Genetic diversity of leafy liverwort species (Jungermanniidae, Marchantiophyta) in Poland: Regional genetic differentiation of leafy liverworts

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Abstract: For each of 8 species of leafy liverworts, 9-10 populations were sampled in 2-3 regions of Poland. In total, 5 regions were taken into account: the Tatra National Park, Bieszczady Mts., Białowieża Forest, Pomeranian Lake District, and Suwałki Lake District. Populations of most of the studied species did not show any correlation between genetic differentiation and geographic distances. Clear differences between regional groups of populations were found in only 2 species. The other species showed a complete or partial lack of genetic differentiation between groups of populations from various geographic regions. Generally, however, mountain populations had greater genetic diversity (H_T, H_S) and coefficient of genetic differentiation (G_{ST}) than lowland populations. In the Tatra National Park all the studied liverworts turned out to be more diverse than in the Bieszczady Mts. Białowieża Forest created a uniform group, standing out markedly from mountainous populations but population in this region had slightly smaller genetic diversity, then in the mountains. In the Pomeranian and Suwałki Lake Districts, genetic diversity of liverworts was significantly lower than in mountains. The decrease in diversity in these regions is a likely consequence of habitat fragmentation (causing population depletion) combined with negative effects of urban development. Habitat fragmentation results in genetic drift and inbreeding depression, which cause a decrease in genetic diversity. In the Pomeranian Lake District the level of total diversity (H_T) and intra-population diversity (H_S) was markedly higher than in the Suwałki Lake District. It may be linked to differences in climate, in the Suwałki Lake District climate is stronger.

Key words: *Bazzania trilobata*, *Trichocolea tomentella*, *Lophozia hatcheri*, *Mylia anomala*, *Lepidozia reptans*, *Calypogeia integristipula*, *Mylia taylorii*, *Tritomaria quinquedentata*, genetic variation, regional variability, habitat fragmentation

Contents

1. Introduction

Liverworts are significant components of many plant communities. Distribution of liverwort species is determined mostly by 2 factors. Firstly, numerous liverwort species are pioneer plants, which can grow even on raw soils. That is why they often colonize bare soils, with minimum competition with higher plants (Szweykowski 1996; Klama 2002, 2003). In such habitats they play an important role in the initial stages of succession (Szweykowski 1996; Stewart 1995). The second factor is their high sensitivity to moisture, as most of liverwort species are hygrophilous, sensitive to drying (Schuster 1966). Consequently, liverworts occupy sites with low competition and high humidity and soil moisture content. Such habitats are widespread in mountains, so liverworts in Poland are most common there.

The Tatra Mountains, have the richest liverwort flora in Poland. This results from the high diversity of habitats, climatic conditions, and history of this mountain range (Szweykowski 1996). In the past, the Tatras were the major refuge for plants with low thermal requirements, migrating to the Western Carpathians during Pleistocene glaciations from other European mountains or even from areas outside Europe. In this region, bedrocks vary widely, but granite and limestone are most common. In spite of degradation of some parts of the Tatras, there are still many places where vegetation is nearly primeval, and in spite of all the changes and deformations, Tatra vegetation is close to natural (Szafer & Zarzycki 1972; Mirek 1996). Similarly, there are still many places with nearly primeval vegetation in the Bieszczady National Park. However, the Bieszczady are part of Eastern Carpathians and during Pleistocene glaciations were located in a greater distance from the ice sheet, even in the coldest period. The flora of the Bieszczady is definitely Eastern Carpathian and clearly differs from the flora of Western Carpathians. Moreover, the Bieszczady, in contrast to the Tatras, are composed mostly of flysch deposits, clays, and sands (Szafer & Zarzycki 1972). Woodlands of both the Tatra and Bieszczady National Parks, because of their natural and often primeval character, are protected as UNESCO Biosphere Reserves. In these woodlands, liverworts are abundant. Thus it is important to assess the variability of liverworts growing in both the areas and determine if there are any clear differences in genetic structure of their local populations in these regions.

In Polish lowlands outside Białowieża Forest, liverworts are very rare, found only in scattered, isolated localities, mostly in Pomeranian Lake District (NW Poland) and Suwałki Lake District (NE Poland). Białowieża Forest, with adjacent forest areas, is the largest stretch of largely natural woodland in the European Plain, closely resembling Europe's primeval forests (Szafer & Zarzycki 1972). Its strong spatial connections with alluvial forests and peatlands is the reason why it is dominated by moist oak-hornbeam forests. The areal contributions of old forest and natural forest stands are high (Faliński 1986; Klama 2002). This is particularly important because forest liverworts most often and most abundantly grow on large fallen logs. They are less common on dead branches and small logs (Klama 2002)

In the Pomeranian Lake District and Suwałki Lake District, the process of disappearance of liverwort flora take place very violently (Szweykowski 1992; Szweykowski & Buczkowska 1996; Klama 2003). This is due to environmental change under the influence of human activity. The major threat for liverworts, e.g. in these regions, is forest fragmentation and exploitation by felling of old trees. This causes changes in microclimate and limitation of microhabitats as well as isolation of the species colonizing them, reduction of population size, and even extinction of some local populations (Klama 2002, 2003; Hanski 2005).

Liverworts are very sensitive to changes of the environment, especially to anthropopressures, because they are characterized by narrow ecological requirements, and therefore even small changes in a microhabitat cause their disappearance. That is why in forest communities exposed to strong human pressure, only few common, the most tolerant species are still found (Steward 1995; Szweykowski & Buczkowska 1996; Klama 2003).

Another factor affecting genetic variation, mostly in transformed habitats, is environmental pollution. The very existence of polluted substrate may stimulate microevolutionary processes and formation of new ecotypes or new ecological forms (Fabiszewski 1986). In some higher plants, different ecological niches are dominated by some specific, best-adapted genotypes (Sahuquillo & Lumaret 1995; Hufford & Mazer 2003).

Perhaps within liverwort species colonizing poor, transformed habitats, some genotypes are adapted to specific environmental conditions.

Because of the fast decline of liverwort species in lowlands, it is extremely important to determine their gene pool and genetic structure. Until recently, biodiversity indices were limited to ecological parameters, such as population dynamics and species richness. Results of genetic research open new perspectives for protection of extremely rare and threatened species (Haig 1998). Most of studies concerning bryophytes (chiefly mosses) indicate that populations found in natural habitats show higher genetic variation than populations occupying sites disturbed by human activity (Akiyama & Hiraoka 1994; Boisselier-Dubayle *et al*. 1995; Wilson & Provan 2003; Spagnuolo *et al*. 2007; Buczkowska *et al*. 2010). By contrast, data on liverwort population biology and genetics, comparing their variability in primeval habitats and in those transformed by human activity, are still scanty. Thus little is known about the true difference in genetic variation of liverworts between these 2 classes of habitats.

The major aims of this study were: (1) to compare genetic diversity of leafy liverwort populations in various parts of Poland; and (2) to determine if the impoverishment of liverwort flora in lowland Poland affects the genetic variation of populations of species growing there.

2. Materials and methods

2.1. Plant material

Samples of 8 species of leafy liverworts (*Bazzania trilobata, Trichocolea tomentella, Lophozia hatcheri, Mylia anomala, Lepidozia reptans*, *Calypogeia integristipula, Mylia taylorii, Tritomaria quinquedentata*) were collected from different regions of Poland. For each of the 8 species, samples were collected from 9-10 populations in 2-3 regions. In total, 5 regions were taken into account: the Tatra National Park (NP), Bieszczady Mts., Białowieża Forest, Pomeranian Lake District, and Suwałki Lake District (Fig. 1, Table 1). Details in sampling and study sites were described in Bączkiewicz (2012, pp. 6-12).
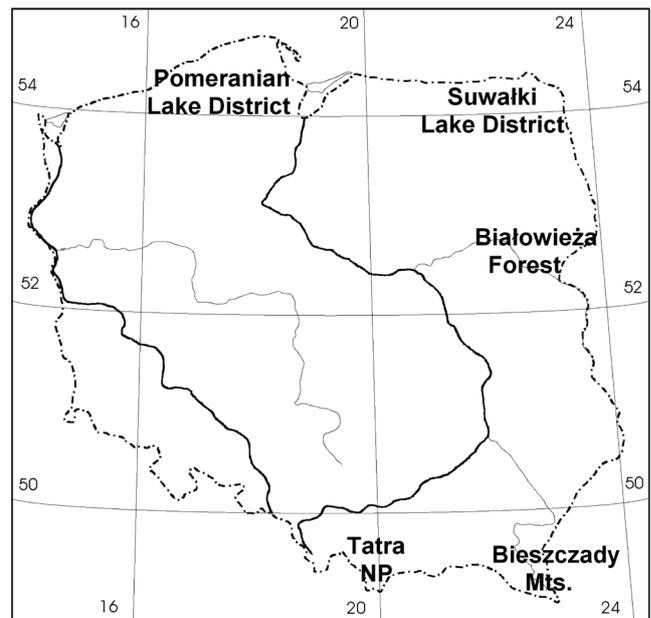
Fig. 1. Five Polish regions from which material was collected: the Tatra National Park, Bieszczady Mts., Białowieża Forest, Pomeranian Lake District, and Suwałki Lake District

2.2. Methods

Isozyme analysis was used to examine the genetic diversity of leafy liverwort populations. Procedure for electrophoresic analysis was described in Bączkiewicz (2012, pp. 12-13).

2.3. Data analysis

To compare the genetic diversity of the 8 leafy liverworts in different regions, for each species the same statistic analysis was performed. Parameters of genotypic diversity within regions were estimated. All collected gametophytes were sorted to detect unique multilocus genotypes (MLGs). Each of the detected distinct MLGs was assumed to be a distinct genet. The proportion of distinguishable genotypes (G/N) was calculated as the number of unique MLGs (G) divided by sample size (Ellstrand & Roose 1987). The mean proportion of G/N was calculated at the population and regional level. The means were tested by the Kruskal-Wallis ANOVA test or the nonparametric Mann-Whitney U test using STATISTICA 7.1 for Windows (StatSoft 2008).

Table 1. Regions where collected samples of 8 species of leafy liverworts

Species	Tatra NP	Bieszczady Mts.	Białowieża Forest	Pomeranian Lake District	Suwałki Lake District
Bazzania trilobata	*	*	*	-	-
Trichocolea tomentella	*	-	-	*	-
Lophozia hatcheri	*	*	-	-	-
Mylia anomala	*	-	-	*	*
Lepidozia reptans	*	-	-	*	*
Calypogeia integristipula	*	*	-	*	-
Mylia taylorii	*	*	-	-	-
Tritomaria quinquedentata	*	*	-	-	-

For each region were estimated: mean number of alleles per locus per region ($A_{reg.}$) and mean per population ($A_{pop.}$) in regions, the percentage of polymorphic loci per region ($P_{reg.}$) and mean per population in each region ($P_{pop.}$). These analyses were performed using POPGENE 1.32 (Yeh *et al*. 2000). The Kruskal-Wallis ANOVA or the Mann-Whitney *U* tests were used to check their statistical significance. Number of rare alleles (frequency < 0.05) and number of private alleles (Slatkin 1985) at the regional level were calculated.

For studied species Nei's (1973, 1978) gene-diversity statistics were calculated: total genetic diversity (H_T) for regions, mean of genetic diversity within population (H_S) in region, coefficient of genetic differentiation (G_{ST}) and gene flow between populations (N_m) in region using POPGENE 1.32. Gene flow (N_m) was estimated indirectly from G_{ST} using the formula $N_m=0.5(1-G_{ST})/G_{ST}$, adapted for haploid organisms (McDermott & McDonald 1993). In bryophytes, this formula is used for both nuclear and chloroplast markers, as the migrating diaspores always comprise a haploid genome, and it is assumed in all cases that the distance of sperm migration is very short, normally not exceeding 10 cm (McLetchie 1996; Korpelainen *et al*. 2005). Pair-wise genetic distances (*D*) and identity (*I*) (Nei 1972) between regions were calculated for each species (using POPGENE 1.32).

AMOVA was used to describe the percentage shares of genetic variation within populations, and between regions in the total genetic variation of species of leafy liverworts. The level of genetic variation among populations was estimated using Φ statistic (analogous to *F* statistic). Significance levels for populations were determined using a permutation test (1000 permutations). An analysis of molecular variance (AMOVA) was done by GenALEx 6.3. (Peakall & Smouse 2006).

3. Results

3.1. Regional genetic diversity of leafy liverworts

3.1.1. *Bazzania trilobata*

Clonal diversity. The genetic variability of *B. trilobata* populations from 3 geographic regions (Tatra NP, Bieszczady Mts., and Białowieża Forest) was compared. Clonal diversity in these regions was similar. The mean *G/N* per population in the Tatra NP was 0.597, in

the Bieszczady Mts. 0.610, and in Białowieża Forest 0.577. A nonparametric Kruskal-Wallis ANOVA test revealed that the mean proportion distinguishable genotypes (G/N) from populations in different regions did not differ statistically significant (H = 0.068, p = 0.97). At the region level the highest genotypic diversity was in the Tatra NP, where G/N was 0.58, in the Bieszczady Mts. slightly less (0.57). In Białowieża Primeval Forest G/N was the lowest (0.50) (Table 2).

Table 2. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Bazzania trilobata* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	27	216	126	0.58	0.597
Bieszczady Mts.	23	184	105	0.57	0.610
Białowieża Forest	28	224	111	0.50	0.577

Isozyme diversity. Genetic diversity at the regional level was the highest in the Tatra NP (Table 3), where the number of alleles was 35. The mean number of alleles per locus ($A_{reg.}$) reached there 2.5 and the percentage of polymorphic loci ($P_{reg.}$) was 85.71%. At the population level, also the mean $A_{pop.}$ and $P_{pop.}$ per population were the highest in the Tatra NP (2.3 and 78.57%, respectively, while in the Bieszczady Mts. $A_{pop.}$=2.1 and $P_{pop.}$=71.42% and in Białowieża Forest $A_{pop.}$=2.2, $P_{pop.}$=61.90%). However, the Kruskal-Wallis ANOVA test showed no statistically significant differences among the 3 regional groups of populations in respect to $A_{pop.}$ (*H*=3.251, *p*=0.20) and $P_{pop.}$ (*H*=3.429, *p*=0.18).

Rare alleles were identified in every region (Table 3). The highest number of rare alleles (7) was detected in the Białowieża Forest. Private alleles at the regional level were found only in the Tatra NP and in Białowieża Forest, as no private alleles were identified in the Bieszczady Mts. In the Tatra NP, alleles *Est-2* and *Est-3* were very frequent in all populations and *Pgi-3* occurred in 2 populations with high frequency, too. In Białowieża Forest, a private allele *Sdh-4* occurred in all studied populations, while it was absent in the mountains. Furthermore, lowland populations differ from mountainous populations also in frequency of some alleles, with typically 2-fold or higher differences. For example, allele

Table 3. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus ($A_{reg.}$) per region and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Bazzania trilobata* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	35	6	3	2.5	85.71	2.30	78.57
Bieszczady Mts.	32	3	0	2.3	78.57	2.10	71.43
Białowieża Forest	34	7	3	2.4	71.43	2.20	61.90

Table 4. Total genetic diversity (H_T±SD), genetic diversity within populations (H_S±SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Bazzania trilobata* within regions

Region	H_T(±SD)	H_S(±SD)	G_{ST}	N_m
Tatra National Park	0.2775 (0.0383)	0.2692 (0.0332)	0.0820	5.3624
Bieszczady Mts.	0.2597 (0.0292)	0.2313 (0.0249)	0.1022	3.5825
Białowieża Forest	0.2449 (0.0605)	0.2417 (0.0574)	0.0356	9.5612

Table 5. Analysis of molecular variance (AMOVA) for *Bazzania trilobata*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	2	0.188	7	$\Phi_{RT} = 0.074$***
Among populations within regions	6	0.287	12	$\Phi_{PR} = 0.122$***
Within populations	622	2.056	81	$\Phi_{PT} = 0.187$***
Among Tatra populations	2	0.212	9	$\Phi_{PT} = 0.090$***
Within Tatra populations	213	2.077	91	
Among Bieszczady populations	2	0.621	12	$\Phi_{PT} = 0.123$***
Within Bieszczady populations	182	2.163	88	
Among Białowieża populations	2	0.081	4	$\Phi_{PT} = 0.043$***
Within Białowieża populations	221	1.948	96	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

Table 6. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Bazzania trilobata*

Region	Tatra NP	Bieszczady Mts.	Białowieża Forest
Tatra National Park	****	0.9765	0.9203
Bieszczady Mts.	0.0238	****	0.9296
Białowieża Forest	0.0830	0.0730	****

Idh-2 had a markedly higher frequency in mountainous populations, while alleles *Acp-1, Gdh-1, Idh-1*, and *Pgm-4* were much more frequent in lowland populations than in the mountains (Table 14 in Bączkiewicz 2012).

The total genetic diversity (H_T) in the geographic regions under study, based on mean allelic frequencies of polymorphic loci over all populations, was the highest in the Tatra NP (0.2775) and the lowest in Białowieża Forest (0.2449). The mean of H_S (genetic diversity within population) per population in the geographic regions was also slightly higher in the Tatra NP (0.2625) than in the other regions, but not significantly (Kruskal-Wallis ANOVA test: *H*=2.756, *p*=0.25). The highest value of the coefficient of genetic differentiation (G_{ST}) was in the Bieszczady Mts. (0.1022) and the lowest in Białowieża Forest (0.0356). Consequently, gene flow between populations (N_m) in the Bieszczady Mts. was the smallest (3.5825) whereas in Białowieża Forest it was the largest (9.5612) (Table 4).

Analysis of molecular variance (AMOVA) showed that only 7% of the total genetic variation was due to differences among regions (Tatra NP, Bieszczady Mts., and Białowieża Forest), while 93% to differences within

regions. Separate analyses for each region revealed that 9% of the total genetic variation within the Tatra NP was due to variation among populations, compared to 12% within the Bieszczady Mts. and 4% within Białowieża Forest (Table 5).

The greatest genetic distance (*D*) was detected between Białowieża Forest and the Tatra NP (0.0830). The highest similarity was between mountainous regions (Tatra NP and Bieszczady Mts., *I*=0.9765), which points to the divergence of Białowieża Forest populations (Table 6). The mean distances between populations within the Tatra NP and Białowieża Forest (0.0475 and 0.0186, respectively) were lower than between regions, but within the Bieszczady Mts. the mean *D* value was much higher (0.1307).

3.1.2. *Trichocolea tomentella*

Clonal diversity. The genetic variability of *T. tomentella* populations from 2 geographic regions (Tatra NP and Pomeranian Lake District) was compared. Clonal diversity was higher in the Tatra NP, where the mean *G/N* per population was 0.534, while within the Pomeranian Lake District it was 0.520. However the nonparametric Mann-Whitney U test, for mean of G/N

Table 7. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Trichocolea tomentella* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	29	232	102	0.44	0.534
Pomeranian Lake District	33	264	108	0.41	0.520

Table 8. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Trichocolea tomentella* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	34	3	5	2.3	66.67	1.96	53.33
Pomeranian Lake District	34	5	6	2.3	66.67	1.90	53.33

from population of both regions did not differ statistically significant (Z=0.612, p=0.54). At the regional level, the value of *G/N* was 0.44 in the Tatra NP and 0.41 in the Pomeranian Lake District (Table 7).

Isozyme diversity. In both regions the same number of alleles ($A_{reg.}$=34) and the same percentage of polymorphic loci $P_{reg.}$=66.67% were found. At the population level the mean $A_{pop.}$ was slighty higher in the Tatra NP (1.96) than in Pomerania (1.90), while the mean $P_{pop.}$ per population was the same ($P_{pop..}$=53.33%) in both regions. The Mann-Whitney *U* test revealed that populations from the Tatra NP and the Pomeranian Lake District did not differ significantly in $A_{pop.}$ (Z=0.122, p=0.90) and $P_{pop.}$ (Z=0.490, p=0.62) (Table 8).

In the Tatra NP, 3 rare and 5 private alleles were found, while in the Pomeranian Lake District, 5 rare and 6 private alleles (Table 8). In the Pomeranian Lake District, all private alleles (with the exception of *PerB-3*) were limited to a single population. In the Tatra NP, by contrast, all the private alleles occurred in several populations (Table 22 in Bączkiewicz 2012).

The total genetic diversity in the geographic regions under study, based on mean allelic frequencies of polymorphic loci, was higher in the Tatra NP (H_T=0.2587) than in the Pomeranian Lake District (H_T=0.2260). The mean of H_S per population in both regions was similar, but in the Tatra NP was slightly higher than in Pomerania, (0.1837 and 0.1792, respectively), but not significantly (Mann-Whitney *U* test: Z=20.367, p=0.71). The coefficient of genetic differentiation within regions was higher in the Tatra NP, where G_{ST}=0.2357. Consequently, gene flow (N_m) between populations within the Tatra NP was lower (2.5407) than in the Pomeranian Lake District (7.6848) (Table 9).

The AMOVA conducted for the *T. tomentella* showed that only 2% of the total genetic variation was due to variation among studied regions and 16% among populations within regions. Separate analyses for each region revealed that 24% of the total genetic variation was due

Table 9. Total genetic diversity (H_T±SD), genetic diversity within populations (H_S±SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Trichocolea tomentella* within regions

Region	H_T (±SD)	H_S (±SD)	G_{ST}	N_m
Tatra National Park	0.2587 (0.0694)	0.1837 (0.0534)	0.2357	2.5407
Pomeranian Lake District	0.2260 (0.0718)	0.1792 (0.0655)	0.0611	7.6848

Table 10. Analysis of molecular variance (AMOVA) for *Trichocolea tomentella*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	1	0.034	2	Φ_{RT} = 0.016***
Among populations within regions	7	0.337	16	Φ_{PR} = 0.166***
Within populations	487	1.695	82	Φ_{PT} = 0.179***
Among Tatra populations	4	0.512	24	Φ_{PT} = 0.241***
Within Tatra populations	227	1.608	76	
Among Pomeranian populations	3	0.173	9	Φ_{PT} = 0.089***
Within Pomeranian populations	260	1.772	91	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

Table 11. Nei's (1978) genetic identities (I, above diagonal) and distances (D, below diagonal) between regional groups of populations of *Trichocolea tomentella*

Region	Tatra NP	Pomeranian Lake District
Tatra National Park	****	0.9783
Pomeranian Lake District	0.0219	****

to variation among populations within the Tatra NP and 9% within the Pomeranian Lake District (Table 10).

The genetic distance (D) between the regions was 0.0219 (Table 11). It was smaller than the mean D between populations within both regions: 0.0901 within the Tatra NP and 0.0311 within the Pomeranian Lake District.

3.1.3. *Lophozia hatcheri*

Clonal diversity. Among the 2 compared geographic regions (Tatra NP and Bieszczady Mts.), clonal diversity was higher in the Tatra NP. In this region the value of G/N (at the regional level) was 0.35 but in the Bieszczady Mts. it was 0.30. The mean G/N per population within the the Tatra region was 0.380 and in the Bieszczady Mts. it was 0.335. This distinction was not statistically significant (Mann-Whitney U test: $Z=0.612$, $p=0.54$) (Table 12).

Table 12. Numbers of patches, plants (N), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (G/N, where G=MLG) of *Lophozia hatcheri* at the region level and at the population level within regions

Region	No. of patches	N	MLG	G/N (reg.)	G/N (pop.)
Tatra National Park	46	368	130	0.35	0.380
Bieszczady Mts.	22	176	53	0.30	0.335

Isozyme diversity. At the regional level, 41 alleles were found in the Tatra NP and 34 alleles in the Bieszczady Mts. (Table 13). The mean number of alleles per locus ($A_{reg.}=3.2$) and the percentage of polymorphic loci ($P_{reg.}=100\%$) were higher in the Tatra NP, too. In the Bieszczady Mts. $P_{reg.}$ reached 92.31%, because locus *Per-B* was monomorphic. Also the mean $A_{pop.}$ and $P_{pop.}$ per population within regions were higher in the Tatra NP ($A_{pop.}=2.10$, $P_{pop.}=69.23\%$) than in the Bieszczady region ($A_{pop.}=1.95$ and $P_{pop.}=63.46\%$). However, the Mann-Whitney U test revealed that populations from both regions did not differ significantly in $A_{pop.}$ ($Z=0.489$, $p=0.62$) and $P_{pop.}$ ($Z=0.490$, $p=0.62$).

In both regions rare and private alleles were detected, including 10 private alleles in the Tatra region and only 3 in the Bieszczady Mts. (Table 13). In the Tatra NP, half of them were found in more than one population (*Idh-4*, *Lap-4*, *MdhB-1*, *PerB-2*, *Sdh-2*), while in the Bieszczady all 3 private alleles were found only in 2 populations: in B-6 (*Got-1* and *Pgd-1*) and in B-9 (*Gdh-1* and *Gdh-1*) (Table 30 in Bączkiewicz 2012).

The total genetic diversity (H_T) in the geographic regions under study, based on mean allelic frequencies of polymorphic loci over all populations, was higher in the Tatra NP (0.2467). Similarly, the mean of genetic diversity within populations (H_S) per population was higher in the Tatra NP (0.2304). In the Bieszczady Mts., H_T and the mean of H_S were 0.2210 and 0.2129, respectively. The Mann-Whitney U test showed no significant differences between the regions in respect to H_S ($Z=0.122$, $p=0.90$). The value of G_{ST} within regions was higher in the Tatra NP (0.2860). Consequently, gene flow (N_m) between populations within regions was lower within the Tatra NP (1.9453) than in the Bieszczady Mts. (2.8662) (Table 14).

The AMOVA conducted for *L. hatcheri* showed a lack of genetic differentiation among regions. Separate analyses for each region revealed that 37% of total genetic variation within the Tatra NP was due to differences among populations, compared to 17% within the Bieszczady Mts. (Table 15).

The genetic distance (D) between the regions was 0.0320 (Table 16). It was lower than the mean D between populations within each region. The mean D among populations within the Tatra NP was 0.1908, and within the Bieszczady Mts. it was 0.1415.

Table 13. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Lophozia hatcheri* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	41	10	10	3.2	100	2.10	69.23
Bieszczady Mts.	34	8	3	2.7	92.31	1.95	63.46

Table 14. Total genetic diversity ($H_T \pm SD$), genetic diversity within populations ($H_S \pm SD$), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Lophozia hatcheri* within regions

Region	$H_T (\pm SD)$	$H_S (\pm SD)$	G_{ST}	N_m
Tatra National Park	0.2467 (0.0515)	0.2304 (0.0238)	0.2860	1.9453
Bieszczady Mts.	0.2210 (0.0615)	0.2129 (0.0454)	0.1485	2.8662

Table 15. Analysis of molecular variance (AMOVA) for *Lophozia hatcheri*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	1	0.000	0	Φ_{RT}= -0.017 n.s.
Among populations within regions	7	0.710	28	Φ_{PR}= 0.282***
Within populations	542	1.810	72	Φ_{PT}= 0.269***
Among Tatra populations	4	0.971	37	Φ_{PT}= 0.368***
Within Tatra populations	283	1.668	63	
Among Bieszczady populations	3	0.405	17	Φ_{PT}= 0.171***
Within Bieszczady populations	259	1.966	83	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, levels of significance n.s. p > 0.05, *** p≤0.001

Table 16. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Lophozia hatcheri*

Region	Tatra NP	Bieszczady Mts.
Tatra National Park	****	0.9685
Bieszczady Mts.	0.0320	****

3.1.4. *Mylia anomala*

Clonal diversity. The genetic variability of *M. anomala* populations from 3 geographic regions (Tatra NP, Pomeranian Lake District, and Suwałki Lake District) was compared. The highest clonal diversity was in the Pomeranian Lake District (mean *G/N* = 0.27 at both the population level and regional level). In the Tatra NP, clonal diversity was slightly lower than in the Pomeranian Lake District (at the population level, mean *G/N*=0.257, and at the regional level, *G/N*=0.23). The lowest values were in the Suwałki Lake District: the mean G/N at the population level was 0.143, and at the regional level it was 0.11 (Table 17). The mean proportion of distinguishable genotypes (*G/N*) from populations in different regions did not differ significantly (Kruskal-Wallis ANOVA test: *H*=6.915, *p*=0.078).

Isozyme diversity. In the Pomeranian Lake District, was found the highest number of alleles (34),

the mean number of alleles per locus ($A_{reg.}$) was 2.0 and the percentage of polymorphic loci ($P_{reg.}$) was 64.71%. The lowest number of alleles (29) was in the Suwałki Lake District found ($A_{reg.}$=1.7 and $P_{reg.}$=41.17%). At the population level, the mean $A_{pop.}$ and $P_{pop.}$ per population within regions were the highest in the Pomeranian Lake District ($A_{pop.}$=1.73, $P_{pop.}$=54.90%). In the Tatra Mts. $A_{pop.}$=1.53 and $P_{pop.}$=43.14, while in the Suwałki Lake District $A_{pop.}$=1.40, $P_{pop.}$=27.45%. The Kruskal-Wallis ANOVA showed no significant differences among the 3 studied regions in respect to $P_{pop.}$ (*H*=5.728, *p*=0.06) and $A_{pop.}$ (*H*=5.445, *p*=0.07) (Table 18).

Rare and private alleles were identified in all the regions. The highest number of rare and private alleles were detected in the Pomeranian Lake District, with 8 rare and 7 private alleles. Four of the private alleles (*Gdh-1*, *Idh-1*, *Idh-3*, *PgdB-1*) occurred in 2 populations of the 3 studied in this region. Remaining private alleles occurred in single populations in this region.

Table 17. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Mylia anomala* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	13	120	28	0.23	0.257
Pomeranian Lake District	24	192	51	0.27	0.270
Suwałki Lake District	30	240	25	0.11	0.143

Table 18. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Mylia anomala* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	32	3	6	1.9	58.82	1.53	43.14
Pomeranian Lake District	34	8	7	2.0	64.71	1.73	54.90
Suwałki Lake District	29	7	5	1.7	41.17	1.40	27.45

Table 19. Total genetic diversity ($H_T \pm$SD), genetic diversity within populations ($H_S \pm$SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Mylia anomala* within regions

Region	$H_T(\pm$SD$)$	$H_S(\pm$SD$)$	G_{ST}	N_m
Tatra National Park	0.1819 (0.0605)	0.0912 (0.0316)	0.2952	1.2531
Pomeranian Lake District	0.1840 (0.0453)	0.1209 (0.0374)	0.0989	5.1234
Suwałki Lake District	0.0978 (0.0289)	0.0513 (0.0135)	0.1367	2.8124

Table 20. Analysis of molecular variance (AMOVA) for *Mylia anomala* populations: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	2	0.314	18	$\Phi_{RT}= 0.180$***
Among populations within regions	6	0.331	19	$\Phi_{PR}= 0.231$***
Within populations	543	1.101	63	$\Phi_{PT}= 0.370$***
Among Tatra populations	2	0.712	34	$\Phi_{PT}= 0.340$***
Within Tatra populations	117	1.379	66	
Among Pomeranian populations	2	0.180	11	$\Phi_{PT}= 0.112$***
Within Pomeranian populations	189	1.614	89	
Among Suwałki populations	2	0.261	27	$\Phi_{PT}= 0.272$***
Within Suwałki populations	237	0.697	73	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p$\leq$0.001

In the Tatra NP, 3 alleles (*MdhA-2, Me-3* and *Sdh-3*) occurred in all studied populations, and alleles *Sdh-3* and *Me-3* occurred with a very high frequency. In the Suwałki Lake District, 5 private alleles were found, but only allele *Pgm-1* occurred in 2 populations in this region. Furthermore, lowland populations differ from mountainous populations by the presence of alleles *Me-1* and *Me-3*. In Tatra NP, allele *Me-1* was absent, whereas in the Pomeranian Lake District it occurred with a frequency up to 46%, and in the Suwałki Lake District with a frequency of 100%. By contrast, allele *Me-3* occurred only in the Tatra NP, with a frequency of over 67% per population (Table 18 and Table 38 in Bączkiewicz 2012).

In the Pomeranian Lake District the total genetic diversity (H_T) was the highest (0.1840). It was slightly lower in the Tatra NP (0.1819) and much lower in the Suwałki Lake District (0.0978). The mean of H_S per population in the geographic regions was the highest in Pomeranian (0.1209), and the lowest in the Suwałki Lake District (0.0513), but the differences were not significant (Kruscal-Wallis ANOVA H=6.489, p=0.39). The highest G_{ST} was in the Tatra NP (0.2952), with the lowest degree of gene flow between populations (N_m=1.2531) (Table 19).

The AMOVA conducted for *M. anomala* showed that only 18% of the total genetic variation was present among regions (Tatra NP, Pomeranian Lake District, and Suwałki Lake District). Separate analyses for each region revealed that 34% of the total genetic variation within the Tatra NP was due to differences among populations, compared to 11% within the Pomeranian

Lake District and 27% within the Suwałki Lake District (Table 20).

The greatest genetic distance was observed between the Tatra NP and the Suwałki Lake District ($D = 0.0884$). The highest similarity was detected between the Tatra NP and the Pomeranian Lake District ($I = 0.9565$) (Table 21). The mean D between populations within regions of the Pomeranian Lake District and the Suwałki Lake District was smaller (0.0297 and 0.0382, respectively) than between regions, but within the Tatra NP it was higher (0.1081).

Table 21. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Mylia anomala*

Region	Tatra NP	Pomeranian Lake District	Suwałki Lake District
Tatra National Park	****	0.9565	0.9154
Pomeranian Lake District	0.0445	****	0.9485
Suwałki Lake District	0.0884	0.0529	****

3.1.5. *Lepidozia reptans*

Clonal diversity. The genetic diversity of *L. reptans* populations from 3 geographic regions (Tatra NP, Pomeranian Lake District, and Suwałki Lake District) was compared. The mean *G/N* per population in the Tatra NP was 0.530, in the Pomeranian Lake District 0.470, and in the Suwałki Lake District 0.423. A nonparametric Kruskal-Wallis ANOVA test revealed that the mean proportion of distinguishable genotypes

Table 22. Numbers of patches, plants (N), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (G/N, where G=MLG) of *Lepidozia reptans* at the region level and at the population level within regions

Region	No. of patches	N	MLG	G/N (reg.)	G/N (pop.)
Tatra National Park	38	304	146	0.48	0.530
Pomeranian Lake District	27	216	91	0.42	0.470
Suwałki Lake District	26	208	82	0.39	0.423

Table 23. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Lepidozia reptans* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	33	10	7	3.0	100	2.40	75.00
Pomeranian Lake District	26	4	0	2.4	81.82	2.20	72.73
Suwałki Lake District	27	4	2	2.5	72.73	2.07	60.61

(G/N) from populations in studied regions differ significantly (H=5.814, p=0.05). The highest clonal diversity at the regional level was in the Tatra NP (G/N=0.48). In the Suwałki Lake District G/N was the lowest (0.39) (Table 22).

Isozyme diversity. At the regional level, the highest number of alleles was found in the Tatra NP (Table 23). In this region the mean number of alleles per locus ($A_{reg.}$) was 3.0 and the percentage of polymorphic loci ($P_{reg.}$) was 100%. In the Suwałki Lake District the percentage of polymorphic loci was the lowest ($P_{reg.}$=72.73%). In the Pomerania and Suwałki Lake District the means number of alleles per locus ($A_{reg.}$) were similar (2.4 and 2.5 respectively). At the population level the mean $A_{pop.}$ and $P_{pop.}$ per population within regions were the highest in the Tatra NP, too (2.40 and 75.00%, respectively), compared to the Pomeranian Lake District ($A_{pop.}$=2.20 and $P_{pop.}$=72.73%) and the Suwałki Lake District ($A_{pop.}$=2.07, $P_{pop.}$=60.61%). However, the Kruskal-Wallis ANOVA test showed no significant differences among the 3 regional groups of populations in respect to $A_{pop.}$(H=2.311, p=0.32) and $P_{pop.}$(H=2.870, p=0.24).

Rare alleles were identified in every region (Table 23). The highest number of them was detected in the Tatra NP (10), while both in the Pomeranian Lake District and the Suwałki Lake District only 4 rare alleles were found. At the regional level, private alleles were found only in the Tatra NP (7) and the Suwałki Lake District (2). No private alleles were identified the Pomeranian Lake District. For the Tatra NP, alleles *Idh-3*, *PgiB-3* and *PgiB-4* were the most characteristic; they

occurred in almost all populations and often with frequency > 0.05 (Table 46 in Bączkiewicz 2012).

The total genetic diversity (H_T) in the geographic regions, based on mean allelic frequencies of polymorphic loci over all populations, was the highest in the Tatra NP (0.2320). In the Suwałki Lake District it was the lowest (0.1811). The mean of H_S per population in geographic regions was also higher in the Tatra NP (0.2186) than in the other regions. In the Suwałki Lake District it was the lowest (0.1658), however, the differences were not significant (Kruscal-Wallis ANOVA test: H=0.622, p=0.73). The highest value of G_{ST} was in the Tatra NP (0.1098), and the lowest the Suwałki Lake District (0.0913). Consequently, gene flow between populations (N_m) was the lowest (4.3643) in the Tatra NP and the largest (5.6477) in the Suwałki Lake District (Table 24).

The AMOVA conducted for *L. reptans* reveales that only 1% of the total genetic variation was due to differences among the 3 regions (Tatra NP, Pomeranian Lake District, and Suwałki Lake District) and 11% among populations within regions. Separate analyses for each region revealed that 12% of the total genetic variation within the Tatra NP was due to differences among populations, compared to 10% within the Pomeranian Lake District and 11% within the Suwałki Lake District (Table 25).

The highest genetic distance (D) was detected between the lowland regions: the Pomeranian and Suwałki Lake Districts (0.0290). The highest similarity was between the Suwałki Lake District and the Tatra NP (I=0.9868) (Table 26). The mean genetic distance between populations within regions was smaller than

Table 24. Total genetic diversity ($H_T \pm$ SD), genetic diversity within populations ($H_S \pm$SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Lepidozia reptans* within regions

Region	H_T(±SD)	H_S(±SD)	G_{ST}	N_m
Tatra National Park	0.2320 (0.0243)	0.2186 (0.0 277)	0.1098	4.3643
Pomeranian Lake District	0.2184 (0.0299)	0.1995 (0.0212)	0.0913	5.6477
Suwałki Lake District	0.1811 (0.0188)	0.1658 (0.0176)	0.1069	4.6535

Table 25. Analysis of molecular variance (AMOVA) for *Lepidozia reptans*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	2	0.023	1	$\Phi_{RT}= 0.013$***
Among populations within regions	7	0.190	11	$\Phi_{PR}= 0.110$***
Within populations	702	1.537	88	$\Phi_{PT}= 0.122$***
Among Tatra populations	3	0.194	12	$\Phi_{PT}= 0.117$***
Within Tatra populations	284	1.467	88	
Among Pomeranian populations	2	0.182	10	$\Phi_{PT}= 0.097$***
Within Pomeranian populations	213	1.686	90	
Among Suwałki populations	2	0.191	11	$\Phi_{PT}= 0.114$***
Within Suwałki populations	205	1.481	89	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

the mean genetic distance between regions. The mean *D* between populations within the Tatra NP was 0.0523, in the Pomeranian Lake District 0.0518, and in the Suwałki Lake District 0.0563.

Table 26. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Lepidozia reptans*

Region	Tatra NP	Pomeranian Lake District	Suwałki Lake District
Tatra National Park	****	0.9745	0.9868
Pomeranian Lake District	0.0258	****	0.9714
Suwałki Lake District	0.0133	0.0290	****

3.1.6. *Calypogeia integristipula*

Clonal diversity. The genetic variability of *C. integristipula* populations from 3 geographic regions (Tatra NP, Bieszczady Mts., and Pomeranian Lake District) was compared. Clonal diversity in these regions was similar. The mean *G/N* per population in the Tatra NP was 0.147, in the Bieszczady Mts. 0.137, and in the Pomeranian Lake District 0.130. The mean proportion of distinguishable genotypes (*G/N*) from populations

in studied regions did not differ significantly (Kruskal-Wallis ANOVA test: *H* = 426, *p* = 0.81). The diversity at the regional lever was identical in the Tatra NP and Bieszczady Mts. (*G/N*=0.14), while in the Pomeranian Lake District it was lower (*G/N* = 0.12) (Table 27).

Isozyme diversity. At the regional level, the highest number of alleles (27) was found in the Tatra NP, with the mean number of alleles per locus ($A_{reg.}$) was 2.3 per region (Table 28). The percentage of polymorphic loci ($P_{reg.}$) in this region was 75.0%. By contrast, in the Pomeranian Lake District, the lowest number of alleles was found (21), and the mean number of alleles per locus ($A_{reg.}$) was 1.8 per region. The percentage of polymorphic loci ($P_{reg.}$) there was 66.67%. Within regions, the mean $A_{pop.}$ and $P_{pop.}$ per population were higher in the Tatra NP (1.60, and 50.0%, respectively) than in the Bieszczady Mts. ($A_{pop.}$=1.47, $P_{pop.}$=41.67%) and in the Pomeranian Lake District ($A_{pop.}$=1.47, *P*=44.44%). However, the Kruskal-Wallis ANOVA showed no statistically significant differences among the 3 regional groups of populations in respect to $A_{pop.}$ (*H*=0.186, *p*=0.29) and $P_{pop.}$ (*H*=92, *p*=0.16).

Rare and private alleles were identified in all the regions (Table 28). The highest number of a rare alleles (5) was

Table 27. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Calypogeia integristipula* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	29	232	33	0.14	0.147
Bieszczady Mts.	25	200	29	0.14	0.137
Pomeranian Lake District	21	168	20	0.12	0.130

Table 28. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Calypogeia integristipula* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	27	2	6	2.3	75.00	1.60	50.00
Bieszczady Mts.	23	5	4	1.9	75.00	1.47	41.67
Pomeranian Lake District	21	2	3	1.8	66.67	1.47	44.44

Table 29. Total genetic diversity ($H_T\pm$SD), genetic diversity within populations ($H_S\pm$SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Calypogeia integristipula* within regions

Region	H_T ($\pm$SD)	H_S ($\pm$SD)	G_{ST}	N_m
Tatra National Park	0.1921 (0.0344)	0.1042 (0.0208)	0.2559	0.9334
Bieszczady Mts.	0.1887 (0.0323)	0.1002 (0.0183)	0.2114	1.8656
Pomeranian Lake District	0.1462 (0.0509)	0.0920 (0.0251)	0.2488	1.0334

Table 30. Analysis of molecular variance (AMOVA) for *Calypogeia intergristipula*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	2	0.120	6	$\Phi_{RT}= 0.065$***
Among populations within regions	6	0.636	34	$\Phi_{PR}= 0.366$***
Within populations	568	1.100	59	$\Phi_{PT}= 0.407$***
Among Tatra populations	2	0.588	34	$\Phi_{PT}= 0.338$***
Within Tatra populations	213	1.151	66	
Among Bieszczady populations	2	0.317	26	$\Phi_{PT}= 0.257$***
Within Bieszczady populations	174	0.917	74	
Among Pomeranian populations	2	1.009	30	$\Phi_{PT}= 0.303$***
Within Pomeranian populations	181	1.216	70	

Explanations: [1]Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

Table 31. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Calypogeia integristipula*

Region	Tatra NP	Bieszczady Mts.	Pomeranian Lake District
Tatra National Park	****	0.8997	0.8901
Bieszczady Mts.	0.1057	****	0.9301
Pomeranian Lake District	0.1165	0.0725	****

detected in the Bieszczady Mts. The number of private alleles was the largest in the Tatra NP (6) and they had a high frequency (>0.100). In contrast, for the Bieszczady Mts., all the 4 private alleles belong to the class of rare alleles (<0.05). In the Pomeranian Lake District, the number of private alleles was the lowest (only 3), but all of them with frequency >0.100. In mountains, both in the Tatra NP and Bieszczady, allele *Acp-1* had a high frequency in all populations (>0.300). The allele was absent in the Pomeranian Lake District (Table 28 and Table 54 in Bączkiewicz 2012).

The total genetic diversity (H_T) and the mean of H_S per population in the geographic regions were the highest in the Tatra NP (0.1921 and 0.1042, respectively) and the lowest in the Pomeranian Lake District (0.1462 and 0.0920, respectively). The differences in respect to means of H_S were not significant (Kruskal-Wallis ANOVA test: *H*=0.36; *p*=0.83). The highest value of G_{ST} was in the Tatra NP (0.2559) and the lowest in the Pomeranian Lake District (0.2114). Gene flow between populations (N_m) was the lowest in the Tatra NP (0.9334) and the highest in the Bieszczady Mts. (1.8656), but in both regions the values were relatively low (Table 29).

The AMOVA conducted for *C. integristipula* showed that only 6% of the total genetic variation was due to dif-

ferences among regions (Tatra NP, Bieszczady Mts., and Pomeranian Lake District). Separate analyses for each region revealed that 34% of the total genetic variation within the Tatra NP was due to differences among populations, compared to 26% within the Bieszczady Mts. and 30% within the Pomeranian Lake District (Table 30).

The highest genetic distance (*D*) was detected between populations from the Tatra NP and Pomeranian Lake District (0.1165). The highest similarity was between the Bieszczady Mts. and Pomeranian Lake District (*I*=0.9301) (Table 31). The mean *D* between populations within the Tatra NP was 0.1256, within the Pomeranian Lake District 0.1659, and within the Bieszczady Mts. 0.0681.

3.1.7. *Mylia taylorii*

Clonal diversity. The genetic variability of *M. taylorii* populations from 2 geographic regions (Tatra NP and Bieszczady Mts.) was compared (Table 32). Clonal diversity at the regional level was much higher in the Tatra NP (*G/N*=0.28) than in the Bieszczady Mts. (*G/N*=0.16). Similarly, the mean *G/N* per population was much higher in the Tatra NP (0.352) than in the Bieszczady Mts. (0.270) but the difference was not significant (Mann-Whitney *U* test: *Z*=1.470, *p*=0.15).

Table 32. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Mylia taylorii* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	50	392	109	0.28	0.352
Bieszczady Mts.	24	200	31	0.16	0.270

Isozyme diversity. In both regions (Tatra NP and Bieszczady Mts.) a similar number of alleles were found: 43 in the Tatra NP and 42 in the Bieszczady Mts. (Table 33). The mean number of alleles per locus ($A_{reg.}$) in the Tatra NP was 2.20 and in the Bieszczady Mts. it was 2.21. The percentage of polymorphic loci ($P_{reg.}$) both in the Tatra NP and the Bieszczady Mts. was high (84.21% and 73.68%, respectively). Mean $A_{pop.}$ and $P_{pop.}$ per population were higher in the Tatra NP (1.74 and 53.68%, respectively), than in the Bieszczady Mts. ($A_{pop.}$=1.52 and $P_{pop.}$=39.47%). However, the Mann-Whitney *U* test revealed that populations from the Tatra NP and the Bieszczady Mts. did not differ significantly in $A_{pop.}$ (*Z*=1.347, *p*=0.18) and $P_{pop.}$ (*Z*=1.347, *p*=0.18).

In both regions, high numbers of rare and private alleles were found: 14 rare and 14 private alleles in the Bieszczady Mts. and 13 rare and 15 private alleles in the Tatra NP (Table 33). The most characteristic allele for the Tatra NP was *Me-1*, identified in all the Tatra NP populations with a frequency of 100%. By contrast, in the Bieszczady Mts. allele *Me-3* reached a frequency of almost 100%. The majority of private alleles were repeated at least two populations: 13 of the 15 private alleles repeated in the Tatra NP populations and 6 of 14 in the Bieszczady Mts. (Table 62 in Bączkiewicz 2012).

The total genetic diversity (H_T) for the geographic regions, based on mean allelic frequencies of polymorphic loci, was higher in the Tatra NP (0.1679) than in the Bieszczady Mts. (0.1519). Similarly, the mean of H_S per population in the geographic regions was higher in the Tatra NP (0.1244), but the difference was not significant (Mann-Whitney *U* test: *Z*=1.347, *p*=0.18). The differentiation between populations within regions was higher in the Bieszczady Mts. (G_{ST}=0.3268) than in the Tatra Mts. (G_{ST}=0.1635). Consequently, gene flow (N_m) between populations within regions was lower in the Bieszczady Mts. (1.0432), than in the Tatra NP (2.5580) (Table 34).

The AMOVA conducted for *M. taylorii* showed that 21% of the total genetic variation was due to differences among the 2 regions (Tatra NP and Bieszczady Mts.). Separate analyses for each region revealed that 17% of the total genetic variation within the Tatra NP was due to differences among populations, compared to 35% within the Bieszczady Mts. (Table 35).

Table 33. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Mylia taylorii* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of privates alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	43	13	15	2.22	84.21	1.74	53.68
Bieszczady Mts.	42	14	14	2.21	73.68	1.52	39.47

Table 34. Total genetic diversity (H_T±SD), genetic diversity within populations (H_S±SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (*Nm*) of *Mylia taylorii* within regions

Region	H_T(±SD)	H_S(±SD)	G_{ST}	N_m
Tatra National Park	0.1679 (0.0356)	0.1244 (0.0250)	0.1635	2.5580
Bieszczady Mts.	0.1519 (0.0384)	0.0833 (0.0175)	0.3268	1.0432

Table 35. Analysis of molecular variance (AMOVA) for *Mylia taylorii*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	1	0.460	21	Φ_{RT}= 0.208***
Among populations within regions	7	0.391	18	Φ_{PR}= 0.223***
Within populations	575	1.361	62	Φ_{PT}= 0.385***
Among Tatra populations	4	0.314	17	Φ_{PT}= 0.174***
Within Tatra populations	394	1.492	83	
Among Bieszczady populations	3	0.575	35	Φ_{PT}= 0.348***
Within Bieszczady populations	181	1.076	65	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

Table 36. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Mylia taylorii*

Region	Tatra NP	Bieszczady Mts.
Tatra National Park	***	0.9942
Bieszczady Mts.	0.0574	***

The genetic distance (*D*) between the regions was 0.0574 (Table 36). The mean *D* between populations within the Tatra NP was lower (0.0364) than between regions, but the mean *D* between populations within Bieszczady Mts. was higher (0.0955).

3.1.8. *Tritomaria quinquedentata*

Clonal diversity. The genetic variability of *T. quinquedentata* populations from 2 geographic regions (Tatra NP and Bieszczady Mts.) was compared. Clonal diversity was higher in the Tatra NP, where the mean *G/N* per population was 0.564, while in the Bieszczady Mts. it was 0.542, but the difference was not significant (Mann-Whitney *U* test: *Z*=0.735, *p*=0.46). At the regional level, in the Tatra NP clonal diversity was higher, too. In this region *G/N* was 0.55 and in the Bieszczady Mts. it was 0.45 (Table 37).

Isozyme diversity. In the Tatra NP, 41 alleles were found, while in the Bieszczady, 34 alleles (Table 38). The mean number of alleles per locus in both regions was high (in the Tatra NP $A_{reg.}$=3.2, in the Bieszczady Mts. $A_{reg.}$=2.6). The percentage of polymorphic loci ($P_{reg.}$) was higher in the Tatra NP (92.31%). Mean $A_{pop.}$ and $P_{pop.}$ per population were higher in the Tatra NP

(2.44 and 75.35%, respectively) than in the Bieszczady Mts. ($A_{pop.}$=2.25 and $P_{pop.}$=73.08%). However, the Mann-Whitney *U* test revealed that the regional groups of populations from the Tatra NP and the Bieszczady Mts. did not differ significantly in $A_{pop.}$ (*Z*=1.225, *p*=0.22) and $P_{pop.}$ (*Z*=1.102, *p*=0.27).

In both regions rare alleles were detected: 9 in the Tatra NP and 8 in the Bieszczady Mts. (Table 38). Private alleles were less numerous: 8 in the Tatra NP and only one in the Bieszczady Mts. (*MdhA-2* in B-8 population). These regions differed in occurrence and frequency of some alleles. Alleles *Hex-1*, *Hex-2*, and *Hex-4* were detected only in the Tatra NP. They occurred in most populations and in the majority at a frequency of > 0.1. In the Bieszczady Mts. only *Hex-3* was detected, with a frequency of 100%. Furthermore, *Acp-3* appeared with a high frequency in all the Tatra populations, while in the Bieszczady Mts. it was found in only one of the 4 studied populations (Table 70 in Bączkiewicz 2012).

The total genetic diversity (H_T) in the geographic regions, based on mean allelic frequencies of polymorphic loci over all populations, was higher in the Tatra NP (0.3616) than in the Bieszczady Mts. (0.2932). Similarly, the mean H_S per population was higher in the Tatra NP (0.2339) than in the Bieszczady Mts. (0.2148). The Mann-Whitney *U* test showed no statistically significant differences among regions in respect to mean H_S (*Z*=1.101, *p*=0.26). The differentiation between populations within regions was higher in the Tatra NP (G_{ST}=0.1821), too. Consequently, gene flow (N_m) between populations within regions was lower

Table 37. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) of *Tritomaria quinquedentata* at the region level and at the population level within regions

Region	No. of patches	*N*	MLG	*G/N* (reg.)	*G/N* (pop.)
Tatra National Park	43	344	188	0.55	0.564
Bieszczady Mts.	34	272	121	0.45	0.542

Table 38. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*) per region ($A_{reg.}$) and per population ($A_{pop.}$) within regions, and percentage of polymorphic loci of *Tritomaria quinquedentata* per region ($P_{reg.}$) and per population ($P_{pop.}$) within regions

Region	No. of alleles	No. of rare alleles	No. of private alleles	$A_{reg.}$	$P_{reg.}$ (%)	$A_{pop.}$	$P_{pop.}$ (%)
Tatra National Park	41	9	8	3.2	92.31	2.44	75.39
Bieszczady Mts.	34	8	1	2.6	84.62	2.25	73.08

Table 39. Total genetic diversity (H_T±SD), genetic diversity within populations (H_S±SD), coefficient of genetic differentiation (G_{ST}), and gene flow between populations per generation (N_m) of *Tritomaria quinquedentata* within regions

Region	H_T(±SD)	H_S(±SD)	G_{ST}	N_m
Tatra National Park	0.3616 (0.0580)	0.2339 (0.0371)	0.1821	2.4045
Bieszczady Mts.	0.2932 (0.0641)	0.2148 (0.0540)	0.0995	4.7381

Table 40. Analysis of molecular variance (AMOVA) for *Tritomaria quinquedentata*: within populations, among populations within regions and among regions

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among regions	1	0.376	14	$\Phi_{RT} = 0.143$***
Among populations within regions	7	0.368	14	$\Phi_{PR} = 0.164$***
Within populations	609	1.880	72	$\Phi_{PT} = 0.283$***
Among Tatra populations	4	0.475	19	$\Phi_{PT} = 0.194$***
Within Tatra populations	341	1.974	81	
Among Bieszczady populations	3	0.221	11	$\Phi_{PT} = 0.112$***
Within Bieszczady populations	268	1.760	89	

Explanations: [1]Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation, Φ_{RT} – variation among regions divided by total variation, Φ_{PR} – variation among populations within regions divided by the sum of variation among populations within regions and variation within populations, Φ_{PT} – the sum of variation among regions and variation among populations divided by total variation, level of significance *** p≤0.001

within the Tatra NP (2.4045) than in the Bieszczady NP (4.7381) (Table 39).

The AMOVA conducted for *T. quinquedentata* showed that 14% of the total genetic variation was due to variation among the 2 regions (Tatra NP and Bieszczady Mts.). Separate analyses for each region revealed that 19% of the total genetic variation within the Tatra Mts. was due to variation among populations, compared to 11% within the Bieszczady Mts. (Table 40).

Table 41. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between regional groups of populations of *Tritomaria quinquedentata*

| Region	Tatra NP	Bieszczady Mts.
Tatra National Park	****	0.8965
Bieszczady Mts.	0.1093	****

The genetic distance (*D*) between the regions was 0.1093 (Table 41). It was lower than the mean *D* between populations within Tatra NP (0.1155), but higher than between populations within Bieszczady Mts. (0.0512).

3.2. Comparison of genetic differentiation of leafy liverworts from different regions of Poland

3.2.1. Clonal diversity

In the studied species no significant differences among regions were found in respect to the mean proportion of distinguishable genotypes (*G/N*) per region (Kruskal-Wallis ANOVA and Mann-Whitney *U* tests). Clonal diversity (*G/N*) was the highest in the Tatra NP for the majority of studied leafy liverwort species (Fig. 2). Only in 2 species the *G/N* values were higher in other regions: in the Pomeranian Lake District in *M. anomala* (both at the population level and at the species level) and in the Bieszczady Mts. in *B. trilobata*, (only at the population level. The *G/N* value in *B. trilobata* at the population level was 0.610 and in *M. anomala* it was 0.270 at both levels. The lowest degree of genotypic diversity was detected in the Suwałki Lake District. In both studied species in this region (*M. anomala* and *L. reptans*), *G/N* was the lowest and amounted to 0.143 and 0.423, respectively. Also the mean genotypic diversity within populations in these species was the lowest (0.11 for *M. anomala* and 0.39 for *L. reptans*).
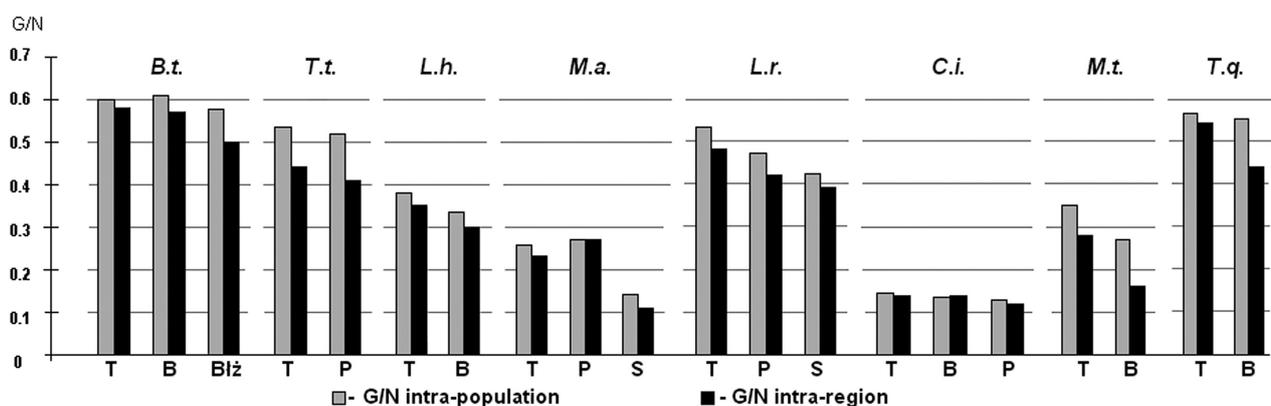
Fig. 2. Mean proportions of distinguishable genotypes (*G/N*) per population within regions and per region for the leafy liverwort species: *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*) in 5 regions: the Tatra National Park (T), Bieszczady Mts. (B), Białowieża Forest (Błż), Pomeranian Lake District (P), and Suwałki Lake District (S)
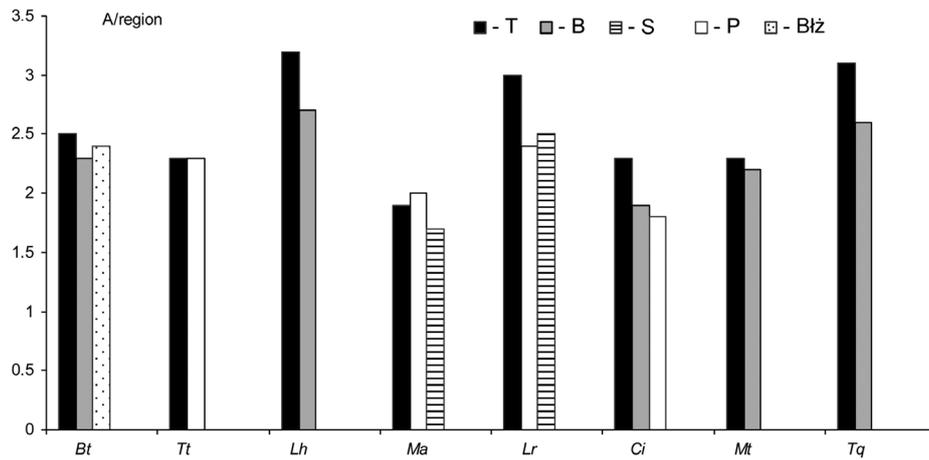
Fig. 3. Mean number of alleles per locus (A_{reg}) at the region level for the leafy liverwort species: *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*) in 5 regions: the Tatra National Park (T), Bieszczady Mts. (B), Białowieża Forest (Błż), Pomeranian Lake District (P), and Suwałki Lake District (S)

3.2.2. Genetic diversity

The greatest number of alleles per locus per region (A_{reg}) was found for the majority of study species in the Tatra NP. Only in *M. anomala* the greatest number of alleles per locus was found in the Pomeranian Lake District (Fig. 3). In the Tatra NP, extremely high A_{reg} was detected in *L. hatcheri* (3.2), *T. quinquedentata* (3.1), and *L. reptans* (3.0). Similarly, the mean $A_{pop.}$ at the population level in regions was the greatest in the Tatra NP in all studied species except for *M. anomala*. The greatest $A_{pop.}$ at the population level (2.4) was detected in *L. reptans* and *T. quinquedentata* (Table 42). The Kruskal-Wallis ANOVA test and the Mann-Whitney

U test showed no statistically significant differences among regions in all studied species in respect to $A_{pop.}$.

Rare alleles were found in all the 5 regions (Fig. 4). The greatest number of rare alleles in species studied in the Tatra NP was detected in *L. hatcheri*, *T. quinquedentata*, and *L. reptans*; in the Pomeranian Lake District in *M. anomala* and *T. tomentella*; in the Bieszczady Mts. in *C. integristipula* and *M. taylorii*; and in Białowieża Forest in *B. trilobata*.

Most of the species had private alleles for each region (Fig. 4). Private alleles were not identified only in the Bieszczady Mts. for *B. trilobata* and in the Pomeranian Lake District for *L. reptans*. The greatest number of

Table 42. The mean number of alleles per locus at the population level (A_{pop}), number of rare alleles per population and number private alleles per population in regions for *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), and *Tritomaria quinquedentata* (*T.q.*)

Species	B.t.	T.t.	L.h.	M.a.	L.r.	C.i.	M.t.	T.q.
Tatra National Park								
A_{pop}	2.3	2.0	2.1	1.5	2.4	1.6	1.7	2.4
Rare alleles/pop.	4.0	2.8	2.8	1.3	5.0	0.0	4.8	5.6
Private alleles/pop.	0.0	0.0	1.0	1.0	1.0	1.0	0.4	0.6
Bieszczady Mts.								
A_{pop}	2.1	n.d.	2.0	n.d.	n.d.	1.5	1.5	2.3
Rare alleles/pop.	4.0	n.d.	1.5	n.d.	n.d.	1.7	3.2	4.5
Private alleles/pop.	0.0	n.d.	0.5	n.d.	n.d.	1.3	2.0	0.2
Białowieża Forest								
A_{pop}	2.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Rare alleles/pop.	3.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Private alleles/pop.	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pomeranian Lake District								
A_{pop}	n.d.	1.9	n.d.	1.7	2.2	1.5	n.d.	n.d.
Rare alleles/pop.	n.d.	3.0	n.d.	2.0	3.3	0.0	n.d.	n.d.
Private alleles/pop.	n.d.	1.3	n.d.	1.0	0.0	0.3	n.d.	n.d.
Suwałki Lake District								
A_{pop}	n.d.	n.d.	n.d.	1.4	2.1	n.d.	n.d.	n.d.
Rare alleles/pop.	n.d.	n.d.	n.d.	1.3	2.3	n.d.	n.d.	n.d.
Private alleles/pop.	n.d.	n.d.	n.d.	1.3	0.7	n.d.	n.d.	n.d.

Explanation: n.d. – no data
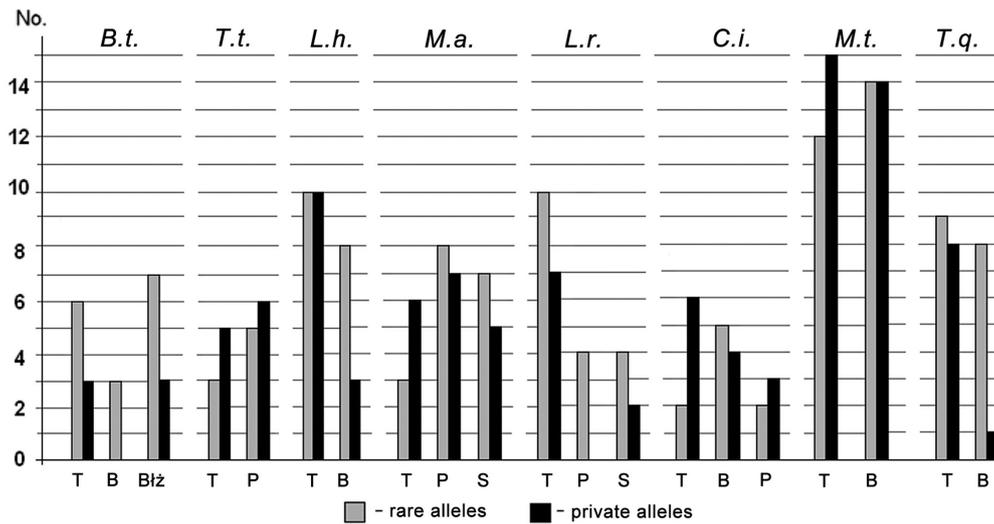
Fig. 4. Numbers of regionally rare and private alleles in the Tatra National Park (T), Bieszczady Mts. (B), Białowieża Forest (Błż), Pomeranian Lake District (P), and Suwałki Lake District (S) for the leafy liverwort species *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), and *Tritomaria quinquedentata* (*T.q.*)

region-specific alleles was detected in the Tatra NP and in the Pomeranian Lake District. In the Tatra NP the number of private alleles was the highest in 5 species (*L. hatcheri*, *L. reptans*, *C. integristipula*, *M. taylorii*, and *T. quinquedentata*), whereas in the Pomeranian Lake District in 2 species (*M. anomala* and *T. tomentella*). The majority of private alleles occurred in different populations in the same region and often had high frequency. For example, in *M. taylorii*, where the greatest number of region-specific alleles was detected, in different populations in the

Tatra NP as many as 13 private alleles per 15 detected occurred in more than one population, while in the Bieszczady Mts., 6 per 13 detected (Table 62 in Bączkiewicz 2012).

Total genetic diversity (H_T) and within-population genetic diversity (H_S) in all the study species except for *M. anomala* were the greatest in the Tatra NP. Only *M. anomala* demonstrated higher genetic diversity (both H_T and H_S) in the Pomeranian Lake District. In the Bieszczady Mts., H_T and H_S values were lower than in the Tatra NP, but higher than in lowland regions, even

Table 43. Total genetic diversity (H_T), genetic diversity within populations (H_S), coefficient of genetic differentiation (G_{ST}) within regions for *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), and *Tritomaria quinquedentata* (*T.q.*)

	B.t.	*T.t.*	*L.h.*	*M.a.*	*L.r.*	*C.i.*	*M.t.*	*T.q.*
				Tatra National Park				
H_T	0.2775	0.2587	0.2467	0.1819	0.2320	0.1921	0.1679	0.3616
H_S	0.2692	0.1837	0.2304	0.0912	0.2186	0.1042	0.1244	0.2339
G_{ST}	0.0820	0.2357	0.2860	0.2952	0.1098	0.2559	0.1635	0.1821
				Bieszczady Mts.				
H_T	0.2597	n.d.	0.2210	n.d.	n.d.	0.1887	0.1519	0.2932
H_S	0.2313	n.d.	0.2129	n.d.	n.d.	0.1002	0.0833	0.2148
G_{ST}	0.1022	n.d.	0.1485	n.d.	n.d.	0.2114	0.3268	0.0955
				Białowieża Forest				
H_T	0.2449	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H_S	0.2417	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
G_{ST}	0.0356	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
				Pomeranian Lake District				
H_T	n.d.	0.2260	n.d.	0.1840	0.2184	0.1462	n.d.	n.d.
H_S	n.d.	0.1792	n.d.	0.1209	0.1995	0.0920	n.d.	n.d.
G_{ST}	n.d.	0.0611	n.d.	0.0989	0.0913	0.2488	n.d.	n.d.
				Suwałki Lake District				
H_T	n.d.	n.d.	n.d.	0.0978	0.1811	n.d.	n.d.	n.d.
H_S	n.d.	n.d.	n.d.	0.0513	0.1658	n.d.	n.d.	n.d.
G_{ST}	n.d.	n.d.	n.d.	0.1367	0.1069	n.d.	n.d.	n.d.

Explanation: n.d. – no data
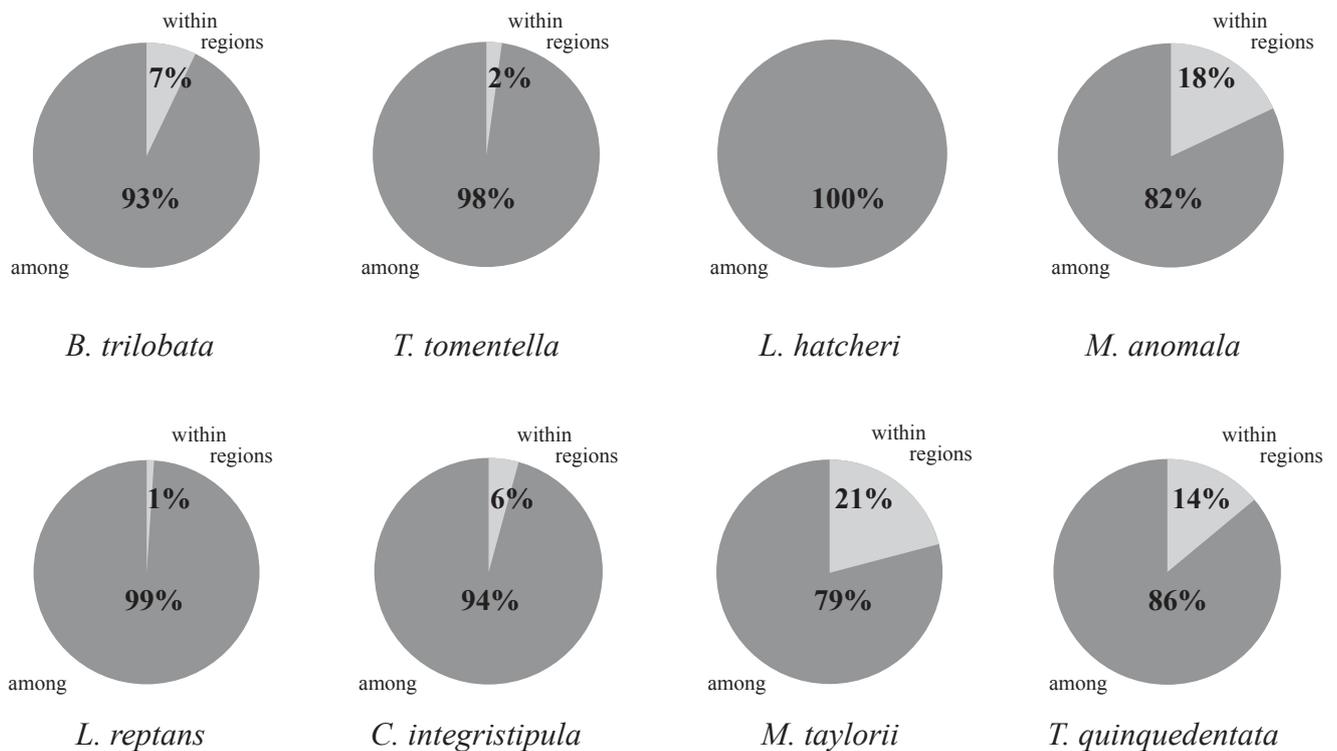
Fig. 5. Analysis of molecular variance (AMOVA) among and within regions for the leafy liverwort species: *Bazzania trilobata, Trichocolea tomentella, Lophozia hatcheri, Mylia anomala, Lepidozia reptans, Calypogeia integristipula, Mylia taylorii*, and *Tritomaria quinquedentata*

in comparison with the primeval Białowieża Forest (in *B. trilobata*). As for northern Polish regions (Pomeranian and Suwałki Lake Districts), higher genetic diversity was confirmed in the Pomeranian Lake District. The values of genetic differentiation (G_{ST}) were greater in the mountains than in lowland areas. In the majority of species of studied leafy liverworts, the greatest G_{ST} value was in the Tatra NP. Only in *B. trilobata* and *M. taylorii* the G_{ST} value was greater in the Bieszczady Mts. The smallest genetic differentiation was detected between primeval Białowieża Forest populations in *B. trilobata* (G_{ST}=0.036). In northern Poland, G_{ST} values were higher in the Suwałki Lake District than in the Pomeranian Lake District in both studied species (*M. anomala* and *L. reptans*) (Table 43).

Analysis of molecular variance (AMOVA) showed that variation among regions (Φ_{RT}) was statistically significant in all the studied species except for *L. hatcheri*, where no differences between regions were found (Fig. 5). The greatest differences between regions were found in *M. taylorii* (Φ_{RT}=0.208). It was the only species where the share of variation among regions was higher than the share of variation among populations within regions in the total genetic variation. In the remaining species the share of diversity among regions was lower than the share of variation among populations within regions in the total genetic diversity of species or was the same, as in *T. quinquedentata*.

4. Discussion and conclusions

The study investigated populations in 5 geographic regions of Poland: the Tatra NP, Bieszczady Mts., Białowieża Forest, and Pomeranian and Suwałki Lake Districts (Fig. 1 and Table 1). Despite the presence of private alleles in different regions and marked differences in frequencies of some alleles, only some of the studied species exhibited significant differentiation between regions (Figs. 9, 11, 13, 15, 17, 19, 21, 23 in Bączkiewicz 2012). A clear division of populations by geographic regions was shown for just 2 species – *M. taylorii* and *T. quinquedentata* – both being fertile and dioecious species with a typically mountainous range (Szweykowski 2006). It must also be noted that *M. taylorii* was the sole species (of all the studied species) in which variation among regions was higher (21%) than among populations within regions (18%) (Table 35). The remaining 6 species demonstrated either a partial population division by regions or none at all.

A complete lack of regional differentiation among populations, in turn, was confirmed for 3 species (*T. Tomentella, L. reptans* and *C. integristipula*) (Figs. 11, 17, 19 in Bączkiewicz 2012). Phenograms for these 3 species are corroborated by the Mantel test that demonstrated no statistically significant correlation between genetic and geographic distances. A partial division of populations by regions was observed in *B.*

trilobata, *L. hatcheri*, and *M. anomala* (Figs. 9, 13, 15 in Bączkiewicz 2012). As for *B. trilobata*, populations from Białowieża Forest created a uniform group in the phenogram, standing out markedly from mountainous populations (Tatra NP and Bieszczady Mts.). In Białowieża Forest may have preserved an old gene pool of liverworts of different origin from those identified in the Tatra NP and Bieszczady Mts. A distinct composition of the gene pool in this area was also observed in other liverwort species (Adamczak *et al.* 2005; Bączkiewicz *et al.* 2008; Buczkowska *et al.* 2010). What is more these regions differences in climate and geology.

A partial or complete lack of genetic differentiation between populations from various geographic regions is a relatively common trait of bryophytes (e.g. Stenøien & Såstad 1999; Freitas & Brehm 2001; Thingsgaard 2001; Van der Velde *et al.* 2001; Gunnarsson *et al.* 2005; Zartman *et al.* 2006; Spagnuolo *et al.* 2007). It is absent even in species that have a rather limited potential for spreading over large distances, as in species that mainly reproduce vegetatively and do not produce gemmae, e.g. in *T. tomentella*. Stenøien and Såstad (1999) investigated species of the genus *Sphagnum*, concluding that there was no differentiation even between continents. The fact that there is no marked genetic differentiation between populations, regions or even continents can be explained by the prevalence of the same somatic mutations of neutral alleles in different populations. The theory is supported by high repeatability of the same genotypes in populations from different regions, which is shown in the present study.

Mountain populations had greater genetic diversity (H_T and H_S) compared to lowland populations (Table 43). In mountain populations in all studied species except for *M. anomala* (for which the highest diversity was detected in the Pomerania region), the highest values of H_T, H_S, number of alleles per locus (*A*), and clonal diversity (*G/N*) in populations were recorded (Fig. 2-4, Table 42). Genetic differentiation (G_{ST}) was also higher in the mountains than in the lowlands (Table 43). For 6 species, G_{ST} values were the highest in the Tatra Mts. (*T. tomentella*, *L. hatcheri*, *M. anomala*, *L. reptans*, *C. integristipula*, *T. quinquedentata*) and for the other 2 species, in the Bieszczady Mts. (*B. trilobata* and *M. taylorii*). High G_{ST} values in mountainous regions are most probably caused by a greater diversity of habitats than in the lowlands and by the different altitudinal zones (Szafer & Zarzycki 1972; Szweykowski 1996). The great diversity of habitats can cause the high number of rare and private alleles in both mountain regions, the Tatra NP in particular, and different altitudinal zones in mountains can hamper the spread of liverworts in these regions.

The Tatra NP turned out to be more diverse than the Bieszczady Mts. in every studied species. Only *B. trilobata* exhibited a slightly more pronounced clonal diversity (*G/N*) in the Bieszczady Mts. (Fig. 2). However, H_S and H_T diversity in the species was higher in the Tatra NP (Table 43). The higher genetic diversity of the Tatra NP populations may be a result of population size (in the Bieszczady Mts., populations were slightly smaller) and differences in geology and climate. The Bieszczady Mts. are a region exhibiting a lower variation of habitats and altitudinal gradient (Szafer & Zarzycki 1972; Szweykowski 1996), which may have resulted in the region's lower genetic diversity. Another factor that may have affected the diversity of liverworts in the 2 locations is the different pre-glacial, glacial, and post-glacial history of both regions (Szweykowski & Buczkowska 1996). Similarly, Pawłowski (1972) already noted differences between the Western and Eastern Carpathians in the spermatophyte flora. The distinctiveness of both regions is confirmed by genetic differences that occurred in some of the studied species, especially in *T. quinquedentata* and *M. taylorii* (Figs. 21, 23 in Bączkiewicz 2012).

In the Pomeranian and Suwałki Lake Districts in this work, genetic diversity of liverworts was significantly lower than in mountains. This finding confirms the results of an earlier study of diversity of ISSR markers in *B. trilobata* (Buczkowska *et al.* 2010). Lower genetic diversity observed in liverwort populations in the Pomeranian and Suwałki Lake Districts can be accounted for by the fragmentation of forests in these regions. As opposed to forest areas in the Białowieża, Tatra, and Bieszczady NPs, forests in the Pomeranian and Suwałki Lake Districts have recently been fragmented because of urban development. Populations currently found in both lake districts are small and rare, probably being remnants of previously much larger populations (Szweykowski 1962; Klama 2002). The fragmentation of habitats results not only in the destruction of a large number of plant stands and a reduced size of the remaining local populations, but also in the depletion of genetic diversity. The process stems mainly from accidental genetic drift combined with inbreeding depression, which has already been demonstrated in a number of studies including bryophytes (Wyatt 1992; Wilson & Provan 2003; Spagnuolo *et al.* 2007; Buczkowska *et al.* 2010).

However, a few studies of bryophytes failed to demonstrate a fall in genetic diversity in fragmented populations compared to continuous populations. For example, Zartman *et al.* (2006) studied the diversity of the leafy liverwort *Radula flaccida* Lindenb. & Gottsche in fragmented and continuous rainforest stretches in the Amazon region. Authors of that study concluded that the effect of population depletion was not linked to a decreased level of population diversity. The mean diversity within populations of both forests was nearly

the same (0.412 and 0.413). Genetic diversity (F_{ST}) in both habitat types was also close to identical (0.19 and 0.18). A similar degree of diversity in populations differing markedly in size in Scandinavia and in Canada has also been detected by RAPD and ISSR markers in *T. tomentella* (Pohjamo *et al.* 2008). It is possible that the absence of differences in genetic diversity among liverworts in fragmented and continuous populations in these works was a consequence of limited degradation of the areas. A similar trend has been identified in *M. anomala*. As the only species included in the study, it did not exhibit reduced genetic diversity in the Pomeranian Lake District compared to the Tatra NP. Surprisingly, it even displayed higher genetic diversity (H_S and H_T) in the former region (Table 43). *M. anomala* populations occur in raised peat-bogs or lakeside boggy areas. Peat-bogs are an example of partially stable habitats. Natural selection pressure in such conditions is weak, which may translate into higher genetic diversity within populations (Szweykowski 1984; Klama 2002). Arguments for this claim are found in reports by other authors who have also noted high levels of genetic diversity in peat-bog bryophytes, e.g. Wilson & Provan (2003) in *Polytrichum commune* (Brid.) Lindb.; Gunnarsson *et al.* (2005) in *Sphagnum angermanicum Hornsch. ex Russ.*, and Thingsgaard (2001) in *S. affine*. Moreover, the majority of peat-bog areas in which *M. anomala* was investigated are formally protected as nature reserves. Consequently, *M. anomala* as the only species studied in the Pomeranian Lake District could be less severely affected by human impact and the associated process of forest fragmentation. It can be concluded, therefore, that the decrease in diversity is a likely consequence of habitat fragmentation (causing population depletion) combined with negative effects of urban development.

Another factor that may have been involved in the decrease in genetic diversity in northern Poland is the post-glacial history of these areas. Northern Poland was affected by the last glacial period, with the retreat of ice occurring ca. 10-12 thousand years ago (*Lindner 2002*). The later period probably saw an intensive recolonization during which some of the alleles could be lost as a result of genetic drift and the founder effect. Results obtained in the present study are consistent with reports concerning the genetic diversity of bryophytes in post-glacial areas (Cromberg 2000; Thingsgaard 2001; Hassel *et al.* 2005). The cited works showed a smaller number of alleles per locus and a reduction of genetic diversity associated with post-glacial migrations.

It is interesting to note that in both studied species in northern Poland (*M. anomala* and *L. reptans*), the level of total diversity (H_T) and intra-population diversity (H_S) was markedly higher in the Pomeranian than in the Suwałki Lake District. However, in the latter, the value of genetic differentiation (G_{ST}) was higher (Table 43). Low isozymatic diversity in that region – compared to other Polish regions – was also found in vascular plants (Szczecińska *et al.* 2006; Celka *et al.* 2010). The phenomenon has not, as yet, been fully explored, but it may be linked to the specific microclimate of the Suwałki Lake District, which is strongly affected by continental climate. The region is one of the coldest parts of Poland. By contrast, the Pomeranian Lake District has a much milder climate with Atlantic influences (Szafer & Zarzycki 1972; Herz 1983). Continental climate is probably related to more severe selection and this, in turn, reduces the level of genetic diversity in plants and increases differentiation of populations. A similar tendency was observed at higher altitudes in the mountains, where gene diversity decreases manifestly with increasing latitude, and differences between populations become more pronounced (Montagnes *et al.* 1993; Cromberg 2000; Thingsgaard 2001).

On the basis of this study and light of literature on this subject, the following conclusions can be drawn:

1. Mountain populations (Tatra NP and Bieszczady Mts.) had greater genetic diversity (H_T and H_S) compared to lowland populations.

2. In the Tatra NP populations were more diverse than the Bieszczady Mts. in every studied species. It may be a result of population size (in the Bieszczady Mts., populations were slightly smaller) and differences in geology and climate.

3. Białowieża Forest created a uniform group, standing out markedly from mountainous populations, but population in this region had slightly smaller genetic diversity, then in the mountains. It may be result of differences in climate and post glacial history of these regions.

4. In the Pomeranian and Suwałki Lake Districts, genetic diversity of liverworts was significantly lower than in mountains. The decrease in diversity in these regions is a likely consequence of habitat fragmentation (causing population depletion) combined with negative effects of urban development. Habitat fragmentation results in genetic drift and inbreeding depression, which cause a decrease in genetic diversity.

5. In the Pomeranian Lake District the level of total diversity (H_T) and intra-population diversity (H_S) was markedly higher than in the Suwałki Lake District. It may be linked to differences in climate.

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