Unconstrained gene flow between populations of a widespread epiphytic lichen *Usnea subfloridana* (Parmeliaceae, Ascomycota) in Estonia

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**Abstract**

Few studies have investigated the genetic diversity of populations of common and widespread lichenized fungi using microsatellite markers, especially the relationships between different measures of genetic diversity and environmental heterogeneity. The main aim of our study was to investigate the population genetics of a widespread and mainly clonally reproducing *Usnea subfloridana* at the landscape scale, focusing on the comparison of lichen populations within hemiboreal forest stands. Particular attention has been paid to the genetic differentiation of lichen populations in two geographically distinct regions in Estonia and the relationships between forest characteristics and measures of genetic diversity. We genotyped 578 *Usnea* thalli from eleven lichen populations using seven specific fungal microsatellite markers. Measures of genetic diversity (allelic richness, Shannon's information index, Nei's unbiased genetic diversity, clonal diversity, the number of multilocus genotypes, the number of private alleles, and the minimum number of colonization events) were calculated and compared between *Usnea* populations. Shared haplotypes, gene flow and AMOVA analyses suggest that unconstrained gene flow and exchange of multilocus genotypes exist between the two geographically remote regions in Estonia. Stand age, mean circumference of the host tree, size of forest site and tree species composition did not show any significant influence on allelic richness, Shannon's information index, Nei's unbiased genetic diversity, clonal diversity, the number of private alleles, and the minimum number of colonization events of *U. subfloridana* populations. Therefore it was concluded that other factors of habitat heterogeneity could probably have a more significant effect on population genetics of *U. subfloridana* populations.

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**1. Introduction**

The genetic diversity, an important part of overall biodiversity, enables evolutionary processes, which provide the raw material for adaptation to changing environments, and ensure healthy populations (Helm et al., 2009; Frankham et al., 2010). The genetic diversity of natural populations results from cumulative effects of historical and present-day processes (Hewitt, 2000; Frankham et al., 2010); the latter include, for example, changes in the current habitat conditions of the environment, which may influence dispersal, growth and vitality of species. Estimating the genetic variability within and among populations, and revealing genetic patterns of populations, improves understanding of the population history, genetic differentiation and gene flow among populations (Werth et al., 2015). Population genetics also contributes to our knowledge of evolutionary processes, ecology, and conservation biology; for example, knowledge of genetic structure and variation of natural populations could be helpful in predicting the population fate in fluctuating environment (e.g., climate change or forest management) or estimating the effective population size of populations (Scheidegger and Werth, 2009; Ouborg, 2009).

Previously published studies regarding genetic structure and diversity of lichen-forming fungi led to different conclusions for each species studied and scale of geographical distribution (e.g., Werth, 2010; Scheidegger et al., 2012; Alors et al., 2017). The genetic diversity of a lichen population could be shaped by different factors of...
environmental heterogeneity. Habitat quality, measured as age or diameter of the host tree, is one of the most important factors affecting the genetic patterns of lichen-forming fungi populations (Otálorá et al., 2011; Scheidegger et al., 2012). For example, Juriado et al. (2011) found a higher genetic diversity in populations of the Lobaria pulmonaria (L.) Hoffm. and more juvenile thalli in old-growth forests compared with managed forests and wooded meadows. Furthermore, different types of disturbance (Werth et al., 2006a), environmental and microclimatic factors (Nadyeina et al., 2014a; Otálorá et al., 2015) could also be significant in explaining the genetic structure and the distribution of gene pools of lichen populations.

Microsatellites or simple sequence repeats (SSR) are considered the most promising markers for investigating the genetic variation and population structure of highly clonal organisms such as lichens (Werth, 2010). The microsatellites are highly polymorphic, species-specific, and selectively neutral markers with a high mutation rate, which provides a more powerful resolution for estimating genetic diversity and variability among populations than former sequence-based method (Selkoe and Toonen, 2006; Werth, 2010). To date, microsatellite primers have been designed for several lichenized fungi (e.g., Prieto et al., 2015; Lutsak et al., 2016; Lagostina et al., 2017) and SSR markers have been successfully applied for studying the genetic diversity, phylogeographic structure, genetic flow and genetic differentiation of lichen populations (e.g., Walser et al., 2003; Otálorá et al., 2011; Nadyeina et al., 2014a). The majority of previous studies which have investigated the population genetic variability of lichen-forming fungi using microsatellite markers have used the threatened, regionally rare or narrowly distributed lichens (e.g., Nadyeina et al., 2014a; Jones et al., 2015; Prieto et al., 2015), but only a few studies have reviewed the microsatellite diversity of common and widely distributed lichenized fungi and genetic structure of their populations (Mansournia et al., 2012; Degtjarenko et al., 2016; Alors et al., 2017).

In the current research we studied the population genetics of a common and widespread lichenized fungus at the landscape scale, focusing on a comparison of lichen populations within hemiboreal forest stands. To achieve this, the genetic variation at seven microsatellite loci in the mycobiont of the epiphytic lichen Usnea subfloridana Stirr. in Estonia, Northern Europe was investigated. The main objectives of this study were: (i) to study the genetic differentiation of U. subfloridana populations in two separate regions of Estonia; and (ii) to investigate the relationships between habitat characteristics and measures of genetic diversity of U. subfloridana populations.

2. Material and methods

2.1. Studied species

U. subfloridana is an epiphytic fruticose macrolichen with a wide distribution across Eurasia, Macaronesia, and North America (Nash et al., 2007; Randlane et al., 2009; Smith et al., 2009). It is very frequent and one of the most commonest Usnea species in Estonia, occurring mostly on Norway spruce (Picea abies), Scots pine (Pinus sylvestris) and Silver birch (Betula pendula), and more rarely on other deciduous trees and lignum (Torra and Randlane, 2007; Randlane et al., 2011). U. subfloridana is not protected locally, and is red-listed in Estonia as Least Concerned (LC) (Randlane et al., 2008). This species reproduces asexually by symbiotic propagules, soralia and isidia, but could also propagate sexually, but specimens with apothecia are very rarely observed (Torra and Randlane, 2007; Randlane et al., 2011). Recent phylogenetic studies indicate that U. subfloridana is not a monophyletic entity but forms an intermixed clade with Usnea florida (L.) Weber ex F.H. Wigg., which is considered the primary, fertile counterpart of the sterile U. subfloridana (e.g., Articus et al., 2002; Saag et al., 2011; Mark et al., 2016). The apotheciate U. florida reproduces exclusively sexually and always lacks vegetative propagules; furthermore, it has distinct ecological requirements, preferring old deciduous trees in areas with a high atmospheric humidity (Randlane et al., 2009; Smith et al., 2009), while U. subfloridana is less ecologically demanding. To date, U. florida has not been recorded from Estonia (Torra and Randlane, 2007).

2.2. Study area

The study area is situated in two separate regions of Estonia, in Põlva County, in the southeastern region (hereafter SE), and in Liää-ne-Viru County, in the northern region (hereafter N) of Estonia, Northern Europe (Fig. 1); the maximum distance between the two studied areas is 184 km. The study area has a characteristic temperate climate with a mean annual temperature of 6 °C, the mean annual precipitation is 672 mm, and the mean wind is 3.7 m/s (Estonian Weather Service, 2018). The vegetation of Estonia belongs to the hemiboreal forest zone, lying in the transitional area, where the southern taiga forest subzone changes into the spruce-hardwood subzone (Ahti et al., 1968; Laasimer and Masing, 1995). The two study sites (SE and N) are both located within the hemiboreal forest zone but in the different vegetation subdivisions. N Estonia being situated in the slightly oceanic to indifferent section, and SE Estonia in the indifferent to slightly continental section according (Ahti et al., 1968) and also in different regions according to the classifications based on sedimentary bedrock (Viiding, 1995) and soils (Reintam, 1962). The study was carried out in P. sylvestris-dominated boreal forests, belonging to the Oxalis–Vaccinium myrtillus, the V. myrtillus, and the Vaccinium vitis-idaea forest site types. These forest types are also widely distributed in other Baltic states (Kairiukštis, 1966; Buss, 1997), in Fennoscandia (Dierßen, 1996), and in northwest Russia (Fedorchuk et al., 2005).

2.3. Sampling

Fieldwork was carried out during the summer of 2011 (in SE Estonia) and the autumn of 2014 (in N Estonia). The potential localities for sampling were chosen from Forest Public Registry maps using comparable forest characteristics from their forest survey (Forest Public Registry, 2017). In total, U. subfloridana populations were sampled from eleven localities; eight populations from SE and three populations from N (Fig 1; Table 1). In each locality or lichen population, 30–62 samples were randomly collected from Norway spruce up to 6 m from the ground using a tree pruner (Table 1). On average, three Usnea thalli were taken from a host tree; if there were less than three thalli, only one or two specimens were sampled, while in other cases more than tree specimens were collected for balancing the sampling. Usnea populations were defined according to the boundaries of forest sites sharing the same values of forest survey data (forest site type, age of trees and proportion of trees in forest stand) according to Forest Public Registry (2017). The tree circumference (BHC) was recorded for each sampled tree at breast height (1.3 m). Other habitat characteristics (stand age, the proportion of pines and birches in forest stands, and size of forest site sharing the same values of data from forest survey) were provided by from Forest Public Registry (2017). Geographical coordinates were recorded per sampled tree with a GPS receiver Garmin GPSMAP 60C.

2.4. Chemical and molecular analyses

All collected Usnea thalli were air dried, cleaned to remove other lichen specimens, and examined under a stereomicroscope. Thin
layer chromatography (TLC) with solvent A (Orange et al., 2001) was used to confirm the identification of collected Usnea species. According to morphology and chemical characteristics (Halonen et al., 1999; Randlane et al., 2009; Clerc, 2011), 578 specimens were identified as *U. subflorida* and used in further molecular analyses; another 79 specimens were removed from the sampling as they belonged to other, similar Usnea species (*Usnea glabrescens* (Vain.) Vain. or *Usnea wasmuthii* Räsanen). Then, the 50 mg of each *U. subflorida* specimen was maintained in 1.5 mL microtubes at −20 °C until the molecular analyses.

The total genomic DNA was extracted using PowerPlant™ Pro DNA Isolation Kit (MO BIO Laboratories, Inc., Qiagen, USA) according to the manufacturer’s protocol. Seven fungal microsatellite loci (Us02, Us03, Us04, Us05, Us06, Us08, and Us09) were amplified in two different multiplex PCR using QIAGEN Multiplex PCR Kit following the instructions described in Törra et al. (2014) and Degtjarenko et al. (2016). Fragment lengths of PCR products were determined on a 3730xl DNA Analyzer (Applied Biosystems) with LIZ-500 as the internal size standard. The alleles were sized and genotyped using GeneMapper® Software v5 (Applied Biosystems).

### 2.5. Statistical analyses

The basic measurements of population statistics (the total number of alleles, mean number of alleles per locus, Nei’s unbiased genetic diversity (*H*), Shannon’s information index (I), allelic richness (A)) for *U. subflorida* populations were calculated in the GenAlex ver 6.5 (Peakall and Smouse, 2012) and the Microsatellite Analyzer ver 2.65 (MSA) (Dieringer and Schlötterer, 2003). The number of private alleles (P) per population were calculated using software HP-Rare (Kalinowski, 2005). Measurements of A and P were standardized using the rarefaction method implemented in the software HP-Rare (Kalinowski, 2005) and MSA (Dieringer and Schlötterer, 2003) respectively. The number of multilocus genotypes (G), the percentage of multilocus genotypes, i.e clonal diversity or genotypic diversity (M: the proportion of different genotypes in the population, G/N), the minimum number of colonization events (C) per population, and total number of multilocus genotypes from all populations were calculated in the software R (R Core Team, 2013) using the R script by Werth et al. (2006a). The number of shared multilocus genotypes between populations was calculated in the software ARLEQUIN ver 3.5 (Excoffier and Lischer, 2010). Hierarchical analyses of molecular variance (AMOVA) with 999 permutations to estimate genetic differentiation were performed using GenAlex ver 6.5 (Peakall and Smouse, 2012). The rate of gene flow (Nm) across seven loci between 11 populations was also estimated using GenAlex ver 6.5 (Peakall and Smouse, 2012). General regression model (GRM) analysis in the STATISTICA ver 7.1 (StatSoft, Inc., 2005) was used to study the relationship between different measurements of genetic diversity (A, I, H, G, M, P, and C) and the characteristics of forest stands. Each population was characterized by the following explanatory variables: (1) stand age (the square root of oldest tree age per forest stand); (2) mean BHC of the host tree per population, values log-transformed; (3) the number of sample size (the square root of collected specimens per population); (4) the size of forest site sharing the same values of forest survey data (forest site type, age of trees and proportion of trees in forest stand); (5) the proportion of pines and birches in forest stands.

### 3. Results

#### 3.1. Genetic variation of *U. subflorida* in Estonia

In total, 66 alleles at seven microsatellite loci in 578 specimens from eleven *U. subflorida* populations were detected (Table 1). All microsatellite loci were polymorphic. The minimum number of alleles was six in locus Us04 and the maximum number of alleles was 14 in locus Us03, and, on average, 3.2–9.5 alleles were found per locus across eleven populations. The mean number of alleles per population was comparatively similar in both regions, varying from 4.9 to 5.4 in populations from N region, and from 5.4 to 7.0 in populations from SE region. We found 283 different multilocus genotypes across 578 specimens in eleven lichen populations. Allelic richness (A) ranged from 4.86 to 6.45 across all lichen populations. Nei’s unbiased genetic diversity (H) varied from 0.58 to 0.65 (Table 1). All lichen populations, except no 3, had private alleles (Table 1). Other detailed measurements of genetic variation per population are given in Table 1. The AMOVA results indicated that most of the total variation (99 %) was found within populations, i.e. among individuals, followed by significant variation among populations within one region (0.5 %; Table 2). The
Overview of the studied Usnea subfloridana populations from the northern (1–3) and the southeastern (4–11) regions of Estonia; sample size, geographical coordinates, forest stand variables, and measurements of genetic diversity.

<table>
<thead>
<tr>
<th>Region</th>
<th>Population</th>
<th>Trees</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Age</th>
<th>BHC</th>
<th>Genetic variation</th>
<th>M</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>1</td>
<td>11</td>
<td>59.54</td>
<td>23.81</td>
<td>97</td>
<td>92.9</td>
<td>0.38</td>
<td>276</td>
<td>9</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>59.53</td>
<td>23.81</td>
<td>146</td>
<td>118.6</td>
<td>0.60</td>
<td>349</td>
<td>9</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>36</td>
<td>59.43</td>
<td>24.17</td>
<td>131</td>
<td>115.6</td>
<td>0.62</td>
<td>349</td>
<td>9</td>
<td>0.22</td>
</tr>
<tr>
<td>Southern</td>
<td>4</td>
<td>11</td>
<td>59.36</td>
<td>23.81</td>
<td>97</td>
<td>92.9</td>
<td>0.58</td>
<td>276</td>
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<td>5</td>
<td>10</td>
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<td>24.17</td>
<td>146</td>
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<td>7</td>
<td>21</td>
<td>59.27</td>
<td>23.81</td>
<td>39</td>
<td>76.8</td>
<td>0.58</td>
<td>276</td>
<td>9</td>
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<td>8</td>
<td>21</td>
<td>59.27</td>
<td>24.17</td>
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<td>76.8</td>
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<td>276</td>
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<td>0.25</td>
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The relationships between different measurements (A, I, H, G, M, P and C) of genetic variation and characteristics of forest stands were studied. The results of GRM showed that stand age, mean BHC of the host tree per population, the number of sample size, the size of forest site sharing the same values of data from forest survey, and proportion of pines and birches in forest stands did not reveal any significant influence on A, I, H, M, P and C (Appendix A.2). There was a statistically significant association between the number of multilocus genotypes (G) and sample size (i.e. the number of collected/studied specimens per population; SS = 321.3; F = 160.6; p = 0.0002; Appendix A.2).

4. Discussion

Seven microsatellite loci were used to study the intra-species genetic diversity of the common and widespread lichen-forming fungus Usnea subfloridana. The genetic differentiation of Usnea populations from two geographically remote regions in Estonia and the relationships between characteristics of forest stands and measures of genetic variation per Usnea subfloridana populations were investigated; the closely clonally distributed specimens exhibited relatively high levels of genetic diversity (H = 0.62; SD = 0.02; Table 1). Populations of the pendulous and clonally reproducing Bryoria capillaris (Ach.) Brodo & D. Hawksw. and B. fuscescens (Gyeln.) Brodo & D. Hawksw. also revealed similar high levels of genetic diversity, H = 0.71 and H = 0.79, respectively (Nadyeina et al., 2014b). Previous studies have also observed high levels of genetic variation in other clonally reproducing cryptogams such as the bryophyte Pleurochaete squarrosa (Brid.) Lindb. (Spagnuolo et al., 2007). The populations of predominantly asexually reproducing lichen-forming fungus L. pulmonaria exhibited slightly lower levels of genetic diversity (H = 0.46; SD = 0.15) in Spain (Otalora et al., 2011) and in Central Europe (Walser et al., 2005; Werth et al., 2006a). Population of strictly outcrossing lichen-forming fungus Parmelia carporrhizans (Taylor) Poelt & Vézda revealed very high levels of genetic diversity (H = 0.74–0.90) and complete absence of clonality in the Mediterranean region (Alors et al., 2017). Our results support the view that closely clonally reproducing species can have comparable high levels of genetic variation, as have normally sexually reproducing species (Vrijenhoek, 1990). Us. subfloridana usually reproduces asexually by symbiotic propagules, soralia and isidia, but rarely bears a few (single or a couple) apothecia as well (Clerc, 2011) indicating the possibility of only limited sexuality. These rare apothecia have also been noticed in Estonian material (Torra and Randlane, 2007), but not in the study samples.

The AMOVA results demonstrated that most of the total genetic variation (99 %) was due to differences among individuals within studied Usnea populations; it also revealed a low proportion (0.5 %) of genetic variation attributed to regional differences (Table 2). High levels of gene flow (Nm = 8.52) or genetic similarity between all studied Us. subfloridana populations was demonstrated. The low dissimilarity could reflect the dominance of clonal spread between populations (Walser, 2004). Moreover, it was found that lichen molecular variation between populations from distinct regions (0.5 %) was also statistically significant (Table 2). The mean gene flow (Nm) for all populations across seven loci was 9.78. The analyses for checking shared haplotypes among populations in the software ARLEQUIN ver 3.5 (Excoffier and Lischer, 2010) revealed that all Usnea populations shared the identical multilocus genotypes with other populations as well as within both regions (Fig 2; Appendix A.1).
showed that populations of predominantly asexually reproducing Usnea subfloridana were highly clonal and structured by limited dispersal capacity of vegetative propagules (Walser, 2004; Werth et al., 2006b; Dal Grande et al., 2012). The effective dispersal distance of L. pulmonaria has been observed to remain very low, ranging from 15 to 30 m (Jüriado et al., 2011) to 140–200 m (Walser, 2004; Werth et al., 2006b). Our results indicated that in case of U. subfloridana, exchange of lichen individuals or their vegetative propagules should exist between the populations even from different regions of Estonia, which are located at almost 200 km from each other. The long distance between northern and southeastern populations did not seem to be a barrier for the dispersal of this species. The morphological peculiarity (growth form of the thallus) probably relevant to the efficient long-range dispersal of pendulous taxa by thallus fragments. Moreover, it has been suggested that drastic events such as storms or long-distance vectors (birds) may also play an important role in the distribution of vegetative propagules that are heavier than sexual propagules (Walser, 2004); for example, Högb erg et al. (2002) suggested that migration of the epiphytic lichen Letharia vulpina (L.) Hue from western North America to Europe occurred via lightweight soredia, overcoming the long distances between continents. Therefore, the long-distance transport of lichen propagules by birds or strong winds and storms seems not impossible in distribution of a common and widespread pendant lichen. Further research should focus on determining the long-range propagule dispersal by pendulous Usnea subfloridana.

Unconstrained gene exchange among populations may also indicate the connectedness of forest patches in different regions, or at least historically. The significance of landscape-scale parameters (including, for example, historic woodland structure or distances from the study area to the nearest contemporary or historic forest) on the colonization and richness of lichens has been demonstrated earlier (Ellis and Coppins, 2007; Randlane et al., 2017). Currently, about half of the territory in Estonia is covered by forests (Raudsaar earlier (1935–1939 according to historical topographic maps (1:50 000) of the Estonian Land Board (2017). It is possible that well-connected populations of lichens shared the individuals or propagules without ecological barriers and accumulated clones over many generations (Frankham et al., 2010).

No significant effect of forest characteristics and other explanatory variables of lichen populations on the minimum numbers of colonization events (C) and the number of private alleles (P) were found in the studied U. subfloridana populations (Appendix A.2). These findings suggest that all observed Usnea populations had developed by multiple independent immigration events from a large, genetically diverse source of populations by rapid clonal spread. Neither the age of forest stands (based on the oldest trees in the stands) nor circumference of host trees had a significant effect (P. Degtjarenko et al. / Fungal Biology 122 (2018) 731–737).

Table 2
Hierarchical analysis of molecular variance (AMOVA) for eleven populations of Usnea subfloridana according to seven microsatellite loci.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance</th>
<th>Percentage</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions</td>
<td>1</td>
<td>4778</td>
<td>0.011</td>
<td>0.5 %</td>
<td>0.014</td>
</tr>
<tr>
<td>Among populations within region</td>
<td>9</td>
<td>25.576</td>
<td>0.012</td>
<td>0.5 %</td>
<td>0.015</td>
</tr>
<tr>
<td>Within populations</td>
<td>567</td>
<td>1253.054</td>
<td>2.210</td>
<td>99 %</td>
<td>0.002</td>
</tr>
<tr>
<td>Total</td>
<td>577</td>
<td>1283.408</td>
<td>2.233</td>
<td></td>
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</tr>
</tbody>
</table>

Bold-faced values of P represent significant effect.
forest stand characteristics (Appendix A.2). Thus the studied lichen populations probably belonged to the same demographic phase. Given the fact that epiphytic lichen richness and abundance generally increase with forest stand age and environmental heterogeneity (McCune, 1993; Marmor et al., 2011), we hypothesized that the genetic variability of *U. subfloridana* populations could be influenced by the habitat maturity (estimated as age of forest stands or circumference of host tree), and tree species composition could be important in maintaining the genetic diversity of epiphyte populations. However, the GRM analyses showed that the age of forest stands and circumference of host tree did not demonstrate a significant effect on any measurement of genetic variability for *U. subfloridana* populations (Appendix A.2), although the positive trend was observed between Nei's unbiased genetic diversity (H) and age of forest stands. These results are consistent with those of Degtjarenko et al. (2016) who did not reveal a significant effect of the average age of lichen phorophyte on the genetic variation of *U. subfloridana* populations. A possible explanation for our result might be that variation in age of a forest stand (92–174; SD = 34.1; Table 1) was not sufficient to reveal a difference between young and old-growth forests; for example, Jüriado et al. (2011) showed that genetic diversity of *L. pulmonaria* populations was significantly higher in habitats of old-growth forests than in managed forests and wooded meadows; furthermore, the relationship between stand age and genetic diversity of lichen populations within the habitats was significantly positive only for wooded meadows. Gjerde et al. (2012) also demonstrated that no significant differences were detected in haplotype richness (based on data from two microsatellite loci) of *L. pulmonaria* collected in either young (40–120 y) or old (140–200 y) forests.

Our observations support the hypothesis that stand age or tree species composition are not of great importance in explaining the genetic patterns of *U. subfloridana*; for example, Boudreault et al. (2009) showed that the relationships between tree age and epiphytic biomass of *Usnea* species was not linear in boreal forest: the biomass of *Usnea* species tended to decrease in 150 y old forest stands. The abundance of *Usnea* thalli could be explained by the availability of branches on spruces, regardless of tree age (Rolstad and Rolstad, 1999). It is also possible that microclimatic heterogeneity (e.g. humidity, wind speed, canopy openness) could have a more consequential influence on *U. subfloridana* populations than habitat age and tree species composition; for instance, annual precipitation has an effect on genetic diversity of *L. pulmonaria* populations in the Iberian Peninsula (Otalora et al., 2015). Further research involving microclimatic measurements may determine the most important factors in shaping the genetic patterns of *U. subfloridana* populations.

5. Conclusions

Our study was aimed at studying the population genetics of the widespread epiphytic *U. subfloridana* in hemiboreal forest stands in Estonia. The results indicated that populations of mainly clonally reproducing *U. subfloridana* exhibited relatively high levels of genetic diversity, revealing a spatially unrestricted dispersal of individuals. Only a negligible genetic differentiation of *U. subfloridana* populations between two geographically remote regions of Estonia was found. Therefore it was concluded that unconstrained gene flow and multilocus genotypes exchange occurs among *U. subfloridana* populations between the two geographical regions or had occurred at least in the past. In our study, the stand age, mean circumference of the host tree, size of forest site sharing the same values of forest survey data and the proportion of birches and pines in the forest stand did not reveal any significant influence on allelic richness (A), Shannon’s information index (I), Nei’s unbiased genetic diversity (H), clonal diversity (M), the number of private alleles (P), and the minimum number of colonization events (C) of *U. subfloridana* populations. We suggest that stand age or tree species composition is not of great importance in explaining the genetic patterns of the pendulous epiphytic lichen *U. subfloridana*.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funbio.2018.03.013.

References


