Biodiv. Res. Conserv. 27: 3-54, 2012

BRC
www.brc.amu.edu.pl

DOI 10.2478/v10119-012-0022-5

Genetic diversity of leafy liverwort species (Jungermanniidae, Marchantiophyta) in Poland: Diversity of leafy liverwort species with various reproductive modes

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Abstract: This monograph presents results of research on genetic diversity of 8 leafy liverwort species differing in reproductive mode. The first 4 species in Poland are regarded as sterile and reproduce only vegetatively: *Bazzania trilobata*, *Trichocolea tomentella, Lophozia hatcheri*, and *Mylia anomala*. The next 4 are fertile, including the monoecious *Lepidozia reptans* and *Calypogeia integristipula* as well as the dioecious *Mylia taylorii* and *Tritomaria quinquedentata*. For each species, 9-10 populations were sampled. In total, 4744 gametophytes from 73 populations were examined by isozyme analysis. The level of their genetic diversity (total, H_T, and within populations, H_S) was high, higher than in thallose liverworts, but comparable to the genetic diversity of mosses or even some species of vascular plants. Thus the traditional opinion that the entire group of liverworts has a much lower level of genetic diversity than mosses is erroneous, as it holds true only for thallose liverworts (Metzgeriidae and Marchantiopsida). My results indicate that the effect of reproductive mode on genetic diversity in leafy liverworts is lower than in vascular plants. Sterile and fertile species of liverworts exhibited similar levels of genetic diversity. Moreover, both groups included species that had both high and low levels of H_T and H_S. In fertile species, monoecious and dioecious species also did not differ significantly in genetic diversity, but dioecious liverworts had slightly higher total diversity (H_T) than monoecious species. In most of the studied leafy liverworts, the share of genetic diversity within populations in the total genetic diversity of species is greater than between populations. The percentage share of variation among populations (Φ_{PT}) in the total genetic variation was correlated with the total genetic diversity of the species (H_T). In species with high H_T, differences between populations tended to be rather small. By contrast, in species with lower H_T, the percentage share of differentiation among populations in the total diversity of species was much higher. My results confirm theory, based on studies by Kimura, that the main causes of genetic diversity of bryophytes are neutral somatic mutations developing in various vegetative parts of plants. The separation of branches or other plant sections with somatic mutations, followed by the growth of new shoots, can increase the level of genetic diversity. The high level of genetic diversity in sterile liverworts indicates that vegetative reproduction has a greater influence on the level of genetic diversity than recombination. My results suggest also that mutation rates are similar in closely related species, but species with a wider ecological range exhibit higher genetic diversity because the variability of habitats can influence the rate and type of somatic mutations. Accordingly, species inhabiting more diverse environments may be more genetically diverse. Patches of the studied species generally consisted of several genotypes (MLGs). Two types of distribution of genotypes in patches were noticed. Patches of species with low total diversity (H_T) were often dominated by 1-2 genotypes, which constituted the major part of a patch. In patches of species with higher H_T, there was no tendency to form patches with predomination of a single genotype. Different genotypes constituted similar proportions of a patch. In all the studied leafy liverwort species there was a high degree of repeatability of the same genotypes (MLGs) in plants from various patches within the same population or in various populations. Probably the main cause of this is the independent repeatability of the same mutations in different specimens.

Key words: Bryophyta, *Bazzania trilobata*, *Trichocolea tomentella, Lophozia hatcheri, Mylia anomala, Lepidozia reptans*, *Calypogeia integristipula*, *Mylia taylorii*, *Tritomaria quinquedentata*, genetic variation, population genetics, sterile and fertile species, population differentiation, breeding system, monoecious, dioecious

Contents

1. Introduction

Liverworts (Marchantiophyta) are a group of bryophytes with ca. 6000 species existing worldwide (Heinrichs *et al.* 2007). Plants of this group are notable for their simple morphology and the dominant haploid phase in their life cycle. Because of haplophase dominance, the process of natural selection affects the haploid genotype directly, causing elimination of all mutations with mild or severe adverse effects. Consequently, no mutations can be masked as heterozygotes, as opposed to diploid organisms. The mechanism is thought to limit potential evolutionary transformations and to be the main reason explaining the slow evolution of bryophytes. Slow evolutionary changes of bryophytes are best evidenced by fossilized forms whose appearance closely resembles contemporary species (Anderson 1963; Schuster 1966; Crum 1972; Krzakowa & Szweykowski 1979; Szweykowski 1984; Innes 1990; Montagnes *et al.* 1993). Consequently, in view of direct selection and slow evolution, which should be correlated with the level of diversity, it was commonly believed until the 1980s that bryophytes had low genetic diversity (Szweykowski 1984). However, later studies (both enzymatic and DNA-based) challenged that view, unexpectedly showing bryophytes to have a high level of genetic diversity. That observation applied in particular to mosses, whose genetic diversity was found to be similar to that of vascular plants (e.g. Stoneburner *et al*. 1991; Montagnes *et al*. 1993; Wyatt 1994; Skotnicki *et al*. 1999; Stenøien & Såstad 1999; Shaw 2000; Van der Velde *et al*. 2001; Cromberg

2002; Grundmann *et al*. 2007; Spagnuolo *et al*. 2007). The findings overturned traditional opinions about the impact of genetic diversity on evolutionary rates in bryophytes (Stenøien & Såstad 1999).

Liverworts in Poland are represented by over 260 species, of which 80% are leafy liverworts (subclass Jungermanniidae) (Szweykowski 2006). Despite it being a large group of species, little is known about the genetic diversity of liverworts. The scarce knowledge is a result of the fact that the majority of studies describing genetic diversity and structure of liverworts performed to date concern thallose liverworts of the class Marchantiopsida and subclass Metzgeriidae (e.g. Zieliński *et al*. 1985; Zieliński 1987; Dewey 1989; Boisselier-Dubayle *et al*. 1995, 1998a; Boisselier-Dubayle & Bischler 1997; Szweykowski & Odrzykoski 1990; Odrzykoski & Szweykowski 1991). Moreover, the majority of existing studies sought to identify genetic markers for related species as the main goal. Genetic diversity was only investigated as a secondary goal. Moreover, most of existing studies were based on a small number of loci. The genetic diversity of thallose liverworts was found in the studies to be low, markedly lower than in mosses. Genetic diversity and structure of leafy liverworts has only been studied in several species to date (Zieliński & Wachowiak-Zielińska 1994; Boisselier-Dubayle *et al*. 1998b; Cromberg 2000a; Buczkowska 2004; Wyatt *et al*. 2005; Adamczak *et al*. 2005; Freitas & Brehm 2001; Pohjamo *et al*. 2008; Buczkowska *et al*. 2010), so results obtained in studies of thallose liverworts have been extrapolated to the entire group of liverworts, promoting the view that the group has a notably low level of genetic diversity. However, as Schuster (1966) argues, leafy liverworts should be more variable genetically than thallose liverworts because their morphology is more complex. On the basis of scanty published data on this subject it is difficult to assess if leafy liverworts are more diverse than thallose liverworts or their genetic diversity is similar. Also, it is not possible to identify patterns describing intra- and inter-population genetic diversity among leafy liverworts.

An important factor determining the level of species diversity is the mode of reproduction. There have been many studies demonstrating the relationship between breeding system and genetic diversity in higher plants. It is believed that dioecious anemophilous species have a higher degree of genetic diversity than monoecious species or species pollinated by animals (Hamrick & Godt 1990). Under this rule, dioecious bryophyte species should display a higher diversity than monoecious species.

It is estimated that ca. 2/3 of all liverwort species, and over 50% of all moss species are dioecious, compared to ca. 4% of angiosperms (Wyatt 1985; Mischler 1988). It has not been fully elucidated, though, whether dioecious bryophyte species are more diverse genetically than monoecious species because the former rarely produce sporophytes. For fertilization to take place, male and female plants must grow in the immediate vicinity because sperm in bryophytes can only spread over several centimetres (Bischler & Boisselier-Dubayle 1997; Wyatt *et al*. 1989a; Hock *et al*. 2008b). Meanwhile, multiple observations show that populations of dioecious species are dominated by entirely female or sterile patches. This, in turn, results in a limited production of sporophytes, while the predominance of vegetative reproduction is expected to lower the level of genetic diversity in the populations (Bowker *et al*. 2000; Bisang & Hedennäs 2005). By contrast, Bengtsson (2003) shows that in stable populations a small number of sporophytes is sufficient for maintaining a relatively large genetic diversity of bryophytes. Monoecious (as opposed to dioecious) species produce large quantities of sporophytes (Longton & Miles 1982; Stenøien & Såstad 2001). There are also mechanisms ensuring cross-fertilization that sustains genetic diversity (Schuster 1966). Many authors investigating the diversity of bryophytes claim that self-fertilization plays a major role among monoecious bryophytes, producing completely identical spores. The effect of the mechanism is similar to vegetative reproduction (Wyatt 1994; Shaw 2000).

Even though there has recently been a range of studies examining the impact of reproduction patterns on the level of genetic diversity of species, the interdependencies that are involved in the process are not entirely clear, and no single universal rule can be formulated (e.g. Mischler 1988; Wyatt *et al.* 1989b; During 1990; Wyatt *et al*. 1992; Wyatt 1994; Cromberg 1996, 1998, 2000b; Stenøien & Såstad 1999, 2001; Van der Velde *et al*. 2001; Bengtsson 2003; Eppley *et al*. 2006; Spagnuolo *et al*. 2007). Moreover, no such data are available for liverworts and it is not known whether – due to the similarity to mosses in terms of life history traits – liverworts have similar models of genetic diversity.

Asexual reproduction is regarded as more common than sexual reproduction among liverworts (Anderson 1963; Longton 1976). All liverworts have an ability to reproduce by thallus regeneration. The tissue of liverworts has a great potential for regeneration, with even tiny thallus fragments (of several cells) being able to grow into a fully fledged new plant. Young plant fragments have the best regeneration potential (Anderson 1980; During 1990; Longton 1997). In addition to reproduction by thallus regeneration, liverworts produce a wide variety of vegetative propagules, the most important of these being gemmae, deciduous leaves, deciduous branches, stolons, and rhizoids (Schuster 1966). Some liverwort species have several systems of vegetative reproduction (During 1990; Newton & Mishler 1994).

Asexual propagation carries a number of important benefits for liverworts: (1) it can begin at an early age, when the plant is not yet able to propagate sexually; (2) plants need less time to produce propagules; (3) it is not seasonal in nature (the period from the development of gametangia to the propagation of spores is about one year); (4) it does not require plants of both sexes to grow at an appropriate distance; (5) it represents an important factor sustaining local populations; and (6) it makes it possible for liverworts to propagate in various environmental conditions, e.g. by filling the gaps remaining after landslides (Jendralski 1955; Szweykowski 1984; During 1990; Klama 2002).

Some bryophytes were found not to produce sporophytes at all, and they reproduce only vegetatively (Longton & Schuster 1983; Mischler 1988). According to estimates prepared for the UK flora, 4% of mosses do not generate sporophytes at all (Longton & Miles 1982). For North America, the corresponding figure is 9% (Lane 1985). Additionally, a considerable group of bryophytes produce sporophytes very rarely; for example, Mischler (1988) reports that 43% of mosses growing in the UK form sporophytes only occasionally. In liverworts the percentage of species not producing sporophytes – or producing them only occasionally – can be even larger than in mosses. In Poland, for instance, ca. 15% of liverworts not producing sporophytes (Szweykowski 2006).

The causes of genetic sterility are varied. For example, sterility can stem from a gene mutation (nuclear or organellar) that damages the process of sexual reproduction (Eckert 2002). Furthermore, evolutionary elimination or limitation of sexual reproduction can occur as a result of adaptation to the environment (Klekowski 1988a, 1988b; Eckert 2002). In some dioecious species there can be entire populations comprising only one sex (Bowker *et al*. 2000). The lack of one sex in a population is also an effect of population dynamics and clonal growth (McLetchie *et al*. 2002). Besides, there are bryophyte species in which one of the sexes has never been identified (Longton & Schuster 1983).

Data concerning the genetic diversity bryophytes with asexual reproduction are very scarce. Asexuality is regarded as a factor that not only reduces genetic diversity (Gemmell 1950; Bengtsson 2003) but also constrains the morphological diversity and range of species, in comparison to species reproducing sexually (Longton & Schuster 1983). Consequently, it should be expected that species that do not propagate sexually will be less diverse than those that do not rely on sexual reproduction. The few existing studies investigating genetic diversity of sterile species of mosses and liverworts at the level of isozymes and DNA suggest that it might be larger than initially assumed, though it is difficult to assess it precisely in relation to fertile species (e.g. Wachowiak-Zielińska & Zieliński 1995; Thingsgaard 2001; Adamczak *et al*. 2005; Pohjamo *et al*. 2008; Buczkowska *et al*. 2010). One of scanty works discussing leafy liverworts compares 2 species of the genus *Ptilidium*, which differ in the mode of reproduction (Adamczak *et al*. 2005): the fertile dioecious species *P. pulcherrimum* (Weber) Vain and the sterile species *P. ciliare* (L.) Hample. The sexually reproducing *P. pulcherrimum* species proved to be more diverse, thus confirming the authors' original assumptions. It is difficult to say whether this is a general rule because the number of studies conducted to date is insufficient to warrant any definite claims. In spite of vast differences in modes of reproduction and reproductive capacity of liverworts, existing data are too limited for drawing general conclusions comparing the genetic diversity of species with various reproductive modes.

The main aim of the study presented here was to describe and compare the genetic diversity of leafy liverwort species differing in methods of reproduction. Isozymatic investigations were performed on 8 leafy liverwort species that are common in Poland and have different modes of reproduction. The first 4 species in Poland are regarded as sterile and reproduce only vegetatively: *Bazzania trilobata* (L.) Gray, *Trichocolea tomentella* (Ehrh.) Dumort., *Lophozia hatcheri* (A. Evans) Steph., and *Mylia anomala* (Hook.) Gray. The other 4 species are fertile, with sporophytes frequently observed in field studies, which suggests that sexual reproduction may play an important part in the reproduction process. Two of the species are monoecious: *Lepidozia reptans* (L.) Dumort., *Calypogeia integristipula* (Steph.); and the others are dioecious: *Mylia taylorii* (Hook.) Gray and *Tritomaria quinquedentata* (Huds.). Furthermore, 3 of the studied species are prolific gemmae producers: *Lophozia hatcheri*, *M. anomala* and *C. integristipula* (Szweykowski 2006).

The study seeks to assess: (1) the level of genetic diversity of leafy liverworts, compared with thallose liverworts and mosses; (2) the correlation between the mode of reproduction and genetic diversity in leafy liverworts; and (3) whether sterile species (propagating only vegetatively) exhibit lower diversity than species that reproduce both vegetatively and sexually.

2. Materials and methods

2.1. Plant material

Samples of 8 species of leafy liverworts that differ in breeding system were collected in Poland.

2.1.1. Species that are sterile in Poland

Bazzania trilobata is dioecious but perianths and sporophytes are very rarely produced, probably only found a few times in south-western Norway (Schuster

Table 1. Collection sites of the studied populations of *Bazzania trilobata* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Dolina Białki (valley)	950	9	72
T-2	Tatra National Park, Dolina Suchej Wody (valley)	1183	9	72
T-3	Tatra National Park, Dolina Kościeliska (valley)	1050	9	72
B-4	Bieszczady Mts., N slope of Mt. Tarnica	1317	9	72
B-5	Bieszczady Mts., N slope of Mt. Krzemień	1280	6	48
B-6	Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	1214	8	64
Błż-7	Białowieża Forest, plot 259, near Hwożna river	152	10	80
Błż-8	Białowieża Forest, plot 343, near Orłówka river	149	8	64
Błż-9	Białowieża Forest, plot 394, near Teremiski village	163	10	80
Total			78	624

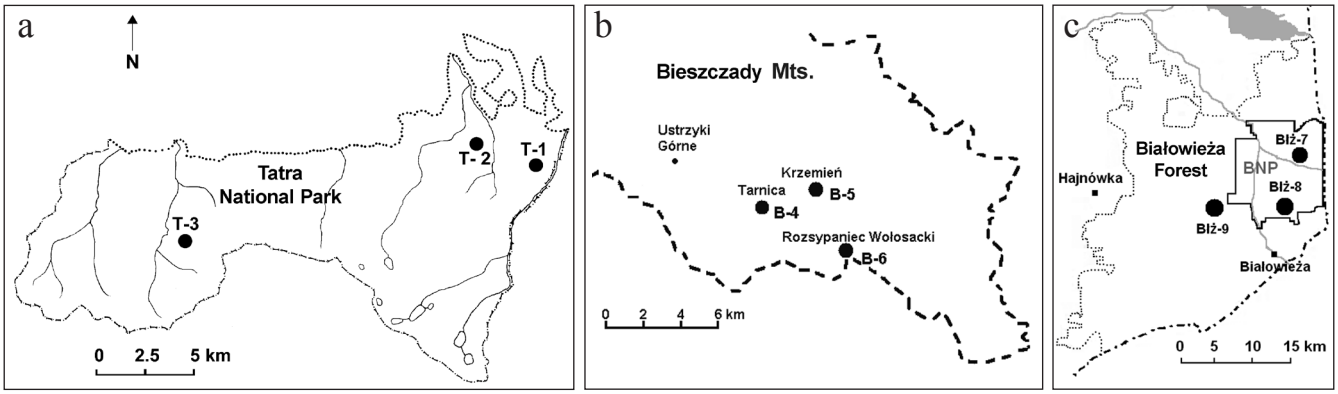

Fig. 1. Location of the studied populations of *Bazzania trilobata* (described in Table 1) in the Tatra National Park (a), Bieszczady Mts. (b), and Białowieża Forest (c)

Table 2. Collection sites of the studied populations of *Trichocolea tomentella* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Dolina Białki (valley)	950	5	40
T-2	Tatra National Park, Las Capowski (forest)	980	8	64
T-3	Tatra National Park, Potok Sichlański (stream)	980	7	56
T-4	Tatra National Park, Potok Chowańców (stream)	1005	5	40
T-5	Tatra National Park, Dolina Suchej Wody (valley)	1194	4	32
P-6	Pomeranian Lake District, bank of Lake Kamień	174	8	64
P-7	Pomeranian Lake District, bank of Lake Orle	185	11	88
P-8	Pomeranian Lake District, Czapliniec Reserve, bank of Lake Książę	154	6	48
P-9	Pomeranian Lake District, bank of Słupia river, near Bytów	95	8	64
Total			62	496

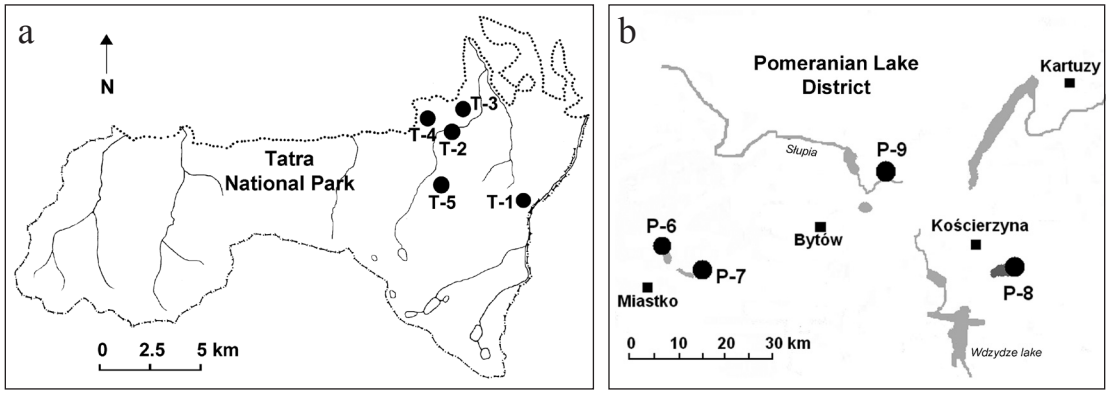

Fig. 2. Location of the studied populations of *Trichocolea tomentella* (described in Table 2) in the Tatra National Park (a) and Pomeranian Lake District (b)

Table 3. Collection sites of the studied populations of *Lophozia hatcheri* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Dolina Suchej Wody (valley)	1183	7	56
T-2	Tatra National Park, Dolina Pańszczyca (valley)	1614	7	56
T-3	Tatra National Park, Dolina 5 Stawów Polskich (valley)	1655	10	80
T-4	Tatra National Park, Dolina Jaworzynka (valley)	1511	6	48
T-5	Tatra National Park, Dolina Roztoki (valley)	1567	5	40
B-6	Bieszczady Mts., N slope of Mt. Tarnica	1319	10	80
B-7	Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	1245	6	48
B-8	Bieszczady Mts., N slope of Mt. Krzemień	1308	7	56
B-9	Bieszczady Mts., N slope of Mt. Kińczyk Bukowski	1185	10	80
Total			68	544

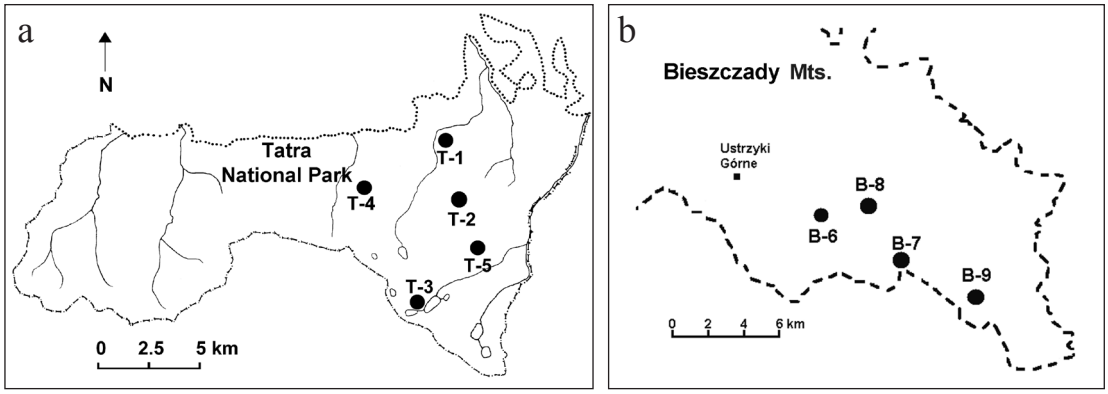

Fig. 3. Location of the studied populations of *Lophozia hatcheri* (described in Table 3) in the Tatra Mts. (a) and Bieszczady Mts. (b)

1969; Damshold 2002). In Poland it has been reported as completely sterile (Szweykowski 2006), but Buczkowska *et al*. (2010) found a few specimens with sporophytes in one colony from Białowieża National Park. It reproduces predominantly asexually by tardily caducous older leaves or by deciduous flagelliform shoots, and lacks gemmae. Widespread in Holarctic regions of Europe, Asia, and North America. In Europe, the species reaches southwards to northern Spain and Portugal, Italy, and Greece (Damshold 2002). In Poland it occurs in all mountains in spruce forests, where it is locally common, and exceptionally found in places situated above the tree line. In lowlands rare, known from scattered sites mainly in northern (Pomeranian Lake District) and NE Poland (Suwałki Lake District, Białowieża Forest) (Klama 2002; Szweykowski 2006). Typically found in shaded places, on decaying logs, on humus or peaty soil, frequently as a pioneer on acid dry rocks and even on the bark of trees.

Trichocolea tomentella is dioecious but plants with sporophytes are extremely rare. For example, in Europe its fertile specimens were collected only once in Sweden (Damshold 2002), though the cited author suggested that they may be overlooked. In North America its sexual reproduction is rare or occasional, too (Schuster 1966). In Poland always sterile (Szweykowski 2006). Gemmae lacking. It reproduces predominantly asexually by clonal regeneration. Suboceanic-mountain distribution in Europe, Asia, North America, and Africa (Damshold 2002). In Polish mountains restricted to the lower montane zone (forest belt) and in the lowlands widespread (Szweykowski 2006). Found on moist decaying humus and mud, sometimes along banks of sheltered small streams; rarely on wet rock walls (Damshold 2002).

Lophozia hatcheri is dioecious but female plants with perianths and sporophytes are rather rare (Damshold 2002). In Poland always sterile (Szweykowski 2006). Plants with gemmae very frequent (Damshold 2002; Szweykowski 2006). It reproduces predominantly asexually by gemmae and clonal regeneration. Bipolar species, with a wide geographic range extending from subarctic-subalpine to arctic-alpine regions of Europe, North and South America, and Asia (Schuster 1969; Damshold 2002). In Poland widespread in all mountain ranges, while infrequent in the lowlands (Szweykowski 2006). Usually found on granite rocks and on acid humus under spruces.

Mylia anomala is dioecious but only occasionally fertile (Paton 1999; Damshold 2002). In Poland always sterile but profusely producing gemmae (Szweykowski 2006). It reproduces predominantly asexually by clonal regeneration and gemmae. Boreal distribution in Europe, North America, and Asia. In Europe distributed from Nordic countries and the UK to Romania and Bulgaria. In Poland widespread in the Tatra Mts. (Szweykowski & Buczkowska 2000), while in the lowlands occur-

Table 4. Collection sites of the studied populations of *Mylia anomala* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Hala Gąsienicowa, Żółta Turnia	1687	6	48
T-2	Tatra National Park, Waksmundzka Młaka (peat-bog)	1368	5	40
T-3	Tatra National Park, N slope of Mt. Ornak	1740	4	32
P-4	Pomeranian Lake District, Lake Duże Sitno	111	8	64
P-5	Pomeranian Lake District, Staniszewskie Błoto Reserve (peat-bog)	215	9	72
P-6	Pomeranian Lake District, Lake Kulkówko	185	7	56
S-7	Suwałki Lake District, Lake Łempis	115	9	72
S-8	Suwałki Lake District, Wielki Mechacz Reserve (peat-bog)	187	10	80
S-9	Suwałki Lake District, Czerwone Bagno Reserve (peat-bog)	132	11	88
Total			69	552

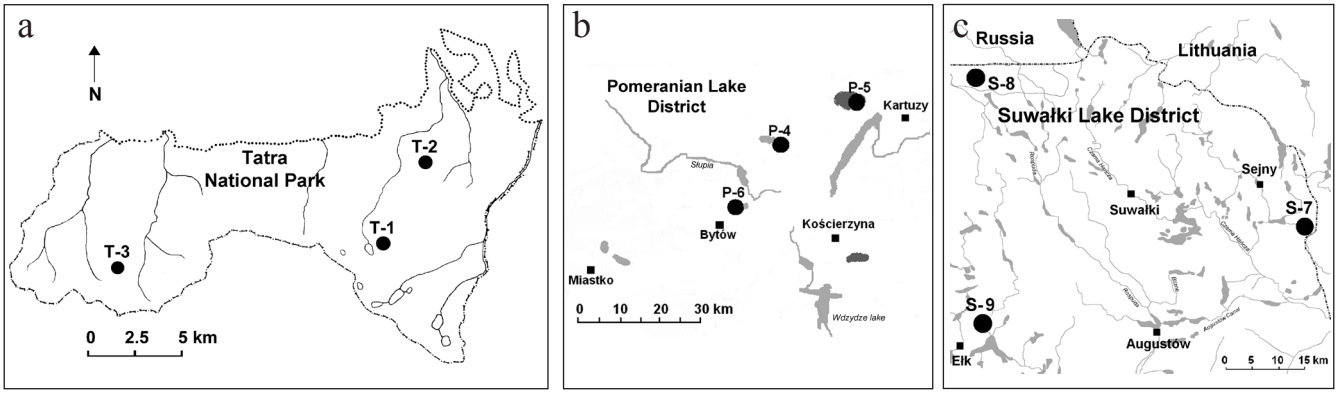

Fig. 4. Location of the studied populations of *Mylia anomala* (described in Table 4) in the Tatra National Park (a) and Pomeranian (b) and Suwałki Lake Districts (c)

ring mainly in northern (Pomerania) and NE Poland (Suwałki Lake District). Very rare in the montane zone but above the tree line growing on peat-bogs among and on *Sphagnum* hummocks (Szweykowski 2006). In the lowlands found on wet peaty banks of lakes or peat-bogs.

2.1.2. Fertile species

Lepidozia reptans frequently produces sporophytes but does not produce gemmae. Mainly monoecious, although sometimes dioecious (Damshold 2002). Circumboreal, absent in the Arctic. In Europe known from the UK and Scandinavia to Spain and Portugal (Damshold 2002). In Poland it is one of the most frequent hepatics, distributed in both lowlands and mountains. In mountains mostly restricted to forests, sporadically recorded in the subalpine zone. In lowlands common in coniferous forests: either primeval, e.g. in Białowieża National Park (Klama 2002) or secondary forest communities, e.g. in Pomerania, Wielkopolska, and Silesia (Szweykowski 2006). Found on moist peaty soil and on shaded forest floor, as a pioneer in various habitats, mainly on decaying logs, as an epiphyte on the bark of trees or directly on soil.

Calypogeia integristipula is monoecious and frequently produces gemmae (Damshold 2002). In Poland in spring frequently fertile. Subboreal-mountain distribution in Europe, North America, and Asia. In Europe known from the UK and Scandinavia to Spain and France (Damshold 2002). In Poland common; in mountains present in all zones but predominantly in the forest belt; in lowlands present in humid forests. Found mostly on rotten wood or on soil.

Mylia taylorii is dioecious (Damshold 2002), in Poland frequently fertile and producing sporophytes regularly. Gemmae extremely rare. In the Polish territory Szweykowski (2006) collected gemmiparous plants only twice. Widespread in suboceanic-montane and circumboreal regions of Europe, Asia, and North America. In Europe distributed from the UK and Scandinavia to the Balkans (Damshold 2002). In Poland recorded in mountains in the montane and subalpine zones. In lowlands found only once, by Winkelmann in 1902 (Szweykowski 2006). In montane forests found mostly on rotten wood; in subalpine zone common on soil and on *Sphagnum-Polytrichum* hummocks.

Tritomaria quinquedentata is dioecious, with frequent male and female plants with perianths and sporophytes, but plants with gemmae very rare (Damshold 2002). In Poland gemmae found only once, in the Bieszczady Mts. (Szweykowski & Buczkowska 1996). Circumpolar species, widespread in boreal-montane regions of Europe, Asia, and North America. In Europe distributed from the UK and Scandinavia to Spain and

Table 5. Collection sites of the studied populations of *Lepidozia reptans* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Dolina Białki (valley)	950	10	80
T-2	Tatra National Park, Dolina Suchej Wody (valley)	1183	10	80
T-3	Tatra National Park, Dolina Kościeliska (valley)	1050	10	80
T-4	Tatra National Park, Dolina Chochołowska (valley)	1184	8	64
P-5	Pomeranian Lake District, forest near Lake Kamień	161	7	56
P-6	Pomeranian Lake District, Czapliniec Reserve (peat-bog)	154	11	88
P-7	Pomeranian Lake District, Staniszewskie Błoto Reserve (peat-bog)	215	9	72
S-8	Suwałki Lake District, forest near Lake Stulpień	115	9	72
S-9	Suwałki Lake District, Augustów Forest	116	8	64
S-10	Suwałki Lake District, forest near Lake Godle	146	9	72
Total			91	728

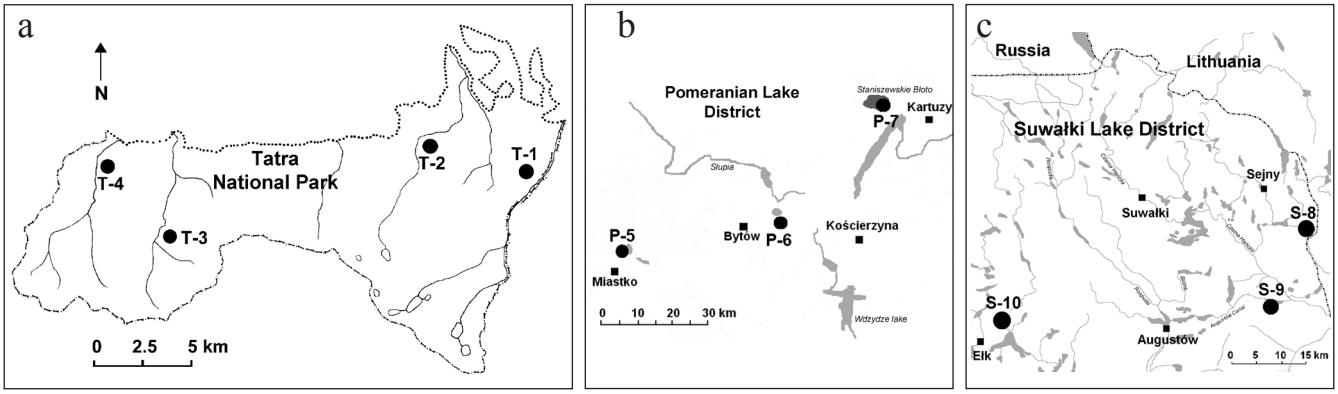

Fig. 5. Location of the studied populations of *Lepidozia reptans* (described in Table 5) in the Tatra National Park (a) and in Pomeranian (b) and Suwałki Lake Districts (c)

Table 6. Collection sites of the studied populations of *Calypogeia integristipula* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park,, Potok Sichlański (stream)	980	10	80
T-2	Tatra National Park, Dolina Białki (valley)	955	10	80
T-3	Tatra National Park, Dolina Suchej Wody (valley)	1187	9	72
B-4	Bieszczady Mts., N slope of Mt. Tarnica	1320	9	72
B-5	Bieszczady Mts., N slope of Mt. Krzemień	1300	8	64
B-6	Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	1215	8	64
P-7	Pomeranian Lake District, Staniszewskie Błoto Reserve (peat-bog)	215	10	80
P-8	Pomeranian Lake District, bank of Lake Duże Sitno	185	5	40
P-9	Pomeranian Lake District, Czapliniec Reserve, bank of Lake Książę	154	6	48
Total			75	600

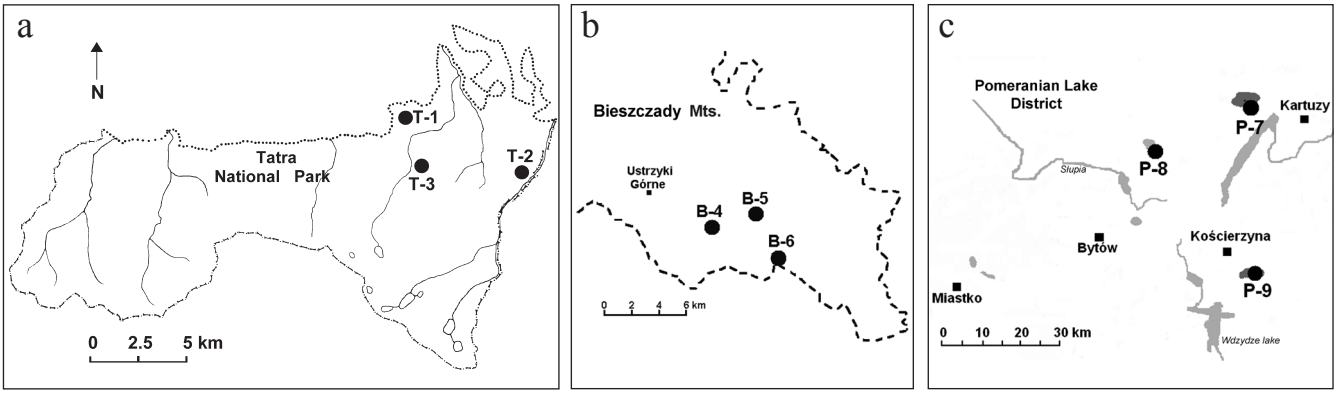

Fig. 6. Location of the studied populations of *Calypogeia integristipula* (described in Table 6) in the Tatra National Park (a), Bieszczady Mts. (b), and Pomeranian Lake District (c)

Table 7. Collection sites of the studied populations of *Mylia taylorii* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park,, Dolina Białki (valley)	950	9	72
T-2	Tatra National Park,, Dolina Suchej Wody (valley)	1200	10	80
T-3	Tatra National Park, Dubrowiska (deforested mountains)	1463	10	80
T-4	Tatra National Park, Dolina Pańszczyca (valley)	1614	10	80
T-5	Tatra National Park, Dolina Kościeliska (valley), Wąwóz Kraków (gully)	1463	10	80
B-6	Bieszczady Mts., Terebowiec stream	698	8	64
B-7	Bieszczady Mts., N slope of Mt. Tarnica	1300	5	40
B-8	Bieszczady Mts., N slope of Mt. Krzemień	1295	6	48
B-9	Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	1111	5	40
Total			73	584

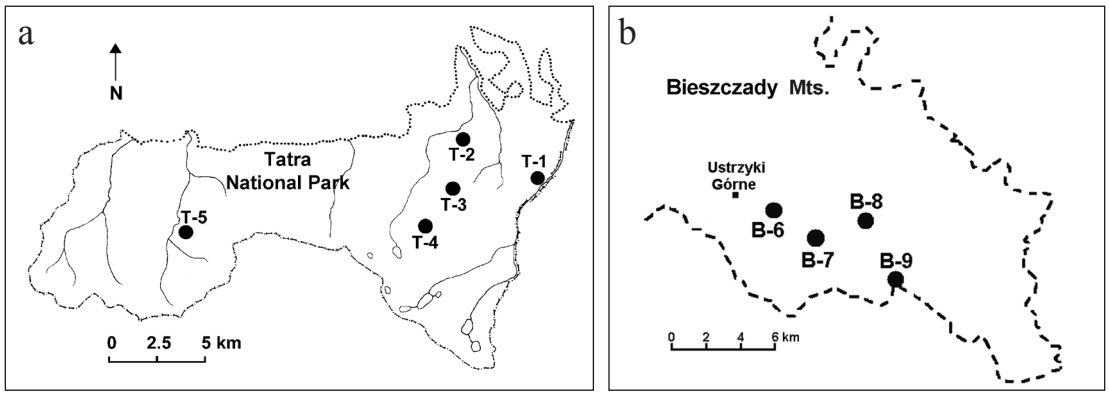

Fig. 7. Location of the studied populations of *Mylia taylorii* (described in Table 7) in the Tatra National Park (a) and Bieszczady Mts. (b)

Table 8. Collection sites of the studied populations of *Tritomaria quinquedentata* with numbers of sampled patches and studied plants

Population	Location	Altitude (m)	No. of patches	No. of plants
T-1	Tatra National Park, Las Capowski (forest)	980	7	56
T-2	Tatra National Park, Dolina Jaworzynki (valley)	1511	10	80
T-3	Tatra National Park, Dolina 5 Stawów Polskich (valley)	1650	6	48
T-4	Tatra National Park, Dolina Kościeliska (valley)	1125	10	80
T-5	Tatra National Park, Dolina Miętusia (valley)	1056	10	80
B-6	Bieszczady Mts., Dolina Górnej Solinki (valley)	748	10	80
B-7	Bieszczady Mts., N slope of Mt. Krzemień	1305	9	72
B-8	Bieszczady Mts., N slope of Mt. Tarnica	1325	6	48
B-9	Bieszczady Mts., NW slope of Mt. Rozsypaniec Wołosacki	1202	9	72
Total			77	616

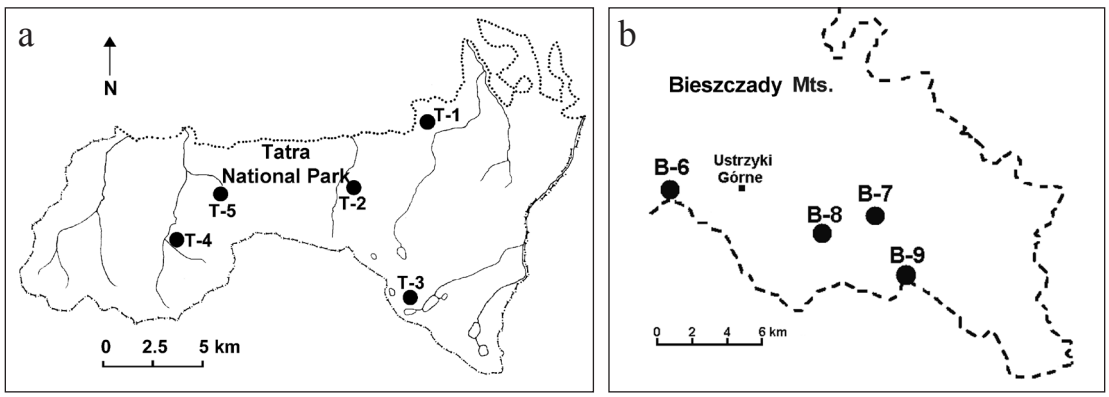

Fig. 8. Location of the studied populations of *Tritomaria quinquedentata* (described in Table 8) in the Tatra National Park (a) and Bieszczady Mts. (b)

France. In Poland widespread in the mountains, locally common, recorded in all altitudinal zones. Currently absent from the North Polish lowlands but some old data date back from the late 19th century (Szweykowski 2006). Found on basic substrates, e.g. sandy, calcareous soil, basic rock walls, and boulders.

2.2. Study sites and sampling

For each of the 8 species of leafy liverworts, samples were collected from 9-10 populations of Poland. Locations of populations of the studied species are presented in Tables 1-8 and in maps (Figs. 1-8). In each population, 5-10 patches were sampled, depending on population size. In total, 593 patches in 73 populations of the 8 species were sampled. Distances between sampled patches were > 5 m. Prior to analysis, each plant was identified according to morphological characters and oil bodies (Müller 1951-1958; Schuster 1966). Plants were stored at 4°C until the beginning of analyses. After isozyme analysis, all samples were deposited as vouchers in the POZW herbarium in Adam Mickiewicz University.

2.3. Electrophoretic analysis

Isozyme analysis was used to examine the genetic and genotypic diversity of leafy liverwort populations. Eight gametophytes per patch were analyzed, so a total of 4744 plants were examined. The methods used for horizontal starch-gel electrophoresis, the staining procedure for isozymes, and genetic interpretation were described by Wendel & Weden (1989). Crude cell extract was prepared by homogenization of a single shoot for PER and ACP in 30 µl of distilled water and for the remaining enzymes in 50 µl of extraction buffer (Gottlieb 1981). The supernatant was absorbed onto 2 mm × 10 mm paper wicks (Whatman 3MM), which were inserted into a 12% starch gel. After separation the

Table 9. List of enzymes studied, their abbreviations (Abbr.), and enzyme commission numbers (E.C.)

Abbr.	Enzyme	E.C.
ACP	Acid phosphatase	3.1.3.2
DIA	Diaphorase	1.8.1.4
EST	Esterase	3.1.1.-
GDH	Glutamate dehydrogenase	1.4.1.2
GOT	Glutamate oxaloacetate transaminase	2.6.1.1
HEX	Hexokinase	2.7.1.1
IDH	Isocitrate dehydrogenase	1.1.1.41
LAP	Leucine aminopeptidase	3.4.1.1.1
ME	Malic enzyme	5.3.1.8
MDH	Malate dehydrogenase	1.1.1.37
MPI	Mannose phosphate isomerase	5.3.1.8
PER	Peroxidase	1.11.1.7
PGD	Phosphogluconate dehydrogenase	1.1.1.44
PGI	Glucose phosphate isomerase	5.3.1.9
PGM	Phosphoglucomutase	5.4.2.2
SDH	Shikimate dehydrogenase	1.1.1.25

Table 10. Buffer systems used for separation of isozymes of studied loci in each species

Species	Buffer systems	Loci
Bazzania trilobata	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, DIA, EST, GDH, GOT, IDH, LAP, MPI, PER
	B. Tris-citrate (pH 7.0)	ME, PGD, PGI, PGM, SDH
Trichocolea tomentella	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, DIA, EST, GDH, GOT, LAP, PER
	B. Tris-histidine (pH 7.0)	MDH, ME, PGD, PGI, PGM, SDH
Lophozia hatcheri	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP,GDH, GOT, LAP, PER
	B. Tris-citrate (pH 7.0)	IDH, MDH
	C. Tris-histidine (pH 7.0)	ME, PGD, SDH
Mylia anomala	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, DIA, GDH, GOT, LAP, MPI, PER
	B. Morpholine-citrate (pH 6.1)	IDH, MDH, ME, PGD, SDH
	C. Tris-histidine (pH 7.0)	PGI, PGM
Lepidozia reptans	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	DIA, GDH, GOT, PGI
	B. Morpholine-citrate (pH6.1)	PGD, SDH
	C. Tris-citrate (pH 7.0)	IDH, ME, PGM
Calypogeia integristipula	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, EST, GDH, GOT, PGI
	B. Morpholine-citrate (pH6.1)	MDH
	C. Tris-citrate (pH 7.0)	ME, PGM, SDH
Mylia taylorii	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, DIA, EST, GDH, GOT, MPI, PER
	B. Morpholine-citrate (pH6.1)	IDH, MDH, ME, PGD, SDH
	C. Tris-histidine (pH 7.0)	PGI, PGM
Tritomaria quinquedentata	A. Tris-citrate (pH 8.2) Lithium-borate (pH 8.3)	ACP, DIA, EST, GDH, GOT, HEX, PER
	B. Tris-histidine (pH 7.0)	IDH, MDH, ME, PGD, SDH

isozymes were detected on the gel slabs. The studied enzyme systems are listed in Table 9. ACP, EST, GOT, LAP, and PER were stained in liquid assay, and the remaining enzymes were stained for 2-3 h at 37°C using the agar-overlay method. The enzymes stained and buffer systems for each species are listed in Table 10.

2.4. Data analysis

To compare the genetic diversity of the 8 leafy liverworts, for each species the same statistic analysis was performed. For each population were estimated: allele frequencies, mean number of alleles per locus (*A*), number of rare alleles (frequency <0.05), number of private alleles (Slatkin 1985), percentage of polymorphic loci (*P*), the mean genetic diversity over loci (H_S) within populations (Nei 1973) by program POPGENE 1.32 (Yeh *et al*. 2000). Means of these statistics for different regions were calculated. The Kruskal-Wallis ANOVA or the Mann-Whitney *U* tests were used to check their statistical significance. To detect the possible effects of selection, the Ewens-Watterson test for neutrality (1000 permutations) (Manly 1985) was performed for each locus. For 8 studied species Nei's (1973, 1978) gene-diversity statistics were used to partition the total genetic diversity (H_T), as well as its components, i.e. genetic diversity within populations (H_S) and the coefficient of genetic differentiation (the relative amount of between population diversity to total diversity – G_{ST}). Separately these statistic were calculated within regions for each species. These analyses were performed using POPGENE 1.32. Correlation between the genetic diversity (H_S) of the population and the population size (*N*) were studied by the Spearman correlation test using STATISTICA program version 7.1 for Windows (StatSoft 2008).

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). Gene flow was estimated using POPGENE 1.32. Pair-wise genetic distances (*D*) and identity (*I*) (Nei 1972) between populations and regions were calculated for each species. On the basis of Nei's (1978) genetic distance matrix, UPGMA phenograms were constructed and principal coordinate analysis (PCA) was performed using statistical software: GenALEx version 6.3. (Peakall & Smouse 2006) and STATISTICA version 7.1 for Windows (StatSoft 2008). The Mantel test was used to assess the correlation between genetic and geographic distances of populations by using GenALEx 6.3. Statistical significance was tested by 1000 permutations. Geographic distances were calculated from latitude and longitude coordinates.

To investigate the genetic structure of populations, an analysis of molecular variance (AMOVA) was done by GenALEx 6.3. AMOVA was used to describe the percentage shares of genetic variation within populations, among 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).

Parameters of genotypic diversity within populations and within regions were also 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. Three different measures for clonal diversity were used in this study. Firstly, the proportion of distinguishable genotypes (*G*/*N*) was calculated as the number of unique MLGs (*G*) divided by sample size (Ellstrand & Roose 1987). The *G*/*N* proportion was calculated for each patch, each population, each region, and for all species jointly. Mean *G*/*N* for all patches in each population and for all populations in each region were calculated to determine if patches within populations and populations within regions were equally variable. 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). Secondly, multilocus genotype diversity was calculated (D_G) as a modification of the Simpson index (Pielou 1969; Ellstrand & Roose 1987), according to the formula:

$$D_G=1-\sum [n_i(n_i-1)]/[N(N-1)]$$
$$(i=1 \text{ to } C)$$

where n_i is the number of individuals (gametophytes) with the same genotype (MLG) *i*, *C* is the number of MLGs, and *N* is sample size. Values of *D* range from 0 to 1, and higher values correspond to greater clonal diversity. Thirdly, genotypic evenness (*E*) scales D_G to the level of polymorphism within the population (Fager 1972; Ellstrand & Roose 1987; Eckert & Barrett 1993):

$$E=(D-D_{min})/(D_{max}-D_{min})$$

where

$$D_{min}=(G-1)(2N-G)/N(N-1) \text{ and } D_{max}=N(G-1)/G(N-1)$$

The nonparametric Mann-Whitney *U* test was used to check statistical significance in respect to: *G*/*N*, *A*, *P*, mean numbers of rare and private alleles, H_S, H_T, and Φ_{PT} between sterile and fertile species and between monoecious and dioecious species.

3. Results

3.1. Genetic diversity of sterile leafy liverworts species

3.1.1. *Bazzania trilobata*

Clonal diversity. Generally, *B. trilobata* patches demonstrated a high level of genotypic diversity (Table 11). The mean number of MLGs (*G*) at the patch level was 5.03. The mean proportion of distinguishable genotypes (*G*/*N*) was 0.623. The highest number of MLGs was observed in population B-4 from the Bieszczady Mts., where the mean *G*/*N* was 0.76. The least diverse patches were observed in population T-1 from the Tatra NP and Błż-9 from Białowieża Forest. For both these populations the mean *G*/*N* in a single patch was 0.53. The number of genotypes (MLGs) detected in patches of *B. trilobata* ranged from 1 to 8 per 8 studied gametophytes. Out of 78 studied patches of this species, only one consisted of a single genotype. It was found in population T-1 (Dolina Białki in the Tatra NP). On the other hand, 8 MLGs were identified in 2 patches: one from T-2 and the other from B-4. The usual number of genotypes detected was 4-5. The number of genotypes within patches of studied populations differed significantly (Kruskal-Wallis ANOVA test: H=19.177, p=0.01).

At the population level of *B. trilobata*, the mean number of genotypes (MLG=41.2) was lower than at the patch level, while the proportion of distinguishable genotypes (*G*/*N*) equalled 0.594 per population. The most diverse was population B-4 from the Bieszczady Mts. (*G*/*N*=0.75), but the least diverse was a population from the same region, B-6 (*G*/*N*=0.52) (Table 12).

The Simpson index (D_G) and evenness index (E) values in all the study populations of *B. trilobata* are high compared to the other leafy liverworts investigated in this study. Values of D_G confirm that the species has high clonal diversity. In all populations of this species, D_G values are similar. The highest D_G value occurred in population B-4 (0.991) and the lowest in T-1 (0.971) (Table 13).

Table 12. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G*/*N*, where *G*=MLG) for populations of *Bazzania trilobata*

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	9	72	39	0.54
T-2	9	72	44	0.61
T-3	9	72	46	0.64
B-4	9	72	54	0.75
B-5	6	48	27	0.56
B-6	8	64	33	0.52
Błż-7	10	80	46	0.58
Błż-8	8	64	39	0.61
Błż-9	10	80	43	0.54
Mean			41.2	0.594

Table 13. Clonal diversity in populations of *Bazzania trilobata*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.971	0.918
T-2	0.982	0.940
T-3	0.985	0.946
B-4	0.991	0.935
B-5	0.974	0.952
B-6	0.973	0.948
Błż-7	0.984	0.961
Błż-8	0.980	0.934
Błż-9	0.974	0.976

Among 624 *B. trilobata* gametophytes under study, only 302 MLGs were detected. The proportion of distinguishable genotypes (*G*/*N*) at the species level was equal to 0.484. Some of the detected MLGs occurred in various patches, while others were only observed once. Sixty-one MLGs (20.2% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 4 populations, including 2 from the Tatra NP populations (T-1, T-2), one from the Bieszczady population (B-4) and one from Białowieża Forest (Błż-8). Overall, the genotype was detected in a total of 6 patches in 9 plants. By contrast, 149 MLGs (49%) were restricted to a single gametophyte. Individual genotypes occurred in 23.9% of studied plants.

Table 11. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G*/*N*, where *G*=MLG) at the patch level in *Bazzania trilobata* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Dolina Białki (valley)	T-1	4.22	0.53
Tatra National Park, Dolina Suchej Wody (valley)	T-2	5.22	0.65
Tatra National Park, Dolina Kościeliska (valley)	T-3	5.33	0.67
Bieszczady Mts., N slope of Mt. Tarnica	B-4	6.11	0.76
Bieszczady Mts., N slope of Mt. Krzemień	B-5	4.83	0.60
Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	B-6	4.50	0.56
Białowieża Forest, plot 259, near Hwożna river	Błż-7	5.40	0.68
Białowieża Forest, plot 343, near Orłówka river	Błż-8	5.00	0.63
Białowieża Forest, plot 394, near Teremiski village	Błż-9	4.80	0.53
Mean		5.046	0.623

Table 14. Allele frequencies at 14 enzyme loci in *Bazzania trilobata* populations

Locus	Allele	Population								
		Tatra National Park			Bieszczady Mts.			Białowieża Forest		
		T-1	T-2	T-3	B-4	B-5	B-6	Błż-7	Błż-8	Błż-9
Acp	1	0.667	0.228	0.320	0.652	0.625	0.554	1.000	1.000	1.000
	2	0.333	0.722	0.680	0.348	0.375	0.446	0.000	0.000	0.000
Dia	1	0.778	1.000	0.889	1.000	1.000	0.492	1.000	1.000	1.000
	2	0.222	0.000	0.111	0.000	0.000	0.508	0.000	0.000	0.000
Est	1	0.025	0.028	0.042	0.000	0.000	0.000	0.000	0.063	0.038
	2	**0.128**	**0.250**	**0.111**	0.000	0.000	0.000	0.000	0.000	0.000
	3	**0.125**	**0.111**	**0.278**	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.722	0.611	0.569	1.000	1.000	1.000	1.000	0.937	0.962
Gdh	1	0.028	0.194	0.181	0.292	0.208	0.062	0.588	0.547	0.338
	2	0.972	0.792	0.806	0.680	0.792	0.938	0.388	0.391	0.625
	3	0.000	0.014	0.014	0.028	0.000	0.000	0.024	0.062	0.037
Got	1	0.333	0.667	0.903	0.556	0.688	0.369	0.400	0.703	0.700
	2	0.667	0.333	0.097	0.444	0.312	0.631	0.600	0.297	0.300
Idh	1	0.167	0.264	0.375	0.305	0.333	0.077	0.638	0.547	0.588
	2	0.736	0.680	0.514	0.675	0.646	0.908	0.250	0.219	0.238
	3	0.097	0.056	0.111	0.020	0.021	0.015	0.112	0.234	0.162
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.012**
Lap	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me	1	0.167	0.167	0.139	0.444	0.167	0.030	0.187	0.234	0.075
	2	0.778	0.736	0.764	0.556	0.770	0.862	0.800	0.750	0.850
	3	0.055	0.097	0.097	0.000	0.063	0.108	0.013	0.016	0.075
Mpi	1	0.667	0.444	0.333	0.333	0.354	0.415	0.250	0.375	0.500
	2	0.333	0.556	0.667	0.667	0.646	0.585	0.750	0.625	0.500
Per	1	0.889	1.000	1.000	1.000	0.667	0.892	1.000	1.000	0.800
	2	0.111	0.000	0.000	0.000	0.333	0.108	0.000	0.000	0.140
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.060**
Pgd	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi	1	0.125	0.000	0.139	0.167	0.083	0.031	0.163	0.000	0.075
	2	0.764	0.806	0.861	0.819	0.917	0.723	0.750	0.891	0.900
	3	**0.111**	**0.153**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.041	0.000	0.014	0.000	0.246	0.087	0.109	0.025
Pgm	1	0.042	0.028	0.028	0.014	0.146	0.062	0.025	0.000	0.063
	2	0.958	0.958	0.861	0.875	0.750	0.877	0.613	0.625	0.675
	3	0.000	0.014	0.111	0.111	0.083	0.046	0.037	0.125	0.075
	4	0.000	0.000	0.000	0.000	0.021	0.015	0.325	0.250	0.187
Sdh	1	0.139	0.083	0.042	0.153	0.250	0.062	0.150	0.203	0.162
	2	0.542	0.723	0.778	0.639	0.542	0.892	0.538	0.469	0.612
	3	0.319	0.194	0.180	0.208	0.208	0.046	0.275	0.297	0.138
	4	0.000	0.000	0.000	0.000	0.000	0.000	**0.037**	**0.031**	**0.088**

Explanation: bold values denote private alleles for regions

Isozyme diversity. A total of 14 loci (*Acp, Dia, Est, Gdh, Got, Idh, Lap, Me, Mpi, Per, Pgd, Pgi, Pgm, Sdh*) were detected in 14 enzyme systems (Table 14). Two loci were monomorphic (*Got* and *Pgm*). A total of 39 alleles were detected in the populations. The number of alleles in a single locus varied from 1 to 4. The most diverse were loci *Est, Idh, Pgi Pgm* and *Sdh*, with 4 alleles each. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci were selectively neutral in the studied populations (Table 15).

The mean percentage of polymorphic loci (*P*) in *B. trilobata* populations was 70.633%. The highest percentage was in population T-1 (*P*=85.70%), while the lowest values were in populations from Białowieża Forest (Błż-7 and Błż-8) and in one population from the Bieszczady Mts. (*P*=57.14%). The mean number of alleles per locus (*A*) per population for all studied loci was

Table 15. Ewens-Watterson test for neutrality for each locus in *Bazzania trilobata* populations

Locus	N	k	Obs. F	L95	U95
Acp	624	2	0.5683	0.5033	0.9968
Dia	624	2	0.8136	0.5065	0.9968
Est	624	4	0.6153	0.3461	0.9715
Gdh	624	3	0.5704	0.4071	0.9904
Got	624	2	0.5152	0.5045	0.9968
Idh	624	4	0.4002	0.3295	0.9747
Lap	624	1	1.0000	---	---
Me	624	3	0.6179	0.3743	0.9904
Mpi	624	2	0.5163	0.5092	0.9968
Per	624	3	0.7249	0.3767	0.9904
Pgd	624	1	1.0000	---	---
Pgi	624	4	0.6893	0.3359	0.9778
Pgm	624	4	0.6369	0.3341	0.9715
Sdh	624	4	0.4693	0.3342	0.9746

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

Table 16. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S ± SD) in *Bazzania trilobata* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S (± SD)
T-1	32	3	0	2.3	85.70	0.2711 (0.1948)
T-2	32	5	0	2.3	71.43	0.2622 (0.2142)
T-3	32	4	0	2.3	78.57	0.2743 (0.2000)
B-4	28	4	0	2.0	64.29	0.1966 (0.2425)
B-5	29	2	0	2.1	71.43	0.2556 (0.2167)
B-6	31	6	0	2.2	78.57	0.2418 (0.1910)
Błż-7	30	5	0	2.1	57.14	0.2167 (0.2492)
Błż-8	29	2	0	2.1	57.14	0.2189 (0.2575)
Błż-9	34	4	2	2.4	71.43	0.2885 (0.2321)
Mean	30.8	3.9	0.22	2.20	70.633	0.24732

2.20, and ranged from 2.0 to 2.4. The greatest number of alleles (34) was found in population Błż-9 (*A*=2.4). The smallest number of alleles (28) was detected in population B-4 (*A*=2.0). Rare alleles were present in all studied populations. The number of rare alleles was the largest (6) in a population from the Bieszczady Mts. (B-6), whereas the lowest number (2) in populations from the Bieszczady Mts. (B-5) and Białowieża Forest (Błż-8). Private alleles (2) were only identified in a single population Błż-9 from Białowieża Forest (Table 16). These were *Idh-4* and *Per-4*, with frequencies of 0.012 and 0.062, respectively (Table 14).

The level of isozymatic diversity of *B. trilobata* was high compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations of *B. trilobata*, reached 0.2993. Also, a high level of genetic diversity within populations (H_S) was noted. It ranged from 0.1966 (B-4) to 0.2885 (Błż-9), with a mean of 0.2473 (Table 16). In the studied populations no statistically significant correlations were detected between genetic diversity within populations (H_S) and population size (*N*) (Spearman test: *R*=-0.1581, *p*=0.68). Coefficient of genetic differentiation, calculated over polymorphic loci among 9 studied populations, was low (G_{ST}=0.0981), associated with high gene flow between populations (N_m=5.912).

Analysis of variance (AMOVA) showed that the genetic differentiation (Φ_{PT}) between *B. trilobata* populations was 0.112. Most of genetic variation (89%) resulted from variation within populations, whereas variation among populations accounted for only 11% (Table 17).

Pair-wise genetic distances (*D*) and similarities (*I*) (Nei, 1978) between 9 populations of *B. trilobata* were computed (Table 18) and on the basis of genetic distances, the UPGMA phenogram was constructed (Fig. 9). Genetic distances ranged from 0.0133 to 0.2524, with a mean of 0.2524. In the phenogram, 2 population groups stand out. The first group contains all the TatraNP populations (T-1, T-2, T-3) and 2 Bieszczady populations (B-4, B-5). The second group comprises 3 lowland populations from Białowieża Forest (Błż-7, Błż-8, Błż-9). The smallest genetic distances

Table 17. Analysis of molecular variance (AMOVA) for *Bazzania trilobata* populations

Source of variation	df	Variance component	Variance (%)	Fixation[1] index
Among populations	2	0.283	11	Φ_{PT}=0.112***
Within populations	622	2.246	89	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 18. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Bazzania trilobata* populations

	T-1	T-2	T-3	B-4	B-5	B-6	Błż-7	Błż-8	Błż-9
T-1	***	0.9527	0.9247	0.9357	0.9533	0.9013	0.8949	0.8952	0.9221
T-2	0.0484	***	0.9844	0.9560	0.9590	0.8535	0.8805	0.8947	0.9080
T-3	0.0783	0.0157	***	0.9539	0.9548	0.8028	0.8825	0.9062	0.9183
B-4	0.0664	0.0450	0.0472	***	0.9266	0.8339	0.9103	0.9229	0.9174
B-5	0.0478	0.0418	0.0462	0.0762	***	0.9082	0.9315	0.9435	0.9662
B-6	0.1039	0.1585	0.1816	0.2196	0.0963	***	0.7877	0.7770	0.8391
Błż-7	0.1111	0.1272	0.1250	0.0940	0.0710	0.2387	***	0.9868	0.9720
Błż-8	0.1107	0.1113	0.0984	0.0802	0.0582	0.2524	0.0133	***	0.9861
Błż-9	0.0811	0.0965	0.0853	0.0862	0.0344	0.1755	0.0284	0.0140	***

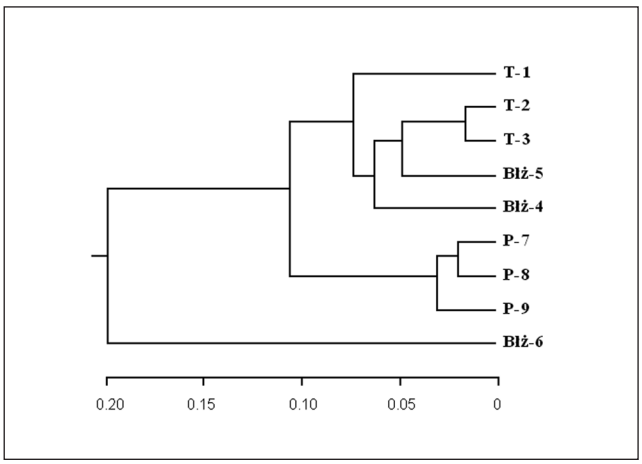

Fig. 9. UPGMA phenogram based on Nei's (1978) genetic distances between *Bazzania trilobata* populations

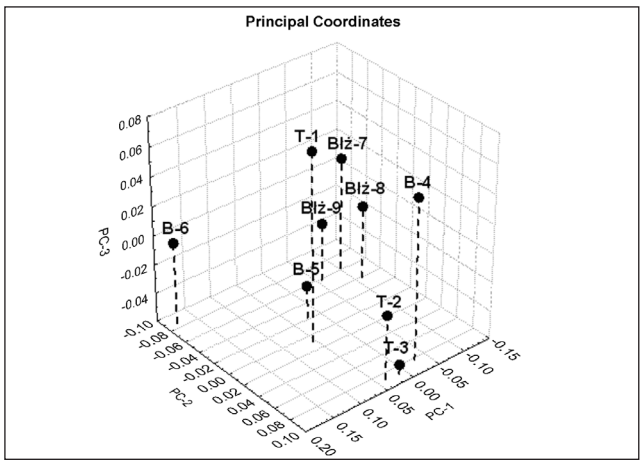

Fig. 10. Three-dimensional scatter plot of principal coordinates analysis (PCA) based on Nei's (1978) genetic distances between populations of *Bazzania trilobata*.
Explained variance: PC1=50.76%, PC2=30.65%, PC3=9.99%

were between these populations from Białowieża Forest. In the phenogram, population B-6 from the Bieszczady Mts. is quite distinct from the other populations. The highest genetic distance between populations was between B-6 and Błż-8. The Mantel test revealed significant correlations between genetic and geographic distances (R=0.293, p=0.01). The phenogram is consistent with the PCA diagram (Fig. 10).

3.1.2. *Trichocolea tomentella*

Clonal diversity. Patches of this species demonstrated a relatively high level of genotypic diversity (Table 19). The mean number of MLGs (G) at the patch level was 4.63, and the mean proportion of distinguishable genotypes (G/N) was 0.578. The most and the least diverse patches were observed in populations from the Tatra NP. In population T-1, G/N was equal to 0.68, whereas in population T-5, G/N was only 0.49. The number of genotypes within patches in the studied populations did not differ significantly (Kruskal-Wallis ANOVA: H=10.031, p=0.53). The number of genotypes (MLGs) detected in patches ranged from 2 to 8 per 8 studied gametophytes. The usual number of genotypes detected in a patch was 4-5. In this species no patch consisted of a single genotype. Eight MLGs were identified in only one patch from population P-7.

The mean number of MLGs at the population level of *T. tomentella* was 28.8, while the mean proportion of distinguishable genotypes (G/N) was 0.528. All populations had a similar level of genotypic diversity. The most and the least diverse populations were in the Pomeranian Lake District. The most diverse was P-6 (G/N=0.63) and the lowest diversity was in P-7 (G/N=0.47) (Table 20).

Table 20. Numbers of patches, plants (N), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (G/N, where G=MLG) for populations of *Trichocolea tomentella*

Population	No. of patches	N	MLG	G/N
T-1	5	40	24	0.60
T-2	8	64	31	0.48
T-3	7	56	28	0.50
T-4	5	40	21	0.53
T-5	4	32	18	0.56
P-6	8	64	40	0.63
P-7	11	88	42	0.47
P-8	6	48	23	0.48
P-9	8	64	32	0.50
Mean			28.8	0.528

Table 19. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (G/N, where G=MLG) at the patch level in *Trichocolea tomentella* populations

Location	Population	MLG	G/N
Tatra National Park, Dolina Białki Valley	T-1	5.4	0.68
Tatra National Park, Las Capowski forest	T-2	4.0	0.50
Tatra National Park, Potok Sichlański (stream)	T-3	4.3	0.54
Tatra National Park, Potok Chowańców (stream)	T-4	4.6	0.58
Tatra National Park, Dolina Suchej Wody (valley)	T-5	4.0	0.49
Pomeranian Lake District, bank of Lake Kamień	P-6	5.0	0.63
Pomeranian Lake District, bank of Lake Orle	P-7	4.9	0.61
Pomeranian Lake District, Czapliniec Reserve, bank of Lake Książę	P-8	4.3	0.54
Pomeranian Lake District, bank of Słupia river, near Bytów	P-9	5.0	0.63
Mean		4.61	0.578

Table 21. Clonal diversity in populations of *Trichocolea tomentella*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.9321	0.6771
T-2	0.9707	0.9526
T-3	0.9560	0.8437
T-4	0.9728	0.9314
T-5	0.9335	0.7781
P-6	0.9876	0.9464
P-7	0.9319	0.9313
P-8	0.9618	0.9093
P-9	0.9641	0.9424

The Simpson index (D_G) and evenness index (E) values in all the study populations of *T. tomentella* are relatively high compared to other studied leafy liverworts. The highest D_G values occurred in Pomeranian population P-6 (0.9876) and the lowest in Pomeranian population P-7 (0.9319) (Table 21).

Only 199 MLGs were detected among 496 *T. tomentella* gametophytes under study, so the proportion of distinguishable genotypes (G/N) at the species level was 0.401. Fifty-one MLGs (25.6% of MLGs) occurred more frequently than in a single patch. The most common genotype was found in 4 populations in both compared regions of Poland: T-2 and T-5 in the Tatra NP and P-6 and P-7 in the Pomeranian Lake District. The genotype was detected in 17 plants from 8 patches. By contrast, 92 MLGs (46.2% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 18.6% of studied plants.

Isozyme diversity. A total of 15 loci (*Acp, Dia, Est, Gdh, Got, Lap, Mdh-A, Mdh-B, Me, Per-A, Per-B, Pgd, Pgi, Pgm, Sdh*) were detected in 13 enzyme systems (Table 22). Two loci were monomorphic (*Got* and *Pgm*). A total of 40 alleles were found in the study populations. The number of alleles in a single locus

Table 22. Allele frequencies at 15 enzyme loci in *Trichocolea tomentella* populations

Locus	Allele	Population								
		Tatra National Park					Pomeranian Lake District			
		T-1	T-2	T-3	T-4	T-5	P-6	P-7	P-8	P-9
Acp	1	0.000	0.000	0.000	0.000	0.000	**0.063**	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	0.922	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	**0.015**	0.000	0.000	0.000
Dia	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.016**
	2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.984
Est	1	0.225	0.141	0.554	0.025	0.219	0.000	0.250	0.250	0.172
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.031**
	3	0.775	0.859	0.446	0.975	0.781	1.000	0.750	0.750	0.797
Gdh	1	0.075	0.000	0.018	0.000	0.000	0.000	0.023	0.000	0.016
	2	0.875	1.000	0.714	0.975	1.000	0.828	0.670	0.917	0.984
	3	0.050	0.000	0.268	0.025	0.000	0.172	0.307	0.083	0.000
Got	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Lap	1	0.800	0.875	0.429	1.000	0.969	1.000	1.000	1.000	1.000
	2	0.200	0.125	0.571	0.000	0.031	0.000	0.000	0.000	0.000
Me	1	0.000	0.797	0.357	1.000	1.000	1.000	1.000	1.000	1.000
	2	**1.000**	**0.203**	**0.643**	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-A	1	0.975	0.891	1.000	1.000	0.800	1.000	1.000	1.000	1.000
	2	**0.025**	**0.109**	0.000	0.000	0.200	0.000	0.000	0.000	0.000
Mdh-B	1	**0.100**	**0.125**	**0.018**	**0.125**	**0.188**	0.000	0.000	0.000	0.000
	2	0.062	0.016	0.054	0.150	0.063	0.125	0.090	0.000	0.032
	3	0.102	0.172	0.089	0.300	0.063	0.281	0.283	0.458	0.188
	4	0.450	0.406	0.536	0.225	0.530	0.350	0.297	0.188	0.516
	5	0.286	0.281	0.304	0.200	0.156	0.244	0.330	0.354	0.266
Per-A	1	0.725	0.969	0.696	0.625	1.000	0.875	0.477	0.750	0.484
	2	0.275	0.031	0.304	0.375	0.000	0.125	0.523	0.250	0.516
Per-B	1	0.000	0.000	0.000	0.000	0.000	**0.484**	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	0.484	0.978	0.938	0.859
	3	0.000	0.000	0.000	0.000	0.000	**0.032**	**0.023**	**0.062**	**0.141**
Pgd	1	0.000	0.031	0.339	0.050	0.000	0.109	0.000	0.146	0.047
	2	0.100	0.344	0.268	0.350	0.156	0.359	0.159	0.396	0.266
	3	0.900	0.609	0.304	0.400	0.438	0.391	0.443	0.312	0.407
	4	0.000	0.016	0.089	0.200	0.406	0.141	0.398	0.146	0.281
Pgi	1	0.000	**0.047**	**0.054**	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.900	0.828	0.946	1.000	0.900	0.907	0.602	0.958	1.000
	3	0.100	0.125	0.000	0.000	0.100	0.093	0.398	0.042	0.000
Pgm	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sdh	1	0.175	0.315	0.607	0.250	0.031	0.047	0.205	0.042	0.016
	2	0.500	0.219	0.304	0.300	0.250	0.391	0.272	0.458	0.281
	3	0.325	0.469	0.000	0.450	0.719	0.562	0.523	0.500	0.703
	4	0.000	0.000	**0.089**	**0.150**	0.000	0.000	0.000	0.000	0.000

Explanation: bold values denote private alleles for regions

varied from 1 to 5. The most diverse was locus *Mdh-B*, in which 5 alleles were identified. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci except *Mdh-2* and *Pgd* were selectively neutral in the studied populations (Table 23).

Table 23. Ewens-Watterson test for neutrality for each locus in *Trichocolea tomentella* populations

Locus	N	k	Obs. F	L95	U95
Acp	496	3	0.9800	0.3996	0.9880
Dia	496	2	0.9960	0.5047	0.9960
Est	496	2	0.8219	0.5029	0.9960
Gdh	496	3	0.6355	0.3957	0.9920
Got	496	3	0.7629	0.3771	0.9920
Lap	496	1	1.0000	—	—
Me	496	2	0.8155	0.5043	0.9960
Mdh-A	496	2	0.9683	0.5016	0.9960
Mdh-B	496	5	0.2695	0.2889	0.9524
Per-A	496	2	0.5879	0.5029	0.9960
Per-B	496	3	0.8109	0.3732	0.9920
Pgd	496	4	0.3024	0.3438	0.9721
Pgi	496	3	0.6728	0.3849	0.9880
Pgm	496	1	1.0000	—	—
Sdh	496	4	0.3661	0.3328	0.9681

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

The mean number of alleles per locus (*A*) per population was 1.93 (Table 24). The greatest number of alleles (31) was found in populations T-2 and T-3 from the Tatra NP (*A*=2.1). The smallest number of alleles (27) was detected in population T-5 from the Tatra Mts. and P-8 from the Pomeranian Lake District (*A*=1.8). The mean percentage of polymorphic loci (*P*) in *T. tomentella* populations was 53.332%. The highest percentage was in 3 populations from the Tatra NP (T-1, T-2, T-3; *P*=60.00%), and the lowest, in population T-4 (*P*=40.00%). Rare alleles were detected in all studied populations. The highest and lowest numbers of rare alleles were found in the Pomeranian Lake District: 6 in population P-9 and only one in P-7. Private alleles were found in 2 populations from the Pomeranian Lake District (P-6, P-9) (Table 24). In P-6, 3 private alleles were present (*Acp-1 Acp-3*, and *PerB-1*) and one of them (*PerB-1*) had a very high frequency (0.484). In P-9, 2 private alleles were found (*Dia-1*, *Est-2*) but with frequency <0.05 (Table 22).

The level of isozymatic diversity of *T. tomentella* was rather high compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.2625. The mean diversity within populations (H_S) was 0.1817, and ranged from 0.1478 (P-9) to 0.2161 (P-6) (Table 24). No statistically significant correlations were detected between genetic diversity (H_S) and population size (*N*) in all studied populations (Spearman test: *R*=0.9449, *p*=0.37). Coefficient of genetic differentiation (G_{ST}), calculated over polymorphic loci among 9 studied populations, was rather low (0.1894), associated with considerable gene flow between populations per generation (N_m=2.1405).

Analysis of molecular variance (AMOVA) showed that the genetic differentiation between *T. tomentella* populations (Φ_{PT}) was 0.174. Most of genetic variation (83%) resulted from variation within populations (Table 25).

Pair-wise genetic distances (*D*) and similarities (*I*) (Nei 1978) between 9 populations of *T. tomentella* were

Table 24. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Trichocolea tomentella* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S (±SD)
T-1	29	2	0	1.9	60.00	0.1914 (0.2444)
T-2	31	5	0	2.1	60.00	0.1827 (0.2433)
T-3	31	2	0	2.1	60.00	0.2021 (0.2559)
T-4	28	3	0	1.9	40.00	0.1695 (0.2408)
T-5	27	2	0	1.8	46.67	0.1726 (0.2703)
P-6	30	3	3	2.0	53.33	0.2161 (0.2882)
P-7	28	1	0	1.9	53.33	0.1847 (0.2652)
P-8	27	2	0	1.8	53.33	0.1680 (0.2513)
P-9	29	6	2	1.9	53.33	0.1478 (0.2967)
Mean	28.9	2.9	0.6	1.93	53.332	0.181662

Table 25. Analysis of molecular variance (AMOVA) for *Trichocolea tomentella* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.356	17	Φ_{PT}=0.174***
Within populations	487	1.695	83	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 26. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Trichocolea tomentella* populations

	T-1	T-2	T-3	T-4	T-5	P-6	P-7	P-8	P-9
T-1	***	0.9514	0.8927	0.9631	0.8726	0.9440	0.9327	0.9690	0.9462
T-2	0.0498	***	0.9132	0.9772	0.9221	0.9609	0.9470	0.9726	0.9608
T-3	0.1135	0.0908	***	0.8834	0.7933	0.8634	0.8753	0.8865	0.8699
T-4	0.0376	0.0230	0.1240	***	0.8945	0.9673	0.9717	0.9891	0.9851
T-5	0.0376	0.0811	0.2316	0.1115	***	0.8942	0.9325	0.8989	0.9007
P-6	0.0577	0.0399	0.1469	0.0332	0.1118	***	0.9486	0.9771	0.9664
P-7	0.0697	0.0545	0.1332	0.0287	0.0699	0.0528	***	0.9704	0.9729
P-8	0.0314	0.0278	0.1204	0.0110	0.1066	0.0231	0.0301	***	0.9811
P-9	0.0553	0.0400	0.1394	0.0150	0.1046	0.0342	0.0274	0.0191	***

computed (Table 26). Genetic distances ranged from 0.0110 to 0.2316 (mean *D*=0.0717). The highest *D* was between populations T-3 and T-5 from the Tatra NP. The highest similarity was between T-4 and P-8 (*I*=0.9891). The Mantel test revealed no significant correlations between genetic and geographic distances (*R*=-0.052, *p*=0.23).

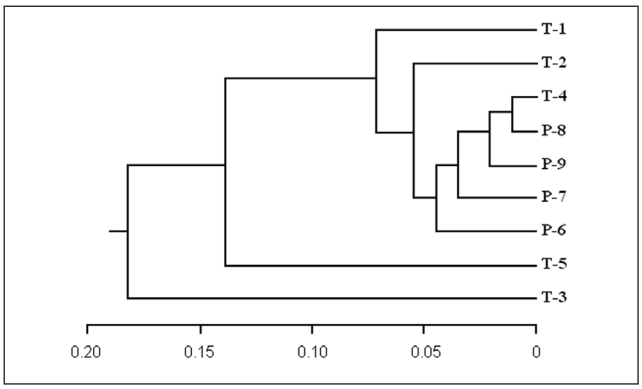

Fig. 11. UPGMA phenogram based on Nei's (1978) genetic distances between *Trichocolea tomentella* populations

In the UPGMA phenogram (Fig. 11), based on Nei's (1978) genetic distances, all the Pomeranian populations make up a single group, which indicates their high similarity. The Tatra populations, by contrast, demonstrate a high diversity and are scattered in the phenogram. One of them (T-4) shows high similarity to the Pomeranian populations. However, populations T-3 and T-5 diverge from all the other studied populations. Populations T-1 and T-2 are intermediate between the Pomeranian populations and divergent the Tatra NP populations, being more similar to the former. The phenogram is consistent with the PCA diagram (Fig. 12).

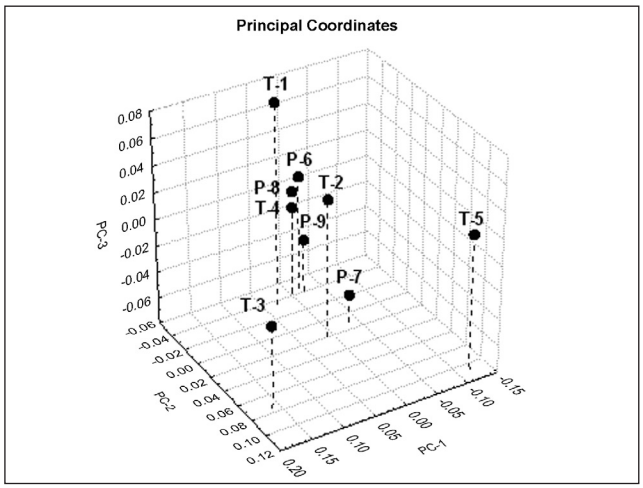

Fig. 12. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between populations of *Trichocolea tomentella*
Explained variance: PC1=43.08%, PC2=25.88%, PC3=11.84%

3.1.3. *Lophozia hatcheri*

Clonal diversity. Patches of this species demonstrated a relatively high level of genotypic diversity (Table 27). The mean number of MLGs (*G*) at the patch level was 4.19, and the proportion of distinguishable genotypes (*G*/*N*) was 0.526. The highest number of

Table 27. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) at the patch level in *Lophozia hatcheri* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Dolina Suchej Wody (valley)	T-1	3.3	0.41
Tatra National Park, Dolina Pańszczyca (valley)	T-2	4.5	0.56
Tatra National Park, Dolina 5 Stawów Polskich (valley)	T-3	5.0	0.63
Tatra National Park, Dolina Jaworzynka (valley)	T-4	3.3	0.42
Tatra National Park, Dolina Roztoki (valley)	T-5	5.0	0.63
Bieszczady Mts., N slope of Mt. Tarnica	B-6	4.0	0.50
Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	B-7	4.0	0.50
Bieszczady Mts., N slope of Mt. Krzemień	B-8	4.1	0.52
Bieszczady Mts., N slope of Mt. Kińczyk Bukowski	B-9	4.5	0.56
Mean		4.19	0.526

MLGs was observed in populations T-3 and T-5, where the mean *G*/*N* in both populations was equal to 0.63. The least diverse patches were identified in population T-1 (Dolina Suchej Wody in the Tatra NP), where the mean *G*/*N* in a single patch was 0.41. The number of genotypes (MLGs) detected in patches of *L. hatcheri* ranged from 1 to 7 per 8 studied gametophytes. The usual number of genotypes detected was 4-5 per patch. Patches consisting of a single genotype were found in only 2 populations: T-2 and T-4. Seven MLGs were identified in only one patch from population T-3, albeit the number of genotypes within patches in studied populations did not differ significantly (Kruskal-Wallis ANOVA test: H=11.637, p=0.17).

The mean number of MLGs at the population level of *L. hatcheri* was 22.22, while the proportion of distinguishable genotypes (*G*/*N*) equalled 0.360. The most and the least diverse populations come from the Tatra NP. The most diverse was T-2 (*G*/*N*=0.48) and the least diverse was T-4 (*G*/*N*=0.23) (Table 28).

Table 28. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G*/*N*, where *G*=MLG) for *Lophozia hatcheri* populations

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	7	56	18	0.32
T-2	7	56	27	0.48
T-3	10	80	34	0.42
T-4	6	48	11	0.23
T-5	5	40	22	0.45
B-6	10	80	28	0.35
B-7	6	48	19	0.40
B-8	7	56	14	0.25
B-9	10	80	27	0.34
Mean			22.22	0.360

The Simpson index (D_G) and evenness index (E) values in all the study populations of *L. hatcheri* are not high compared to other studied leafy liverworts. The highest D_G value occurred in population B-9 from the Bieszczady Mts. (0.9807) and the lowest in T-1 from the Tatra NP (0.8292) (Table 29).

Table 29. Clonal diversity in populations of *Lophozia hatcheri*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.8292	0.8003
T-2	0.9461	0.9280
T-3	0.9509	0.9433
T-4	0.9732	0.9423
T-5	0.8833	0.8765
B-6	0.9693	0.9489
B-7	0.9610	0.9516
B-8	0.9247	0.9609
B-9	0.9807	0.9733

Among 544 *L. hatcheri* gametophytes under study, only 178 MLGs were detected. The proportion of distinguishable genotypes (*G*/*N*) at the species level was equal to 0.327. Sixty-two MLGs (34.8% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 3 of the Bieszczady populations (B-6, B-7 and B-8). Overall, the genotype was detected in a total of 8 patches in 17 plants. By contrast, 49 MLGs (27.5% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 27.5% of studied plants.

Isozyme diversity. A total of 13 loci (*Acp, Gdh, Got, Idh, Lap, Mdh-A, Mdh-B, Me, Per-A, Per-B, Per-C, Pgd, Sdh*) were detected in 10 enzyme systems (Table 30). All studied loci were polymorphic. A total of 44 alleles were detected in the study populations. The number of alleles in a single locus varied from 2 to 5. The most diverse were loci *Mdh-B* and *Pgd*, with 5 alleles each. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci were selectively neutral in the studied populations (Table 31).

The mean percentage of polymorphic loci (*P*) in the *L. hatcheri* populations was 66.667% (Table 32). The highest percentage was in population T-5, where all loci were polymorphic (*P*=100%), while the lowest, in population T-4 (*P*=30.77%). The greatest number of alleles per population (32) was found in population T-5 (mean number of alleles per locus *A*=2.5). In contrast, the smallest number (18) was detected in population T-4 (*A*=1.4). Both populations come from the Tatra NP. The mean number of alleles per locus (*A*) per population, for all studied loci, was 2.03. In 5 studied populations rare alleles were found, but 2 populations from the Tatra NP (T-2 and T-4) and 2 populations from the Bieszczady Mts. (B-7 and B-8) apparently lacked them. The number of rare alleles was the largest (6) in population T-3 from the Tatra NP. In 5 populations, 8 private alleles were found: in 3 populations from the Tatra NP (T-2, T-3, T-5) and 2 from the Bieszczady Mts. (B-6, B-9). Four private alleles had a high frequency, mostly >0.100: *PerC-4* (0.137) in T-3, *Gdh-1* (0.200) in B-9, and *Sdh-1* (0.089) in T-2. Remaining 4 private alleles belonged to the class of rare alleles, with frequency <0.05: *Got-1* (B-6), *MdhB-5* (T-3), *Lap-3* (T-5), and *PerC-1* (T-5) (Table 30).

The level of isozymatic diversity of *L. hatcheri* was relatively high compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.2504. H_S varied from 0.1663 (T-4) to 0.2788 (T-2), with a mean of 0.22165 (Table 32). In the studied populations no statistically significant correlations were detected between genetic diversity within populations (H_S) and population size (*N*) (Spearman test: R=0.6065,

Table 30. Allele frequencies at 13 enzyme loci in *Lophozia hatcheri* populations

Locus	Allele	Population								
		Tatra National Park					Bieszczady Mts.			
		T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
Acp	1	0.938	0.768	0.737	1.000	0.325	0.338	1.000	0.607	0.550
	2	0.062	0.232	0.263	0.000	0.675	0.662	0.000	0.393	0.450
Gdh	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.200**
	2	1.000	0.768	0.650	1.000	0.900	1.000	0.872	1.000	0.800
	3	0.000	0.232	0.350	0.000	0.100	0.000	0.127	0.000	0.000
Got	1	0.000	0.000	0.000	0.000	0.000	**0.025**	0.000	0.000	0.000
	2	0.000	0.000	0.362	0.375	0.100	0.025	0.191	0.000	0.000
	3	1.000	1.000	0.638	0.625	0.900	0.950	0.809	1.000	1.000
Idh	1	0.031	0.214	0.050	0.000	0.025	0.100	0.191	0.000	0.375
	2	0.031	0.429	0.538	0.375	0.975	0.300	0.340	1.000	0.600
	3	0.797	0.161	0.412	0.625	0.000	0.400	0.468	0.000	0.025
	4	**0.141**	**0.196**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap	1	0.938	0.482	1.000	1.000	0.900	0.725	1.000	1.000	1.000
	2	0.062	0.232	0.000	0.000	0.000	0.200	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	**0.050**	0.000	0.000	0.000	0.000
	4	0.000	**0.286**	0.000	0.000	**0.050**	0.000	0.000	0.000	0.000
Mdh-A	1	0.203	0.071	0.888	1.000	0.850	0.163	0.362	0.625	0.888
	2	0.797	0.929	0.112	0.000	0.150	0.763	0.638	0.375	0.112
Mdh-B	1	0.000	0.000	**0.025**	0.000	**0.100**	0.000	0.000	0.000	0.000
	2	0.203	0.643	0.188	0.000	0.125	0.313	0.383	0.321	0.413
	3	0.797	0.357	0.562	0.813	0.775	0.475	0.404	0.679	0.587
	4	0.000	0.000	0.175	0.187	0.000	0.137	0.213	0.000	0.000
	5	0.000	0.000	**0.050**	0.000	0.000	0.000	0.000	0.000	0.000
Me	1	0.031	0.054	0.000	0.000	0.000	0.000	0.277	0.000	0.125
	2	0.000	0.643	0.038	0.000	0.900	0.125	0.255	0.482	0.350
	3	0.969	0.303	0.962	1.000	0.100	0.850	0.468	0.518	0.525
Per-A	1	0.000	0.000	0.000	0.000	0.100	0.075	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	0.900	0.925	1.000	1.000	1.000
Per-B	1	1.000	1.000	0.838	1.000	0.900	1.000	1.000	1.000	1.000
	2	0.000	0.000	**0.162**	0.000	**0.100**	0.000	0.000	0.000	0.000
Per-C	1	0.000	0.000	0.000	0.000	**0.025**	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.075	0.075	0.000	0.000	0.000
	3	1.000	1.000	0.863	1.000	0.900	0.925	1.000	1.000	1.000
	4	0.000	0.000	**0.137**	0.000	0.000	0.000	0.000	0.000	0.000
Pgd	1	0.000	0.000	0.000	0.000	0.000	**0.025**	0.000	0.000	**0.050**
	2	0.172	0.179	0.313	0.167	0.050	0.213	0.000	0.000	0.375
	3	0.594	0.303	0.675	0.708	0.600	0.525	0.575	0.947	0.488
	4	0.234	0.275	0.012	0.125	0.250	0.212	0.319	0.054	0.087
	5	0.000	0.143	0.000	0.000	0.100	0.025	0.106	0.000	0.000
Sdh	1	0.000	**0.089**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	**0.357**	0.000	0.000	**0.100**	0.000	0.000	0.000	0.000
	3	0.000	0.089	0.275	0.000	0.000	0.000	0.085	0.000	0.263
	4	0.219	0.412	0.038	0.000	0.425	0.488	0.234	0.571	0.325
	5	0.781	0.053	0.687	1.000	0.475	0.450	0.681	0.429	0.225

Explanation: bold values denote private alleles for regions

Table 31. Ewens-Watterson test for neutrality for each locus in *Lophozia hatcheri* populations

Locus	N	k	Obs. F	L95	U95
Acp	551	2	0.5182	0.5033	0.9964
Gdh	551	4	0.7610	0.3381	0.9713
Got	551	3	0.7939	0.3973	0.9928
Idh	551	5	0.3167	0.2786	0.9467
Lap	551	4	0.8023	0.3269	0.9748
Mdh-A	551	3	0.4371	0.3904	0.9892
Mdh-B	551	5	0.4164	0.3039	0.9431
Me	551	3	0.5276	0.3842	0.9892
Per-A	551	2	0.9644	0.5043	0.9964
Per-B	551	2	0.9200	0.5054	0.9964
Per-C	551	4	0.9259	0.3380	0.9748
Pgd	551	5	0.4203	0.2853	0.9433
Sdh	551	5	0.3714	0.2902	0.9430

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

p=0.08). The value of G_{ST} was not high (0.2659), associated with rather low gene flow between populations (N_m=1.2731). Analysis of molecular variance (AMOVA) showed that the genetic differentiation between *L. hatcheri* populations (Φ_{PT}) was 0.275. Most of genetic variation (ca. 73%) resulted from variation within populations (Table 33).

Pair-wise genetic distances (D) and similarities (I) (Nei, 1978) between 9 populations of *L. hatcheri* were computed (Table 34). Genetic distances ranged from 0.1019 to 0.2948 with a mean of 0.1908. The highest genetic distance was between populations T-5 from the Tatra NP and B-8 from the Bieszczady Mts. (D=0.2948). The smallest distance (D=0.0255) was between 2 populations from the Tatra NP: T-3 and T-4. However, the

Table 32. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Lophozia hatcheri* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S(±SD)
T-1	24	3	0	1.9	61.54	0.1870 (0.1666)
T-2	31	0	1	2.4	69.23	0.2788 (0.2317)
T-3	30	6	2	2.3	84.62	0.2481 (0.2042)
T-4	18	0	0	1.4	30.77	0.1663 (0.2006)
T-5	32	5	2	2.5	100.0	0.2718 (0.1656)
B-6	30	4	1	2.3	84.62	0.2544 (0.2264)
B-7	26	0	0	2.0	61.54	0.2029 (0.2769)
B-8	19	0	0	1.5	46.15	0.1902 (0.2357)
B-9	26	2	1	2.0	61.54	0.2039 (0.2826)
Mean	26.2	2.2	0.78	2.03	66.667	0.22165

Table 33. Analysis of molecular variance (AMOVA) for *Lophozia hatcheri* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.686	27	Φ_{PT} = 0.275***
Within populations	542	1.810	73	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 34. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Lophozia hatcheri* populations

	T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
T-1	***	0.8441	0.8318	0.8437	0.7278	0.8211	0.8209	0.8226	0.7956
T-2	0.1620	***	0.8439	0.8284	0.8320	0.8304	0.8443	0.8863	0.8272
T-3	0.1841	0.1610	***	0.9091	0.8189	0.8526	0.8834	0.8454	0.8189
T-4	0.1699	0.1683	0.1019	***	0.7483	0.8029	0.8293	0.8511	0.8053
T-5	0.2890	0.1785	0.1878	0.2890	***	0.6597	0.8509	0.7095	0.7487
B-6	0.1971	0.1859	0.1594	0.2526	0.3411	***	0.9088	0.8792	0.8116
B-7	0.1974	0.1573	0.1240	0.1871	0.2615	0.1122	***	0.9478	0.8451
B-8	0.1953	0.1207	0.1561	0.1613	0.2948	0.1528	0.1287	***	0.8698
B-9	0.2630	0.1897	0.1766	0.2165	0.2894	0.1403	0.1702	0.1446	***

Mantel test revealed significant correlations between genetic and geographic distances (R=-0.209, p=0.01)

On the basis of genetic distances (Nei 1978) the UPGMA phenogram was constructed (Fig. 13). All the Bieszczady populations make up a single group, which indicates their high similarity. By contrast, the populations from the Tatra NP demonstrate a high differentiation and create a scattered group in the graph. The most distinct population was T-5, but T-1 and T-2 also showed marked dissimilarity. Besides, populations T-3 and T-4 were similar to populations from the Bieszczady Mts. The phenogram is consistent with the PCA diagram (Fig. 14).

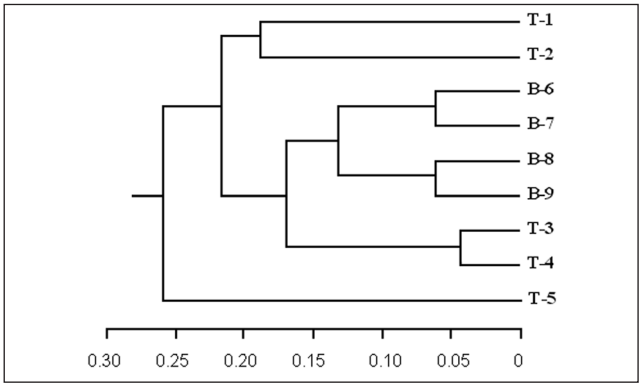

Fig. 13. UPGMA phenogram based on Nei's (1978) genetic distances between *Lophozia hatcheri* populations

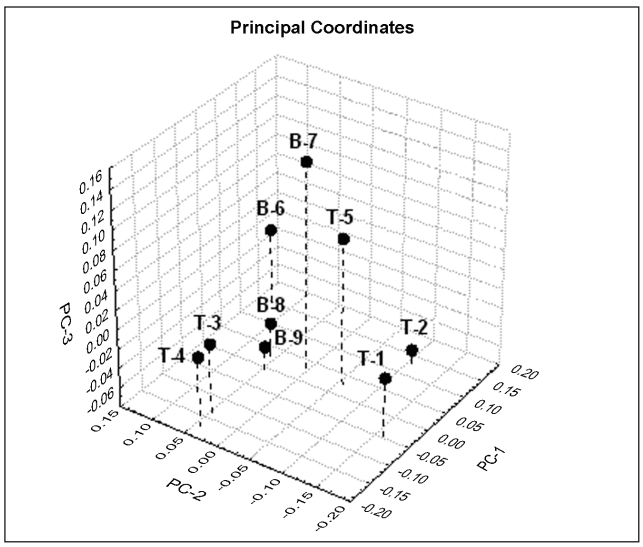

Fig. 14. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between *Lophozia hatcheri* populations
Explained variance: PC1=40.14%, PC2=28.51%, PC3=13.91%

3.1.4. *Mylia anomala*

Clonal diversity. Patches of this species demonstrated a low level of genotypic diversity. The mean number of MLGs (*G*) at the patch level was 2.66, and the proportion of distinguishable genotypes (*G*/*N*) was 0.321 (Table 35). The highest number of MLGs were observed in populations T-2 and T-3 from the Tatra NP, where the mean *G*/*N* was equal to 0.37. The least diverse patches were identified in population S-7 from the Suwałki Lake District (*G*/*N*=0.20). The number of genotypes (MLGs) detected in patches of *M. anomala* ranged from 1 to 6 per 8 studied gametophytes. There were 12 patches consisting of a single genotype: 10 in the Suwałki Lake District and 2 in the Pomeranian Lake District. Patches consisting of 6 MLGs were identified in only 2 populations from the Pomeranian Lake District. The usual number of genotypes detected was 4-5, albeit the number of genotypes within patches in studied populations did not differ significantly (Kruskal-Wallis ANOVA test: H=12.615, p=0.181).

The Simpson index (D_G) and evenness index (E) values in all the study populations of *M. anomala* are low compared to other investigated leafy liverworts. The values of D_G and E confirm that the species has a low clonal diversity. The highest D_G values occurred in population P-5 (0.9128) from the Pomeranian Lake District, while the lowest in S-7 (0.4782) from the Suwałki Lake District (Table 37).

Ninety MLGs were detected among 552 studied *M. anomala* gametophytes. The proportion of distinguishable genotypes (*G*/*N*) at the species level was equal to 0.163. It is one of the least diverse liverworts studied. Thirty-eight MLGs (42.2% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 5 populations, including all populations from the Suwałki Lake District (S-7, S-8 , S-9) and 2 from the Pomeranian Lake District (P-4, P-6). Altogether, this genotype was detected in 91 plants from 17 patches. By contrast, only 22 MLGs (49% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 3.99% of studied plants.

Table 35. Mean number of identified multilocus (MLG) and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) at the patch level in *Mylia anomala* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Hala Gąsienicowa, Żółta Turnia	T-1	3.2	0.36
Tatra National Park, Waksmundzka Młaka (peat-bog)	T-2	3.0	0.37
Tatra National Park, N slope of Mt. Ornak	T-3	3.0	0.37
Pomeranian Lake District, Lake Duże Sitno	P-4	2.5	0.31
Pomeranian Lake District, Staniszewskie Błota Reserve (peat-bog)	P-5	2.6	0.32
Pomeranian Lake District, Lake Kulkówko	P-6	2.7	0.34
Suwałki Lake District, Lake Łempis	S-7	1.6	0.20
Suwałki Lake District, Wielki Mechacz Reserve (peat-bog)	S-8	2.1	0.26
Suwałki Lake District, Czerwone Bagno Reserve (peat-bog)	S-9	3.2	0.36
Mean		2.66	0.321

Clonal diversity within populations was low. The mean number of MLGs at the population level of *M. anomala* was only 13.1, and the proportion of distinguishable genotypes (*G*/*N*) was 0.223. The most diverse was population P-6 from the Pomeranian Lake District (*G*/*N*=0.34), and the least diverse was population S-7 from the Suwałki Lake District (*G*/*N*=0.10) (Table 36).

Table 36. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) in populations of *Mylia anomala*

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	6	48	15	0.31
T-2	5	40	11	0.27
T-3	4	32	6	0.19
P-4	8	64	15	0.23
P-5	9	72	17	0.24
P-6	7	56	19	0.34
S-7	9	72	7	0.10
S-8	10	80	12	0.15
S-9	11	88	16	0.18
Mean			13.1	0.223

Isozyme diversity. A total of 17 loci (*Acp, Dia, Gdh, Got, Idh, Lap, Mdh-A, Mdh-B, Me, Mpi, Per, Pgd-A, Pgd-B, Pgi-A, Pgi-B, Pgm, Sdh*) were detected in 14 enzyme systems (Table 38). Three loci were monomorphic (*Lap, Mpi* and *Per*). A total of 45 alleles were detected in the study populations. The number of alleles

Table 37. Clonal diversity in populations of *Mylia anomala*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.8963	0.8907
T-2	0.8756	0.8841
T-3	0.7742	0.8486
P-4	0.8442	0.8129
P-5	0.9128	0.9253
P-6	0.9084	0.8665
S-7	0.4782	0.4551
S-8	0.7074	0.6683
S-9	0.8968	0.9189

Table 38. Allele frequencies at 17 enzyme loci in *Mylia anomala* populations

Locus	Allele	Population								
		Tatra National Park			Pomeranian Lake District			Suwałki Lake District		
		T-1	T-2	T-3	P-4	P-5	P-6	S-7	S-8	S-9
Acp	1	0.312	0.550	0.875	0.188	0.056	0.286	0.000	0.337	0.523
	2	0.688	0.450	0.125	0.812	0.944	0.714	1.000	0.663	0.420
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.057**
Dia	1	1.000	0.975	1.000	0.922	1.000	0.643	1.000	1.000	1.000
	2	0.000	0.025	0.000	0.078	0.000	0.357	0.000	0.000	0.000
Gdh	1	0.000	0.000	0.000	**0.047**	0.000	**0.054**	0.000	0.000	0.000
	2	1.000	1.000	1.000	0.953	1.000	0.946	1.000	1.000	1.000
Got	1	0.396	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.080
	2	0.604	1.000	1.000	0.969	0.931	1.000	1.000	1.000	0.920
	3	0.000	0.000	0.000	0.000	**0.069**	0.000	0.000	0.000	0.000
Idh	1	0.000	0.000	0.000	0.000	**0.056**	**0.196**	0.000	0.000	0.000
	2	1.000	1.000	1.000	0.969	0.944	0.804	1.000	1.000	1.000
	3	0.000	0.000	0.000	**0.031**	**0.083**	0.000	0.000	0.000	0.000
Lap	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-A	1	0.979	0.953	0.973	1.000	1.000	1.000	1.000	1.000	1.000
	2	**0.021**	**0.047**	**0.027**	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-B	1	0.354	0.775	0.000	0.703	0.306	0.250	0.000	0.000	0.000
	2	0.646	0.180	1.000	0.297	0.611	0.750	1.000	1.000	1.000
	3	0.000	**0.045**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	**0.083**	0.000	0.000	0.000	0.000
Me	1	0.000	0.000	0.000	0.094	0.403	0.464	1.000	1.000	1.000
	2	0.250	0.325	0.000	0.906	0.597	0.536	0.000	0.000	0.000
	3	**0.750**	**0.675**	**1.000**	0.000	0.000	0.000	0.000	0.000	0.000
Mpi	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Per	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-A	1	**0.292**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	**0.069**	0.000	0.000	0.000	0.000
	3	0.708	1.000	1.000	1.000	0.931	1.000	1.000	1.000	1.000
Pgd-B	1	0.000	0.000	0.000	**0.031**	**0.083**	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.057**
	3	**0.333**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4	0.000	0.150	0.219	0.000	0.070	0.107	0.042	0.075	0.273
	5	0.667	0.850	0.781	0.969	0.847	0.943	0.958	0.925	0.670
Pgi-A	1	1.000	1.000	1.000	1.000	1.000	1.000	0.931	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	**0.069**	0.000	0.000
Pgi-B	1	0.312	0.200	0.250	0.406	0.556	0.321	0.000	0.125	0.330
	2	0.396	0.400	0.350	0.344	0.222	0.107	0.056	0.125	0.125
	3	0.000	0.400	0.000	0.000	0.000	0.143	0.000	0.200	0.000
	4	0.292	0.000	0.000	0.250	0.222	0.429	0.944	0.550	0.545
Pgm	1	0.000	0.000	0.000	0.000	0.000	0.000	**0.028**	**0.012**	0.000
	2	1.000	1.000	1.000	1.000	1.000	1.000	0.861	0.988	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	**0.111**	0.000	0.000
Sdh	1	0.000	0.000	0.344	0.016	0.028	0.196	0.000	0.038	0.080
	2	0.333	0.675	0.562	0.984	0.972	0.804	1.000	0.962	0.920
	3	**0.667**	**0.325**	**0.094**	0.000	0.000	0.000	0.000	0.000	0.000

Explanation: bold values denote private alleles for regions

in a single locus varied from 1 to 5. The most diverse was *Pgd-A* locus, in which 5 alleles were identified. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci except PGI-2 were selectively neutral in studied populations (Table 39).

The mean percentage of polymorphic loci (*P*) in populations was 41.829% (Table 40). The highest percentage was in population P-4 (*P*=58.82%) and the lowest in population S-7 (*P*=23.53%). The mean number of alleles per locus (*A*) per population, for all studied loci, was 1.56. The greatest number of alleles (30) was found in population P-5 (mean number of alleles per locus *A*=1.8). The smallest number of alleles (22) was detected in population S-7 (*A*=1.3). In all studied populations (except P-6 and S-9), rare alleles were present. The number of rare alleles was the largest (5) in population P-4 from the Pomeranian Lake District. Private alleles were found in 5 populations, mostly in P-5. Most of them had a frequency > 0.05. Two private alleles, *Pgd A-1* and *Pgd B-3*, occurred in population T-1 with high frequencies: 0.292 and 0.333, respectively (Table 38).

The level of isozymatic diversity of *M. anomala* compared to other studied liverworts was low. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.1897.

Table 39. Ewens-Watterson test for neutrality for each locus in *Mylia anomala* populations

Locus	N	k	Obs. F	L95	U95
Acp	552	3	0.5614	0.4016	0.9892
Dia	552	2	0.9102	0.5048	0.9964
Gdh	552	2	0.9540	0.5032	0.9964
Got	552	3	0.8867	0.3964	0.9928
Idh	552	3	0.9194	0.3789	0.9928
Lap	552	1	1.0000	—	—
Mdh-A	552	2	0.9964	0.5044	0.9964
Mdh-B	552	4	0.6200	0.3284	0.9749
Me	552	3	0.4399	0.4052	0.9948
Mpi	552	1	1.0000	—	—
Per	552	1	1.0000	—	—
Pgd-A	552	3	0.9162	0.3751	0.9892
Pgd-B	552	5	0.7245	0.2979	0.9466
Pgi-A	552	2	0.9785	0.5048	0.9964
Pgi-B	552	4	0.2704	0.3358	0.9749
Pgm	552	3	0.9573	0.4024	0.9892
Sdh	552	3	0.6808	0.3987	0.9892

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

H_S ranged from 0.0414 (S-7) to 0.1310 (P-5), with a mean of 0.08672 (Table 40). In the studied populations no statistically significant correlations were detected between genetic diversity within populations (H_S) and population size (N) (Spearman test: R=0.2378, p=0.51). The value of G_{ST}, calculated over polymorphic loci among 9 studied populations, was high (0.3402), associated with a low level of gene flow between populations (N_m=0.9697).

Analysis of molecular variance (AMOVA) showed that the genetic differentiation between *M. anomala* populations (Φ_{PT}) was 0.338. Most of genetic variation (66%) resulted from variation within populations, whereas the variation among populations accounted for 34% (Table 41).

Pair-wise genetic distances (D) and similarities (I) (Nei, 1978) between 9 populations of *M. anomala* were computed (Table 42). Genetic distances ranged from 0.2048 to 0.0102 (mean D=0.09284). The highest genetic distance was between populations T-3 from the Tatra NP and S-7 from the Suwałki Lake District. The smallest genetic distance (the highest genetic similarity) was between 2 populations from the Suwałki Lake District: S-7 and S-8. The Mantel test revealed significant correlations between genetic and geographic distances (R=0.535, p<0.01).

The UPGMA phenogram (Fig. 15) based on Nei's (1978) genetic distances between 9 populations of *M. anomala*, demonstrates 2 distinct population groups. One encompasses all the Pomeranian populations (P-4, P-5, P-6), which exhibit very high genetic similarity, and 2 more distinct the Tatra NP populations (T-1 and T-2).

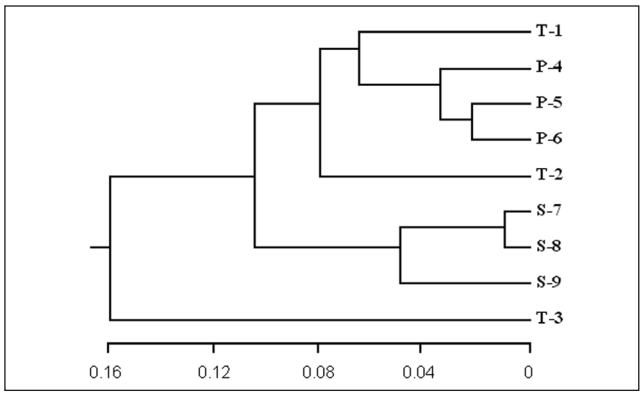

Fig. 15. UPGMA phenogram based on Nei's (1978) genetic distances between *Mylia anomala* populations

Table 40. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (A), percentage of polymorphic loci (P), and allelic diversity within populations (H_S±SD) in *Mylia anomala* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	A	P (%)	H_S (±SD)
T-1	27	1	2	1.6	52.94	0.1002 (0.2427)
T-2	27	3	1	1.6	41.18	0.1078 (0.2261)
T-3	23	1	0	1.4	29.41	0.0557 (0.1583)
P-4	28	5	0	1.7	58.82	0.1231 (0.1931)
P-5	30	1	3	1.8	52.94	0.1310 (0.2046)
P-6	28	0	0	1.7	52.94	0.1088 (0.2243)
S-7	22	2	2	1.3	23.53	0.0414 (0.0995)
S-8	24	2	0	1.4	29.41	0.0537 (0.1365)
S-9	25	0	2	1.5	29.41	0.0588 (0.2100)
Mean	26.0	1.7	1.1	1.56	41.829	0.08672

Table 41. Analysis of molecular variance (AMOVA) for *Mylia anomala* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.561	34	Φ_{PT} = 0.338***
Within populations	543	1.101	66	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 42. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Mylia anomala* populations

	T-1	T-2	T-3	P-4	P-5	P-6	S-7	S-8	S-9
T-1	***	0.9152	0.8590	0.9518	0.9429	0.9253	0.8709	0.8931	0.8878
T-2	0.0886	***	0.9197	0.9558	0.9261	0.9169	0.9345	0.8702	0.8899
T-3	0.1520	0.0837	***	0.8543	0.8499	0.8711	0.8148	0.8607	0.8833
P-4	0.0494	0.0452	0.1575	***	0.9773	0.9575	0.8831	0.9018	0.8839
P-5	0.0588	0.0768	0.1627	0.0229	***	0.9775	0.9345	0.9491	0.9365
P-6	0.0776	0.0867	0.1380	0.0435	0.0227	***	0.9476	0.9655	0.9503
S-7	0.1382	0.1802	0.2048	0.1243	0.0678	0.0538	***	0.9899	0.9324
S-8	0.1131	0.1390	0.1500	0.1034	0.0522	0.0351	0.0102	***	0.9663
S-9	0.1190	0.1166	0.1241	0.1234	0.0656	0.0510	0.0700	0.0343	***

The second group includes all 3 lowland populations from the Suwałki Lake District (S-7, S-8, S-9). Population T-3 from the Tatra Mts., though, is quite distinct from the other populations. The phenogram shows high similarities of populations within the Pomeranian and Suwałki Lake Districts and high genetic differentiation of the Tatra NP populations. The phenogram is consistent with the PCA diagram (Fig. 16).

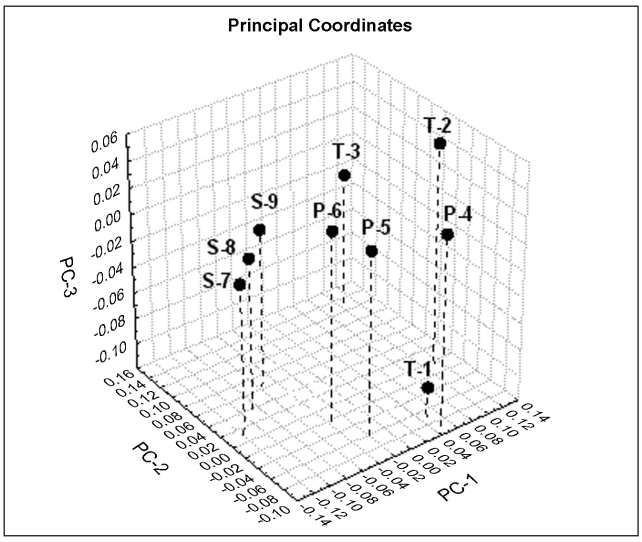

Fig. 16. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between populations of *Mylia anomala*
Explained variance: PC1=43.29%, PC2=31.51%, PC3=10.99%

3.2. Genetic diversity of fertile leafy liverwort species

3.2.1. *Lepidozia reptans*

Clonal diversity. Generally *L. reptans* patches demonstrated a relatively high level of genotypic diversity (Table 43). The mean number of MLGs (*G*) at the patch level was 4.54, and the proportion of distinguishable genotypes (*G*/*N*) was 0.569. The highest number of MLGs was observed in populations T-2 and T-3 from the Tatra NP, where the mean *G*/*N* was equal to 0.64. The least diverse patches were identified in population S-10 from the Suwałki Lake District, where *G*/*N*=0.49. The number of genotypes (MLGs) of *L. reptans* detected in patches ranged from 2 to 8 per 8 studied gametophytes. The usual number of genotypes detected was 4-5 per patch. In this species no patch consisted of a single genotype. Eight MLGs were identified only in one patch in population T-1 (Dolina Białki in the Tatra NP). The number of genotypes within patches in studied populations did not differ significantly (Kruskal-Wallis ANOVA test: H=10.031, p=0.53).

All populations had a similar level of genotypic diversity. The mean number of MLGs was 34.9 per population, while the proportion of distinguishable genotypes (*G*/*N*) was 0.480. The most diverse was population T-2 from the Tatra NP (*G*/*N*=0.58). The least diverse was population S-10 from the Suwałki Lake District (*G*/*N* =0.38) (Table 44).

Table 43. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) at the patch level in *Lepidozia reptans* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Dolina Białki (valley)	T-1	4.7	0.59
Tatra National Park, Dolina Suchej Wody (valley)	T-2	5.1	0.64
Tatra National Park, Dolina Kościeliska (valley)	T-3	5.1	0.64
Tatra National Park, Dolina Chochołowska (valley)	T-4	4.5	0.56
Pomeranian Lake District, forest near Lake Kamień	P-5	4.6	0.58
Pomeranian Lake District, Czapliniec Reserve (peat-bog)	P-6	4.4	0.55
Pomeranian Lake District, Staniszewskie Błoto Reserve (peat-bog)	P-7	4.6	0.58
Suwałki Lake District, forest near Lake Stulpień	S-8	4.1	0.51
Suwałki Lake District, Augustów Forest	S-9	4.4	0.55
Suwałki Lake District, forest near Lake Godle	S-10	3.9	0.49
Mean		4.54	0.569

Table 44. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) in populations of *Lepidozia reptans*

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	10	80	38	0.48
T-2	10	80	46	0.58
T-3	10	80	40	0.50
T-4	8	64	36	0.56
P-5	7	56	27	0.48
P-6	11	88	38	0.43
P-7	9	72	36	0.50
S-8	9	72	33	0.46
S-9	8	64	28	0.43
S-10	9	72	27	0.38
Mean			34.9	0.480

The Simpson index (D_G) and evenness index (E) values in all the study populations of *L. reptans* are high compared to other investigated leafy liverworts. These values confirm that the species has high clonal diversity. In all populations D_G values are similar. The highest D_G value occurred in the Tatra population T-2 (0.9832) and the lowest in the Suwałki Lake District population S-8 (0.9425) (Table 45).

Table 45. Clonal diversity in populations of *Lepidozia reptans*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.9472	0.8827
T-2	0.9832	0.9586
T-3	0.9797	0.9932
T-4	0.9653	0.9319
P-5	0.9656	0.9433
P-6	0.9804	0.9741
P-7	0.9800	0.9761
S-8	0.9425	0.8583
S-9	0.9697	0.9415
S-10	0.9664	0.9347

Among 728 *L. reptans* gametophytes under study, only 238 MLGs were detected. The proportion of distinguishable genotypes (*G*/*N*) at the species level was equal to 0.327. Seventy-three MLGs (30.7% of MLGs)

Table 46. Allele frequencies at 11 enzyme loci in *Lepidozia reptans* populations

Locus	Allele	Population									
		Tatra National Park				Pomeranian Lake District			Suwałki Lake District		
		T-1	T-2	T-3	T-4	P-5	P-6	P-7	S-8	S-9	S-10
Dia	1	0.000	0.000	0.000	**0.016**	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.035	0.000	0.000	0.015	0.089	0.170	0.264	0.042	0.000	0.000
	3	0.965	1.000	1.000	0.969	0.911	0.818	0.736	0.958	0.984	1.000
	4	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.016	0.000
Gdh	1	**0.088**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.100	0.200	0.000	0.172	0.392	0.303	0.500	0.111	0.234	0.208
	3	0.587	0.525	0.750	0.422	0.179	0.489	0.472	0.583	0.735	0.723
	4	0.225	0.275	0.250	0.406	0.429	0.205	0.028	0.306	0.031	0.069
Got	1	0.000	0.000	0.016	0.047	0.000	0.023	0.023	0.153	0.000	0.000
	2	1.000	1.000	0.984	0.953	1.000	0.977	0.977	0.694	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.153**	0.000	0.000
Idh	1	0.000	0.100	0.016	0.000	0.000	0.091	0.083	0.000	0.000	0.000
	2	1.000	0.838	0.953	0.906	1.000	0.909	0.917	1.000	1.000	1.000
	3	0.000	**0.062**	**0.031**	**0.094**	0.000	0.000	0.000	0.000	0.000	0.000
Me	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.014**
	2	0.875	0.613	0.938	0.766	0.750	0.909	0.778	0.917	0.625	0.489
	3	0.063	0.150	0.047	0.234	0.232	0.068	0.180	0.083	0.375	0.497
	4	0.062	0.027	0.015	0.000	0.018	0.023	0.042	0.000	0.000	0.000
Pgd-A	1	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	**0.012**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgd-B	1	0.000	0.000	0.047	0.016	0.018	0.023	0.069	0.000	0.031	0.000
	2	0.062	0.400	0.078	0.031	0.089	0.534	0.292	0.000	0.250	0.097
	3	0.938	0.600	0.875	0.953	0.893	0.443	0.639	1.000	0.789	0.903
Pgi-A	1	0.488	0.550	0.313	0.875	0.375	0.580	0.653	0.583	0.500	0.597
	2	0.137	0.300	0.281	0.125	0.143	0.034	0.181	0.319	0.172	0.222
	3	0.375	0.150	0.406	0.000	0.482	0.386	0.166	0.098	0.328	0.181
Pgi-B	1	0.125	0.200	0.500	0.375	0.000	0.432	0.125	0.250	0.313	0.428
	2	0.738	0.588	0.094	0.563	1.000	0.568	0.875	0.750	0.687	0.572
	3	0.000	**0.025**	**0.250**	**0.015**	0.000	0.000	0.000	0.000	0.000	0.000
	4	**0.137**	**0.187**	**0.156**	**0.047**	0.000	0.000	0.000	0.000	0.000	0.000
Pgm	1	1.000	1.000	0.984	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	0.000	0.000	**0.016**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sdh	1	0.000	0.012	0.000	0.016	0.000	0.000	0.000	0.306	0.047	0.028
	2	0.112	0.150	0.171	0.202	0.125	0.000	0.209	0.222	0.266	0.097
	3	0.788	0.700	0.563	0.313	0.214	0.546	0.444	0.153	0.281	0.722
	4	0.100	0.138	0.266	0.469	0.661	0.454	0.347	0.319	0.406	0.153

Explanation: bold values denote private alleles for regions

occurred more frequently than in a single patch. The most common genotype was found in 7 populations in 3 regions of Poland: in the Tatra NP (T-1, T-3, T-4), Pomeranian Lake District (P-6, P-7), and Suwałki Lake District (S-8, S-9). It was detected in 35 plants from 14 patches. By contrast, 103 MLGs (43.3% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 14.2% of studied plants.

Isozyme diversity. All 11 examined enzyme loci of *L. reptans* (*Dia, Gdh, Got, Idh, Me, Pgd-A, Pgd-B, Pgi-A, Pgi-B, Pgm, Sdh*) were polymorphic (Table 46). A total of 36 alleles were detected in the study populations. The number of alleles in a single locus varied from 2 to 4. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci were selectively neutral in the studied populations (Table 47).

Table 47. Ewens-Watterson test for neutrality for each locus in *Lepidozia reptans* populations

Locus	N	k	Obs. F	L95	U95
Dia	712	4	0.8696	0.3367	0.9777
Gdh	712	4	0.3933	0.3429	0.9777
Got	712	3	0.9081	0.3835	0.9944
Idh	712	3	0.8926	0.3786	0.9944
Me	712	4	0.6099	0.3299	0.9805
Pgd-A	712	2	0.9972	0.5095	0.9972
Pgd-B	712	3	0.6528	0.3778	0.9916
Pgi-A	712	3	0.4091	0.3826	0.9916
Pgi-B	712	4	0.4669	0.3455	0.9777
Pgm	712	2	0.9972	0.5046	0.9972
Sdh	712	4	0.3616	0.3356	0.9750

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

The mean percentage of polymorphic loci (*P*) in the *L. reptans* populations was 70.003%. The highest values were in populations T-3, T-4, P-6, and P-7 (*P*=81.82%), and the lowest in populations T-5 and S-10 (*P*=54.55%). The mean number of alleles per locus per population was high (for all studied loci *A*=2.25). The greatest number of alleles (27) was found in population T-2, T-3, and T-4 from the Tatra NP (*A*=2.5). The smallest number of alleles (only 22) was detected in populations P-5 from the Pomeranian Lake District and S-10 from the Suwałki Lake District (*A*=2.0) (Table 48). In all studied populations rare alleles were found. Rare alleles were most numerous (8) in a population from the Tatra NP (T-4) and the least numerous (only one rare allele) in populations from the Tatra NP (T-1) and from the Suwałki Lake District (S-8). Private alleles were found in 6 populations (T-1,T-2, T-3, T-4, S-8 and S-7) (Table 48). Two of private alleles had a high frequency: *Got-3* (0.153) in population S-8 and *Gdh-1* (0.088) in population T-1. Remaining 4 of them (*Dia-1*, *Me-1, PgdA-2*, *Pgm-2*) belonged to the class of rare alleles (frequency <0.05) (Table 46).

The level of isozymatic diversity of *L. reptans* was rather high compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.2416. The mean diversity within populations (H_S) was 0.19708, and ranged from 0.1373 (S-9) to 0.2486 (T-3) (Table 48). In all studied populations no statistically significant correlations were detected between genetic diversity (H_S) and population size (*N*) (Spearman test: R=0.3741, p=0.23). Coeffition of genetic differentiation, calculated over polymorphic loci among 10 studied populations, was rather low (G_{ST}=0.1225), associated with high gene flow between populations (N_m=5.5810).
Analysis of molecular variance (AMOVA) showed that the genetic differentiation (Φ_{PT}) between *L. reptans*

Table 48. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Lepidozia reptans* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S (±SD)
T-1	24	1	1	2.2	63.64	0.1867 (0.2380)
T-2	27	4	1	2.5	72.73	0.2150 (0.2674)
T-3	27	7	1	2.5	81.82	0.2486 (0.2686)
T-4	27	8	1	2.5	81.82	0.2243 (0.2472)
P-5	22	2	0	2.0	54.55	0.1554 (0.2587)
P-6	25	5	0	2.3	81.82	0.2182 (0.2411)
P-7	25	3	0	2.3	81.82	0.2250 (0.2292)
S-8	23	1	1	2.1	63.64	0.1748 (0.2764)
S-9	23	4	0	2.1	63.64	0.1373 (0.2724)
S-10	22	2	1	2.0	54.55	0.1855 (0.2710)
Mean	24.5	3.7	0.6	2.25	70.003	0.19708

Table 49. Analysis of molecular variance (AMOVA) for *Lepidozia reptans* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	9	0.207	12	Φ_{PT} = 0.118***
Within populations	702	1.537	88	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 50. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Lepidozia reptans* populations

	T-1	T-2	T-3	T-4	P-5	P-6	P-7	S-8	S-9	S-10
T-1	***	0.9723	0.9539	0.9486	0.9363	0.0466	0.9609	0.9499	0.9565	0.9631
T-2	0.0281	***	0.9397	0.9481	0.9160	0.9614	0.9669	0.9285	0.9604	0.9703
T-3	0.0472	0.0621	***	0.9320	0.8789	0.9333	0.9177	0.9277	0.9407	0.9437
T-4	0.0528	0.0532	0.0705	***	0.9418	0.9406	0.9588	0.9702	0.9601	0.9505
P-5	0.0659	0.0878	0.1291	0.0599	***	0.9282	0.9479	0.9399	0.9471	0.8967
P-6	0.0549	0.0394	0.0690	0.0612	0.0745	***	0.9729	0.9190	0.9609	0.9279
P-7	0.0399	0.0336	0.0858	0.0421	0.0535	0.0274	***	0.9499	0.9731	0.9473
S-8	0.0514	0.0742	0.0751	0.0302	0.0620	0.0845	0.0514	***	0.9558	0.9277
S-9	0.0445	0.0404	0.0612	0.0407	0.0544	0.0399	0.0273	0.0452	***	0.9693
S-10	0.0376	0.0302	0.0580	0.0508	0.1090	0.0749	0.0542	0.0750	0.0312	***

populations was 0.118. Most of genetic variation (88%) resulted from variation within populations, whereas the variation among populations accounted for only 12% (Table 49).

Pair-wise genetic distances (*D*) and similarities (*I*) (Nei, 1978) between 10 populations of *L. reptans* were computed (Table 50). Genetic distances ranged from 0.1299 to 0.0273 (mean *D*=0.05647). The highest genetic distance was between population T-3 from the Tatra NP and P-5 from the Pomeranian Lake District. The highest genetic similarity was between 2 populations from the Suwałki Lake District: S-7 and S-9. The Mantel test revealed no significant correlations between genetic and geographic distances (*R*=0.085, *p*=0.22).

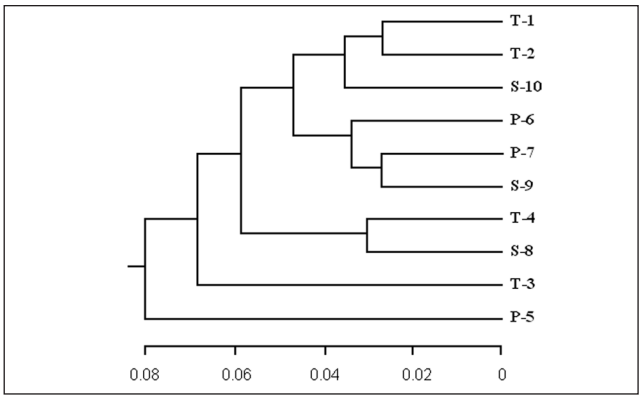

Fig. 17. UPGMA phenogram based on Nei's (1978) genetic distances between *Lepidozia reptans* populations

In the UPGMA phenogram (Fig. 17), based on Nei's (1978) genetic distances, 3 population groups and 2 populations exhibiting high separateness stand out. The first group comprises 3 populations: 2 from the Tatra NP (T-1 and T-2) and one from the Suwałki Lake District (S-10). The second group includes 2 populations from the Pomeranian Lake District (P-6 and P-7) and one population from the Suwałki Lake District (S-9). The third group consists of 2 populations, one from the Tatra NP (T-4) and one from the Suwałki Lake District (S-8). Two separated populations were T-3 (Tatra Mts., Dolina Kościeliska) and P-5 (Pomeranian Lake District, forest near Lake Stulpień). Thus the phenogram did not group the populations according to geographic distribution. The phenogram is consistent with the PCA diagram (Fig. 18).

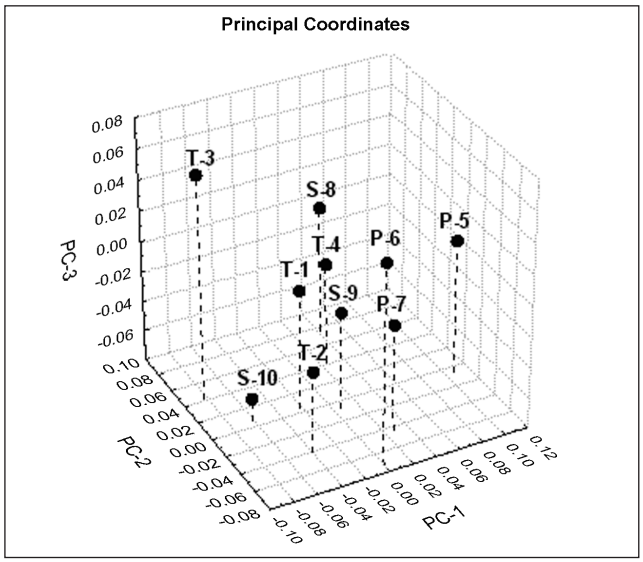

Fig. 18. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between populations of *Lepidozia reptans*
Explained variance: PC1=33.52%, PC2=22.47%, PC3=15.37%

3.2.2. *Calypogeia integristipula*

Clonal diversity. Patches of this species demonstrated a low level of genotypic diversity (Table 51). The mean number of MLGs (*G*) at the patch level was 2.43, and the proportion of distinguishable genotypes (*G*/*N*) was 0.294. The highest number of MLGs was observed in population B-5 from the Bieszczady Mts. (*G*/*N*=0.38). The least diverse patches were identified in population P-8 from the Pomeranian Lake District (*G*/*N*=0.22).

The number of genotypes (MLGs) detected in patches of *C. integristipula* ranged from 1 to 5 per 8 studied gametophytes. In all populations some patches consisted of a single genotype. The number of such patches was the largest (12) in population T-1. In many patches consisting of a few MLGs, one of them

Table 51. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) at the patch level in *Calypogeia integristipula* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Potok Sichlański (stream)	T-1	2.5	0.25
Tatra National Park, Dolina Białki (valley)	T-2	2.4	0.30
Tatra National Park, Dolina Suchej Wody (valley)	T-3	2.4	0.30
Bieszczady Mts., N slope of Mt. Tarnica	B-4	2.8	0.35
Bieszczady Mts., N slope of Mt. Krzemień	B-5	3.0	0.38
Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	B-6	2.4	0.30
Pomeranian Lake District, Staniszewskie Błota Reserve (peat-bog)	P-7	2.4	0.30
Pomeranian Lake District, bank of Lake Duże Sitno	P-8	2.0	0.22
Pomeranian Lake District, Czapliniec Reserve, bank of Lake Książę	P-9	2.0	0.25
Mean		2.43	0.294

dominated. The number of genotypes within patches in studied populations differed significantly (Kruskal-Wallis ANOVA test: H=17.159, p=0.029).

At the population level, the mean number of genotypes (MLGs) of *C. integristipula* was low, and equalled 9.6. The proportion of distinguishable genotypes (*G*/*N*) was 0.138. The most and the least diverse population came from the Pomeranian Lake District. The most diverse was population P-7 (Staniszewskie Błoto Reserve), where *G*/*N* was 0.19, and the least diverse was P-9 (Czapliniec Reserve), where *G*/*N* was 0.08 (Table 52).

Table 52. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) in populations of *Calypogeia integristipula*

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	10	80	12	0.15
T-2	10	80	14	0.17
T-3	9	72	9	0.12
B-4	9	72	10	0.14
B-5	8	64	10	0.16
B-6	8	64	7	0.11
P-7	10	80	15	0.19
P-8	5	40	5	0.12
P-9	6	48	4	0.08
Mean			9.6	0.138

The Simpson index (D_G) and evenness index (E) values in all the study populations of *C. integristipula* are low compared to other leafy liverworts. This finding confirms that the species has low clonal diversity. The highest D_G values occurred in population T-2 (0.8975) and the lowest in P-9 (0.6348) (Table 53).

Table 53. Clonal diversity in populations of *Calypogeia integristipula*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.8066	0.8892
T-2	0.8975	0.9663
T-3	0.8279	0.8935
B-4	0.7660	0.7832
B-5	0.8869	0.9147
B-6	0.7123	0.9259
P-7	0.8934	0.8732
P-8	0.8526	0.8921
P-9	0.6348	0.7957

Among 600 *C. integristipula* gametophytes under study, only 68 MLGs were detected. The proportion of distinguishable genotypes (*G*/*N*) at the species level was equal to 0.113. Some of the detected MLGs occurred in various patches, while others were only observed once. Thirty-one MLGs (45.6% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 6 populations, including one population from the Tatra NP (T-3), all populations from the Bieszczady Mts. (B-4, B-5, B-6) and 2 populations from the Pomeranian Lake District (P-7, P-9). Overall, the genotype was detected in a total of 23 patches in 73 plants. By contrast, only 13 MLGs (19.1% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 2.2% of studied plants.

Isozyme diversity. A total of 12 loci (*Acp, Est, Gdh, Got, Mdh-A, Mdh-B, Me, Pgd-A, Pgd-B, Pgi, Pgm, Sdh*) were detected in 10 enzyme systems of *C. integristipula* populations (Table 54). All studied loci were polymorphic. A total of 35 alleles were detected in the study populations. The number of alleles in a single locus varied from 2 to 6. The most diverse was locus *Pgi*, in which 4 alleles were identified. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci were selectively neutral in the studied populations (Table 55).

The mean percentage of polymorphic loci in the populations was low (P=45.370%). The highest percentage was in population B-5 (P=75.00%) and the lowest, in populations B-6 and P-9 (P=16.67%). The greatest number of alleles (22) was found in population B-5, where the mean number of alleles per locus (A) was 1.8 (Table 56). Only in one population (B-5 from the Bieszczady Mts.) rare alleles were found, but they were numerous (5). The mean number of alleles per locus (A) per population, for all studied loci, was 1.5. Private alleles at the population level were detected in 4 populations (T-1, T-2, B-5, and P-7) (Table 54). Half of the 8 detected private alleles had a high frequency (>

Table 54. Allele frequencies at 12 enzyme loci in *Calypogeia integristipula* populations

Locus	Allele	Population								
		Tatra National Park			Bieszczady Mts.			Pomeranian Lake District		
		T-1	T-2	T-3	B-4	B-5	B-6	P-7	P-8	P-9
Acp	1	0.571	1.000	0.300	1.000	0.360	0.344	0.000	0.000	0.000
	2	0.429	0.000	0.700	0.000	0.640	0.656	1.000	1.000	1.000
Est	1	0.000	0.000	0.000	0.000	0.000	0.000	**0.100**	**0.600**	0.000
	2	**0.429**	**0.440**	**0.311**	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.571	0.560	0.689	1.000	1.000	1.000	0.900	0.400	1.000
Gdh	1	1.000	1.000	1.000	1.000	0.953	1.000	0.850	0.400	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	**0.150**	**0.600**	0.000
	3	0.000	0.000	0.000	0.000	**0.047**	0.000	0.000	0.000	0.000
Got	1	0.000	0.000	0.000	0.000	**0.047**	0.000	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	0.953	1.000	1.000	1.000	1.000
Mdh-A	1	0.000	**0.338**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	1.000	0.662	1.000	1.000	1.000	1.000	0.900	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	**0.100**	0.000	0.000
Mdh-B	1	0.000	0.000	0.488	0.156	0.391	0.000	0.000	0.400	0.000
	2	1.000	1.000	0.388	0.844	0.609	1.000	0.900	0.600	1.000
	3	0.000	0.000	0.124	0.000	0.000	0.000	0.100	0.000	0.000
Me	1	**1.000**	**0.712**	**0.300**	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.700	1.000	1.000	1.000	0.613	0.475	1.000
	3	0.000	0.288	0.000	0.000	0.000	0.000	0.387	0.525	0.000
Pgd-A	1	0.786	0.750	1.000	1.000	0.594	0.625	0.425	0.400	0.000
	2	0.000	0.250	0.000	0.000	0.000	0.000	0.575	0.600	1.000
	3	0.214	0.000	0.000	0.000	0.406	0.375	0.000	0.000	0.000
Pgd-B	1	0.000	0.000	0.000	0.000	**0.047**	0.000	0.000	0.000	0.000
	2	0.000	**0.250**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	1.000	0.750	1.000	1.000	0.953	1.000	1.000	1.000	1.000
Pgi	1	**0.143**	**0.350**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2	0.857	0.563	0.850	0.281	0.953	1.000	1.000	1.000	1.000
	3	0.000	0.000	0.150	0.500	0.047	0.000	0.000	0.000	0.000
	4	0.000	0.087	0.000	0.219	0.000	0.000	0.000	0.000	0.000
Pgm	1	0.000	0.000	0.000	0.000	**0.047**	0.000	0.000	0.000	0.000
	2	1.000	1.000	1.000	0.672	0.422	1.000	1.000	0.600	0.796
	3	0.000	0.000	0.000	0.328	0.531	0.000	0.000	0.400	0.204
Sdh	1	0.143	0.300	0.245	0.125	0.188	0.000	0.537	0.550	0.408
	2	0.643	0.700	0.725	0.875	0.812	1.000	0.453	0.450	0.592
	3	**0.214**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Explanation: bold values denote private alleles for regions

0.100): *MdhA-1* (T-2), *MdhA-3* (P-7), *PgdB-2* (T-2), *Sdh-3* (T-1). Remaining 4 private alleles belonged to the class of rare alleles (frequency <0.05): *Gdh-3*, *Got-1, PgdB-1, Pgm-1*. They were found in population B-5 (Table 54).

The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations of *C. integristipula*, was low (0.2047). Also, a low level of genetic diversity within populations (H_S) was noted. H_S ranged from 0.0437 (P-9) to 0.1259 (P-7), with a mean of 0.09882 (Table 56). No statistically significant correlations were detected between genetic diversity within populations (H_S) and population size (N) in the studied populations (Spearman test: R=0.017, p=0.97). Coefficient of genetic differentiation, calculated over polymorphic loci among 9 studied populations, was high (G_{ST}=0.3985), associated with very low gene flow between populations per generation (N_m=0.7547).

Table 55. Ewens-Watterson test for neutrality for each locus in *Calypogeia integristipula* populations

Locus	N	k	Obs. F	L95	U95
Acp	593	2	0.5563	0.5034	0.9966
Est	593	3	0.8159	0.4045	0.9899
Gdh	593	3	0.8765	0.3984	0.9933
Got	593	2	0.9899	0.5031	0.9966
Mdh-A	593	3	0.8817	0.3804	0.9933
Mdh-B	593	4	0.6246	0.3274	0.9733
Me	593	6	0.3762	0.2608	0.9150
Pgd-A	593	3	0.6760	0.3812	0.9899
Pgd-B	593	3	0.8533	0.3858	0.9899
Pgi	593	4	0.6327	0.3401	0.9733
Pgm	593	5	0.6745	0.2768	0.9473
Sdh	593	3	0.5787	0.3879	0.9899

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

Analysis of molecular variance (AMOVA) showed that the genetic differentiation (Φ_{PT}) between *C. integristipula* populations was 0.397. Most of genetic variation (60%) resulted from variation within populations, whereas the variation among populations accounted for 40%. Intra-population variation was lower than in other species (Table 57).

Pair-wise genetic distances (D) and similarities (I) (Nei 1978) between 9 populations of *C. integristipula* were computed (Table 58). Genetic distances ranged

Table 56. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Calypogeia integristipula* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S(±SD)
T-1	18	0	1	1.5	41.67	0.1135 (0.2262)
T-2	20	0	2	1.7	58.33	0.1050 (0.2290)
T-3	19	0	0	1.6	50.00	0.0942 (0.2274)
B-4	17	0	0	1.4	33.33	0.0889 (0.2133)
B-5	22	5	4	1.8	75.00	0.1251 (0.2103)
B-6	14	0	0	1.2	16.67	0.0867 (0.1791)
P-7	19	0	0	1.6	58.33	0.1259 (0.2312)
P-8	19	0	1	1.6	58.33	0.1028 (0.2189)
P-9	14	0	0	1.2	16.67	0.0473 (0.1608)
Mean	18	0.6	0.9	1.5	45.37	0.09882

Table 57. Analysis of molecular variance (AMOVA) for *Calypogeia intergristipula* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.726	40	Φ_{PT} = 0.397***
Within populations	568	1.100	60	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 58. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Calypogeia integristipula* populations

	T-1	T-2	T-3	B-4	B-5	B-6	P-7	P-8	P-9
T-1	***	0.9206	0.8840	0.7989	0.8168	0.8711	0.8764	0.5895	0.8437
T-2	0.0827	***	0.8430	0.7574	0.7734	0.8318	0.8097	0.5587	0.7857
T-3	0.1233	0.1708	***	0.9304	0.9370	0.9409	0.9281	0.6873	0.9481
B-4	0.2245	0.2778	0.0721	***	0.9240	0.9257	0.8994	0.6248	0.9528
B-5	0.2023	0.2569	0.0651	0.0790	***	0.9533	0.8882	0.6368	0.9420
B-6	0.1380	0.1842	0.0609	0.0773	0.0479	***	0.9079	0.6060	0.9587
P-7	0.1320	0.2111	0.0747	0.1060	0.1185	0.0966	***	0.7282	0.9621
P-8	0.5284	0.5822	0.3749	0.4703	0.4512	0.5008	0.3171	***	0.6522
P-9	0.1699	0.2411	0.0533	0.0484	0.0597	0.0421	0.0387	0.4274	***

from 0.0387 to 0.5822 (mean *D*=0.19742). The highest genetic distance was between population T-2 from the Tatra NP and P-8 from the Pomeranian Lake District. The highest genetic similarity was between 2 Pomeranian populations: P-7 and P-9. The Mantel test revealed no significant correlations between genetic and geographic distances (*R*=0.192, *p*=0.15).

The UPGMA phenogram (Fig. 19), based on Nei's (1978) genetic distance displays 2 distinctive groups. One consists of 6 populations: one from the Tatra NP (T-3), all populations from the Bieszczady Mts. (B-4, B-5, B-6) and 2 populations from Pomerania (P-7, P-9). The second group comprises 2 genetically similar the

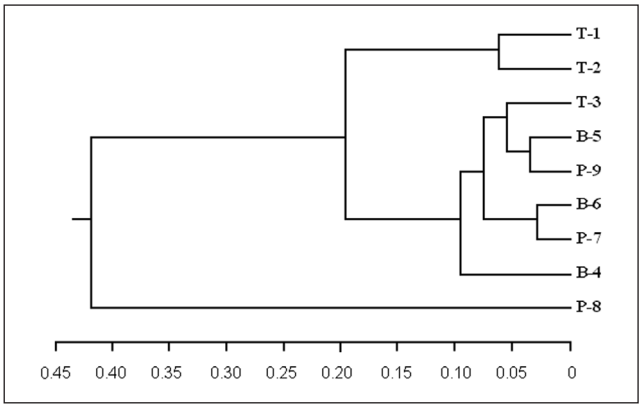

Fig. 19. UPGMA phenogram based on Nei's (1978) genetic distances between *Calypogeia integristipula* populations

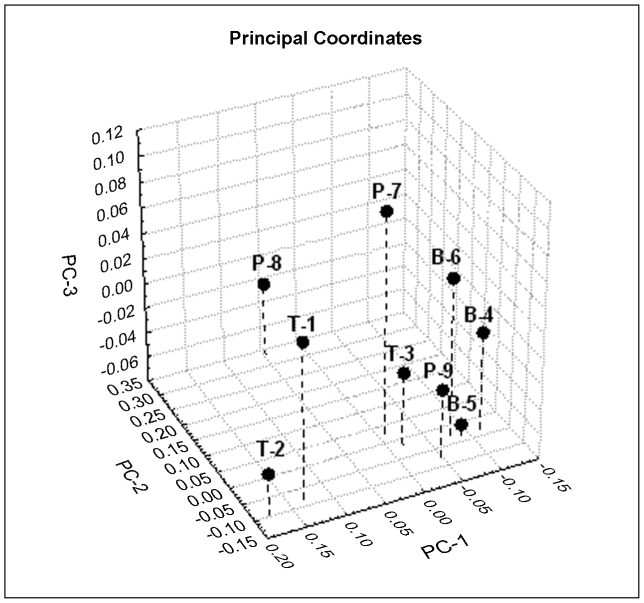

Fig. 20. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between population of *Calypogeia integristipula*
Explained variance: PC1=49.52%, PC2=29.95%, PC3=8.05%

Tatra NP populations (T-1, T-2). Population P-8 from the Pomeranian Lake District (Lake Czarne), is the most distinct. The phenogram shows the lack of division of populations depending on geographic regions.

Moreover, there is no clear differentiation between mountainous and lowland populations . The phenogram is consistent with the PCA diagram (Fig. 20).

3.2.3. *Mylia taylorii*

Clonal diversity. Patches of this species demonstrated a low level of clonal diversity (Table 59). The mean number of MLGs (*G*) at the patch level was 3.21, and the proportion of distinguishable genotypes (*G*/*N*) was 0.397. The highest and the lowest numbers of MLGs were observed in populations from Bieszczady Mts. The highest value was in population B-8 (*G*/*N*= 0.46), whereas the lowest in B-6 and B-7 (*G*/*N* =0.33). The number of genotypes (MLGs) detected in patches ranged from 1 to 7 per 8 studied gametophytes. Usually 4-5 MLGs per patch were found. The number of genotypes within patches in the studied populations did not differ significantly (Kruskal-Wallis ANOVA test: H=5.3906, p=0.75). Seven patches consisting of only one genotype were found. All of them came from the Tatra NP (populations T-1, T-2, T-3, T-4, T-5). By contrast, in 3 patches as many as 7 MLGs were identified per 8 analyzed plants. These patches came from 2 populations (T-1 and T-4) from the Tatra NP.

Numbers of genotypes (MLGs) at the population level of *M. taylorii* were low and the mean equalled 21.1 MLGs per population. The mean proportion of distinguishable genotypes (*G*/*N*) was 0.316. The most diverse was population T-2 from the Tatra NP (*G*/*N*= 0.43) and the least diverse was population B-7 from the Bieszczady Mts. (*G*/*N*=0.20) (Table 60).

The Simpson index (D_G) and evenness index (E) values in all the study populations of *M. taylorii* are low compared to other studied leafy liverworts. Generally, clonal diversity in the Tatra NP populations was higher than in the Bieszczady Mts. The highest D_G values occurred in population T-2 (0.9589) and the lowest in B-7 (0.7667) (Table 61).

In all populations of *M. taylorii* only 168 MLGs were detected per 584 studied gametophytes. The proportion of distinguishable genotypes (*G*/*N*) at the species level was 0.288. Thirty-two MLGs (19.1% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 9 plants from 8 patches growing in the 4 Tatra NP populations (T-1, T-3, T-4, T-5). By contrast, 68 MLGs (40.5% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 11.6% of studied plants.

Isozyme diversity. All 19 examined enzyme loci of M. taylorii (*Acp, Dia-A, Dia-B, Est-A, Est-B, Gdh, Got, Idh, Mdh-A, Mdh-B, Me, Mpi, Pgd-A, Pgd-B, Pgi-A*

Table 59. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G*/*N*, where *G*=MLG) at the patch level in *Mylia taylorii* populations

Location	Population	MLG	*G*/*N*
Tatra National Park, Dolina Białki (valley)	T-1	3.1	0.39
Tatra National Park, Dolina Suchej Wody (valley)	T-2	3.5	0.44
Tatra National Park, Dubrowiska (deforested mountains)	T-3	2.9	0.36
Tatra National Park, Dolina Pańszczyca (valley)	T-4	3.8	0.42
Tatra National Park, Dolina Kościeliska (valley), Wąwóz Kraków (gully)	T-5	3.2	0.40
Bieszczady Mts., Terebowiec stream	B-6	2.6	0.33
Bieszczady Mts., N slope of Mt. Tarnica	B-7	2.6	0.33
Bieszczady Mts., N slope of Mt. Krzemień	B-8	3.7	0.46
Bieszczady Mts., N slope of Mt. Rozsypaniec Wołosacki	B-9	3.5	0.44
Mean		3.21	0.397

Table 60. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G*/*N*, where *G*=MLG) in *Mylia taylorii* populations

Population	No. of patches	*N*	MLG	*G*/*N*
T-1	9	72	21	0.29
T-2	10	80	34	0.43
T-3	10	80	26	0.33
T-4	10	80	28	0.35
T-5	10	80	29	0.36
B-6	8	64	15	0.23
B-7	5	40	8	0.20
B-8	6	48	17	0.35
B-9	5	40	12	0.30
Mean			21.1	0.316

Table 61. Clonal diversity in populations of *Mylia taylorii*: Simpson's diversity index (D_G) and evenness index (E)

Population	D_G	E
T-1	0.8889	0.8413
T-2	0.9589	0.9262
T-3	0.9275	0.8959
T-4	0.9470	0.9374
T-5	0.9310	0.8825
B-6	0.8120	0.7750
B-7	0.7667	0.7724
B-8	0.8990	0.8448
B-9	0.9154	0.9268

Pgi-B, Pgi-C, Pgm, Sdh) were polymorphic (Table 62). A total of 57 alleles were detected in the studied populations. The number of alleles in a single locus varied from 2 to 4. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci were selectively neutral in the studied populations (Table 63).

Table 62. Allele frequencies at 19 enzyme loci in *Mylia taylorii* populations

Locus	Allele	Population								
		Tatra National Park					Bieszczady Mts.			
		T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
Acp	1	0.000	0.000	0.000	0.000	**0.139**	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	**0.031**	0.000	**0.163**	0.000
	3	0.833	1.000	1.000	1.000	0.760	0.969	1.000	0.837	1.000
	4	**0.167**	0.000	0.000	0.000	**0.101**	0.000	0.000	0.000	0.000
Dia-A	1	1.000	0.900	1.000	0.909	1.000	1.000	1.000	1.000	1.000
	2	0.000	**0.100**	0.000	**0.091**	0.000	0.000	0.000	0.000	0.000
Dia-B	1	0.000	0.000	**0.100**	**0.091**	**0.045**	0.000	0.000	0.000	0.000
	2	1.000	1.000	0.900	0.909	0.955	1.000	1.000	1.000	1.000
Est-B	1	**0.014**	**0.013**	0.000	**0.012**	0.000	0.000	0.000	0.000	0.000
	2	0.972	0.987	0.950	0.988	0.960	1.000	1.000	1.000	1.000
	3	**0.014**	0.000	**0.050**	0.000	**0.040**	0.000	0.000	0.000	0.000
Est-C	1	**0.014**	**0.138**	**0.150**	**0.386**	0.000	0.000	0.000	0.000	0.000
	2	0.972	0.862	0.800	0.489	0.975	1.000	1.000	1.000	1.000
	3	**0.014**	0.000	**0.050**	**0.125**	**0.025**	0.000	0.000	0.000	0.000
Gdh	1	0.000	**0.066**	0.000	**0.023**	**0.020**	0.000	0.000	0.000	0.000
	2	1.000	0.953	1.000	0.977	0.980	0.906	0.899	0.838	1.000
	3	0.000	0.000	0.000	0.000	0.000	**0.063**	**0.101**	**0.163**	0.000
	4	0.000	0.000	0.000	0.000	0.000	**0.031**	0.000	0.000	0.000
Got	1	1.000	1.000	1.000	1.000	1.000	0.969	1.000	0.837	1.000
	2	0.000	0.000	0.000	0.000	0.000	**0.031**	0.000	**0.163**	0.000
Idh	1	0.000	0.000	0.000	0.000	0.000	**0.109**	0.000	0.000	0.000
	2	1.000	1.000	1.000	1.000	1.000	0.860	1.000	1.000	1.000
	3	0.000	0.000	0.000	0.000	0.000	**0.031**	0.000	0.000	0.000
Mdh-A	1	1.000	0.988	0.900	1.000	1.000	1.000	1.000	0.959	1.000
	2	0.000	0.012	0.100	0.000	0.000	0.000	0.000	0.041	0.000
Mdh-B	1	0.042	0.163	0.275	0.239	0.101	0.016	0.525	0.163	0.125
	2	0.597	0.437	0.713	0.671	0.570	0.766	0.475	0.816	0.500
	3	0.361	0.400	0.000	0.034	0.329	0.187	0.000	0.021	0.375
	4	0.000	0.000	0.012	0.056	0.000	0.031	0.000	0.000	0.000
Me	1	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	0.000	0.000	0.000	0.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.163**	0.000
	3	0.000	0.000	0.000	0.000	0.000	**1.000**	**1.000**	**0.838**	**1.000**
Mpi	1	1.000	0.687	0.800	0.818	1.000	1.000	1.000	1.000	1.000
	2	0.000	**0.313**	**0.200**	**0.182**	0.000	0.000	0.000	0.000	0.000
Pgd-A	1	1.000	0.500	1.000	1.000	1.000	1.000	1.000	0.429	1.000
	2	0.000	**0.500**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.571**	0.000
Pgd-B	1	0.014	0.100	0.000	0.012	0.000	0.000	0.000	0.102	0.000
	2	0.986	0.900	1.000	0.898	0.899	1.000	1.000	0.408	1.000
	3	0.000	0.000	0.000	**0.090**	**0.101**	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.490**	0.000
Pgi-A	1	0.861	0.738	1.000	1.000	1.000	1.000	0.925	0.959	0.813
	2	0.139	0.262	0.000	0.000	0.000	0.000	0.075	0.041	0.187
Pgi-B	1	0.208	0.450	0.325	0.773	0.684	0.187	0.175	0.286	0.750
	2	0.792	0.425	0.625	0.091	0.291	0.750	0.825	0.551	0.250
	3	0.000	**0.125**	**0.050**	**0.136**	**0.025**	0.000	0.000	0.000	0.000
	4	0.000	0.000	0.000	0.000	0.000	**0.063**	0.000	**0.163**	0.000
Pgi-C	1	0.000	0.137	0.100	0.352	0.595	0.000	0.000	0.000	0.063
	2	0.958	0.788	0.588	0.648	0.265	1.000	0.750	1.000	0.812
	3	0.000	0.000	0.000	0.000	0.000	0.000	**0.250**	0.000	**0.125**
	4	**0.042**	**0.075**	**0.312**	0.000	0.000	0.000	0.000	0.000	0.000
Pgm	1	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.031
	2	1.000	0.950	1.000	1.000	1.000	1.000	0.975	1.000	0.969
	3	0.000	0.000	0.000	0.000	0.000	0.000	**0.025**	0.000	0.000
Sdh	1	0.000	0.025	0.313	0.000	0.051	0.125	0.000	0.041	0.031
	2	1.000	0.975	0.687	1.000	0.949	0.875	1.000	0.816	0.969
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.142**	0.000

Explanation: bold values denote private alleles for regions

Table 63. Ewens-Watterson test for neutrality for each locus in *Mylia taylorii* populations

Locus	N	k	Obs. F	L95	U95
Acp	592	4	0.8681	0.3354	0.9733
Dia-A	592	3	0.9733	0.3980	0.9933
Dia-B	592	3	0.7702	0.4063	0.9899
Est-B	592	2	0.9474	0.5045	0.9966
Est-C	592	2	0.9474	0.5045	0.9966
Gdh	592	3	0.7903	0.3719	0.9899
Got	592	4	0.8229	0.3378	0.9766
Idh	592	2	0.8682	0.5030	0.9966
Mdh-A	592	4	0.4292	0.3393	0.9732
Mdh-B	592	4	0.4631	0.3437	0.9733
Me	592	3	0.9799	0.3885	0.9899
Mpi	592	3	0.8465	0.3910	0.9899
Pgd-A	592	2	0.9635	0.5051	0.9966
Pgd-B	592	4	0.4506	0.3324	0.9832
Pgi-A	592	4	0.9471	0.3528	0.9733
Pgi-B	592	2	0.9668	0.5039	0.9966
Pgi-C	592	3	0.9700	0.3873	0.9899
Pgm	592	3	0.5680	0.3920	0.9899
Sdh	592	2	0.8260	0.5062	0.9966

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

The mean percentage of polymorphic loci (*P*) in the *M. taylorii* populations was 47.368% (Table 64). The highest percentage was in population T-2 from the Tatra NP (*P*=73.68%) and the lowest in populations B-7 and B-9 from the Bieszczady Mts. (*P*=31.58%). The mean number of alleles per locus (*A*) per population for all studied loci was 1.64. The greatest number of alleles (36) was found in population T-2 from the Tatra NP (*A*=1.9), while the smallest was detected in population B-7 (25) from the Bieszczady Mts. (*A* = 1.3).

In all studied populations rare alleles were found. Their number was the highest (7) in a population from the Tatra NP (T-1), and the lowest (only one rare allele) in a population from the Bieszczady Mts. (B-7). Private alleles, at the population level, were found in 5 populations (T-2, T-5, B-6, B-7, and B-8). Seven of the 10 identified private alleles had a high frequency (> 0.100): *PgdA-2* (T-2); *Acp-1* (T-5); *Idh-1* (B-6); *Me-2*, *PgdA-2, PgdA-3*, *PgdB-4*, *Sdh-3* (B-8). Remaining 3 belonged to the class of rare alleles (frequency <0.05): *Gdh-4*, *Idh-3* (B-6), *Pgm-3* (B-7) (Table 62).

The level of isozymatic diversity of *M. taylorii* was low compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.1878. Also, a low level of genenetic diversity within populations (H_S) was noted. The mean H_S was 0.1061, and ranged from 0.0511 (B-7) to 0.1740 (T-2) (Table 64). In the studied populations no statistically significant correlations were detected between genetic diversity (H_S) and population size (*N*) (Spearman test: *R*=0.4852, *p*=0.18). Coefficient of genetic differentiation, calculated over polymorphic loci among 9 studied populations, was rather high (G_{ST}=0.3281), associated with a low level of gene flow between populations. The mean number of migrants per population (N_m) was 1.0238.

Analysis of molecular variance (AMOVA) showed that the genetic differentiation between *M. taylorii* populations (Φ_{PT}) was 0.312. Most of genetic variation (69%) resulted from variation within populations (Table 65).

Pair-wise genetic distances (*D*) and similarities (*I*) (Nei 1978) between 9 populations of *M. taylorii* were computed (Table 66). Genetic distances ranged from 0.1312 to 0.0255, with a mean of 0.07260. The highest genetic distance was between populations T-2 and B-6. The highest genetic similarity was between 2 populations from the Bieszczady Mts.: B-7 and B-9 (*I*=0.9748). The Mantel test revealed significant correlations between genetic and geographic distances (*R*=0.806, *p*<0.01).

Table 64. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Mylia taylorii* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S(±SD)
T-1	30	7	0	1.6	42.11	0.0828 (0.1467)
T-2	36	4	1	1.9	73.68	0.1740 (0.2209)
T-3	32	4	0	1.7	47.37	0.1181 (0.2003)
T-4	34	4	0	1.8	52.63	0.1373 (0.1989)
T-5	32	5	1	1.7	52.63	0.1101 (0.2035)
B-6	31	6	3	1.6	36.84	0.0859 (0.1499)
B-7	25	1	1	1.3	31.58	0.0511 (0.1488)
B-8	34	4	4	1.8	57.89	0.1260 (0.2073)
B-9	27	2	0	1.4	31.58	0.0703 (0.1734)
Mean	31.2	4.1	1.1	1.64	47.368	0.10610

Table 65. Analysis of molecular variance (AMOVA) for *Mylia taylorii* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.617	31	Φ_{PT}= 0.312***
Within populations	575	1.361	69	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

Table 66. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Mylia taylorii* populations

	T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
T-1	***	0.9715	0.9738	0.9540	0.9636	0.8940	0.9352	0.9162	0.9312
T-2	0.0289	***	0.9608	0.9617	0.9565	0.8770	0.9102	0.9011	0.9218
T-3	0.0371	0.0445	***	0.9717	0.0287	0.9235	0.9048	0.8862	0.9256
T-4	0.0471	0.0391	0.9633	***	0.0374	0.8938	0.9052	0.8911	0.9257
T-5	0.0265	0.0400	0.9662	0.0344	***	0.8973	0.9262	0.8973	0.9124
B-6	0.1120	0.1312	0.1084	0.1123	0.0796	***	0.9483	0.9107	0.9443
B-7	0.0669	0.0941	0.0766	0.0997	0.1001	0.0531	***	0.9514	0.9748
B-8	0.0875	0.1042	0.1083	0.1153	0.1208	0.0935	0.0498	***	0.9467
B-9	0.0713	0.0814	0.0916	0.0772	0.0773	0.0573	0.0255	0.0548	***

On the basis of genetic distances (Nei 1978) the UPGMA phenogram was constructed (Fig. 21). In the phenogram, 2 population groups stand out. The first group contains all the Tatra NP populations and the second contains all the Bieszczady populations. Populations from the Tatra NP exhibit a greater degree of similarity than populations from the Bieszczady Mts. In the Tatra NP group the greatest similarity was noted for population pairs T-1 (Dolina Białki) with T-3 (Dubrowiska), and T-5 (Wąwóz Kraków) with T-4 (Dolina Pańszczyca), while in the Bieszczady Mts., for populations B-7 (*N* slope of Mt. Tarnica) with B-8 (*N* slope of Mt. Krzemień). Population B-6 (Bieszczady Mts., Terebowiec stream) is the most distinct. The phenogram groups populations according to geographic distribution and provides evidence for a marked differentiation between the Tatra NP and Bieszczady populations. The phenogram is consistent with the PCA diagram (Fig. 22).

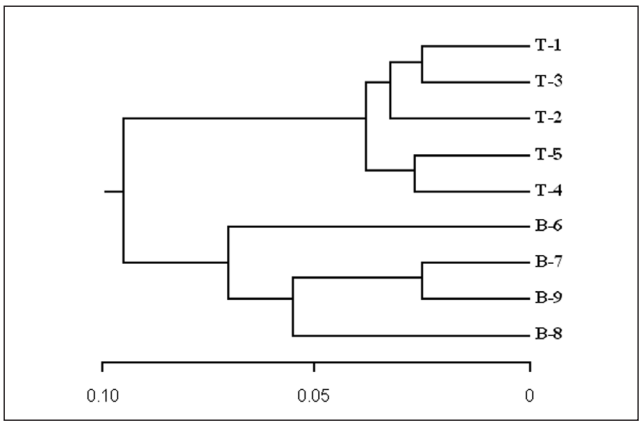

Fig. 21. UPGMA phenogram based on Nei's (1978) genetic distances between *Mylia taylorii* populations

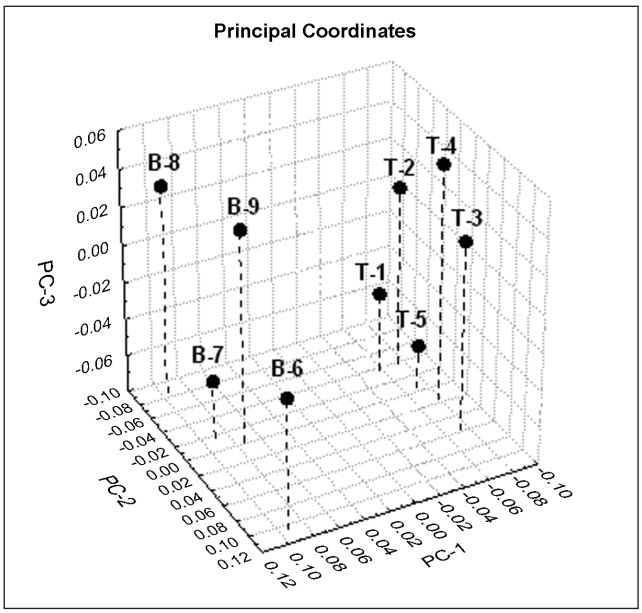

Fig. 22. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between *Mylia taylorii* populations
Explained variance: PC1=47.92%, PC2=21.31%, PC3=10.44%

3.2.4. *Tritomaria quinquedentata*

Clonal diversity. Patches of this species demonstrated a high level of clonal diversity (Table 67). At the patch level, the mean number of MLGs (*G*) was 4.97 per patch. The mean proportion of distinguishable genotypes (*G*/*N*) was 0.620. Among the studied populations, the highest and the lowest numbers of MLGs in patches were observed in populations from the Bieszczady Mts.: B-9 (*G*/*N*=0.76) and B-6 (*G*/*N*=0.54). The number of genotypes (MLGs) detected in patches ranged from 1 to 8 per 8 studied gametophytes. Usually, 4-5 genotypes per patch were detected. The number of genotypes within patches in studied populations did not differ significantly (Kruskal-Wallis ANOVA test: H=12.4787, p=0.13). Only one patch consisted of a single genotype. The patch came from population T-1. On the other hand, 8 MLGs per patch were identified in 2 patches: one from T-5 and the other from B-9 population.

All populations of *T. quinquedentata* had a high genotypic variability. The mean number of MLGs at the population level was 37.7, while the proportion of distinguishable genotypes (*G*/*N*) equalled 0.554. The most diverse was population T-3 (Dolina 5 Stawów Polskich in the Tatra NP), where *G*/*N* reached 0.69. The least diverse was population T-1 from the same region (Las Capowski), where *G*/*N* equalled 0.45 (Table 68).

The Simpson index (D_G) and evenness index (E) values in all the study populations of *T. quinquedentata* are very high compared to other leafy liverworts. It

Table 67. Mean number of identified multilocus genotypes (MLG) and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) at the patch level in *Tritomaria quinquedentata* populations

Location	Population	MLG	*G/N*
Tatra National Park, Las Capowski (forest)	T-1	4.9	0.61
Tatra National Park, Dolina Jaworzynki (valley)	T-2	5.2	0.65
Tatra National Park, Dolina 5 Stawów Polskich (valley)	T-3	5.3	0.66
Tatra National Park, Dolina Kościeliska (valley)	T-4	4.8	0.60
Tatra National Park, Dolina Miętusia (valley)	T-5	4.6	0.57
Bieszczady Mts., Dolina Górnej Solinki (valley)	B-6	4.3	0.54
Bieszczady Mts., N slope of Mt. Krzemień	B-7	4.8	0.60
Bieszczady Mts., N slope of Mt. Tarnica	B-8	4.7	0.59
Bieszczady Mts., NW slope of Mt. Rozsypaniec Wołosacki	B-9	6.1	0.76
Mean		4.97	0.62

Table 68. Numbers of patches, plants (*N*), identified multilocus genotypes (MLG), and proportion of distinguishable genotypes (*G/N*, where *G*=MLG) in *Tritomaria quinquedentata* populations

Population	No. of patches	*N*	MLG	*G/N*
T-1	7	56	25	0.45
T-2	10	80	45	0.56
T-3	6	48	33	0.69
T-4	10	80	43	0.54
T-5	10	80	46	0.58
B-6	9	72	38	0.53
B-7	10	80	43	0.54
B-8	6	48	26	0.54
B-9	9	72	40	0.56
Mean			37.7	0.554

confirms that the species has high clonal diversity. Most of populations have similar D_G values. The highest D_G values occurred in population B-7 (0.9817) and the lowest in B-8 (0.9058) (Table 69). In total, 294 MLGs were detected among 616 *T. quinquedentata* gametophytes. The proportion of distinguishable genotypes (*G*/*N*) at the species level equalled 0.477. Forty-nine MLGs (16.7% of MLGs) occurred more frequently than in a single patch (both in the same population and in different populations). The most common genotype was found in 3 populations, including 2 from the Bieszczady populations (B-6 and B-7), and from the Tatra NP population (T-5). Overall, the most common genotype was detected in 13 plants from 5 patches. By contrast, 162 MLGs (52.3% of MLGs) were restricted to a single gametophyte. Individual genotypes occurred in 26.3% of studied plants.

Table 69. Clonal diversity in populations of *Tritomaria quinquedentata*: Simpson's diversity index (D_G) and evenness index (*E*)

Population	D_G	*E*
T-1	0.9578	0.9342
T-2	0.9816	0.9548
T-3	0.9814	0.9080
T-4	0.9810	0.9617
T-5	0.9807	0.9476
B-6	0.9598	0.8799
B-7	0.9817	0.9614
B-8	0.9058	0.7163
B-9	0.9660	0.8838

Isozyme diversity. A total of 11 loci (*Acp, Dia, Gdh, Got, Hex, Idh, Mdh-A, Mdh-B, Me, Per-A, Per-B, Pgd, Sdh*) were detected in 13 enzyme systems (Table 70). All loci were polymorphic. A total of 42 alleles were detected in the study populations. The number of alleles in a single locus varied from 1 to 4. The most variable loci were *Dia, Gdh, Hex, Idh, Me* and *Pgd*, with 4 alleles each. The Ewens-Watterson test for neutrality showed that allele frequencies at all loci except *Pgd* were selectively neutral in the studied populations (Table 71). The mean number of alleles per locus (*A*) per population, for all studied loci, was 2.36. The greatest number of alleles (38) was found in population T-3 (*A*=2.9), whereas the smallest number of alleles (only 25) was detected in population T-1 (*A*=1.9). The mean percentage of polymorphic loci (*P*) in populations was 74.359%. The highest *P* values were found in populations T-3, T-4, and T-5 (*P*=84.62%) from the Tatra NP, while the lowest was in population T-1 (*P*=46.15%), from the Tatra NP, too. In all studied populations rare alleles were found. They were most numerous (7 rare alleles) in 2 populations from the Tatra NP (T-2 and T-3). In 3 studied populations (T-3, T-5, B-8), private alleles were found (Table 72): 2 of them (*Gdh-4*, *Me-4*) with frequency < 0.05, and the other 2 (*Dia-4*, *MdhA-2*) with frequency ≥ 0.1 (Table 70).

The level of isozymatic diversity of *T. quinquedentata* was high compared to other studied liverworts. The total genetic diversity (H_T), based on mean allelic frequencies of polymorphic loci over all populations, was 0.3679. Also, a high level of genetic diversity within populations (H_S) was noted, with a mean of 0.22541, and varied between 0.1221 (T-1) and 0.2804 (T-3) (Table 72). In all studied populations no statistically significant correlations were detected between genetic diversity (H_S) and population size (*N*) (Spearman test: *R*=0.4171, *p*=0.21). The coefficient of genetic differentiation, calculated over polymorphic loci among 9 studied populations, was relatively high (G_{ST}=0.2276). The mean number of migrants per population (N_m) was 1.6966, indicating a considerable degree of gene flow between populations.

Table 70. Allele frequencies at 13 enzyme loci in *Tritomaria quinquedentata* populations

Locus	Allele	Population								
		Tatra National Park					Bieszczady Mts.			
		T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
Acp	1	0.143	0.000	0.120	0.000	0.000	0.100	0.000	0.000	0.000
	2	0.571	0.038	0.160	0.900	0.700	0.900	0.778	1.000	1.000
	3	0.286	0.962	0.720	0.100	0.300	0.000	0.222	0.000	0.000
Dia	1	0.000	0.050	0.160	0.038	0.012	0.050	0.028	0.042	0.042
	2	1.000	0.925	0.840	0.962	0.888	0.900	0.972	0.958	0.875
	3	0.000	0.025	0.000	0.000	0.000	0.050	0.000	0.000	0.083
	4	0.000	0.000	0.000	0.000	**0.100**	0.000	0.000	0.000	0.000
Gdh	1	0.232	0.275	0.240	0.163	0.212	0.088	0.028	0.146	0.292
	2	0.732	0.563	0.700	0.763	0.775	0.812	0.972	0.854	0.667
	3	0.036	0.162	0.020	0.074	0.013	0.100	0.000	0.000	0.042
	4	0.000	0.000	**0.040**	0.000	0.000	0.000	0.000	0.000	0.000
Got	1	0.000	0.000	0.000	0.025	0.000	0.012	0.069	0.021	0.028
	2	1.000	1.000	1.000	0.975	1.000	0.988	0.931	0.979	0.972
Hex	1	**1.000**	**0.175**	**0.340**	**0.538**	**0.275**	0.000	0.000	0.000	0.000
	2	0.000	**0.788**	**0.560**	**0.462**	**0.137**	0.000	0.000	0.000	0.000
	3	0.000	0.038	0.020	0.000	0.288	1.000	1.000	1.000	1.000
	4	0.000	0.000	**0.080**	0.000	**0.300**	0.000	0.000	0.000	0.000
Idh	1	0.054	0.050	0.080	0.038	0.000	0.012	0.014	0.042	0.000
	2	0.286	0.163	0.080	0.100	0.075	0.225	0.069	0.417	0.000
	3	0.660	0.788	0.800	0.862	0.925	0.762	0.917	0.542	0.972
	4	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.028
Mdh-A	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.875	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.125**	0.000
Mdh-B	1	0.161	0.275	0.140	0.112	0.163	0.212	0.111	0.250	0.069
	2	0.554	0.275	0.560	0.500	0.312	0.413	0.444	0.354	0.556
	3	0.286	0.450	0.300	0.388	0.525	0.375	0.445	0.396	0.375
Me	1	0.000	0.236	0.400	0.213	0.163	0.287	0.389	0.271	0.125
	2	0.000	0.075	0.140	0.450	0.337	0.275	0.139	0.437	0.778
	3	1.000	0.689	0.420	0.338	0.500	0.438	0.472	0.292	0.098
	4	0.000	0.000	**0.040**	0.000	0.000	0.000	0.000	0.000	0.000
Per-A	1	0.000	0.000	**0.100**	0.000	**0.050**	0.000	0.000	0.000	0.000
	2	1.000	1.000	0.900	1.000	0.950	1.000	1.000	1.000	1.000
Per-B	1	0.411	0.238	0.060	0.000	0.325	0.600	0.556	0.333	0.000
	2	0.589	0.762	0.920	0.775	0.675	0.400	0.444	0.667	1.000
	3	0.000	0.000	**0.020**	**0.225**	0.000	0.000	0.000	0.000	0.000
Pgd	1	0.036	0.025	0.200	0.100	0.150	0.012	0.083	0.125	0.042
	2	0.071	0.275	0.400	0.825	0.637	0.188	0.250	0.000	0.319
	3	0.875	0.625	0.026	0.025	0.200	0.438	0.486	0.708	0.458
	4	0.018	0.075	0.140	0.050	0.013	0.362	0.181	0.167	0.181
Sdh	1	0.000	0.038	0.120	0.225	0.037	0.225	0.125	0.021	0.014
	2	1.000	0.800	0.680	0.725	0.888	0.612	0.736	0.812	0.681
	3	0.000	0.162	0.200	0.050	0.075	0.162	0.139	0.167	0.306

Explanation: bold values denote private alleles for regions

Table 71. Ewens-Watterson test for neutrality for each locus in *Tritomaria quinquedentata* populations

Locus	N	k	Obs. F	L95*	U95*
Acp	618	3	0.5430	0.4045	0.9903
Dia	618	4	0.8561	0.3465	0.9712
Gdh	618	4	0.6105	0.3331	0.9712
Got	618	2	0.9650	0.5044	0.9968
Hex	618	4	0.3472	0.3292	0.9744
Idh	618	4	0.6925	0.3331	0.9775
Mdh-A	618	1	1.0000	—	—
Mdh-B	618	3	0.3763	0.3762	0.9935
Me	618	4	0.3611	0.3464	0.9775
Per-A	618	2	0.9713	0.5047	0.9968
Per-B	618	3	0.5518	0.3894	0.9903
Pgd	618	4	0.3364	0.3385	0.9744
Sdh	618	3	0.6143	0.4011	0.9903

Explanations: N – number of plants, k – number of alleles, L95 – lower limit of 95% confidence interval, U95 – upper limit of 95% confidence interval

Analysis of molecular variance (AMOVA) showed that the genetic differentiation between *T. quinquedentata* populations (Φ_{PT}) was 0.235. Most of genetic variation (77%) resulted from variation within populations, whereas the variation among populations accounted for only 23% (Table 73).

Pair-wise genetic distances (*D*) and similarities (*I*) (Nei 1978) between 9 populations of *T. quinquedentata* were computed (Table 74). Genetic distances ranged from 0.0158 to 0.0255 (mean *D*=0.14639). The highest genetic distance was between populations T-2 and B-9. The smallest distance was between 2 populations from the Bieszczady Mts.: B-6 and B-7. The Mantel test revealed significant correlations between genetic and geographic distances (R=0.599, p< 0.01).

Table 72. Numbers of identified alleles, rare alleles, private alleles, mean number of alleles per locus (*A*), percentage of polymorphic loci (*P*), and allelic diversity within populations (H_S±SD) in *Tritomaria quinquedentata* populations

Population	No. of alleles	No. of rare alleles	No. of private alleles	*A*	*P* (%)	H_S(±SD)
T-1	25	3	0	1.9	46.15	0.1221 (0.2530)
T-2	32	7	0	2.5	76.92	0.2344 (0.2300)
T-3	38	7	2	2.9	84.62	0.2804 (0.2356)
T-4	31	6	0	2.4	84.62	0.2570 (0.2181)
T-5	33	5	1	2.5	84.62	0.2760 (0.2491)
B-6	31	5	0	2.4	76.92	0.2479 (0.2499)
B-7	29	3	0	2.2	76.92	0.2327 (0.2413)
B-8	28	4	1	2.2	76.92	0.1823 (0.2536)
B-9	28	6	0	2.2	61.54	0.1966 (0.2459)
Mean	30.6	5.1	0.4	2.36	74.359	0.22541

Table 73. Analysis of molecular variance (AMOVA) for *Tritomaria quinquedentata* populations

Source of variation	df	Variance component	Variance (%)	Fixation Index[1]
Among populations	8	0.577	23	Φ_{PT} = 0.235***
Within populations	609	1.880	77	

Explanations: [1] Φ_{PT} (analogous to F_{ST}) – variation among populations divided by total variation. Level of significance *** p≤0.001

On the basis of genetic distances (Nei 1978) the UPGMA phenogram was constructed (Fig. 23). In the phenogram, 2 population groups stand out. The first group contains all populations from the Tatra NP (T-1, T-2, T-3, T-4, T-5). The second group contains all populations from the Bieszczady Mts. (B-6, B-7, B-8, B-9). Populations from the Bieszczady Mts. show greater similarity to each other than populations from the Tatra NP. Within the Bieszczady Mts. the highest similarity is

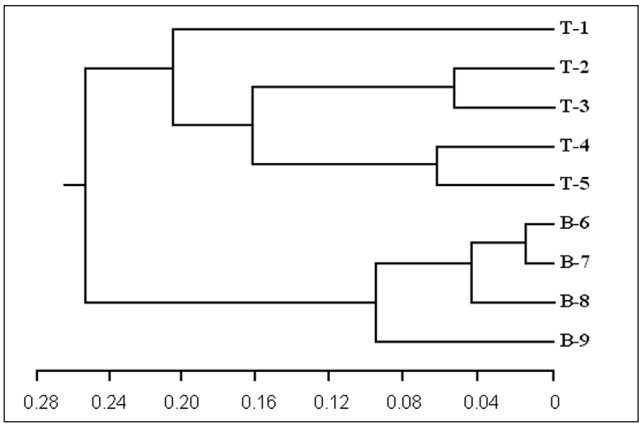

Fig. 23. UPGMA phenogram based on Nei's (1978) genetic distances between *Tritomaria quinquedentata* populations

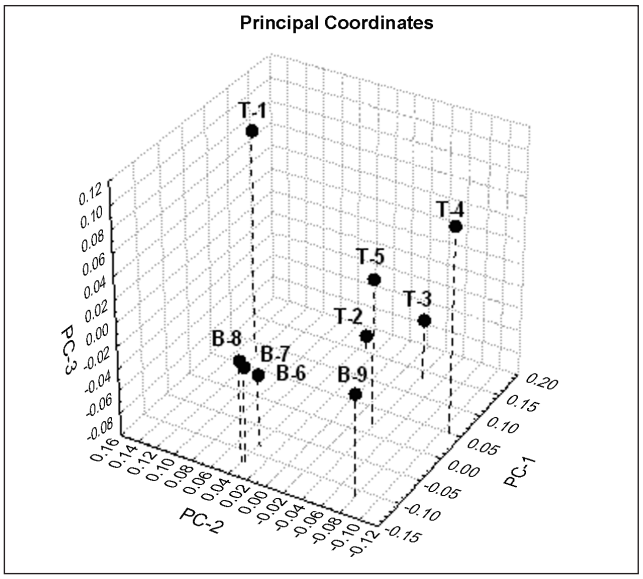

Fig. 24. Three-dimensional scatter plot of PCA based on Nei's (1978) genetic distances between *Tritomaria quinquedentata* populations
Explained variance: PC1=51.97%, PC2=21.31%, PC3=14.61%

Table 74. Nei's (1978) genetic identities (*I*, above diagonal) and distances (*D*, below diagonal) between *Tritomaria quinquedentata* populations

	T-1	T-2	T-3	T-4	T-5	B-6	B-7	B-8	B-9
T-1	***	0.8641	0.8595	0.8443	0.8851	0.8415	0.8257	0.8335	0.7676
T-2	0.1461	***	0.9581	0.8388	0.8842	0.8208	0.7817	0.7813	0.7583
T-3	0.1514	0.0428	***	0.9111	0.9205	0.8408	0.8037	0.7986	0.8181
T-4	0.1692	0.1758	0.0931	***	0.9527	0.8503	0.8417	0.8312	0.8754
T-5	0.1221	0.1231	0.0828	0.0485	***	0.9275	0.9063	0.8935	0.9028
B-6	0.1726	0.1975	0.1734	0.1622	0.0753	***	0.9843	0.9586	0.9194
B-7	0.1915	0.2463	0.2186	0.1723	0.0984	0.0158	***	0.9701	0.9256
B-8	0.1821	0.2468	0.2250	0.1849	0.1126	0.0423	0.0303	***	0.9441
B-9	0.2645	0.2766	0.2008	0.1331	0.1022	0.0841	0.0774	0.0575	***

observed between populations B-6 and B-7 (*N* slope of Mt. Krzemień and Dolina Górnej Solinki, respectively). In the Tatra NP the most distinct was population T-1 (Las Capowski). By contrast, in this region 2 subgroups show higher genetic similarity: T-2 with T-3 (Dolina Jaworzynki and Dolina 5 Stawów Polskich, respectively) and T-4 with T-5 (Dolina Kościeliska and Dubrowiska, respectively). The phenogram groups populations according to geographic distribution and illustrates a clear divergence between populations from both regions. The phenogram is consistent with the PCA diagram (Fig. 24).

3.3. Comparison of genetic diversity of sterile and fertile leafy liverworts

3.3.1. Clonal diversity

The mean proportion of distinguishable genotypes (*G*/*N*) for all studied leafy liverwort species was 0.4910 at the patch level, 0.4014 at the population level, and 0.3225 at the species level. At these levels, mean values of *G*/*N* were not significantly higher for sterile species than for fertile species (Mann-Whitney *U* test: Z=0.443, p=0.66; Z=0.918, p=0.36; and Z=0.577, p=0.56, respectively). For sterile species, *G*/*N* values at the patch, population, and species levels were 0.5120, 0.4312, and 0.3438, and for fertile species were 0.4700, 0.3715, and 0.3012, respectively (Fig. 25). Moreover, the mean *G*/*N* of 2 monoecious species (*C. integristipula* and *L. hatcheri*) was slightly higher than the mean *G*/*N* of 2 dioecious species (*M. taylorii* and *T. quinquedentata*), the difference was not significant (Mann-Whitney *U* test: Z=-0.387, p=0.70).

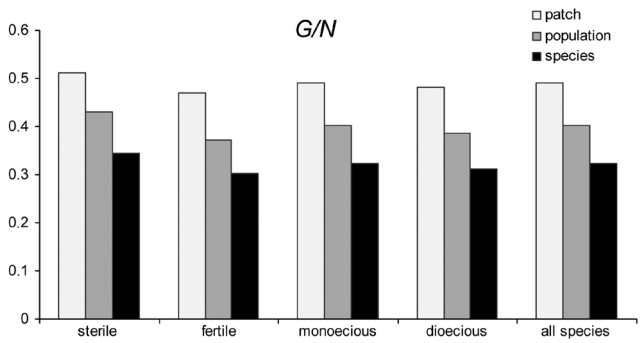

Fig. 25. Mean proportion of distinguishable genotypes (*G*/*N*) at the patch, population and species level for the sterile species and for the fertile species; with division of fertile species into monoecious, and the mean for all the studied species

However, some of the studied species differ significantly in the mean proportion of distinguishable genotypes (*G*/*N*) at the level of patches and populations (Kruskal-Wallis ANOVA test: H=58.897, p<0.01; H=59.03, p<0.01). The most diverse species were *B. trilobata* and *T. quinquedentata* (Fig. 26). In both species the proportion of *G*/*N* was the highest at the level of patches (0.623; 0.620), populations (0.594; 0.554) and species (0.484; 0.477), respectively. However, the 2 species are very different as regards the mode of reproduction: *B. trilobata* is sterile in the Polish territory, while *T. quinquedentata* is often fertile, dioecious. Neither of the species produces gemmae. By contrast, the lowest clonal diversity was observed in *C. integristipula* and *M. anomala*. In these species the proportion of *G*/*N* was 0.294 and 0.321 at the patch level, 0.138 and 0.223 at the population level and 0.113 and 0.163 at the species level, respectively. These 2 species differ in the mode of reproduction, too: *C. integristipula* is monoecious, often producing sporophytes, while *M. anomala* in Poland reproduces only vegetatively. Interestingly, both species produce abundant gemmae.

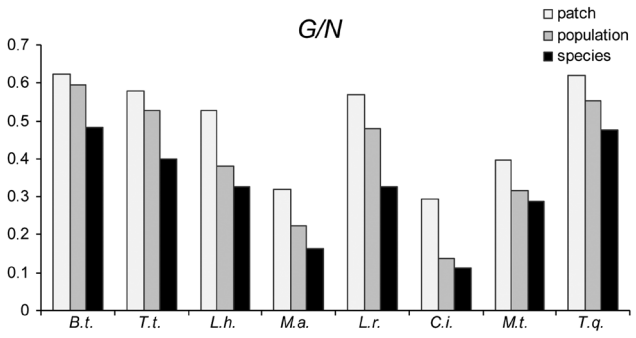

Fig. 26. Mean proportion of distinguishable genotypes (*G*/*N*) at the patch, population and species level for all the studied leafy liverwort species: *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*)

Genetic diversity in patches was proportional to the genetic diversity of the species as a whole, i.e. in species with higher genetic diversity, patches exhibited higher genetic diversity, too , whereas in species with lower genetic diversity, most patches exhibited very low genetic diversity, too (Fig. 26). Furthermore, the *G*/*N* value was always the highest at the patch level and the lowest at the species level. This was caused by high repeatability of the same genotypes in different patches, both within the same population and across populations. The greatest number of repeated genotypes was identified in low-diversity species.

In nearly all studied species – except *T. quinquedentata* and *L. reptans* – there were patches in which only one genotype (MLG) was detected. The greatest numbers of patches with a single genotype were identified in *C. integristipula* (13 patches), *M. anomala* (12 patches), and *M. taylorii* (7 patches). In the remaining species, patches with a single genotype were extremly rare, found only in 1-2 of all studied patches of a species. On the other hand, in 6 patches as many as 8 MLGs per 8 study gametophytes were identified (*G*/*N*=1.0).

They were found in 4 most diverse species: *B. trilobata* (2 patches), *T. tomentella* (one patch), *L. reptans* (one patch), and *T. quinquedentata* (2 patches). Usually, 4-5 MLGs per patch were detected in *B. trilobata*, *T. tomentella*, *L. hatcheri*, *L. reptans*, and *T. quinquedentata*, and 2-3 MLGs per patch in *M. anomala*, *C. integristipula*, and *M. taylorii*.

Two patterns of distribution of genotypes in patches can be distinguished. In the first pattern, patches were dominated by one genotype (MLG and the remaining genotypes were much rarer. This pattern was mainly found in species characterized by low genetic diversity (*C. integristipula, M. anomala*, and *M. taylorii*). In the second pattern, patches had no dominant genotype and consisted of a larger number of genotypes occupying similar proportions of a patch. This pattern of genotype distribution in patches was observed in species with higher genetic diversity, while predominance of any single genotype was very rare.

3.3.2. Genetic diversity

The mean number of alleles per locus (*A*) for all the studied species of leafy liverworts at the population level (1.93) and species level (2.99) was high. In both groups of species (sterile and fertile), the mean *A* at the population level was similar (1.92 and 1.93, respectively). At the species level, the mean *A* was higher for fertile species (3.10), compared to sterile (2.88). In the majority of fertile species, *A* values at the level of species were at least 3.0. In sterile species, they generally were below 3.0. Nevertheless, the sterile and fertile species do not differ significantly in mean *A* at the population and species level (Mann-Whitney *U* test: Z=-1.188, p=0.23; and Z=-1.010, p=0.31, respectively). The highest number of alleles per locus (*A*) at the population level was detected in *T. quinquedentata* (2.36), and the lowest in *C. integristipula* (1.50). Both species belong to the group of fertile species. The highest *A* at the species level was detected in *L. hatcheri* (3.4), and the lowest in *M. anomala* (2.6). Both species are sterile and produce abundant gemmae (Fig. 27).

Fertile species have higher numbers of rare and private alleles at the level of populations and species (Fig. 28), but the differences are not significant (Mann-Whitney *U* test for rare alleles: Z=-0.722, p=0.47; and Z=-144, p=0.54, respectively; for private alleles:

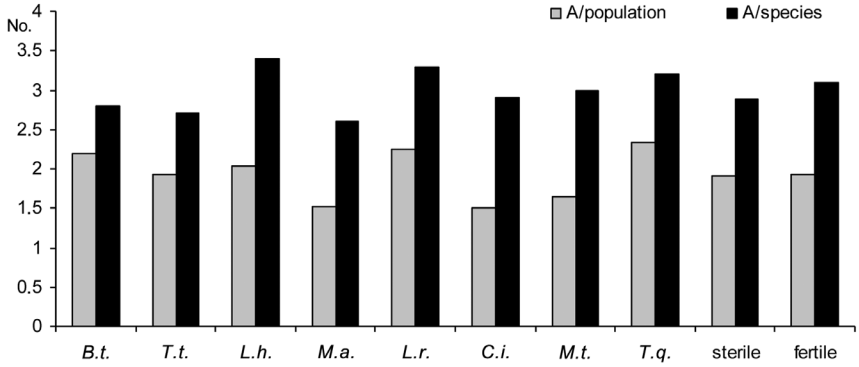

Fig. 27. Mean number of alleles per locus (*A*) at the population level and at the species level for all the studied species of leafy liverworts *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*); with division of species into sterile (*B.t.*, *T.t.*, *L.h.*, *M.a.*) and fertile (*L.r.*, *C.i.*, *M.t.*, *T.q.*)

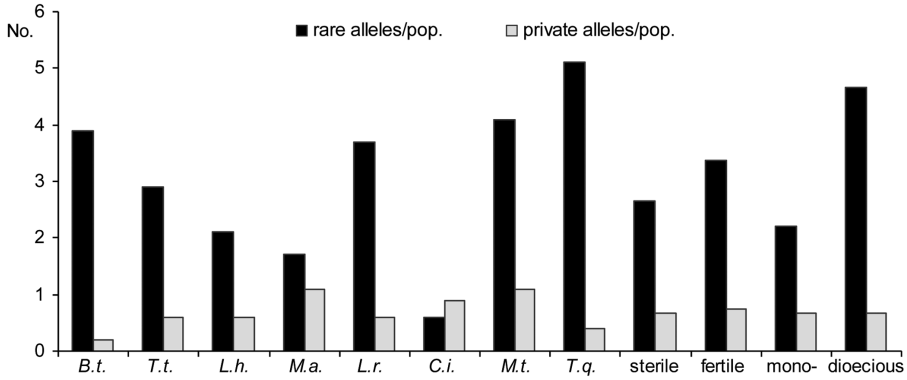

Fig. 28. Mean number of rare and private alleles per population for all the studied species of leafy liverworts: *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*); with division of species into fertile (*L.r.*, *C.i.*, *M.t.*, *T.q.*) and sterile (*B.t.*, *T.t.*, *L.h.*, *M.a.*) and subdivision of fertile species into monoecious (*L.r., C.i.*) and dioecious (*M.t., T.q.*)

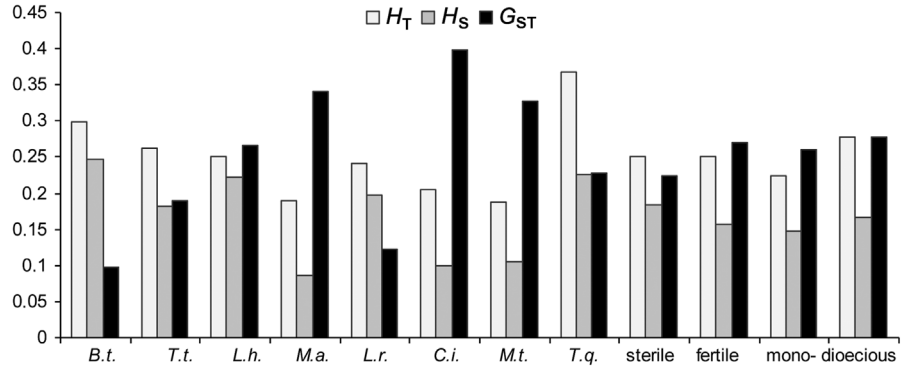

Fig. 29. Total genetic diversity (H_T), genetic diversity within populations (H_S), and the coefficient of genetic differentation (G_{ST}) for all the studied species of leafy liverworts: *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatcheri* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), *Tritomaria quinquedentata* (*T.q.*); with division of species into fertile (*L.r.*, *C.i.*, *M.t.*, *T.q.*) and sterile (*B.t.*, *T.t.*, *L.h.*, *M.a.*)

Z=0.144, *p*=0.88; and *Z*=-0.433, *p*=0.67, respectively). The greatest number of rare alleles at the population level was found in *T. quinquedentata* (5.1), while the smallest in *C. integristipula* (0.6). At the species level, the greatest number was found in *M. taylorii* (26) and the smallest in *B. trilobata* (6). However, in *B. trilobata*, the mean number of rare alleles per population was 3.9, i.e. relatively high compared to other species. This disparity stems from the fact that the same alleles were rare in some populations but frequent in others. The greatest number of private alleles per population (1.1) was detected in both *Mylia* species: *M. anomala* (sterile) and *M. taylorii* (fertile). The mean number of alleles per locus (*A*) at the level of species was identical for monoecious and dioecious species (3.1). However, at the population level, it was slightly higher for dioecious (1.99) compared to monoecious species (1.87). Dioecious species also had a greater number of rare alleles. In monoecious species, there were 11.5 rare alleles per species and 2.25 per population. In dioecious species, there were on average 19.5 rare alleles per species and 4.6 per population. The Mann-Whitney *U* test showed that the monoecious and dioecious species do not differ significantly in mean number of rare and private alleles at the population level (*Z*=-1.162, *p*=0.25; and *Z*=0.387, *p*=0.70, respectively) and at the species level (*Z*=-1.122, *p*=0.23; and *Z*=-0.775, *p*=0.44, respectively).

The mean total genetic diversity (H_T) determined for all the studied species of leafy liverworts was high, reaching 0.25049. What is more, the mean level of total genetic diversity for fertile and sterile species was nearly identical: 0.25048 and 0.25050, respectively (Fig. 29). *T. quinquedentata* was proven to be the most diverse species of all the species under study, with H_T equal to 0.3679. It is a fertile dioecious species, which does not produce gemmae. The lowest total diversity was found in 2 species belonging to the same genus, though differing in the mode of reproduction: the fertile *M. taylorii* (0.1878) and the sterile but producing gemmae *M. anomala* (0.1897). The mean within-population diversity (H_S) for all investigated species was 0.17059. For sterile species it was higher (0.18433) than for fertile ones (0.15685). The highest H_S was found in 2 species: *B. trilobata* (0.2473) and *T. quinquedentata* (0.2254). By contrast, the lowest H_S was detected in *C. integristipula* (0.0988). Furthermore, low H_S was also detected in both *Mylia* species: *M. anomala* (0.0867) and *M. taylorii* (0.1061). The Mann-Whitney *U* test showed that the sterile and fertile species do not differ significantly in respect to H_T (*Z*=0.433, *p*=0.67) and H_S (*Z*=0.144, *p*=0.89). Within studied fertile species, the mean H_T and H_S values were higher for dioecious species (*M. taylorii*, *T. quiquendentata*) than for monoecious species (*L. reptans*, *C. integristipula*). Mean values of H_T for dioecious and monoecious species were 0.278 and 0.223, respectively, while H_S reached 0.166 and 0.148, respectively. These differences between these groups of species were not significant (Mann-Whitney *U* test). It indicates that fertile species exhibit a slightly higher degree of H_T and H_S diversity, but both groups contain species with high and low diversity. The mean coefficient of genetic differentiation (G_{ST}) was slightly higher for fertile species (0.26918) than for sterile species (0.22340). The highest values of G_{ST} were identified in less diverse species: *C. integristipula* (0.3985), *M. taylorii* (0.3281), and *M. anomala* (0.3402). In contrast, the lowest value was identified in *B. trilobata* (0.0981), which belongs to the group of the most diverse species. Within fertile species, G_{ST} values for dioecious species were higher (0.278) than for monoecious species (0.261) (Fig. 29).

Analysis of molecular variance (AMOVA) showed that in all studied species of leafy liverworts most of genetic variation of species was within populations: 60-89% of total variation (Fig. 30). In species with high H_T,

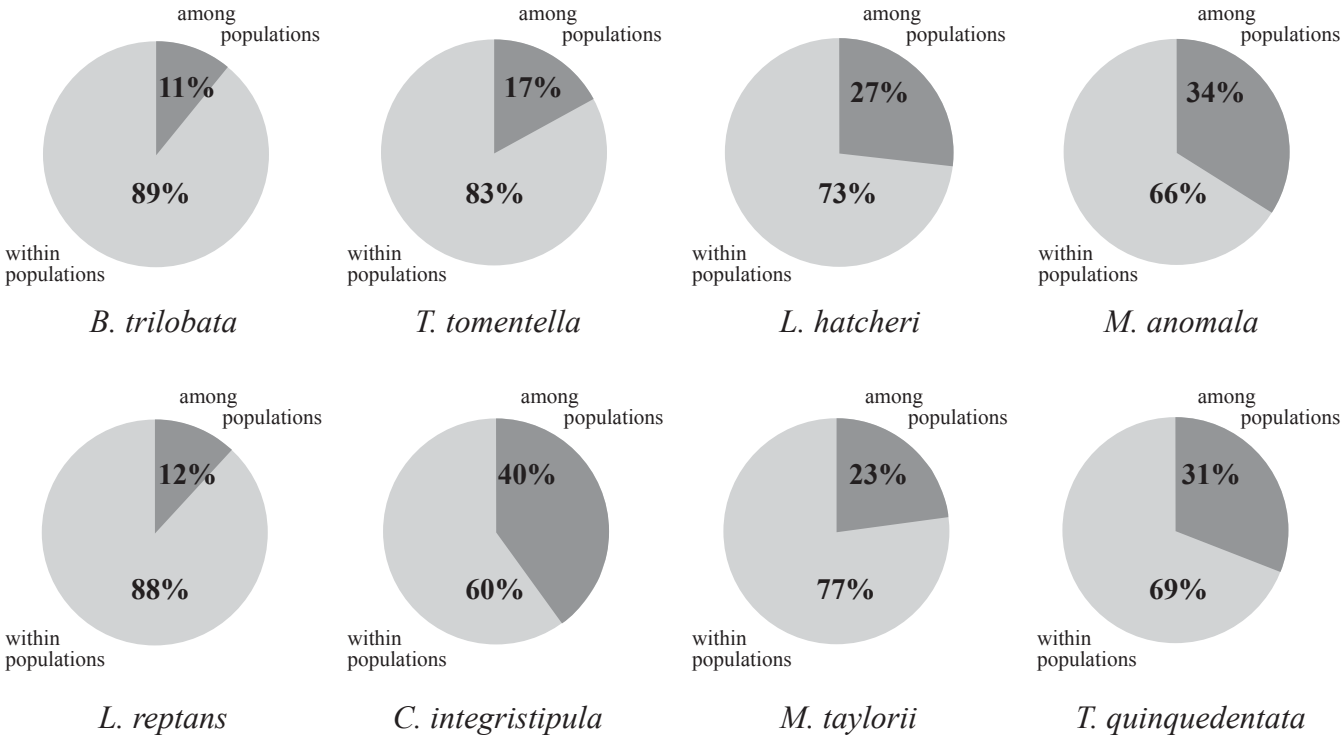

Fig. 30. Percentage contribution of genetic diversity among and within populations to total genetic diversity in the leafy liverwort species *Bazzania trilobata* (*B.t.*), *Trichocolea tomentella* (*T.t.*), *Lophozia hatchery* (*L.h.*), *Mylia anomala* (*M.a.*), *Lepidozia reptans* (*L.r.*), *Calypogeia integristipula* (*C.i.*), *Mylia taylorii* (*M.t.*), and *Tritomaria quinquedentata* (*T.q.*)

Table 75. Levels of genetic diversity in species of thallose and leafy liverworts with various modes of reproduction

Species	Markers	H_T	H_S	G_{ST}	Φ_{PT}	Reference
Thallose, dioecious, fertile						
Asterella liukiuensis	isozymes	0.272	0.064	0.681	n.d.	Itouga *et a*l.(2002)
Conocephalum conicum A	isozymes	n.d.	0.044	0.231	n.d.	Odrzykoski (1986)
Conocephalum conicum J	isozymes	0.151	0.142	0.045	n.d.	Kim *et al*. (1996)
Conocephalum conicum L	isozymes	n.d.	0.025	0.232	n.d.	Odrzykoski (1986)
Conocephalum conicum S	isozymes	n.d.	0.012	0.276	n.d.	Odrzykoski (1986)
Conocephalum conicum T	isozymes	0.217	0.090	0.584	n.d.	Akiyama & Hiraoka (1994)
Conocephalum japoniocum	isozymes	0.041	0.012	0.062	n.d.	Itouga *et al*. (1999)
Marchantia chenopoda	isozymes	0.298	0.223	0.244	n.d.	Moya (1993)
Pellia nessiana	isozymes	n.d.	0.031	n.d.	n.d.	Zieliński (1987)
Aneura pinguis A	isozymes	0.1441	0.0297	0.7939	n.d.	Bączkiewicz & Buczkowska (2005)
Aneura pinguis B	isozymes	0.1173	0.0319	0.7283	n.d.	Bączkiewicz & Buczkowska (2005)
Aneura pinguis C	isozymes	0.1287	0.0081	0.9372	n.d.	Bączkiewicz & Buczkowska (2005)
Thallose, monoecious or mostly monoecious but sometimes dioecious, fertile						
Preissia quadrata	isozymes	0.211	0.015	0.928	n.d.	Boisselier-Dubayle & Bischler (1997)
Riccia dictyospora A	isozymes	n.d.	0.024	n.d.	n.d.	Dewey (1989)
Riccia dictyospora B	isozymes	n.d.	0.076	n.d.	n.d.	Dewey (1989)
Riccia dictyospora C	isozymes	n.d.	0.072	n.d.	n.d.	Dewey (1989)
Pellia epiphylla (S)	isozymes	n.d.	n.d.	0.026	n.d.	Zieliński (1987)
Rebulia hemisphaerica	isozymes	n.d.	0.059	0.702	n.d.	Boisselier-Dubayle *et al.* (1998a)
Leafy, dioecious, fertile						
Porella canariensis	RAPD	n.d.	n.d.	n.d.	0.603	Freitas & Brehm (2001)
Ptilidium pulcherrimum	isozymes	0.168	0.155	0.374	n.d.	Adamczak *et al.* (2005)
Leafy, monoecious, fertile						
Calypogeia fissa	isozymes	0.294	0.015	0.891	0.514	Buczkowska (2004)
Caylpogeia azurea	isozymes	0.065	n.d.	n.d.	n.d.	Buczkowska (in preparation)
Leafy, only with asexual reproduction or extremely rarely fertile						
Ptilidium ciliare	isozymes	0.099	0.0736	0.263	n.d.	Adamczak *et al*. (2005)
Bazzania trilobata	ISSR	0.308	0.307	n.d.	0.532	Buczkowska *et al*. (2010)
Trichocolea tomentella	ISSR	n.d.	0.184	n.d.	0.404	Pohjamo *et al.* (2008)
	RAPD	n.d.	0.0934	n.d.	n.d.	

Explanations: H_T – total genetic diversity, H_S – genetic diversity within population, G_{ST} – coefficient of genetic differentiation, Φ_{PT} (analogous to F_{ST} and G_{ST}) – interpopulation differentiation, n.d. – no data

the percentage contribution of genetic variation among populations to total variation was much lower than in species with lower H_T. For example, the lowest percentage contribution of genetic variation among populations was in the highly variable *B. trilobata* (11%) and *L. reptans* (12%). By contrast, in species with low H_T (*C. integristipula*, *M. anomala*, *M. taylorii*), the contribution of genetic differences between populations was higher, ranging between 31% in *M. taylorii* and 40% in *C. integristipula*. In these species the fixation index was the highest (> 0.300). In spite of this, in all studied species the genetic differentiation among populations (Φ_{PT}) was statistically significant. AMOVA with division into sterile species and fertile species revealed no significant differences in Φ_{PT} (Mann-Whitney *U* test: Z=1.354; p=0.56).

4. Discussion and conclusions

4.1. Genetic diversity of leafy liverworts vs. thallose liverworts and mosses

The level of genetic diversity of leafy liverworts under study was high. The range of intra-population genetic diversity (H_S) of the study species varied between 0.0988 and 0.2473 (Fig. 29). It is thus much higher than in thallose liverworts but comparable to the genetic diversity of mosses (Tables 75 and 76) or even some species of vascular plants (Hamrick & Godt 1990). H_S values in thallose liverworts usually are considerably lower than 0.100, with only a few known exceptions, e.g. *Conocephalum conicum* species *J* (L.) Dum. (0.142) (Kim *et al*. 1996) and *Marchantia chenopoda* (L.) (0.223) (Bischler & Boisselier-Dubayle 1997). In contrast, H_S values in leafy liverworts and mosses are usually either higher than or close to 0.100. Furthermore, studies investigating the diversity of DNA markers (RAPD and ISSR) in leafy liverworts – like *Bazzania trilobata* (Buczkowska *et al*. 2010), *Trichocolea tomentella* (Pohjamo *et al*. 2008), and *Mannia fragrans* Balb. Frye & L. Clark (Hock *et al*. 2008a, 2009) – demonstrate genetic diversity levels that are similar to those of mosses. The findings show that in terms of H_S, leafy liverworts resemble mosses rather than thallose liverworts.

Total genetic diversity (H_T) at the level species in thallose liverworts, however, is not that low compared to leafy liverworts (Fig. 29, Table 75). The level of H_T

Table 76. Levels of genetic diversity in species of mosses with various modes of reproduction

Species	Markers	H_T	H_S	G_{ST}	F_{ST}	Reference
Mosses: dioecious, fertile						
Brachythecium rivulare	RAPD	0.2765	0.2166	0.2168	n.d.	Zhu *et.al*.(2007)
Breutelia pendula	isozymes	n.d.	n.d.	n.d.	0.5584	Eppley *et al*. (2006)
Hylocomiun splendens	isozymes	0.274	0.209	0.073	n.d.	Cromberg *et al.* (1997)
Hypnodendrom arcuatum	isozymes	n.d.	n.d.	n.d.	0.6089	Eppley *et al*. (2006)
Hypoptergium filiculaeforme	isozymes	n.d.	n.d.	n.d.	0.0873	Eppley *et al*. (2006)
Leucodon sciuroides	isozymes	n.d.	0.036-0.084	n.d.	0.088	Cromberg (2000b)
Macromitrium longines	isozymes	n.d.	n.d.	n.d.	0.5010	Eppley *et al*. (2006)
Meesia triquetra	isozymes	0.151	0.082	0.454	n.d.	Montagnes *et al*. (1993)
Plagiomnium ellipticum	isozymes	0.143	0.091	0.166	n.d.	Wyatt *et al*. (1992)
Plagiomnium insigne	isozymes	0.164	0.065	0.334	n.d.	Wyatt *et al*. (1992)
Plytrichadelphus magellanicus	isozymes	n.d.	n.d.	n.d.	0.5498	Eppley *et al*. (2006)
Polytrichum commune	isozymes	0.025	0.025	n.d.	n.d.	Van der Velde & Bijlsma (2000)
Polytrichum formosum	isozymes	0.038	0.034	n.d.	n.d.	Van der Velde & Bijlsma (2000
Polytrichum formosum	isozymes	0.038	n.d.	n.d.	0.099	Van der Velde *et al*.(2001)
Polytrichum juniperium	isozymes	0.166	0.123	n.d.	n.d.	Van der Velde & Bijlsma (2000
Polytrichum piliferum	isozymes	0.105	0.094	n.d.	n.d.	Van der Velde & Bijlsma (2000
Polytrichum uliginosum	isozymes	0.026	0.023	n.d.	n.d.	Van der Velde & Bijlsma (2000
Sphagnum affine	isozymes	n.d.	0.122	n.d.	0.512	Thingsgaard (2001)
Sphagnum rubellum	isozymes	0.116	0.071	0.112	n.d.	Cromberg (1998)
Mosses: monoecious, fertile						
Acrocladium chlamydophyllum	isozymes	n.d.	n.d.	n.d.	0.4860	Eppley *et al*. (2006)
Funaria hygrometrica	isozymes	n.d.	n.d.	n.d.	0.6015	Eppley *et al*. (2006)
Plagiomnium medium (2n)	isozymes	0.328	0.277	0.128	n.d.	Wyatt *et al*. (1992)
Rhynchostegium tenuifolium	isozymes	n.d.	n.d.	n.d.	0.3672	Eppley *et al*. (2006)
Syntrichia antarctica	isozymes	n.d.	n.d.	n.d.	0.7840	Eppley *et al*. (2006)
Sphagnum capillifolium	isozymes	0.144	0.118	0.152	n.d.	Cromberg (1998)
Tortula muralis	isozymes	n.d.	n.d.	n.d.	0.5829	Eppley *et al.* (2006)
Mosses: only with asexual reproduction or extremely rare fertile						
Pleurochaete squarrosa	isozymes	0.193	0.108	0.443	n.d.	Grundmann *et al.* (2007)
Pleurochaete squarrosa	ISSR	n.d.	n.d.	n.d.	0.411	Spagnuolo *et al.* (2007)

Explanations: H_T – total genetic diversity, H_S – genetic diversity within population, G_{ST} (analogous to F_{ST}) – coefficient of genetic differentiation, n.d. – no data

diversity in both groups of liverworts is more similar than H_S, though slightly lower in thallose liverworts. The H_T range in thallose liverworts is between 0.041 and 0.298, and in leafy liverworts, between 0.065 and 0.3679 (in isozymes). The fact that both groups are more similar in terms of H_T than H_S is a consequence of different models of population diversity in these liverwort groups. The majority of thallose liverwort species exhibit a lower level of intra-population genetic diversity (H_S) and great differences between populations. By contrast, leafy liverworts generally show greater H_S and lower differences between populations.

It is interesting to note that thallose liverwort species of the subclass Metzgeriidae (class Jungermanniopsida), such as *Aneura pinguis* (L.) Dum. or *Pellia* species, are characterized by a similar level of genetic diversity and a similar model of population diversity to thallose liverworts of the class Marchantiopsida, even though in evolutionary aspects they are closer to leafy liverworts (Schuster 1966; Heinrichs *et al*. 2007) (Table 75). A lower level of genetic diversity among thallose liverworts is probably a feature linked to their simpler, more primitive morphological characteristics, as pointed out by Schuster (1966). Consequently, the traditionally held view (Stoneburner *et al*. 1991; Boisselier-Dubayle *et al*. 1995) that the entire group of liverworts has a much lower level of intra-population diversity than mosses is in fact erroneous. It holds true only for thallose liverworts (Marchantiopsida and Metzgeriidae), but not for leafy liverworts (Jungermanniidae).

4.2. Relationship between mode of reproduction and genetic diversity in leafy liverworts

Leafy liverworts investigated in the present study did not exhibit significant differences in total diversity (H_T) between sterile and fertile species. Mean H_T values calculated for both groups were nearly identical (Fig. 29). Likewise, H_S values determined for both groups were similar – with mean H_S in sterile species slightly higher (0.1706) than in fertile species (0.1568). Moreover, both groups included species that had both high and low levels of genetic diversity (H_T and H_S). Therefore, the conclusion arises that whether a species has high or low diversity is not related to fertility of the species. Unfortunately, the hypothesis can only be supported by few existing studies investigating the level of diversity of bryophytes that are either completely sterile or reproduce sexually very rarely. One of them is the study by Krzakowa & Szweykowski (1979), examining peroxidase diversity in *Plagiochila asplenoides*, a leafy liverwort species which is classified as sterile in the territory of Poland (Szweykowski 2006). The study provided evidence for high genetic diversity. Unfortunately, no similarly high diversity was confirmed for other enzymatic loci, which proved to be mainly monomorphic (Zieliński & Wachowiak-Zielińska 1994). Low diversity of *P. asplenoides*, however, was not confirmed in studies based on RAPD and ISSR markers (unpublished data). Diversity detected in the studies was in fact comparable to diversity of other species of leafy liverworts, which are characterized by high isozymatic and DNA-level diversity, like *B. trilobata* (Buczkowska *et al*. 2010) and *T. tomentella* (Pohjamo *et al*. 2008). Another example of high genetic diversity in a species that produces sporophytes only occasionally is the moss *Pleurochaete squarrosa*. High genetic diversity was demonstrated both in isozymes and in ISSR and cpDNA markers (Grundmann *et al*. 2007; Spagnuolo *et al.* 2007).

In the study presented here, fertile monoecious, and dioecious species also exhibited similar genetic diversity, but the mean total diversity (H_T) for dioecious species was about 0.055 higher than for monoecious species (Fig. 29). Anyway, the most diverse (*T. quinquedentata*) and the least diverse (*M. taylorii*) species were dioecious. Researchers who investigated moss species differ in their opinions on genetic variation in monoecious and dioecious mosses. Some authors (e.g. Wyatt *et al*. 1992) believe that higher diversity is demonstrated in monoecious species, whereas others, in dioecious species (e.g. Cromberg 1996). My results and other studies of bryophytes indicate that there is no simple correlation between modes of reproduction and the level of genetic diversity in bryophytes. Moreover, it seems that the effect of reproduction mode on genetic diversity in bryophytes is lower than in vascular plants (Hamrick & Godt 1990).

4.3. Factors affecting diversity levels in bryophytes

Factors affecting the genetic diversity of bryophytes are an interesting research problem. Why similarly high diversity levels are observed both in sterile species (e.g. *B. trilobata*, *T. tomentella*) and in fertile species (e.g. *T. quinquedentata*, *L. hatcheri*) and why monoecious and dioecious species demonstrate similar genetic diversity?

My result confirm theory, based on studies by Kimura (Kimura 1968; Kimura & Ohta 1971), that the main causes underlying genetic diversity of bryophytes are neutral (or nearly neutral) somatic mutations developing in various vegetative parts of plants (e.g. Mischler 1988; Wyatt 1994; Skotnicki *et al*. 1999; Stenøien 1999; Wolf *et al*. 2000; Cromberg *et al*. 2003; Van der Velde & Bijlsma 2003; Pohjamo *et al*. 2008). Bryophytes, and liverworts in particular, have a high frequency of clonal reproduction (Anderson 1963; Longton 1976). New gametophytes usually arise by means of thallus regeneration or vegetative propagules. The separation of branches or other plant sections with somatic mutations, followed by the growth of new shoots, can increase the

level of genetic diversity. Even if the mutation rate is low, as plants grow, the number of mitotic divisions occurring in vegetative plant sections is so great that it can explain high genetic diversity levels (Mischler 1988). The process can account for the correlation observed between asexual reproduction patterns in liverworts and their high level of genetic diversity. It can also explain high genetic diversity observed among sterile liverworts, such as *B. trilobata*, *L. hatcheri* and *T. tomentella*. In light of this, it seems that in liverworts vegetative reproduction has more influence on the level of genetic diversity than recombination. It thus follows that the level of genetic diversity in leafy liverworts is a function of the somatic mutation rate and, consequently, of factors like the capacity of species to accumulate somatic mutations and to spread, plant lifespan, and accidental genetic drift. Moreover, these factors (accumulation of somatic mutations, capacity to spread, plant lifespan scale) can also – at least partially – account for the fact that different species exhibit various levels of genetic diversity. Wyatt *et al*. (1993) suggested that low diversity observed among some species can be related to factors that go back to the Ice Age. At that time alleles could have been "lost" in refugia as a consequence of genetic drift, as the populations were shrinking. In the post-glacial era, as ice sheets retreated and ice-free areas were rapidly colonized, the level of genetic diversity of some species might have been reduced as a result of the bottleneck (Hewitt 1996). Because liverworts are pioneering plants and have a high colonization rate (Szweykowski 1984; Klama 2002; Snäll *et al*. 2005), it is a likely assumption that they spread across large distances at that time. Given favourable conditions, relatively small colonies could have grown into large populations which, however, retained their low diversity (Hewitt 1996). The mechanism can be responsible for low genetic diversity observed in species like *M. taylorii* or *C. integristipula* – even though they include both sexual and vegetative reproduction patterns. However is not unlikely, that the real cause of low diversity in some species of liverworts may be completely another.

A theory proposed by Zukerkandl & Pauling (1965) states that mutation rates are similar in closely related species. That theory is confirmed by patterns observed in 2 species of the genus *Mylia*: *M. taylorii* (fertile) and *M. anomala* (sterile). They both show low levels of genetic diversity, but the fertile species *M. taylorii* has slightly higher diversity (H_T and H_S) than the sterile species *M. anomala*. Similar genetic diversity patterns were also demonstrated in species of the genus of the genus *Calypogeia* (unpublished data) and *Ptilidium* (Adamczak *et al*. 2005). The mean genetic diversity of both species is moderate, though again slightly higher in the fertile species *P. pulcherrimum* than in the sterile species *P. ciliare*. Both genera, *Mylia* and *Ptilidium*, show a higher level of genetic diversity in their fertile species. It needs to be added, though, that the fertile species grow in more variable environments. *M. anomala* has a wider range (being found both in the mountains and in lowland areas) but it is confined exclusively to peatlands. In contrast, *M. taylorii*, even though it grows in the mountains, can be found in forests and in the subalpine zone, on rotten wood and also in peatlands. By the same token, *P. pulcherrimum* – compared to *P. ciliare* – is ecologically more versatile, as it grows frequently as an epiphyte on the bark of trees, on rotten wood, and directly on rocks (Szweykowski 2006).

It is difficult to pinpoint a single factor affecting the level of genetic diversity: fertility or greater environmental variability. Both factors are likely to be involved in the process. A study by Wyatt *et al.* (1989b), on related species of the moss genus *Plagiomnium*, shows that environmental versatility of the species is a factor that influences genetic diversity of species more prominently than recombination in respect of differential selection pressures between populations. The 2 most diverse species examined in the present study (*T. quinquedentata* and *B. trilobata*) indeed grow in very versatile habitats and in different climatic conditions, so this confirms Wyatt's theory. *T. quinquedentata* is growing on basic substrates, such as sandy, calcareous soil, basic rock walls, and boulders, but it occurs in all altitudinal zones. *B. trilobata* occurs on shaded places, on decaying logs, on humus or peaty soil, and as a pioneer on acid dry rocks and even on the bark of trees. It is growing in mountains and in lowland regions.

It should thus be concluded that related species of leafy liverworts probably have a similar rate of mutations. However, species with a wider ecological range exhibit higher genetic diversity because the variability of habitats can influence the rate and type of somatic mutations. Accordingly, species inhabiting more diverse environments may be more genetically diverse. In fertile species (*P. pulcherrimum* or *M. taylorii*) recombination is yet another factor promoting higher genetic diversity.

4.4. Intra-patch diversity

Patches of the study species generally were not formed by single clones but usually consisted of several genotypes (MLGs). The mean number of genotypes in patches was markedly smaller in species of low diversity than in species of higher diversity – regardless of the method of reproduction (Fig. 26). The most diverse patches were found in *B. trilobata* and *T. quinquedentata*, and the least diverse patches, in *M. anomala* and *C. integristipula*. This indicates that the general level of diversity in patches was directly proportional to the total genetic diversity of species (H_T).

Patches consisting of a single genotype were very rare, but they were still detected in nearly all study

species (except *T. tomentella* and *L. reptans*), even in the most diverse ones. In species with low diversity the number of single-genotype patches was much greater. By nevertheless, very diverse patches, where *G*/*N* reached 1.0, were even less numerous. Overall, there were only 6 of them in a total of 593 patches under analysis. They were only found in 4 most diverse species: *B. trilobata*, *T. quinquedentata*, *T. tomentella*, and *L. reptans*. Most patches consisted of several genotypes, usually 2-5 MLGs.

As mentioned above, 2 types of distribution of genotypes in patches were noted. Patches of species with low genetic diversity (*C. integristipula*, *M. taylorii*, *M. anomala*) were often dominated by 1-2 genotypes that constituted most of a patch (plants for analysis were collected uniformly from the entire patch). In patches of species with higher H_T diversity there was no tendency for predomination of single genotype, and different genotypes constituted similar proportions of a patch. Dominant genotypes in patches of these species were encountered very infrequently.

The predomination of individual genotypes in patches was also noted in other bryophytes, e.g. in *Preissia quadrata* (Scop.) Nees (Boisselier-Dubayle & Bischler 1997), *Mania fragrans* (Hock *et al*. 2008b), *Sphagnum rubellum* (Wils.) (Cromberg 1998). In all these species, however, the cited authors detected low diversity at the species level. That finding, in turn, confirms that the predomination of a single genotype in a patch is typically associated with species of low genetic diversity.

The level of genetic diversity in patches can be affected by a number of factors. First of all, individual patches could be formed by a different number of spores (Krzakowa & Szweykowski 1979; Szweykowski 1984; Show 2000). Secondly, patches could be formed with diaspores found in the soil. Diaspores (spores, vegetative propagules, and thallus fragments) can remain in soil for a long time and be an important source of new genotypes (e.g. During *et al*. 1988; Jonsson 1993; Hock *et al*. 2008a). The diaspore bank in soil can function as a "genetic memory" and represent an evolutionary potential of the population. Thanks to diaspores present in soil after a periodic disappearance of liverworts in a given area (Krzakowa & Szweykowski 1979; Szweykowski 1984; Klama 2002), newly emerged patches can exhibit a high degree of diversity from the very outset. The next 2 factors are patch age and recombination level (the latter for fertile species). The rate of recombination per locus in bryophytes is very low, close to zero, both in monoecious and dioecious species (Stenøien 1999; Stenøien & Såstad 2001). Furthermore, it is thought that a major part of spores released by bryophytes fall into the same patch where (due to high density and lack of room) the germination rate for new spores is very low (Longton & Schuster 1983; Roads & Longton 2003; Hock *et al*. 2008b) Finally, if it were to be assumed that the main factor responsible for diversity in bryophytes are somatic mutations, diversity identified in patches would also depend on the number of mutations in the thalli and on the capacity of the species to spread by thallus regeneration and vegetative propagules. This hypothesis can be corroborated by the fact that in the study species – both sterile and fertile – a large part of genotypes (MLGs) in patches demonstrated a high degree of similarity. The genotypes only differed in 1-2 genes. Since liverworts reproduce mostly vegetatively (Anderson 1963; Longton 1976), there are grounds to assume that gametophytes with such slight changes can come from a single individual – with variation resulting from somatic mutations.

4.5. Repeatability of the same genotypes in various patches

In all studied species there was a high degree of repeatability of the same genotypes (MLGs). Genotypes repeated not only within the same patch but also in various patches within the same population and even in various populations. Sometimes the populations were located many kilometres apart. For example, in *L. reptans* or *C. integristipula* the most common genotype was identified in all the geographic regions in which samples were taken (the populations were located both in the mountains and in lowland regions) (Figs. 5-6). In species characterized by low diversity, the repeatability of genotypes was much greater than in species with a high degree of diversity. The most common genotypes occurred in many plants. For example, in *M. anomala* the most common genotype was detected in 91 specimens, in 17 patches from 5 populations (located in the Pomeranian and Suwałki Lake Districts). The genotype was identified in 16.5% of all gametophytes under study. A markedly lower repeatability was noted in more diverse species. For example, in *B. trilobata* the most popular genotype was found in only 10 gametophytes from 2 populations, while in *T. quinquedentata* the most common genotype was detected in as many as 5 populations, but only in 13 plants.

There can be several causes of repeatability of the same genotypes in various populations. It can, for example, be a consequence of gene flow over large distances. The theory of large gene flow (in the form of diaspores) over considerable distances, however, has been losing popularity recently (Lienert 2004). In the case of bryophytes, the majority of diaspores spread across a distance of several – to around a dozen – centimetres from parent plants, whereas only some of them travel over larger distances (e.g. Miles & Longton 1992; Bischler & Boisselier-Dubayle 1997; Hock *et*

al. 2008a). Numerous studies of bryophytes show that repeatability of genotypes is common, as it has been observed in many species (e.g. Wachowiak-Zielińska & Zieliński 1995; Shaw & Srodon 1995; Cromberg *et al*. 1997; Thingsgaard 2001; Adamczak *et al*. 2005; Wyatt *et al*. 2005). For example, the most common genotype for *Sphagnum affine* Ren. & Card. was identified in 68% of populations (Thingsgaard 2001). In the leafy liverwort *Porella platyphylla* (L.) Pfeiff. it was found in 7-26 MLGs in a population of ca. 100 individual plants (Wyatt *et al*. 2005). Similarly high repeatability of MLGs has been noted in 2 species of the genus *Ptilidium*. In *P. ciliare* a total of 15 MLGs were identified in 8 populations, whereas in *P. pulcherrimum* a total of 25 MLGs were found in 11 populations growing many kilometres apart (Adamczak *et al*. 2005). In view of these facts, the transport of thallus fragments and other diaspores on such a large scale, over large distances, seems rather improbable. It is certain that some of the repeated genotypes could be a result of an accidental appearance of the same alleles in different specimens, especially if the genotype is composed of high-frequency alleles. Otherwise, the repetition of genotypes can also stem from historical links existing between different populations. The causes discussed above, however, fail to account fully for the fact that the phenomenon is so widespread.

In my opinion, probability the main cause of high genotype repeatability rates is the independent repeatability of the same mutations in different specimens. Because different species have varying degrees of diversity, it can be linked to different tolerance of species to genome mutations. Low-diversity species exhibit a lower tolerance to new mutations, so the repeatability of the same mutations in different patches seems greater in this group of species.

4.6. Inter-population differentiation in liverworts

Many studies investigating bryophytes have shown that there are 2 basic patterns of genetic variation. The majority of bryophytes exhibit a pattern where in the total genetic diversity of species the share of genetic diversity within populations is greater than between populations (e.g. Wyatt *et al.* 1989a; Cromberg *et al*. 1997; Van der Velde *et al*. 2001; Itouga *et al*. 2002; Bączkiewicz & Buczkowska 2006; Grundmann *et al*. 2007; Zhu *et al*. 2007; Buczkowska *et al*. 2010). The second pattern, encountered less frequently, is the opposite, i.e. the share of genetic diversity between populations is greater than the share of diversity within populations in the total genetic diversity of species (e.g. Zieliński & Wachowiak-Zielińska 1994; Bischler & Boisselier-Dubayle 1997; Boisselier-Dubayle & Bischler 1997; Cromberg 1998; Freitas & Brehm 2001; Buczkowska 2004). My studies and some of the earlier investigations on leafy liverworts show that in this group the first pattern predominates (Adamczak *et al*. 2005; Bączkiewicz & Buczkowska 2006; Pohjamo *et al*. 2008; Buczkowska *et al*. 2010). The second pattern has only been identified rarely, e.g. in *Calypogeia fissa* (Buczkowska 2004).

The percentage share of differentiation between populations (Φ_{PT}) (Fig. 30) in the total genetic diversity of species of leafy liverworts was largely correlated with the level of genetic diversity of the species (H_T) (Fig. 29). In species with high total genetic diversity (H_T), differences between populations tend to be rather small. The smallest percentage share of differentiation among populations in the total diversity of species was noted in *B. trilobata* (11%) and in *L. reptans* (12%). In species with lower total genetic diversity, the percentage share of differentiation among populations in the total diversity of species was much higher, between 31% and 40% (*C. integristipula*, *M. taylorii* and *M. anomala*).

The correlation between the level of total genetic diversity (H_T) with the percentage share of differentiation between population is also noted in thallose liverworts. Thallose liverworts exhibit low genetic diversity (e.g. Zieliński 1984; Stoneburner *et al*. 1991; Boisselier-Dubayle & Bischler 1997; Itouga *et al*. 2002), lower than leafy liverworts (Table 75). Consequently, the inter-population differentiation is here high and in this group the second pattern of genetic diversity is usually observed. The authors who detected large differences between thallose liverwort populations explained them typically with limited spreading of spores, major role played by asexual reproduction, and genetic drift. These explanations seem improbable because populations of leafy liverworts are affected by the same factors. Moreover, some of the species of leafy liverworts are sterile or reproduce sexually very infrequently (e.g. *B. trilobata*, *T. tomentella*), and yet the first model is dominant. In the second pattern of diversity, a big role in high genetic differentiation between populations can be played by adaptation to the microhabitat (Wyatt *et al*. 1989b; Stenøien 1999). Large differences among populations result from adaptation to different environmental conditions and effects of different selection pressures. The theory can hold true if there are large differences between environments or niches, while gene flow is limited. What is more, in this case, selection affects genes that influence morphological and physiological traits rather than neutral enzymatic genes, and the majority of isozymatic loci in bryophytes (Stenøien 1999), including leafy liverworts (Tables: 15, 23, 31, 39, 63, 47, 55, 63, 71), are selectively neutral. Currently, there is no evidence supporting the claim that environmental adaptation plays an important role in the differentiation of bryophytes, since the adaptative role is rather due to

high phenotypic plasticity, specific to this plant group (Bischler Boisselier-Dubayle 1997; Cromberg *et al*. 1997).

However, the role of environmental adaptation in the differentiation of bryophytes cannot be ruled out completely because genes that are neutral in one environment can become less favourable or completely unfavourable in other environmental conditions. The theory can be corroborated by the existence of private alleles that are characteristic for populations or even whole geographic regions (Fig. 22). In leafy liverworts a higher number of private alleles were identified in low-diversity species. It is debatable whether they are involved in environmental adaptation, but private alleles certainly contribute to the larger differences in low-diversity species, compared to species displaying higher diversity.

4.7. Conclusions

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

1. The level of genetic diversity of leafy liverworts is much higher than in thallose liverworts and comparable to the genetic diversity of mosses or even some species of vascular plants.
2. The effect of reproduction mode on genetic diversity in leafy liverworts is lower than in vascular plants, because sterile and fertile species exhibited similar level of genetic diversity and within fertile species monoecious and dioecious also exhibited similar genetic diversity.
3. The main causes underlying genetic diversity of bryophytes are neutral (or nearly neutral) somatic mutations developing in various vegetative parts of plants. The separation of branches or other plant sections with somatic mutations, followed by the growth of new shoots, can increase the level of genetic diversity.
4. Related species have a similar rate of mutations, however, species with a wider ecological range exhibit higher genetic diversity because the variability of habitats can influence the rate and type of somatic mutations.
5. In leafy liverworts is a high degree of repeatability of the same genotypes – not only within the same patch but also in various patches within the same population and even in various populations. Probably the main cause of high genotype repeatability rates is the independent repeatability of the same mutations in different specimens.
6. The majority of leafy liverworts exhibit a pattern where in the total genetic diversity of species the share of genetic diversity within populations is greater than between populations.
7. Patches of leafy liverworts generally were not formed by single clones but usually consisted of several genotypes. The mean number of genotypes in patches was markedly smaller in species of low total genetic diversity (H_T) than in species of higher diversity – regardless of the method of reproduction. Patches with predomination of a single genotype are typically associated with species of low genetic diversity.

Acknowledgements. I sincerely thank Dr Katarzyna Buczkowska for extensive help in this study and for many valuable remarks. I am also grateful to Prof. Lech Urbaniak and Prof. Wiesław Prus-Głowacki for interest in my work and many valuable comments. I wish to thank my colleagues from the Department of Genetics at the Adam Mickiewicz University and Institute of Experimental Biology for helpful discussions. I thank Prof. Henryk Klama for help in field research. I would like to thank Ms Patrycja Gonera for technical assistance and Mrs Sylwia Ufnalska for improvement of the English manuscript. I thank the Directors of the Tatra National Park, Białowieża National Park, and Bieszczady National Park for support provided during the field work. Finally, I thank my family for their support. This work was partly supported by grant no. 0503/PO4/2005/29 from the Ministry of Science and Higher Education, Warsaw, Poland.

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