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.

**Keywords**: forest age, genetic diversity, lichenized fungi, microsatellites, population genetics

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; Frankman 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 our 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 2010).
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 (Otalora et al. 2011; Scheidegger et al. 2012). For example, Jüriado 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; Otalora 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 the 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, gene flow and genetic differentiation of lichen populations (e.g., Walser et al. 2003; Otalora et al. 2011; Nadyeina et al. 2014a). The majority of previous studies which have considered 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* Stirt. 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

*Usnea 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 (Tõrra and Randlane 2007; Randlane et al. 2011). *Usnea 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 (Tõrra 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 *U. 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 (Tõrra 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 Lääne-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. 1986; 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. 1986) 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 *Pinus sylvestris*-dominated boreal forests, belonging to the *Oxalis–Vaccinium myrtillus*, the *Vaccinium myrtillus*, and the *Vaccinium vitis-idaea* forest site types. These forest types are also widely distributed in other Baltic states (Kairiūkštis 1966; Bušs 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
A 36 maps using comparable forest characteristics from their forest survey (Forest Public Registry
2017). In total, Usnea 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 three 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. subfloridana and used in further molecular analyses;
another 79 specimens were removed from the sampling as they belonged to other, similar Usnea
species (U. glabrescens (Vain.) Vain. or U. wasmuthii Räsanen). Then, the 50 mg of each U.
subfloridana 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 Tõrra 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 Usnea subfloridana 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 version 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 *Usnea subfloridana* in Estonia

In total, 66 alleles at seven microsatellite loci in 578 specimens from eleven *Usnea subfloridana*
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 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 8.52. 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).

3.2 Genetic diversity of Usnea subfloridana in relation to forest stand heterogeneity

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 U. subfloridana populations
were investigated; the mainly 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 *Lobaria 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 *Parmelina carporrhizans* (Taylor) Poelt & Vězda 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 mainly clonally reproducing species can have comparable high levels of genetic variation, as have normally sexually reproducing species (Vrijenhoek 1990).

*Usnea 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 (Tõrra 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 *U. subfloridana* populations was demonstrated. The low dissimilarity could reflect the dominance of clonal spread between populations (Walser 2004). Moreover, it was found that lichen populations shared common identical multilocus genotypes between all populations and also between the two regions (Fig. 2; Appendix A.1). The maximum geographical distance among the populations sharing the identical multilocus genotypes was 183 km, which was observed between population no 2 from N region and population no 4 from SE region of Estonia.

Lichen-forming fungi reproducing via ascospores generally have long distance dispersal, while clonally distributed species via isidia/soredia or thallus fragments may have a restricted dispersal and distinct genetic structure (Werth 2010; Werth et al. 2006b). Previous SSR-based studies on population genetics of lichenized fungi showed that populations of predominantly asexually reproducing *Lobaria pulmonaria* 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 15–30 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, Högberg 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 *U. 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 et al. 2016); prior to agricultural
activity (ca. 3000 years ago) this figure was most probably more than 80%, while in the 1920–
1930s it was the most restricted (Pärt 2011). Our study areas in both SE and N Estonia belong to
forested territories that have existed since 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 on C and P. We also recorded high values (0.77−0.90; SD=0.04; Table 1) of clonal diversity (M) in *U. subfloridana* populations and found no significant relationships between the clonal diversity and 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 years) or old (140–200
years) 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
year 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 *Lobaria*
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 *Usnea*
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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**References**


Figure legend

Fig 1 – Distribution map of *Usnea subfloridana* in Estonia (marked with light grey squares), and study populations (dark grey circles) in the southeastern and the northern regions of Estonia.

Fig 2 – Counts of shared haplotypes between populations of *Usnea subfloridana* in the southeastern (SE) and the northern regions (N) of Estonia; the thickness of lines reflects the number of shared haplotypes between populations.
Table 1 – 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 variation. Populations, the number of population; Specimens, the number of collected thalli per population; Trees, the number of host trees from which thalli were collected in each population; Latitude, latitudinal coordinates of the centre of forest site; Longitude, longitudinal coordinates of the centre of forest site; Age, the stand age (based on the oldest trees in the stands); BHC, mean circumference (cm) of the host tree per population (measured from each sampled tree at breast height 1.3 m); Betula, proportion of *Betula pendula* in each forest site; Pinus, proportion of *Pinus sylvestris* in each forest site; Size, the size of forest site (ha) sharing the same values of forest survey (age, proportion of trees in forest stands, forest site type); H, Nei’s unbiased genetic diversity per population; A, standardized allelic richness per population; I, Shannon’s information index per population; G, the number of multilocus genotypes per population; M, clonal diversity per population; C, the minimum number of colonization events per population; P, the number of private alleles per population.

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<td>0.81</td>
<td>0.85</td>
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<td>Size</td>
<td>0.7</td>
<td>1.9</td>
<td>4.3</td>
<td>13.6</td>
</tr>
<tr>
<td>Genetic variation</td>
<td>H</td>
<td>0.58</td>
<td>0.60</td>
<td>0.63</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>5.33</td>
<td>4.86</td>
<td>5.26</td>
<td>6.22</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>1.16</td>
<td>1.16</td>
<td>1.23</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>38</td>
<td>27</td>
<td>31</td>
<td>46</td>
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<tr>
<td></td>
<td>M</td>
<td>0.83</td>
<td>0.90</td>
<td>0.86</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.25</td>
<td>0.18</td>
<td>0.0</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Table 2 – Hierarchical analysis of molecular variance (AMOVA) for eleven populations of *Usnea subfloridana* according to seven microsatellite loci. Bold-faced values of P represent significant effect.

<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>4.778</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>
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<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>
<td></td>
</tr>
</tbody>
</table>
Highlights

- Microsatellite diversity of *Usnea subfloridana* was studied in hemiboreal forests.
- Populations of *U. subfloridana* exhibited high levels of genetic diversity.
- Populations from remote regions of Estonia shared common multilocus genotypes.
- A high level of gene flow must have occurred between *U. subfloridana* populations.