosystem with a primeval character. We had the opportunity to analyze these issues in the best preserved area in Europe — Bialowieża Forest (Fig. 1), known for its primeval character and the presence of the entire spectrum of forest ecological niches (Cieśliński et al., 1996a, b).

Lichenicolous fungi are only one group of fungi inhabiting lichen thalli. There is also a large and still not fully explored fungal group growing inside lichen hosts and referred to as endolichenic fungi. They are divided depending on their symptoms (obvious or lack) on lichen hosts. Lichenicolous fungi are symptomatic and often cause damage and discolorations of lichen thalli or induce the formation of galls on the lichen hosts, or at least the formation of reproductive structures, e.g., pycnidia, sclerotia or ascomata, are visible. The lack of clear fungal signs on the lichen thallus can mean that these fungi may be present inside of the thallus as asymptomatic fungi (Fleischhacker et al., 2015; Fernández-Mendoza et al., 2017; Diederich et al., 2018a), as in some cases their presence was also detected in visually uninfected thalli (Fernández-Mendoza et al., 2017). However it is not completely clear if they were present as deposits of diaspores beneath or on the surface of thalli. On the other hand, the presence of endolichenic fungi, which occur asymptotically in the lichen thalli, can be detected only by molecular methods and culturing (Petrini et al., 1990; Girlanda et al., 1997; Fleischhacker et al., 2015; Zhang et al., 2015; Muggia et al., 2016; Wang et al., 2016; Fernández-Mendoza et al., 2017; Muggia and Grube, 2018). Their origin and relationships with lichen-forming mycobionts and lichenicolous fungi are still not fully understood and need further studies on a more global scale. They are also considered as an important evolutionary link to the plant-associated endophytes (Arnold et al., 2009; U’Ren et al., 2010; Fleischhacker et al., 2015; Muggia et al., 2016). In our work we focussed only on lichenicolous fungi, which are clearly noticeable on lichen thalli. Studies involving the analyses of only symptomatic fungi, omitting the entire variety of fungi that do not reveal their presence and are in general difficult and time-consuming to study in large number of samples, are common in lichenology and mycology (e.g., Diederich et al., 2018a and literature cited therein; Merinero and Gauslaa, 2018; Zhurbenko and Ohmura, 2018; Iršenaitė et al., 2019).

Based on the current information about the higher specialization of lichenicolous fungi than lichens, and the strong connection between lichen-associated fungi and lichen hosts (Lawrey and Diederich, 2003; U’Ren et al., 2012; Zhang et al., 2015), as well as the dependence of lichen-associated fungi on the habitat conditions (Lawrey and Diederich, 2003; U’Ren et al., 2012), we expected that the occurrence of lichenicolous fungi in forest communities depends on both the presence of the lichen host and the specific forest community, which may be adopted as a proxy of microclimatic conditions. In turn, lichens, as an important component of forest ecosystems, are highly connected with tree species and the substrate on which they grow (Cieśliński et al., 1995). Therefore, our hypotheses were: (i) lichen hosts are more specialized than lichenicolous fungi in relation to the species of tree and substrate they inhabit, (ii) lichenicolous fungi are more specialized than their lichen hosts in relation to forest communities due to their specific microclimate requirements. The main objectives of our study were to explore the relationships between lichenicolous fungi and lichen hosts, and to compare the connection between both lichenicolous

Fig. 1. Study area in Bialowieża National Park (Bialowieża Forest) divided into 144 permanent plots with various forest communities: T-C Tilio-Carpinetum, C-A Circaeo-Alnetum, P-Q Pino-Quercetum, Q-P Querco-Piceetum, P-P Peucedano-Pinetum, C. e-A Carici elongatae-Alnetum.
fungi and lichen hosts, and forest communities, tree species, and substrates.

2. Materials and methods

2.1. Study area

Białowieża Forest is situated in the north-eastern part of Poland, between 23°31′–24°21′ E longitude and 52°29′–52°57′ N latitude. It is the best preserved forest area in the European lowland (Sabatini et al., 2018), characterized by high species richness of plants, animals and fungi, high structural complexity and long-lasting continuity of biological processes. Białowieża National Park (BNP), situated in the heart of Białowieża Forest (Fig. 1), protects the oldest and best preserved parts of the forest excluded from direct human interference since 1921. BNP differs from the rest of the Białowieża Forest by its higher species richness, greater age of trees, more complicated spatial structure, and higher variability of tree age: from the youngest to the oldest. It is also outstanding because of the presence of trees reaching the end of their natural lifespan, exposed roots of fallen trees, and deadwood in diverse degrees of decomposition, etc. The high degree of naturalness and specific microclimate of the centuries-old forests of BNP is also reflected in the shape and great species diversity of lichen biota (Ciesliński et al., 1996a; Ertz et al., 2018; Lubek et al., 2018).

2.2. Study design

The research was conducted May to October 2014 and 2015, on 144 permanent plots (Faliński and Mutenko, 1997). 100 × 100 m each (Fig. 1). The study area is representative for the entire Białowieża Forest in terms of shape of the most common forest communities: fertile oak-linden-hornbeam forest (T-C), streamside alder-ash forest (C-A), pine-oak mixed forest (P-Q), moist oak-spruce forest (Q-P), mesic (spruce)-pine forest (C-A), and alder carr (Alnobetula-Aceretum). These communities differ in terms of structure and climatic conditions (Table 1). The investigated forest communities comprise various species of trees (Fig. 2), which differ in bark hardness, structure, water capacity, content of chemical compounds and pH (Pinus sylvestris – pH 3.4–3.8, Picea abies – pH 3.8–4.5, Quercus robur – pH 3.7–5.0, Alnus glutinosa – pH 4.2–5.0, Populus tremula – pH 3.9–7.9, Carpinus betulus – pH 4.6, Tilia sp. – pH 4.8–6.2, Fraxinus excelsior – pH 5.2–6.8; after Barkman, 1958). These habitat properties are important factors shaping lichen species occurrence. The investigated area is also characterized by a high variety of substrates usually absent in transformed or managed forests, e.g. bark of trunks and branches of fallen trees, wood of trunks and branches of fallen trees, wood of small stumps, bark of dead standing trees and wood of dead standing trees.

In 2014–2015 two researchers worked on each plot for 3–4 h, investigating all possible microhabitats (see also Lubek et al., 2018). A magnifying hand lens with a light was used for the detection of symptomatic lichenicolous fungi, and samples for laboratory identification were taken. On each research plot all species of lichen (epiphytic, epizylic and epigeic groups) and lichenicolous fungi on all available phorophytes and substrates were recorded (Supplementary Table 1).

For each species of lichen we recorded: (i) type of forest community, (ii) species of phorophyte, (iii) type of substrate. The same information plus host species of lichen were recorded for all lichenicolous fungi. Two datasets were created for analyses – (i) lichenicolous fungi and (ii) host lichens – for fungi examined, to explore their distribution in relation to habitat. Species difficult to identify were marked as cf. or aff. (lichenicolous fungi) or spp. (lichens) on the basis of visible features. The fungi growing on un-identified lichen species, in the ‘lichenicolous fungi’ database, were used to analyze fungi ecological preferences for phorophytes, substrates and forest communities. In the ‘lichen hosts’ database, only lichen hosts identified to the species level were used.

The nomenclature of lichen species follows Faitynowicz and Kossowska (2016) and lichenicolous fungi – Diederich et al. (2018a). The collected material is deposited in the lichen herbarium of Jan Kochanowski University in Kielce (KTC) and the University of Gdańsk (UGDA).

2.3. Data analysis

To explore the relationships between (i) lichenicolous fungi and lichen hosts, the quantitative interaction matrix (network) based on the frequency of fungi and lichens (the number of plots, with species’ records) was constructed using the plotweb::bipartite() function (Dormann et al., 2008, 2018). The same approach was adopted to compare interactions between fungi and lichens and: (ii) forest community, (iii) tree species and (iv) substrate. Thus, we developed seven interaction matrices for: lichenicolous fungi and lichen hosts, lichenicolous fungi and forest communities, lichen hosts and forest communities, lichenicolous fungi and tree species, lichen hosts and tree species, lichenicolous fungi and substrates, and lichen hosts and substrates. To check whether there were significant differences between the number of interactions of lichens and lichenicolous fungi with a forest community, tree species and substrate, we used the Chi-squared (X²) test with the highest level of significance set at $P = 0.05$.

To determine the degree of complementary specialization of lichenicolous fungi to lichen hosts, as well as specialization of lichenicolous fungi and lichen hosts for each species in each of seven networks, we calculated the Kullback-Leibler distance $d'$ using the bipartite::specieslevel() function (Dormann et al., 2008, 2018). The index $d'$ is a deviation from the expected probability distribution, assuming that all species interact with their partners in proportion to their observed frequency totals (Blüthgen et al., 2006, 2007). Apart from the diversity of lichenicolous fungi and lichen hosts, the Kullback-Leibler distance $d'$ also considers the fact that species may specialize on the most common host, forest community, tree species or substrate. Thus, index $d'$ is relatively insensitive to asymmetric specialization, and estimates the degree of specificity in the species' realized niche (Blüthgen et al., 2006; Blüthgen, 2010; Albrecht et al., 2013). Index $d'$ ranges from 0 to 1, where 0 means the high generalization of species (low specialization level), and 1 the highest possible specialization of species to the exact host, forest community, tree species or substrate (Blüthgen et al., 2006). To check whether there were significant differences between the specialization $d'$ of lichenicolous fungi and lichen hosts to forest community, tree species and substrate, we used a paired $t$-test with the maximum level of significance set at $P = 0.05$.

To compare compositional differences between lichen hosts and their lichenicolous fungi, as well as compositional differences of fungi and lichens across forest communities, tree species and substrates, for each of seven configurations, we calculated the mean Bray-Curtis dissimilarity index using the vegan::vegdist() function, and used the ordination techniques implemented in the vegan package (Oksanen et al., 2018). A preliminary Detrended Correspondence Analysis (DCA; vegan::decorana()/function) performed separately for lichenicolous fungi and their lichen hosts, lichenicolous fungi and forest communities, lichen hosts and forest communities, lichenicolous fungi and tree species, lichen hosts and tree species, lichenicolous fungi and substrates, and lichen hosts
and substrates, showed low values of gradient length (<3 SD units). Therefore, in the next step for each of seven configurations we performed the PCA ordination using the vegan::ndet() function. However, after the visualization of results, in each configuration we found an inadequate distribution of sites (in case of ordinations performed for fungi sites were: lichen hosts, forest communities, tree species and substrates; in case of lichen hosts as sites we adopted: forest communities, tree species and substrates) and species, i.e. placing both sites and species in the same place of the ordination space. Therefore, the Non-metric Multidimensional Scaling (NMDS; vegan::metaMDS() function) was used as the most appropriate method for exploring differences in species composition for each configuration. Prior to the NMDS, to avoid biases connected with the uneven ranges among variables (in the case of: lichenicolous fungi vs. lichen hosts, lichenicolous fungi vs. tree species and lichen hosts vs. forest communities), data were normalized using the vegan::decostand() function. We calculated the mean Bray-Curtis dissimilarity index to compare compositional differences between forest communities, tree species and substrates focusing separately on lichenicolous fungi and lichen hosts, for each of seven configurations. All analyses were performed using R software (R Core Team, 2018). To find whether the number of lichenicolous fungi species depended on the richness of hosts at the plot level, we used linear regression.

3. Results

Most of the lichenicolous fungi species were highly specialized to their hosts: 83% of species were associated with a single lichen genus or even with only one species of lichen host (Figs 3 and 4; Supplementary Table 1). The network analysis based on the species’ frequency (only species registered on at least 5 plots were included in the analysis) revealed 105 interactions between 68 studied lichen taxa (62 species, and 6 taxa at genus level) and 24 species of lichenicolous fungi (Fig. 3). Comparing networks constructed for lichens and lichenicolous fungi separately, and depending on forest community (Fig. 5A–B), tree species (Fig. 5C–D) and substratum (Fig. 5E–F), we found that in each combination the number of interactions of lichenicolous fungi (Fig. 5) was significantly higher ($X^2 = 117.01, P<0.001; X^2 = 226.23, P<0.001$ and $X^2 = 160.69, P<0.001$, respectively) than the number of interactions of lichenicolous fungal species (114, 121, 118, respectively). This is the result of a greater diversity and more frequent occurrence of lichen species than fungi in the studied area (Supplementary Tables 2 and 3).
Specialization of lichenicolous fungi to the forest community (mean $d' = 0.10$) was significantly higher than specialization of lichen species (mean $d' = 0.03$, $t = 4.00$, $P < 0.01$; Supplementary Tables 2 and 3; Fig. 6). The NMDS analysis did not reveal strong relationships of lichen host species to the forest community (Fig. 7A; compare Fig. 5), while some lichenicolous fungal species were associated with the specific forest community (Fig. 7B; compare Fig. 5).

Specialization of lichenicolous fungi to tree species (mean $d' = 0.18$) was significantly higher than specialization of lichen species (mean $d' = 0.12$, $t = 4.54$, $P < 0.05$; Supplementary Tables 2 and 3; Fig. 6), but these relationships were not clearly visible using NMDS, except for species associated with Scots pine (Fig. 7C and D; compare Fig. 5).

Specialization of lichenicolous fungi to substrate (mean $d' = 0.17$) was significantly higher than specialization of lichen species (mean $d' = 0.11$, $t = 2.34$, $P < 0.01$; Supplementary Tables 2 and 3; Fig. 6). The NMDS ordination for lichen hosts revealed several groups of substrates connected with specific species (Fig. 7E; compare Fig. 5), while in the case of lichenicolous fungi there were more and well defined groups of species grouped around specific substrates (Fig. 7F; compare Fig. 5).

The mean specialization of lichenicolous fungi on lichen hosts was several times higher (mean $d' = 0.79$) in comparison to their specialization to substrates, tree species or forest communities, with the highest specialization ($d' > 0.96$) reported for Clypeococcum hypocenomycis, Monodictys epilepraria, Taeiniolella caespitosa, Taeiniolella delicata and Tremella lichenicola (Supplementary Table 2). Species composition of lichenicolous fungi strongly differed between lichen host species (mean $BC = 0.93$; Fig. 8). Assemblages of lichenicolous fungi differed more than assemblages of lichen host species in all three analyses: forest communities, tree species and substrates (Fig. 8). Despite high statistical significance ($P < 0.001$), we found only a weak relationship between richness of fungi and their lichenicolous hosts ($R^2 = 0.08$; Supplementary Fig. 1).

4. Discussion

4.1. Lichenicolous fungi and their hosts

Lawrey and Diederich (2003) estimated that 99% of lichenicolous fungal species are associated with only a single lichen genus. In our study specialization was lower: 83% of lichenicolous fungi were associated with a single lichen genus or even with one species of lichen host (Figs 3 and 4). This difference is not high, taking into account the relatively low level of knowledge on the
ecology of lichenicolous fungi. Only a few species of lichenicolous fungi were generalists and occurred on many different lichen host species belonging to different, often distantly related genera, e.g. *Lichenocodium erodens*, *Lichenostigma maurei*, *Lichenostigma alpinum* (Figs 3 and 4; Supplementary Table 2). Some highly specialized fungi, e.g. *C. hypocenomyces*, *T. delicata* (despite being known from very different host genera, in the investigated area was noted only on *Ropalospora viridis*; see also Heuchert et al., 2018) and *T. lichenicola*, despite being associated with only one, but common, host species were very common in the investigated area (62, 78, 58

Fig. 5. Networks based on the frequency of species showing interactions of lichen hosts and lichenicolous fungi with: (A–B) forest communities, (C–D) tree species and (E–F) substrates; A, C, E refer to the lichen hosts and B, D, F to lichenicolous fungi. For full species names, forest communities and tree species names see Materials and methods, and Supplementary Tables 1–3.

Fig. 6. Comparison of species specialization index $d'$ calculated for lichen hosts and lichenicolous fungi depending on forest community, tree species and substrate. Box covers the 95% confidence range. The thick horizontal line is the median. Black points are outliers. Red and blue backgrounds around boxes are Kernel density plots.
However, most lichenicolous fungi connected with a single host species were very rare (Supplementary Table 1). Lawrey and Diederich (2003) reported that a high number of lichenicolous fungi are very rare in their range. In our study, Skyttea nitschkei, recorded only from one forest type (P-Q) on Thelotrema lepadinum, was very rare despite the commonness of its host, recorded on several tree species – hornbeams, lindens, oaks, ashes and alders – and in several forest types: T-C, C-A and C-e-A. The occurrence of host species did not always correlate with the presence of its parasite, even if both are common. These relationships can be traced on Figs 3 and 5. Thus, frequency of lichenicolous fungi does not depend on the frequency of their potential lichen hosts. Parasites are usually less frequent than their hosts, which is associated with their high ecological requirements. In our opinion lichenicolous fungi, just like stenotopic lichens, need specific habitats. S. nitschkei demands not only the proper host growing in ancient woodland with a long historical continuity of forest cover (Diederich et al., 2018b) but also showed high habitat selectivity. Majewski (1971) reported similar limitations of the occurrence of fungi (Peronosporales, Erysiphaceae, Uredinales, Ustilaginales) parasitising herb plants. The presence of parasitic fungi depended not only on the host plant, but they showed high specificity to habitat conditions.

On the one hand, lichenicolous fungi showed high selectivity to the host, but on the other hand some sensitivity to infections by lichenicolous fungi is specific to lichen species (Figs 3 and 4). This is probably due to the differences in the resistance of lichens to infections, associated with the level of concentration of carbon-based records, respectively; Supplementary Table 1). However, most lichenicolous fungi connected with a single host species were very rare (Supplementary Table 1). Lawrey and Diederich (2003) reported that a high number of lichenicolous fungi are very rare in their range. In our study Skyttea nitschkei, recorded only from one forest type (P-Q) on Thelotrema lepadinum, was very rare despite the commonness of its host, recorded on several tree species – hornbeams, lindens, oaks, ashes and alders – and in several forest types: T-C, C-A and C-e-A. The occurrence of host species did not always correlate with the presence of its parasite, even if both are common. These relationships can be traced on Figs 3 and 5. Thus, frequency of lichenicolous fungi does not depend on the frequency of their potential lichen hosts. Parasites are usually less frequent than their hosts, which is associated with their high ecological requirements. In our opinion lichenicolous fungi, just like stenotopic lichens, need specific habitats. S. nitschkei demands not only the proper host growing in ancient woodland with a long historical continuity of forest cover (Diederich et al., 2018b) but also showed high habitat selectivity. Majewski (1971) reported similar limitations of the occurrence of fungi (Peronosporales, Erysiphaceae, Uredinales, Ustilaginales) parasitising herb plants. The presence of parasitic fungi depended not only on the host plant, but they showed high specificity to habitat conditions.

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secondary compounds in the thallus, which provides protection against various biotic and abiotic stressors (Asplund and Merinero, 2018). About 69% of analysed lichen species were inhabited by only one species of lichenicolous fungus (Supplementary Table 1). Only 6% of lichen species (Cladonia coniocraea, Cladonia digitata, Hypogymnia physodes, Lecanora pulicaris, Parmelia sulcata) were associated with more than 5 lichenicolous fungi. Different groups of lichen species show differences in being specific hosts to lichenicolous fungi (Girlanda et al., 1997; Lawrey and Diederich, 2003). However, this rule is not universal as some species, infected by only one lichenicolous fungus in our study area, are inhabited by other species of fungi or even by several species in other regions. This could be related to the high specialization of lichenicolous fungi to habitat conditions. Some of the studies dealing with lichenicolous fungi, endolichenic fungi and free growing fungi, and their phylogenetic relationships, reported that lichen thalli may constitute a transitional stage of the occurrence for fungi, evidenced by the isolation of these fungi from lichen thalli (Girlanda et al., 1997; Divakar et al., 2015; Zhang et al., 2015). Some lichens, at the initial stage of thallus development, even grow on lichen hosts as parasites or saprobionts (e.g. Timdal, 1986; Friedl, 1987; Ott et al., 2011). We observed one species of lichen (identified as Fidelia aff. leucopellaesus) in the study area which probably grew as a parasite or saprobiont on the thalli of Loxospora elatina. This species has not been recorded in the literature as lichenicolous so far, and further investigation is needed to settle if it represents a separate species or just a young stage of F. leucopellaesus. On plots where F. aff. leucopellaesus was found, often typical F. leucopellaesus grew on the same tree phorophytes.

4.2. Specialization to forest communities

Our hypothesis that lichenicolous fungi are more specialized than their lichen hosts in relation to forest communities was confirmed. Most of the fungi growing on lichens showed high preference for T-C, C. e-A and C-A forest communities (Figs 5B and 7B) — habitats with a humid microclimate, more fertile soils and higher tree canopy cover (Table 1). These types of forest hosted the greatest number of lichenicolous fungal species, and some exclusive species also occurred there (compare Supplementary Table 1). Similar dependencies were found by Majewski (1971) for parasitic fungi growing on herb plants. Despite the fact that the greatest species diversity of plants and potential hosts was recorded in coniferous forests, he recorded the greatest number of plant parasitic fungal species in forest communities of C-A and T-C where high air humidity fosters infection of potential hosts by parasites (Majewski, 1971).

Lawrey and Diederich (2003) put forward a question about dependence between the diversity of the lichen community and the diversity of lichenicolous fungi. They assumed that measures of species diversity should be correlated for lichens and lichenicolous fungi. A greater diversity of lichen species should serve as the basis for the occurrence of a higher variety of species of lichenicolous fungi. However, our results indicate that this is not the only important factor (Supplementary Fig. 1). Plots with the greatest species diversity of lichens rarely overlapped with the plots with the greatest species diversity of lichenicolous fungi. Probably, besides the number of lichen species, the diversity of fungi is significantly influenced by other factors, e.g. the specific microclimate of the habitat. Our results suggest that humidity is of great importance: the greatest numbers of lichenicolous fungi species were found in the habitats with the highest humidity, soil fertility and primary production, i.e. in C-A and C. e-A (Table 1; compare Zarnowiecki, 1995). This dependence is visible not only for lichenicolous fungi, but also to some extent for lichens. Thus, the large diversity of lichen species was not directly translated to the richness of lichenicolous fungi species. Similar results were obtained by Jonsson and Jonsell (1999), who discovered that correlation in species richness among different organism groups (e.g. vascular plants, bryophytes, epiphytic lichens, wood-inhabiting fungi) in Swedish spruce forests was not relevant, and total species richness was correlated with several habitat factors, mainly particular substrates and degree of forestry impact.

Most species of lichenicolous fungi did not show a clear preference for a specific forest community (Figs 5B and 7B). However, there were several species that showed high specialization: C. hypocenomycis, Bachmanniomyces punctum and Epicladonia sandstedel to P-P, T. delicata and M. epilepria to T-C. Several species also showed greater preferences for humid communities of C-A and C. e-A, e.g. L. alpinum, Microcalicium dissemintatum, Vouauxiella lichenicolica, Lichenostigma chloroterata and T. lichenicola. The similarity of species composition and frequency of lichenicolous fungi divided the studied forest communities into two groups, and it was greater between forest types within the group of deciduous forest communities (C-A, C. e-A and T-C) and within the group of coniferous forest communities (P-Q, Q-P and P-P) than between them (Fig. 7B). These groups of forests are characterized by similar habitat components, such as low or intermediate humidity, less fertile soils and lower tree canopy cover (Table 1; Fig. 2). A similar relationship was also found by Majewski (1971) for plants. In the case of lichen hosts, these relationships were shaped similarly but less clearly (Fig. 7A).

4.3. Specialization to tree species

The primeval ecosystems of Bialowieza Forest offer high diversity of microhabitats important for lichens, many of which are not found in transformed anthropogenic forests (Ciesliński et al., 1995). One of the most important substrates is the bark of various tree species, which differs in structure, hardness, water capacity and pH value, which are additionally modified by the age of trees (Barkman, 1958). Therefore, the main core of the lichen biota in the investigated area is built by species developing on the bark of trees (Fig. 5C, E and 7C, E). This is a microhabitat potentially important for lichenicolous fungi as well. We found the greatest number of lichenicolous fungal species on lichen hosts growing on the bark of living trees (Fig. 5D, F and 7D, F). The greatest number of fungal species was associated with the bark of alder (Fig. 5D), which occurs mainly in humid C-A and C. e-A communities (Fig. 2; Table 1), despite the fact that the largest number of species of potential lichen hosts was found on hornbeam (Fig. 5C) typically occurring in the drier (in comparison with C-A and C. e-A) T-C community (Fig. 2; Table 1).

Lichenicolous fungi, similarly to lichens, showed preferences for a particular tree species (Fig. 7C and D), but not always to the same tree species as their hosts. The lichenicolous fungus Arthrorhaphis aeruginosa was strongly associated with alder (Figs 5D and 7D) but its host lichen Cladonia coniocraea was most frequent on birch (Figs 5C and 7C). Another species, Tenuiella punctata, was associated with hornbeam and alder, but its host lichens Graphis pulvulentula and Graphis betulina were most frequent on rowan, linden and hazel. This is probably the effect of the high specialization of lichenicolous fungi to forest communities and their demands for high air humidity, which seems to limit their distribution only to specific, more humid forest communities and the tree species that build them.

The biota of lichenicolous fungi caused the aggregation of tree species into two clearly distinguished groups – (1) hazel, ash, hornbeam, lime and (2) alder, birch, spruce and oak (Fig. 7D) – dominated by trees of higher and lower pH of bark, respectively.
Comparison to lichen hosts (Fig. 5E and F and 7E, F). Our hypothesis that lichen hosts are more specialized than the lichenicolous fungi, in relation to the species of tree and substrate they inhabit, was not confirmed. Fungi growing on lichens clearly formed several groups including species associated with different types of substrates, starting from the bark of living branches and shrubs, through to the bark of the trunks of fallen, living and dead trees, dead wood, ending at the foot of living and dead trees (Fig. 7F). The case of lichen species was similar, but the groups were less clear. Lichenicolous fungi were clearly associated with particular microhabitats, differing by environmental factors, e.g. humidity (e.g. foot of trees vs. bark of twigs), light exposure (e.g. bark of twigs vs. bark of fallen trees) and pH of substrates (wood vs. bark). The greatest species diversity of lichenicolous fungi was recorded for: the bark of living trees, the bark of twigs and branches of fallen trees, and the wood of trunks and branches of fallen trees. The above analyses, including substrates as well as different species of trees and forest communities, indicate that the notable factor shaping fungal biota, in addition to moisture, may be the pH of the substrate, the content of micronutrients in the substrate, or exposure to light. Due to the lack of detailed data on the chemical and physical character of the substrates (e.g. pH value, water capacity, nutrients contained) distinguished as being significant, it was not possible to analyze the dependence of occurrence lichenicolous fungi on a specific substrate.

5. Conclusions

Our results give an insight into the interactions between lichenicolous fungi and their lichen hosts, and the environmental factors affecting them in forest ecosystems. We found lichenicolous fungi to be more specialized than their lichen hosts: species diversity and frequency of these fungi depend on forest community, tree species and substrate to a greater degree than their hosts did. Apart from the high specificity of lichenicolous fungi towards the lichen hosts, the next factors that most influenced the species composition of the fungi were substrate and forest community. Our study is the first to indicate ecological dependencies between associations of lichenicolous fungi and their hosts, microhabitats and forest communities in primeval forest ecosystem representative for lowland temperate Europe. Our results can be used as a model tool for assessment of the distribution of lichenicolous fungi in other forest ecosystems.

Author contributions

B.J. designed the research; A.L. and M.K. elaborated the main objectives of the study and collected the data; P.C. and A.L. analysed data; A.L. prepared the manuscript, with contributions of all other authors.

Additional information

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Supplementary data

Supplementary data can be found online at https://doi.org/10.1016/j.funeco.2019.100866.

References


Lücking, R., 1999. Foliicolous lichens and their lichenicolous fungi from Ecuador, with a comparison of lowland and montane rain forest. Wilddenowia 299–335. ISSN 0511-9618.


Ratyńska, H., Wojterska, M., Brzęg, A., Koćak, M., 2010. Multimedia Encyclopedia ZbiorowiskaRolnychPolski([NFOSiGW,UKW,IET].[Amultimediacaencyclo-


