VILNIUS UNIVERSITY

INA ŠPAKAITĖ

MORPHOLOGY, ECOLOGY AND PHYLOGENY OF CYANOBACTERIA BELONGING TO GENERA *NOSTOC* AND *DESMONOSTOC* IN LITHUANIA

Summary of doctoral dissertation

Biomedical sciences, botany (04B)

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The dissertation will be defended at the public session held by the Council for Biomedical Sciences on 12th September 2014 at 2:00 p.m. in the Great auditorium of the Faculty of Natural Sciences of Vilnius University.

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VILNIAUS UNIVERSITETAS

INA ŠPAKAITĖ

LIETUVOS *NOSTOC* IR *DESMONOSTOC* GENČIŲ MELSVABAKTERIŲ MORFOLOGIJA, EKOLOGIJA IR FILOGENIJA

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Disertacija bus ginama viešame Biomedicinos mokslo krypties tarybos posėdyje 2014 m. rugsėjo mėn. 12 dieną 14:00 val. Vilniaus universiteto Gamtos mokslų fakulteto Didžiojoje auditorijoje.

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INTRODUCTION

Cyanobacteria are one of the world's oldest photosynthetic prokaryotic organisms with great ecologic significance to various terrestrial and aquatic ecosystems (WHITTON & POTTS, 2000). One of the main directions and issues relating to the scientific studies of cyanobacteria is the knowledge of cyanobacteria diversity and taxonomy (KOMÁREK, 2011). *Nostoc* Vaucher ex Bornet et Flahault 1886 species can be found in all of the Earth's continents, often spread abundantly in terrestrial and aquatic ecosystems, and are among the main organisms in most extreme and nutrient-poor habitats (GEITLER, 1932; POTTS, 2000). Cyanobacteria *Desmonostoc* Hrouzek et Ventura are, apparently, also widely spread all over the world, mostly found in various terrestrial habitats, except for arctic or hot desert regions (HROUZEK et al., 2013). *Nostoc* and *Desmonostoc* cyanobacteria fixate molecular nitrogen, can grow in nitrogen-low environment, and are source of nitrogen compounds for plants and algae in terrestrial and aquatic ecosystems. They also have various symbiotic relationships with plants (*Gunnera*, cycadean, moss), while *Nostoc* species have such relationships with fungi too (*Geosiphon pyriformis*), and they are phycobionts of lichens (DODDS et al., 1995; HROUZEK et al., 2013).

Modern complex (phylogenetic and morphological) research methods have led to the conclusion that *Nostoc* genus is heterogeneous (GEITLER, 1932), i.e. made of several different monophyletic groups that should be distinguished as new genera (LACHANCE, 1981; HROUZEK et al., 2005; KOMÁREK, 2010 a). Following the phylogenetic studies of *Nostoc* genus, ŘEHÁKOVÁ et al. (2007) described the cyanobacterial genus *Mojavia* Řeháková et Johansen, and later HROUZEK et al. (2013) distinguished genus *Desmonostoc*. The monophyletic group of *Nostoc* species is typically indicated as *Nostoc commune* or *Nostoc sensu stricto* group in the phylogenetic analysis (ŘEHÁKOVÁ et al., 2007; CUZMAN et al., 2010; HROUZEK et al., 2013). According to the data of KOMÁREK (2010 a), about 60 morphological *Nostoc* species and strains are known so far, most of which have not been precisely described. Identification of *Nostoc* species still remains problematic due to the heterogenic feature of the genus, insufficient features to characterise the species, complicated life cycle which is rarely described in classical studies. Most of the *Nostoc* species are critical and require more detailed taxonomic studies (GEITLER, 1932; ELENKIN 1938; KONDRATYEVA & KISLOVA, 2002; KAŠTOVSKÝ et al., 2010). For most scientists the main objective of the study is the complex research of cyanobacterial diversity and taxonomy based on the morphological, molecular, and ecological methods.

In Lithuania, no detailed studies of the cyanobacterial genus *Nostoc* have been carried out so far, therefore very little data is collected on the diversity, biology, and ecology of *Nostoc* species. Sparse information on the studies of the Lithuanian *Nostoc* species can be found in early works of botanists (GILIBERT, 1781; S. B. JUNDZIŁŁ, 1791; 1811; J. JUNDZIŁŁ, 1830; PABRIEŽA, 1900; SOKOŁOWSKA-RUTKOWSKA, 1932;

MATUSZKIEWICZÓWNA, 1934). Afterwards terrestrial *Nostoc* species were analysed by POCIENĖ (1960; 1961; 1981), POCIENĖ & INDRAŠYTĖ (1977). Several freshwater *Nostoc* species are mentioned in the studies performed by JANKAVIČIŪTĖ (1996) and KAROSIENĖ & KASPEROVIČIENĖ (2009). And also, no phylogenetic research of *Nostoc* and other *Nostocales* species have been carried out.

The aim of the study was to investigate the morphology, ecology and phylogeny of the cyanobacteria belonging to genera *Nostoc* and *Desmonostoc* in Lithuania.

Objectives of the study:

1) to collect samples of *Nostoc* and *Desmonostoc* species in different regions of Lithuania;
2) to identify and analyse *Nostoc* and *Desmonostoc* samples collected and stored at Vilnius University;
3) to perform a morphological analysis of *Nostoc* and *Desmonostoc* species, to evaluate stability of the morphological features and their diagnostical suitability;
4) to analyse the reproduction and developmental cycles of *Nostoc* and *Desmonostoc* species;
5) to isolate *Nostoc* and *Desmonostoc* strains and perform their morphological analysis;
6) to compile an expanded checklist of Lithuanian *Nostoc* and *Desmonostoc* species based on the original morphometric data and pictures;
7) to apply a molecular approach to the diversity of *Nostoc* and *Desmonostoc* species;
8) to investigate the ecology of *Nostoc* and *Desmonostoc* species *in situ*.

Statements for defence.

1. Diversity of *Nostoc* species in Lithuania is wider than it was presumed before this study.
2. Using of natural populations and cultures in morphological analysis of species of *Nostoc* and *Desmonostoc* genus is significant for identification of species and evaluation of suitability of diagnostic morphological features. Morphological features of *Nostoc* and *Desmonostoc* species are sufficient to identificate only some of cyanobacterial species.
3. Lithuanian *Nostoc* species are not only morphologically, but also phylogenetically heterogenic organisms.

Scientific novelty of the study. For the first time in Lithuania, detailed investigations were carried out on the diversity, taxonomy, biology and ecology of freshwater and terrestrial *Nostoc* and *Desmonostoc* species. Different types of samples (natural population and cultures) were analysed to obtain an accurate diagnosis of the species. An expanded checklist was compiled of 40 *Nostoc* and 2 *Desmonostoc* taxa with original morphological and ecological data as well as pictures. Ten freshwater and terrestrial *Nostoc* species, two *Nostoc* species forms and two *Desmonostoc* species were recorded in Lithuania for the first time. During the research, 17 *Nostoc* and two *Desmonostoc* strains were isolated. The molecular-phylogenetic approaches were applied to study the diversity and taxonomy of *Nostoc* and *Desmonostoc* species in Lithuania.

Significance of the study. The complex research of the *Nostoc* and *Desmonostoc* genera cyanobacteria provided new data on the diversity and ecology of algae in Lithuania. An expanded checklist of cyanobacteria *Nostoc* and *Desmonostoc* may be included into the Lithuanian Algae Flora Book. The detailed analyses of morphological features and morphometrical data, abundant pictures of important features and stages of life cycles, and ecological data are important for the *Nostoc* and *Desmonostoc* species identification. The morphological, molecular-phylogenetic and ecological analyses reveal a heterogenity of Lithuanian *Nostoc* species and are important and useful for further taxonomic studies of *Nostocales* cyanobacteria. Isolated *Nostoc* and *Desmonostoc* strains may be used in chemotaxonomic and biochemical approaches of cyanobacteria.

Presentation of the results. The results of the research were presented at five international scientific conferences: „Mokslas – Lietuvos ateitis“ (Science – Future of Lithuania) (Vilnius, Lithuania, 2009); „Research and Conservation of Biological Diversity in Baltic Region“ (Daugavpils, Latvia, 2009); „XI Young Systematists‘ Forum“ (London, United Kingdom, 2009); „Taxonomy the Queen of Science – the Beauty of Algae“ (Krakow, Poland, 2010); „18th Symposium of the International Association for Cyanophyte Research“ (České Budějovice, Czech Republic, 2010).

Volume and structure of the disertation. The dissertation is written in Lithuanian and includes Abbreviations, Introduction, Literature review, Research material and methods, Results, Discussion, Conclusions, References (197), Appendix (49 figures, 1 table). The dissertation is iliustrated with 18 figures, 9 tables. Volume of the dissertation is 228 pages (excluding appendix).

MATERIALS AND METHODS

The research was carried out in 2005 and in 2009–2011. The material of collected cyanobacteria by other researchers (1980–2010) was used as well. The material was collected in 21 administrative districts of Lithuania. A total of 230 planktonic, metaphytic, benthic and 62 terrestrial (soil, epiphytic) algae samples were collected and analysed. Cyanobacteria *Nostoc* and *Desmonostoc* were investigated in 49 freshwater (25 lakes and reservoirs, 13 ponds, 9 rivers, one spring and wetland) and 60 land habitats (roadsides, tracks, meadows, littoral of water bodies and other habitats). The collected samples were preserved with 40% formaldehyde (4% concentration in the final sample) and some of terrestrial samples were desiccated. The temperature, pH and conductivity of water bodies were measured *in situ* with a portable universal metres (HANNA HI991300, HANNA HI98127 and HANNA DisT WP 3).

Twenty samples of freshwater and terrestrial cyanobacteria were cultured in spring water at room temperature and natural light. Nineteen cyanobacterial strains were isolated from samples collected in 14 different habitats (11 freshwater and three terrestrial). The

isolation of cyanobacteria was performed on agar-solidified (1.0% w/v) BG-11_0 media (RIPPKA et al., 1979). The enrichment of 25 cyanobacterial samples (14 freshwater and 11 terrestrial) was carried out in liquid media BG-11_0 applying molecular fingerprinting (TGGE) and morphological approaches. The enrichement of cultures and isolation of cyanobacteria was performed at the Institute of Ecosystem Study (National Research Council, Firenze, Italy) in collaboration with Dr. S. Ventura, Dr. C. Mascalchi, Dr. C. Sili and at the Department of Botany and Genetics (Vilnius University).

The morphological analysis of *Nostoc* and *Desmonostoc* species was carried out by analysing different type specimens: natural population (NP), spring water cultures (SW), enrichment cultures (EC), and strains (S). All samples were examined using Olympus CH40, Olympus BX51 light microscopes, and Olympus SZ61 stereomicroscope. The morphological features of cyanobacteria were microphotographed using Nikon E 4500, QImaging Micropublicher 3.3 RTV and Imaging Go-3 digital cameras. For identification of *Nostoc* and *Desmonostoc* species the following references were used: GEITLER (1932), ELENKIN (1938), HOLLERBACH et al. (1953), DESIKACHARY (1959), KONDRATYEVA (1968), WHITTON (2002), PEREIRA et al. (2005) and HROUZEK et al. (2005; 2013). The following features were analysed to describe the morphology of cyanobacteria: size, shape, consistence, mucilaginous envelope, surface and colour of colonies; length, shape and arrangement of filaments; types and colour of mucilaginous sheaths; shape, size, granulation and colour of vegetative cells; gas vesicles; shape and size of heterocytes; akinete-like cells (AKLC); shape, size and granulation of akinetes; abundance of akinetes within trichomes; solitary cells, hormogonia and other reproductive structures. Overall, 100 or less were used measurements for vegetative cells, heterocytes and akinetes dimensions.

The statistical data analysis was performed using PAST 2.03 software package (HAMMER et al., 2001). Kruskal-Wallis criterion, ANOSIM test, cluster and principal component analyses (PCA), non-metric multidimensional scaling (nMDS) model were employed to analyse the data of the research.

The diversity of 25 collected cyanobacterial samples and 16 strains (13 freshwater and 3 terrestrial) were investigated using molecular fingerprint (TGGE, *Temperature gradient gel electrophoresis*) and 16S rRNR gene sequence analyses respectively. The total community DNA of natural samples and strains DNA were extracted with PowerPlant^{TM} DNA Isolation Kit (Mo Bio Laboratories Inc.).

The cyanobacterial 16S rRNA gene fragment (approximately 420 bp) was amplified by the polymerase chain reaction applying TGGE analysis. The first and second PCR were performed with cyanobacterial specific primers CYA359F+GC and CYA781R (a) (NÜBEL et al., 1997). The TGGE technique was used with a TGGE MAXI systema (Biometra, Germany) following the manufacturer's instructions. The temperature gradient for sequence-specific separation of PCR products was 42–52 ^{o}C. Individual TGGE bands were cut from gel (WATANABE et al., 1998) and amplified with the same primers. The

CYA359F primer was modified by taking out the clamp (GC-rich 5' end). TGGE band sequences were determined by BMR Genomics (Padua, Italy) using primers CYA359F+GC and CYA781R (a).

The 16S rRNA gene sequences of cyanobacterial strains were obtained from a DNA fragment amplified with universal primers 16S27F and cyanobacterial specific primer 23S30R (EDWARDS et al., 1989; TATON et al., 2003). Sequencing of the full-length 16S rRNA gene was carried out with primers 16S979F, 16S544R and 16S1092R (HROUZEK et al., 2005) by BMR Genomics.

The chromotograms were analysed by the gauntlet HRED/HRAP/CONSED (GORDON et al., 2004) for both TGGE and 16S rRNA analyses. The consensus sequences were imported in ARB (LUDWIG et al., 2004), which was used for the subsequent analytical steps. Most similar sequences included in the analysis were retrieved from SILVA database (http://www.arb-silva.de, PRUESSE et al., 2007). The SINA aligner from the same website was also used to produce the sequence alignment for the ARB software. The phylogenetic relationships of the sequences were constructed by neighbour-joining (NJ; TGGE) and maximum parsimony (MP, 16S rRNA analysis) methods using the ARB software. NJ and MP trees were bootstrapped 1000 times.

GENERAL CHARACTERISTICS OF HABITATS

The investigation of cyanobacteria diversity and ecology was carried out in various Lithuanian freshwater and land habitats. The water temperature, pH, and conductivity were measured *in situ*. Mostly cyanobacteria was identified in 1.66–582.2 ha lakes and reservoirs (51 % of the total water bodies) with pH ranged between 6.0 and 8.7, water temperature – 16.0–25.0 ^{0}C, and conductivity – 78–390 µS/cm. Small ponds and wetland (0.0004–0.52 ha; 28.6 %) indicated water pH to be at 7.1–8.4, temperature at 17.0–26.0 ^{0}C, while conductivity varied from 220 to 580 µS/cm. The investigated first-third order of rivers and spring (20.0 % of the total water bodies) had water pH ranging between 7.5 and 8.6, temperature of 10.0–21.0 ^{0}C, and conductivity of 200–375 µS/cm.

During the research, samples of terrestrial *Nostoc* and *Desmonostoc* cyanobacteria were collected in different geographic areas in Lithuania with different climate characteristics (average annual temperature 5.5–7.5 ^{o}C, average annual precipitation 550–800 mm) (BASALYKAS et al., 1958; BUKANTIS,1994) and different types of soil (from sandy to loam soil) (LIEKIS et al., 2001).

RESULTS AND DISCCUSION

Diversity of *Nostoc* and *Desmonostoc* species

During present study 20 *Nostoc* species and two intraspecific taxa were identified – *N. caeruleum*, *N. carneum*, *N. commune*, *N. edaphicum*, *N. ellipsosporum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. kihlmanii*, *N. linckia*, *N. paludosum*, *N. passerinianum*, *N. piscinale*, *N. pruniforme*, *N. punctiforme*, *N.* cf. *sphaericum*, *N.* cf. *sphaeroides*, *N. spongiaeforme*, *N. verrucosum*, *N.* cf. *wollnyanum*, *N. cuticulare* f. *ligericum* and *N. cuticulare* f. *polymorphum*. And also, 18 examples were identified to the genus level because of several reasons: sparse samples, their discrepancy to the taxa described in the sources of literature, or insufficient morphological features. Out of the previously known 11 *Nostoc* species and intraspecific taxa in Lithuania (GILIBERT, 1781; J. S. JUNDZIŁŁ, 1791; 1811; B. JUNDZIŁŁ, 1830; PABRIEŽA, 1900; SOKOŁOWSKA-RUTKOWSKA, 1932; MATUSZKIEWICZÓWNA, 1935; POCIENĖ & INDRAŠYTĖ, 1977; POCIENĖ, 1960; 1961; 1981; JANKAVIČIŪTĖ, 1996; KAROSIENĖ & KASPEROVIČIENĖ, 2009), only *N. microscopicum* and *N. punctiforme* f. *populorum* were not recorded in the present study. Compared to the data obtained in the cyanobacteria studies in other countries of the world (SKINNER & ENTWISLE, 2001; WILLÉN, 2001; WHITTON, 2003; PATOVA & DEMINA, 2007; UZUNOV et al., 2008; BOSTOCK & HOLLAND, 2010; DEGTEVA et al., 2010; KAŠTOVSKÝ et al., 2010; KOZHEVNIKOV & KOZHEVNIKOVA, 2011; BAKIEVA et al., 2012; CĂRĂUȘ, 2012), a high diversity of *Nostoc* species was detected in Lithuania.

Twelve *Nostoc* species and intraspecific taxa (*N. carneum*, *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. edaphicum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. passerinianum*, *N. piscinale*, *N.* cf. *sphaericum*, *N. spongiaeforme*, *N.* cf. *wollnyanum*), *Desmonostoc* genus including two taxa (*D. muscorum* and *Desmonostoc* sp.) were recorded for the first time in Lithuania (Fig. 1–3, 16 l, m, 17 a–c, e, g–l).

Based on the existing research data, it can be presumed that the diversity of *Nostoc* and *Desmonostoc* species in Lithuania could be even higher than it is determined at the present time. We believe that the detailed morphological and molecular studies of cyanobacterial natural populations and newly isolated strains would enable to identify specimens on the species level and to specify the taxonomy of the species. In some cases new *Nostoc*, *Desmonostoc* or other genera species could be described.

A checklist of all *Nostoc* and *Desmonostoc* (40 *Nostoc*, 2 *Desmonostoc* taxa) cyanobacteria is compiled with the authentical data of biology (morphology, morphometry and reproduction), ecology and photographs of morphological features.

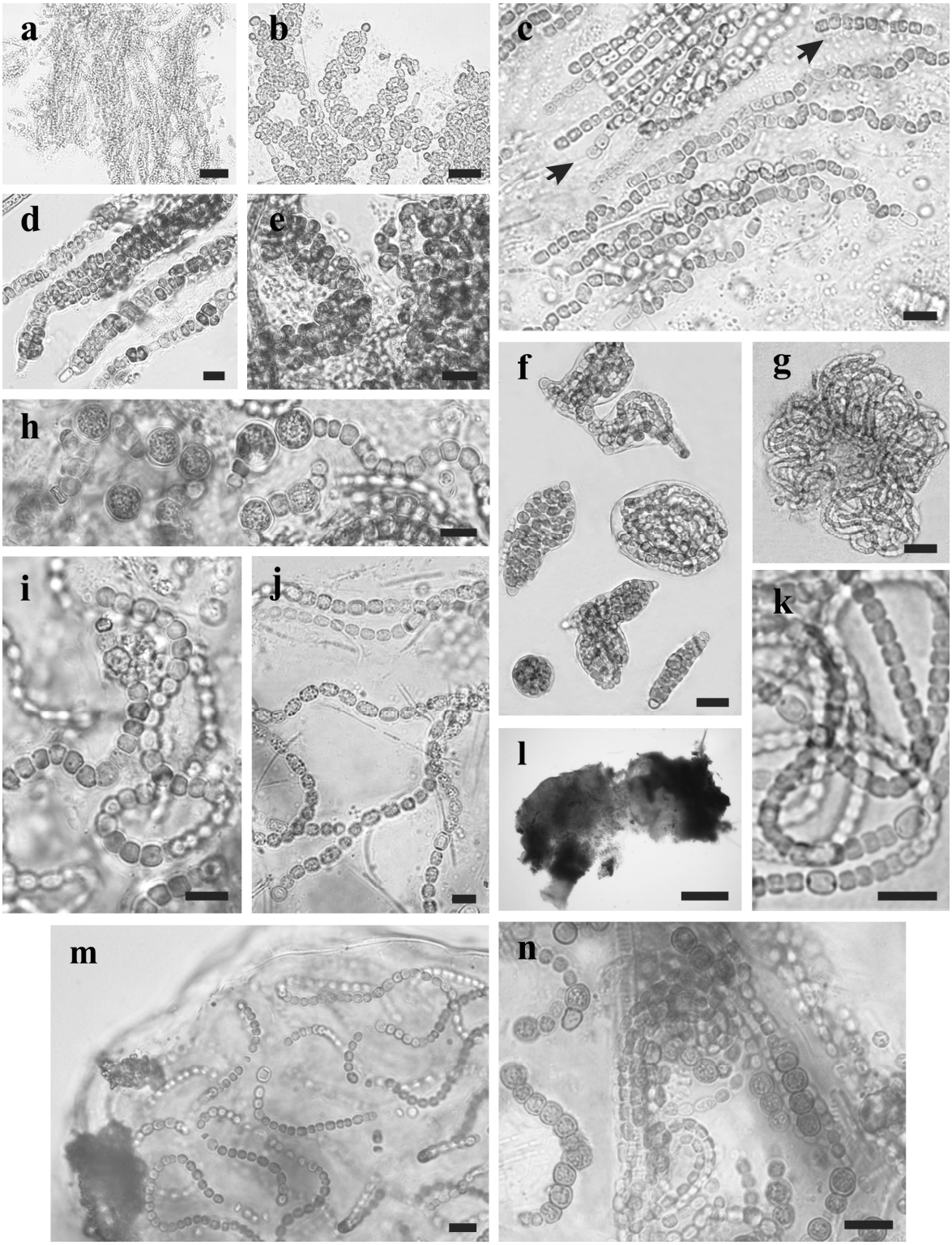


Fig. 1. *Nostoc cuticulare* f. *ligericum* (a–c, NP; d, SW), *N. cuticulare* f. *polymorphum* (e, h, i, SW), *N. edaphicum* (f, g, k, BG), *N. passerinianum* (j, k, NP), *N.* cf. *humifusum* (m, n, NP); a – film-like conglomeration of colonies, b, d – colonies of various stages, c – hormogonia (➔) and their subsequent developmental stage, e, m – fragment of colonies, f – various young colonies, g – microscopic colony, h, n – akinetes in chains, i, j, k – vegetative trichomes, l – macroscopic colony (scale bars: c, d, h–j, k–n – 10 µm, b, e, f – 20 µm, a, g – 40 µm, l – 2 mm) Abbreviations: NP – natural population, SW – sample of spring water, BG – sample of medium BG11

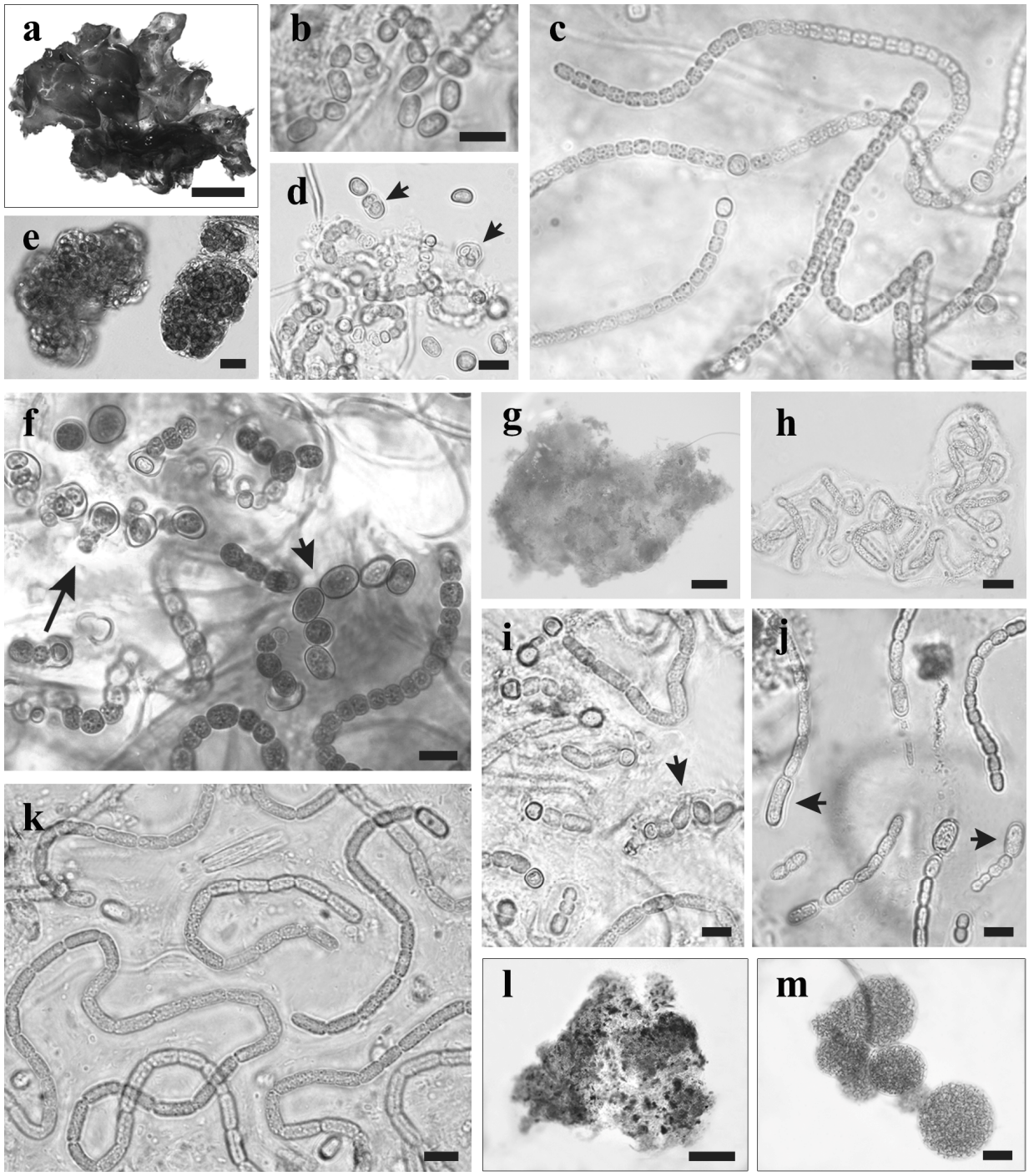


Fig. 2. *Nostoc* cf. *foliaceum* (a–c, NP; d, e, BG), *N.* cf. *sphaericum* (f, BG), *N.* cf. *wollnyanum* (g–i, k, SW; j, NP), *N. piscinale* (l, m, NP); a, g, l – macroscopic colonies, b – akinetes in chain, c, k – vegetative trichomes, d – trichomes, akinetes and germinated akinetes (➔), e, h, m – young colonies in various shapes, f, j – akinetes (➔), germinated akinetes (⟶), i – development of trichomes from hormogonium (scale bars: b–d, f, i–k,– 10 µm, e, h – 20 µm, m – 200 µm, l – 500 µm, g – 1 mm, a – 2 mm) Abbreviations: NP – natural population, SW – sample of spring water, BG – sample of medium BG11

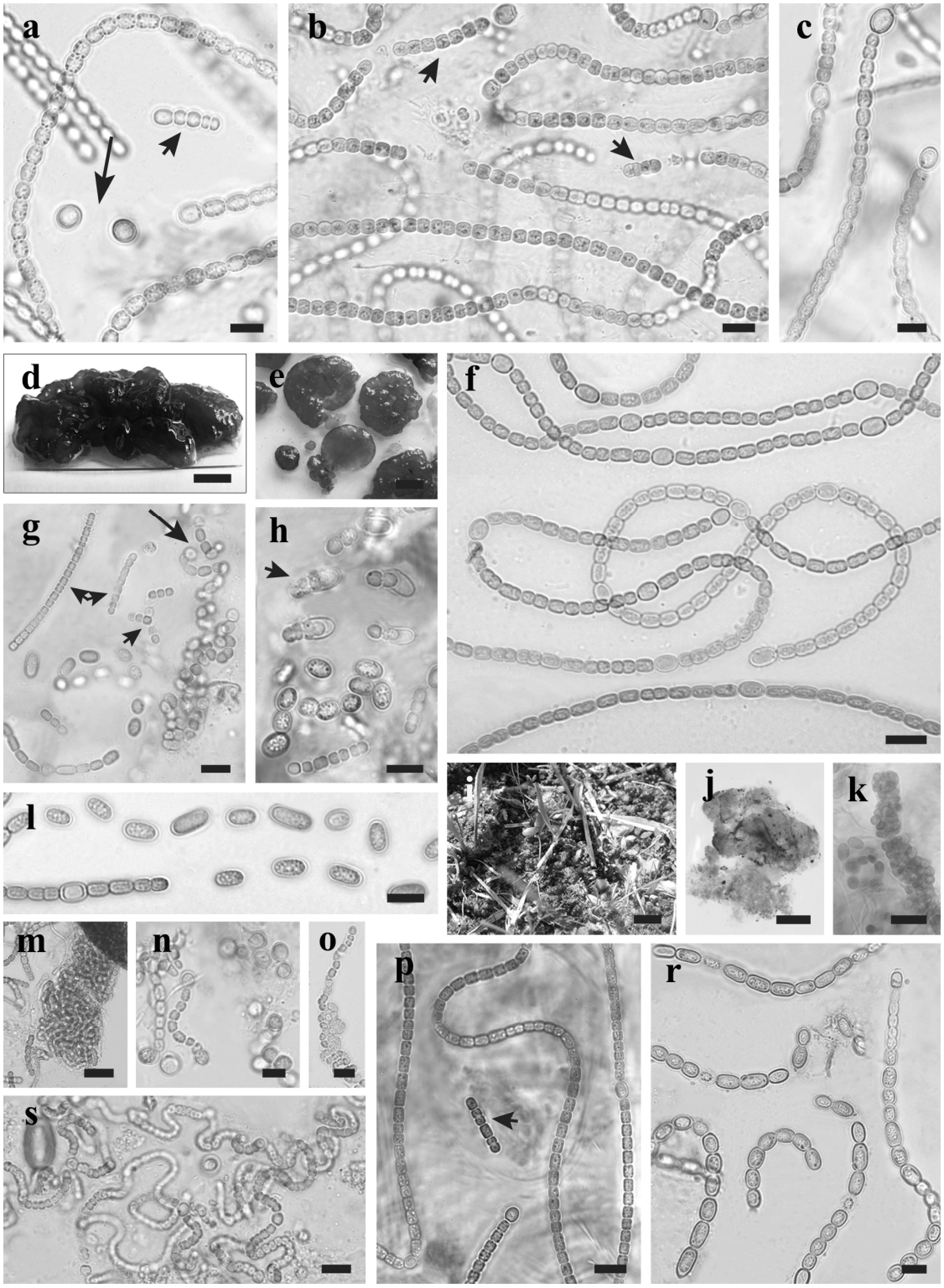


Fig. 3. *Nostoc piscinale* (a–c, NP), *N. spongiaeforme* (d–h, l, NP), *Desmonostoc muscorum* (i, j, r, NP; k, BG; p, SW), *N. entophytum* (m–o, s, NP); a–c, f, p, s – vegetative trichomes, akinetes (➞) and hormogonia (a, b, p, ➛), g – hormogonia (➛), aseriate colony (➞), h – germinated akinetes (➛), k – young colony consists of akinetes, m – microscopic colony, n – akinetes, o – trichome with akinete-like cells, d, e, j, i – macroscopic colonies (i – in nature), l, r – akinetes in chains (scale bars: a–c, f–h, l, n–s – 10 μm, k, m – 20 μm, j – 2 mm, d – 1 cm, e, i – 2 cm) Abbreviations: NP – natural population, SW – sample of spring water, BG – sample of medium BG11

Morphological analysis of *Nostoc* and *Desmonostoc* species

The cluster analysis (UPGMA) of morphological features of *Nostoc* and *Desmonostoc* species (generalised data of NP, SW, BG and S samples) revealed a strong morphological heterogeneity of *Nostoc* s. l. genus and a morphological similarity of some *Nostoc* species to *Desmonostoc* species. The *Nostoc* and *Desmonostoc* species and their different types of examples are composed of three major clusters (I, II, III) in the dendrogram (Fig. 4).

Different morphology of irregularly expanded colonies is characteristic to *Nostoc* and *Desmonostoc* species of the **first cluster (I)** (Fig 4.). The specific morphological features of the species in Group A (I cluster) are the following: 1) usually macroscopic, soft gelatinous or firm consistency, irregularly expanded colonies (Fig. 2 a, g, 3 d, j, 16 l, 17 e); 2) flexuous, loosely or densely entangled trichomes; 3) barrel-shaped or cylindrical vegetative cells (Fig. 2 c, k, 3 f, p, 17 a, g, h), oval and cylindrical akinetes (Fig. 2 b, g, j, 3 h, l, r, 17 a, h, i). *D. muscorum* and *Desmonostoc* sp. did not build a separate cluster in the dendrogram but they merged with morphologically similar *Nostoc ellipsosporum* and *Nostoc* sp. 5 species. Species of both *Desmonostoc* and *Nostoc* genera form usually soft, gelatinous colonies, long, flexuous and loosely entangled trichomes, akinetes into long chains and diffused mucilaginous sheaths (Fig. 3 j, p, r, 17 e, g-i). Further detailed (morphological and phylogenetic) studies of *N. ellipsosporum*, and *Nostoc* sp. 5 that are morphologically similar to *Desmonostoc* species and of *D. muscorum* are required that could be used as the basis to evaluate their phylogenetic relations and distinctive features of the genera. Different types of samples of *N.* cf. *wollnyanum* species built a separate cluster, because of the exceptionally long cylindrical vegetative cells and akinetes (Fig. 2 j, k) that are characteristic to their species. Group B (I cluster) consists of a few *Nostoc* species and most *Nostoc* taxa that are identified to a genus level. These species are characterized by various morphological features of colonies, vegetative trichomes and cells, and by a variety of reproductive structures. Only few common features are typical to the species in this group: 1) irregularly expanded, soft, gelatinous and mucilaginous colonies (Fig. 1 g, l, 2 l, 15 k, 16 d, e); 2) flexuous and loosely entangled trichomes in diffused mucilagine (Fig. 1 j, m, 2 b, 15 m, 17 a, f, j, k); 3) akinetes are usually not formed.

Several morphological similarities were determined in *Nostoc* species of the **second cluster** (II) (Fig. 4): 1) colonies are usually irregularly expanded, film-like and attached to the substrate colonies (Fig. 1 a); 2) characterized by aseriate stages (Fig. 1 b, d, e, 3 m, o); 3) trichomes are strongly, sometimes screw-shaped curved or straigth (Fig. 1 a, e, 17 f). A common feature of *Nostoc* species of the **third cluster** (III) is solid, spherical to oblong or irregularly expanded, with firm surface layer colonies (Fig. 15 a, e, h).

The cultivation of natural populations in spring water or nutrition medium and isolation of strains in many cases provided some important information for the

identification and description of species. And also, this research method made it possible to assess the stability of features used in diagnostic of *Nostoc* species and their importance more accurately. According to MATEO et al. (2011), the research of strains is important to the description of species when macroscopic colonies of species are not found in the natural environment.

Based on the morphological analysis of different types of samples, a stability of morphological features was determined for some species of both genera (*N. caeruleum*, *N. cuticulare* f. *polymorphum*, *N. entophytum*, *N. punctiforme*, *N.* cf. *wollnyanum*, *Nostoc* sp. 3, *Nostoc* sp. 8, *Nostoc* sp. 9, *Nostoc* sp. 10, *Nostoc* sp. 11, *Nostoc* sp. 13 and *Nostoc* sp. 18 and *D. muscorum*). Samples of different types of other species are distant in the dendrogram and are included in different clusters (Fig. 4). An individual variability of morphological features was determined in these species (*N. carneum*, *N. commune*, *N.* cf. *foliaceum*, *N. kihlmanii*, *N. linckia*, *N. piscinale*, *N.* cf. *sphaericum*, *Nostoc* sp. 6 and *Nostoc* sp. 17). Differences in the morphological features (colonies, trichomes, cells) and developmental cycles of natural populations and culture were determined during the research. The studies performed by MATEO et al. (2011) showed that the composition of the nutrition medium determine a variability of morphological features of the same *Nostoc* strain. Various ecophysiological and morphological changes that are not necessarily consistent with the characteristics of natural populations can occur when growing strains in standart conditions (KOMÁREK, 2006). We presume that a complex morphological analysis of natural populations and strains of *Nostoc* species is significant to anaccurate description of species, as well as to the diversity and ecology studies of cyanobacteria.

Based on the research into *Nostoc* and *Desmonostoc* species, some of the species are attributable to the group of species that can be identified by classical morphological descriptions. These species are *N. edaphicum*, *N. caeruleum*, *N. commune* (?), *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. ellipsosporum*, *N. kihlmanii*, *N. passerinianum*, *N. pruniforme*, *N. punctiforme*, *N. verrucosum* and *Desmonostoc muscorum*. The morphological features of these Lithuanian species (in most cases natural populations were taken into account) matched the descriptions provided in the sources of literature (GEITLER 1932; ELENKIN 1938; HOLLERBACH et al., 1953; DESIKACHARY, 1959; KONDRATYEVA, 1968), while other species also demonstrated a stability of morphological features in samples of different types (natural populations, cultures). The morphological descriptions of other species that are presented in other types of literature sources (GEITLER 1932; ELENKIN 1938; HOLLERBACH et al., 1953; DESIKACHARY, 1959; KONDRATYEVA, 1968) may not be sufficient or accurate for the identification of species. Such are 11 species: *N. carneum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. linckia*, *N. paludosum*, *N. piscinale*, *N. spongiaeforme*, *N.* cf. *sphaericum*, *N.* cf. *sphaeroides* and *N.* cf. *wollnyanum*. The taxonomic status of most of these species is uncertain and there is very little information about them. And also, a number of *Nostoc*

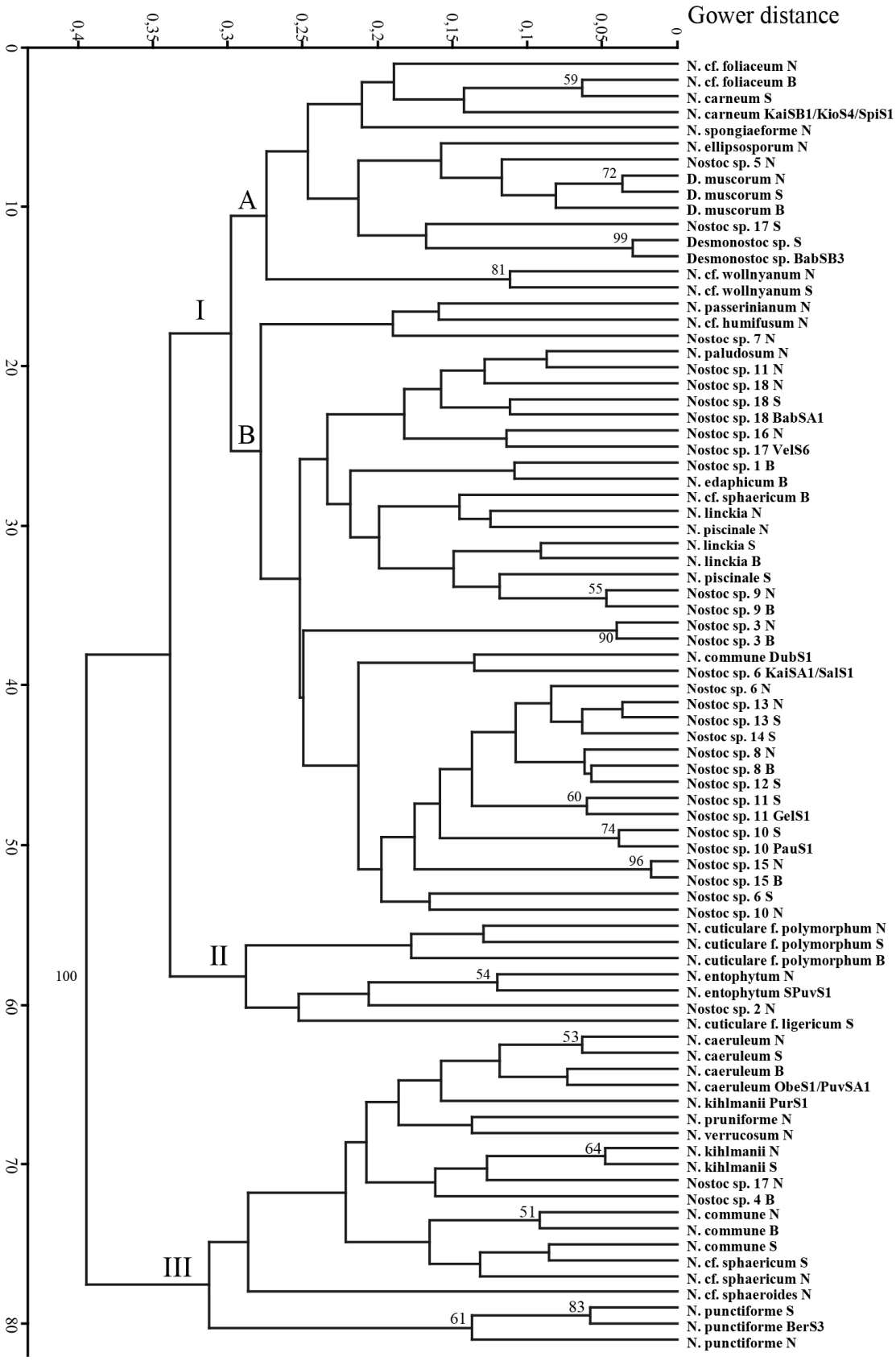


Fig. 4. Cluster analysis (UPGMA method) of *Nostoc* and *Desmonostoc* cyanobacteria based on morphological data. Numbers near nodes indicate bootstrap values ≥50 %. Abbreviations: N – natural population, S – sample of spring water, B – sample of medium BG11, BerS3 and other – strains

species are critical, morphologicaly similar and difficult to identify (GEITLER, 1932; KOMÁREK, 2010 a). Some confusion in taxonomy of *Nostoc* species is also brought about by some of the scientists differently treating *Nostoc* species. *N. spongiaeforme* is treated as a synonym to *N. carneum* species, *N. piscinale* – as a synonym of *N. linckia*, *N. cuticulare* and *N. entophytum* – as synonyms of *N. paludosum*, and *N. wollnyanum* – as a synonym of *N. ellipsosporum* with reference to KAŠTOVSKÝ et al. (2010). However, these above mentioned species and their synonyms were treated as separate species in our study as some morphological differences were determined between them.

Several morphological differences of *N. spongiaeforme* and *N. carneum* were determined during the study of Lithuanian populations. Young and adult *N. carneum* colonies were irregularly expanded, with firm surface layer, trichomes usually were curved and closely entangled, and akinetes were oval (Fig. 16 l, m, 17 a-c). *N. spongiaeforme* colonies usually had a diffused surface layer, trichomes were flexuous and loosely entangled, akinetes were cylindrical (Fig. 3 f, l). Statistically reliable differences ($p < 0.05$) among all measurements of cells of *N. carneum* and *N. spongiaeforme* were determined. GEITLER (1932) notes that *N. wollnyanum* is not distinguished from *N. ellipsosporum* by any distinct morphological features, except shorter akinetes. Lithuanian *N.* cf. *wollnyanum* vegetative cells and akinetes were narrower and longer (Fig. 2 k) than those in *N. ellipsosporum* species ($p<0.05$). Moreover, *N.* cf. *wollnyanum* vegetative cells were cylindrical with rounded ends (Fig. 2 k), and akinetes were found in colonies sparsely and solitary (Fig 2 j), while *N. ellipsosporum* akinetes were observed in long chains. Comparing the Lithuanian populations of *N. linckia* and *N. piscinale*, cells were wider and longer ($p<0.05$; except length of akinetes), flexuous and loosely entangled trichomes in *N. piscinale* species (Fig. 3 a-c). *N. piscinale*, *N. linckia*, *N. carneum* and *N. spongiaeforme* are characterized by long chains of akinetes (GEITLER, 1932; ELENKIN, 1938; KONDRATYEVA, 1968), however in the Lithuanian populations, *N. piscinale* were found to form solitary oval akinetes (Fig. 3 a). GEITLER (1932) defined *N. sphaeroides*, *N. caeruleum* and *N. kihlmanii* as separate but morphologically similar species. ELENKIN (1938) and MOLLENHAUER et al. (1999) refer to *N. sphaeroides* and *N. kihlmanii* species as synonyms of *N. caeruleum*. The Lithuanian *N. kihlmanii* vegetative cells, heterocytes and akinetes were larger ($p<0.05$) (Fig. 15 f, g) than those of *N. caeruleum* species (Fig. 15 i). Besides, the akinetes of *N. kihlmanii* are rounded (Fig. 15 g), while those of *N. caeruleum* are oval. The width is generally greater than the length in vegetative cells and heterocytes of *N. kihlmanii*, contrary to *N. caeruleum* cells. The results of this study showed that vegetative cells with gas vesicles are characteristic to *N. caeruleum*, *N. kihlmanii* and *N. sphaeroides* species. We presume that gas vesicles feature is characteristic not solely to *N. kihlmanii* species as it is stated in the sources of literature (GEITLER, 1932). Gas vesicles could be one of the distinctive features for the group of *N. caeruleum*, *N. kihlmanii* and *N. sphaeroides* species. We presume that the species that

are considered as synonyms are most probably separate species and their names should be in use for the time being.

Morphological features of *Nostoc* and *Desmonostoc* species: vegetative and reproductive structures, developmental cycles

The morphological analysis of species belonging to *Nostoc* and *Desmonostoc* genera reveals several important diagnostic morphological features of these species. The shape and consistence of mature colonies may be a distinctive feature of *Nostoc* and *Desmonostoc* species. The analysis of the Lithuanian *Nostoc* species identified a stability of the morphology of colonies in different types of samples. *N. caeruleum* and *N. kihlmanii* colonies are spherical of solid consistence (Fig. 15 e, h), while colonies *N. carneum* and *D. muscorum* are soft, gelatinous and lumpy (fig 3 i, j). The colonies represent a steady diagnostic feature that remains in the samples of both natural populations and cultures. However, some studies claim that the form of *Nostoc* strains colonies is influenced by the composition of the medium and by the cultivation conditions (LAZAROFF & VISHNIAC, 1964; KANTZ & BOLD, 1969). The present studies also identified *Nostoc* species where the consistence of colonies varied not only in different types of samples but also among the populations (*N. commune*, *N. ellipsosporum*, *N.* cf. *foliaceum*, *N.* cf. *sphaericum*).

The sinuous nature of trichomes and their arrangement in the colony could also be a rather significant distinctive feature of *Nostoc* species. HROUZEK et al. (2013) claim that the distinctive feature of *Nostoc* species is a particularly dense arrangement of trichomes in colonies, wheareas one of the distinctive features of *Desmonostoc* species is long, widely flexuous and usually loosely entangled trichomes. The features of the Lithuanian *Desmonostoc* cyanobacteria trichomes corresponded to the statements of HROUZEK et al. (2013). The trichomes in mature colonies of most Lithuanian *Nostoc* species are flexuous and closely or loosely entangled (Fig. 1 j, m, 2 c, k, 3 b, f, 15 i, l, 16 a, f, k). However, e.g. *N. entophytum*, *N. cuticulare* f. *polymorphum*, *N. cuticulare* f. *ligericum*, *N. kihlmanii* and *N. punctiforme* were distinguished by highly curved and densely entangled trichomes (Fig. 1 a-e, f, g, 15 a, c, d). The true branching trichome was observed in several Lithuanian *Nostoc* species (*Nostoc* sp. 6, *Nostoc* sp. 10 and *Nostoc* sp. 13). This phenomenon was described in a number of scientific papers (LAZAROFF & VISHNIAC, 1962; MOLLENHAUER, 1970; MATEO et al., 2011). According to KOMÁREK (2010 a), the true branching could be just an abnormal phenomenon of the trichomes.

In their studies of the soil *Nostoc* strains HROUZEK et al. (2005) identified that mucilaginous sheath features are possibly stable in the strains and could be a significant diagnostic feature. During the studies of Lithuanian populations, the individual mucilaginous sheaths and the surface layer of colonies were assessed separately. After summarising all Lithuanian *Nostoc* and *Desmonostoc* species, the trichomes were defined to be most frequently distributed in diffused colourless mucilage, while

individual mucilaginous sheaths are formed only in rarer cases. The structure and colour of the mucilaginous sheaths varies when comparing different samples or even different colonies within the sample. The analysis of strains belonging *Nostoc* and *Desmonostoc* genera identified a steady shape of mucilaginous sheaths; diffused and colourless mucilaginous sheaths were formed in most cases (Fig, 15-17). However, the analysis of the mucilaginous surface layer of colonies identified a variation of this feature. We believe that the surface layer of colonies might be a more significant diagnostic feature than the individual mucilaginous sheaths.

The variety of the development cycles of *Nostoc* (and *Desmonostoc*) species was described by LAZAROFF and VISHNIAC (1961; 1962), MOLLENHAUER (1986), KONDRATYEVA & KISLOVA (2002), HROUZEK et al. (2005) and MATEO et al. (2011). The studies of the Lithuanian populations confirmed the results of the previous studies that a variety of reproduction structures (akinetes, hormogonia, solitary cells, proliferation of colonies) and of development cycles are characteristic to cyanobacteria of *Nostoc* genus. The Lithuanian *Desmonostoc* cyanobacteria were described as reproducing by akinetes and hormogonia. During the studies most of the Lithuanian *Nostoc* and *Desmonostoc* species came out to be characteristic of both sporogenic (akinetes formed) and heterocitic (hormogonia formed) cycles. And also, the studied *Nostoc* species are characteristic of the development cycles where solitary cells and proliferating colonies are formed. Structures of reproduction and spreading – aseriate stages, round or oblong shaped colonies with a firm mucilaginous surface layer – are formed in the development cycle of both genera. Akinete-like cells are identified only to *Nostoc* species. The studies of the Lithuanian cyanobacteria showed that the development cycle of many of the *Nostoc* and *Desmonostoc* species is a variable characteristic, i.e. it varies within the populations and most likely depends also on the growing conditions. On the other hand, the species are characterised by the stability of the reproduction structures and development cycle, i.e. the same reproduction structures and development stages are formed in different types of samples. The study undertaken by MATEO et al. (2011) showed that the composition of medium influences the trichome shapes, cell measurements of *Nostoc* and *Desmonostoc* strains but not their development cycles. The study level of the life cycle is relevant to the taxonomy of both *Nostoc* and *Desmonostoc* species; therefore further studies of these cyanobacteria life cycles are necessary.

The data of the studies revealed that 37 *Nostoc* and *Desmonostoc* species (88.1 % of the total number of taxa; Fig. 5) reproduce by hormogonia. In most of the species hormogonia formation was determined to be a stable feature characteristic of all types of the samples (natural populations, cultures). And also, all the studied strains formed hormogonia (Fig. 15 j, m, o, 16 a, c, f, h, k, 17 g). HROUZEK et al. (2005; 2013) provided similar study results in respect of the terrestrial *Nostoc* (and *Desmonostoc*) strains – out of fourteen studied strains hormogonia were not identified only in two strains. And also,

it is not only the case of hormogonia formation but also their morphological type, and further hormogonia development that are relevant to the authors. Other scientists (MATEO et al., 2011) evaluated the size and number of the hormogonia cells in *Nostoc* strains. According to HROUZEK et al. (2005), hormogonia formation, their morphological features could be used to distinguish the *Nostoc* species or species groups. However, MOLLENHAUER (1986 b) claims that the morphological features of hormogonia are of little significance to taxonomy as different species form hormogonia of similar morphology. KANTZ & BOLD (1969) identified that the hormogonia formed by all of the studied of *Nostoc* strains are similar to *N. punctiforme* hormogonia and only their development stages are different. We believe that hormogonia formation may be an insignificant distinctive feature of *Nostoc* species as most of the studied species formed hormogonia. However, the shape of hormogonia and the way of their further development could be important diagnostic features. In present studies different shapes of hormogonia were identified in *Nostoc* species (straight, short or long, spiral; Fig. 3 a, b, g, p, 15 j, m, o, 16 a, c, f, h, k) that develop into new vegetative trichomes (Fig. 1 c, 15 o, 16 h) or into various structures of young colonies (Fig 15 j, r). As no uniform opinion exists about the significance of the diagnostic feature of hormogonia, the studies of hormogonia formation and development should be significant in further studies of *Nostoc* taxonomy.

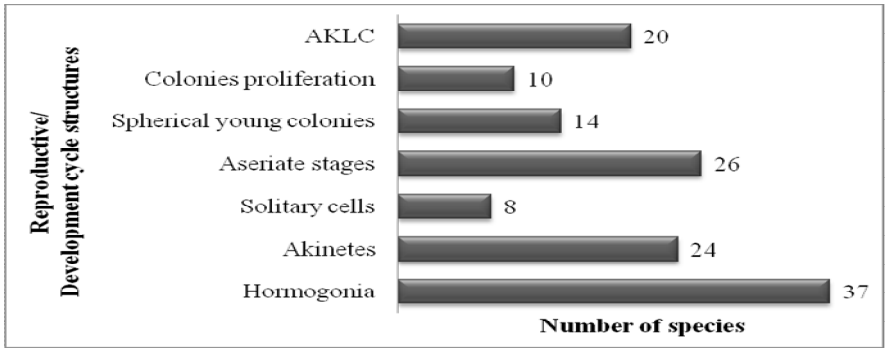


Fig. 5. Abundance of reproductive and development cycle structures of *Nostoc* and *Desmonostoc* species

The morphological features of akinetes (size, shape, wall structure, number in trichome, formation type, etc.) are significant in the taxonomy of both *Nostoc* and *Desmonostoc* genera and their species (GEITLER, 1932; KOMÁREK & ANAGNOSTIDIS, 1989; SANT'ANNA et al., 2007; HROUZEK et al., 2013). Reproduction by akinetes was identified for more than half of the analysed to *Nostoc* and *Desmonostoc* species (24 species, 57.1 % of the total number of taxa; Fig. 5). Akinetes abundantly and in long chains were observed in some species (*N. carneum*, *N. cuticulare* f. *ligericum*, *N. ellipsosporum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. linckia*, *N. passerinianum*, *N.* cf. *sphaericum*, *N. spongiaeforme*, *Nostoc* sp. 2, *Nostoc* sp. 5, *D. muscorum* and *Desmonostoc* sp.; Fig. 1 h, n, 2 b, f, 3 l, r, 17 a, i). Most of these species

fell into cluster I (group A) in the dendrogram to morphological features (Fig. 4). Only solitary formed akinetes were observed in the colonies of other *Nostoc* species (*N. caeruleum*, *N. commune*, *N. kihlmanii*, *N. pruniforme*, *N. piscinale*, *N.* cf. *sphaeroides*, *N. verrucosum*, *N.* cf. *wollnyanum*, *Nostoc* sp. 17 and *Nostoc* sp. 18; Fig, 2 j, 3 a, 15 g, l). These species fell into different clusters in the dendrogram, but most of the species composed cluster III (Fig. 4). We believe that formation or non-formation of akinetes and their abundance in the trichome could be a significant diagnostic feature of studied cyanobacteria. Morphological and phylogenetic analysis of strains showed that strains belonging to *Nostoc commune* cluster (Fig. 13) did not form akinetes (*N. punctiforme* BerS3, *Nostoc* sp. 6 KaiSA1 and SalS1, *Nostoc* sp. 10 PauS1, *Nostoc* sp. 11 GelS1) or formed them sparsely (*N. kihlmanii* PurS1, *N. commune* DubS1; Fig 15 g, l). However, other strains falling under different clusters of the phylogenetic tree (Fig. 13) formed akinetes variously. *Desmonostoc* sp. BabSB3 and BabSB4, *N. carneum* KaiSB1, KioS4, KioS5 and *N. entophytum* SPuvS1 strains formed akinetes abundantly and in long chains (Fig. 17 a, i), but none were observed in *Nostoc* sp. 17 VelS6 and *Nostoc* sp. 18 BabSA1 strains. A similar akinetes formation/non-formation trend was observed in natural populations of species, except for *Nostoc* sp. 17 and *Nostoc* sp. 18, whereas akinetes were found in natural populations of these species.

GEITLER (1932) and MOLLENHAUER et al. (1999) claim that akinetes are not characteristic for *N. caeruleum* and *N. kihlmanii* species and that *N. commune* do not form them or are formed very rarely. However, akinetes of these species were observed in the samples of Lithuanian cyanobacteria. *N. kihlmanii* akinetes were observed in the strain (Fig. 15 g) but were not found in the natural populations. In case of the *N. caeruleum*, *Nostoc* sp. 17 and *Nostoc* sp. 18 it was just the opposite. The studies of the Lithuanian cyanobacteria and the literature data indicate that akinete formation is not observed in all of the *Nostoc* species. Therefore, further complex studies of the species would allow to identify the formation patterns of akinetes of different species as well as their diagnostic significance in the *Nostoc* taxonomy.

Another diagnostic feature of *Nostoc* and *Desmonostoc* species is the structure and colour of the akinetes wall. Differences in the akinetes wall colour are determined only to individuals of *Nostoc* species in natural populations. Most of the cyanobacteria formed akinetes in a colourless wall, and only akinetes of the *Nostoc* sp. 2 and *N.* cf. *humifusum* species had a brown wall. The wall of the akinetes of all strains belonging to *Nostoc* and *Desmonostoc* genera is colourless. No morphological differences of akinete walls structure were determined in the Lithuanian *Nostoc* and *Desmonostoc* species (and strains) – the wall of all cyanobacteria akinetes was smooth. However, according to literature data (GEITLER, 1932, SANT'ANNA et al., 2007), the structure of akinetes wall may be a distinctive feature for some of *Nostoc* species (*N. gelatinosum*, *N. alatosporum*). Lithuanian *Nostoc* and *Desmonostoc* species (and strains) formed finely granulated or granule-free akinetes. During the study it was observed that granules occur

mostly at the beginning of akinetes formation and decrease before their division. Before germination of akinetes, their cytoplasm becomes homogenous as during the cell division process the cyanophycin granules are used as the source of nitrogen (FLORES & HERRERO, 2010). We presume that the granular structure of akinetes is a varying feature that depends on the akinete development stage and should not be considered as the diagnostic feature of species.

Reproduction by solitary cells (cocci) is also characteristic of some of *Nostoc* species (8 species, 19.0 % of the total number of taxa; Fig. 5). The solitary cells formed by *Nostoc* species were also described by MOLLENHAUER (1970; 1986 b), GAO & YE (2003) and SANT'ANNA et al. (2007). The studies of Lithuanian cyanobacteria supported the previous studies (ELENKIN, 1938; ABDELAHAD & BAZZICHELLI, 1989) suggesting that one of the main ways of reproduction for *N. punctiforme* is by solitary cells (Fig. 15 b). In other colonies of *Nostoc* species (*N. caeruleum*, *N. carneum*, *N. commune*, *N.* cf. *sphaericum*, *Nostoc* sp 6 and *Nostoc* sp. 10; Fig. 15 n, p, s) solitary cells were observed from which new vegetative trichomes or young colonies were formed. These solitary cells are similar to vegetative cells and are some sort of one cell hormogonia.

Proliferation and fragmentation of colonies was identified in 10 *Nostoc* species (19.5 % of the total number of the taxa; Fig. 5). According to some sources of literature (BORNET & FLAHAULT, 1888; KANTZ & BOLD, 1969; MOLLENHAUER, 1970, 1986 b; POTTS, 2000; DENG et al., 2008), *N. caeruleum*, *N. commune*, *N. pruniforme*, *N. punctiforme*, *N. sphaeroides* and *N. zetterstedtti* cyanobacteria reproduce by proliferation of colonies.

Studies of Lithuanian *Nostoc* species confirmed that proliferation (and fragmentation of colonies) is characteristic for species (*N. caeruleum*, *N. commune* and *N. punctiforme*) for which this reproduction was already known, and also revealed that proliferation is characteristic to seven other species – *N. edaphicum*, *N. kihlmanii*, *N. paludosum*, *N.* cf. *sphaericum*, *N. spongiaeforme*, *N. verrucosum* and *Nostoc* sp. 17 species. Fragmentation of young colonies (oblong shaped in most cases) into smaller round colonies was frequently observed in *N. caeruleum*, *N. commune*, *N. edaphicum*, *N. kihlmanii*, *N. punctiforme* and *N.* cf. *sphaericum*. Fragmentation of colonies was observed to be more characteristic for young oblong shaped colonies formed from long hormogonia.

Aseriate stage structures (26 species, 61.9 % of the total number of taxa) and round or oblong shaped colonies with a firm mucilaginous surface layer were defined in the life cycle of a considerable part of Lithuanian species belonging to *Nostoc* and *Desmonostoc* genera (14 species, 33.3 %; Fig. 5). The aseriate structures as a significant distinctive feature of species or strains were described by LAZAROFF & VISHNIAC (1961; 1962), KANTZ & BOLD (1969), HROUZEK et al. (2003), MATEO et al. (2011). HROUZEK et al. (2013) note that aseriate colonies are formed by *Desmonostoc* cyanobacteria very rarely and sparsely. Aseriate structures were observed in *Desmonostoc* species sparsely in

comparison with *Nostoc* species. Aseriate structures are characteristic for *N. caeruleum*, *N. commune*, *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. entophytum*, *N.* cf. *foliaceum*, *N. punctiforme*, *N.* cf. *sphaericum* and other species (Fig. 1 b, d, f, 2 e, 3 m, o, 15 c, j, r, 16 b).

Akinete-like cells (AKLC, Fig. 3 o, g) are characteristic of half of the described Lithuanian *Nostoc* species (20 species, 47.6 % of the total number of taxa; Fig. 5) and they were formed by half of the studied *Nostoc* strains (Fig 16 g, i). They were not observed in the samples of *Desmonostoc* species. HROUZEK et al. (2013) also note that *Desmonostoc* strains form akinete-like cells only in the initial development stages contrary to *Nostoc* cyanobacteria. At the moment, no sufficient data exists on the significance of akinete-like cells for the *Nostoc* species identification; therefore the future studies are necessary and might be significant to the cyanobacteria taxonomy.

Ecology of *Nostoc* and *Desmonostoc* species

New to Lithuania *Nostoc* species and *Desmonostoc muscorum* are known from some other European countries (Bulgaria, the Czech Republic, Denmark, Spain, Israel, the United Kingdom, Sweden, Romania, Russia) (GEITLER, 1932; ELENKIN, 1938; KONDRATYEVA, 1968; WILLÉN, 2001; MATEO et al., 2003; WHITTON et al., 2003; DOUTELERO et al., 2004; SERRANO et al., 2004; BÁRBARA et al., 2005; UZUNOV et al., 2008; KAŠTOVSKÝ et al., 2010; CĂRĂUŞ, 2012; GUIRY & GUIRY, 2013; HROUZEK et al., 2013). The study reveals that the most common terrestrial cyanobacteria in Lithuania is *N. commune* (51 localities), although it was found only in two localities before (MATUSZKIEWICZÓWNA, 1934; Herbarium of Vilnius University (WI)). According to MOLLENHAUER et al. (1999), the distribution of *N. commune* in Europe is varied – species is frequent in some countries, while it is rare or extinct due to decline of typical habitats in others countries (central Europe). However, according to more recent data *N. commune* is common in the Czech Republic (KAŠTOVSKÝ et al. 2010). Other identified *Nostoc* species are less frequent in Lithuania: *N. caeruleum* were found in 13 localities, *N. piscinale* – 9, *N. spongiaeforme* – 7, *N. verrucosum* – 6, and *N. cuticulare* f. *polymorphum* – 5. Fourteen identified *Nostoc* species and intraspecific taxa, as well as *D. muscorum* were found very rarely – in no more than four localities. According to KAŠTOVSKÝ et al. (2010), *N. caeruleum* is a rare species in the Czech Republic, however, in Lithuania it was recorded most often. Cyanobacterium *N. pruniforme* is probably rare in Europe, it was mostly found in West and North Europe (MOLLENHAUER et al., 1999; WILLÉN, 2001; WHITTON et al., 2003; TÄUSCHER, 2011; CĂRĂUŞ, 2012), while in some regions of Russia this species is included into the Red Data Book (PATOVA & DEMINA, 2007; KOMULAINEN, 2009; DEGTEVA et al., 2010). *N. pruniforme* was found in two lakes in the present study. VITONYTĖ (2014) also recorded this species in benthos of two Lithuanian rivers. MOLLENHAUER et al. (1999) state that *N. caeruleum*, *N. commune*, *N. pruniforme* and *N. verrucosum* populations declined in

Europe due to anthropogenic activities, agriculture and increase of waters eutrophication. KAŠTOVSKÝ et al. (2010) described *N. carneum*, *N. edaphicum*, *N. ellipsosporum*, *N. kihlmanii* and *N. sphaericum* species as rare and very rare in the Czech Republic. These species were recorded also rare in Lithuania. Newly recorded in Lithuania *N.* cf. *foliaceum*, *N.* cf. *humifusum* and *N. passerinianum* species, according to KAŠTOVSKÝ et al. (2010) were not found in the Czech Republic in recent 50 years. In some cases, data on occurrence of species is several decades old or insufficient, so it does not give precise information about species in Europe or the world.

Most of *Nostoc* and *Desmonostoc* species recorded in the present study are hydrophytic (27 species, 67.5 % of the total number of taxa), while aerophytic species (11 species, 26.0 %) were less common. *N. caeruleum*, *N. entophytum* and *N. passerinianum* were found in both aquatic and terrestrial habitats. The highest number of hydrophytic species (30 species, 71.4 %) was recorded in lentic ecosystems and only three species (7.1 %) developed in lotic ecosystems. Nine *Nostoc* and *Desmonostoc* species (21.4 %) were found in temporaly damp habitats – coastlines of water bodies and close to water bodies, while seven *Nostoc* species (16.6 %) were found in meadows, trampled grass and roadsides (Fig. 6, Table 1).

New information on *Nostoc* and *Desmonostoc* species ecology was established during the studies in Lithuania. *N. kihlmanii*, *N. punctiforme* and *N.* cf. *sphaeroides* species were found in water bodies, whereas until recently these species were only found in soil (POCIENĖ, 1960; 1961; 1981; POCIENĖ & INDRAŠYTĖ, 1977). Terrestrial *N. passerinianum* and *N. wollnyanum* species (GEITLER, 1932; HOLLERBACH et al., 1953), were also found in epiphyton of water bodies in Lithuania. Hydrophytic cyanobacteria *N. entophytum* (GEITLER, 1932) and *N. caeruleum* (MOLLENHAUER et al., 1999) were recorded both in terrestrial (coastline of water bodies) and in aquatic habitats. *N. caeruleum* is described as aquatic and terrestrial species in previous Lithuanian references (SOKOŁOWSKA-RUTKOWSKA, 1932; POCIENĖ, 1960; 1961). It is possible that cyanobacteria *N. caeruleum* and *N. entophytum* can grow also in terrestrial, but wet habitats.

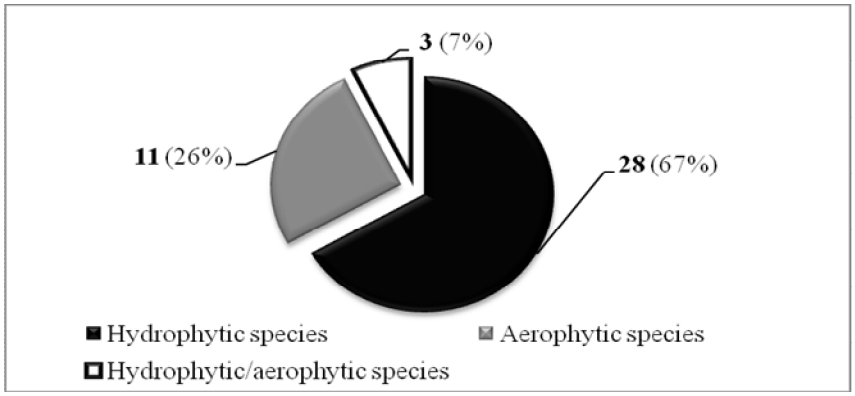


Fig. 6. Distribution of *Nostoc* and *Desmonostoc* species according to ecological groups

The present study of Lithuanian *Nostoc* and *Desmonostoc* populations confirmed the literature data (GEITLER, 1932; ELENKIN, 1938; KONDRATYEVA, 1968; DODDS et al., 1995), that most of *Nostoc* species grow in periphyton (23 species, 54.8 % of the total number of taxa) and metaphyton (19 species, 45.2 %). Only three (7.1 %) *Nostoc* species (*N. caeruleum*, *N. linckia* and *N. piscinale*; Fig. 7) were found in plankton. *Desmonostoc* sp. was recorded in metaphyton of lake. According to the literature data (HROUZEK et al., 2013), *Desmonostoc* cyanobacteria are usually found on land, but seldom in periphyton also. Similarly to the data of other literature sources (GEITLER, 1932; KONDRATYEVA, 1968), *N. cuticulare* f. *polymorphum* was typical to epiphyton of lakes, while *N. spongiaeforme* was common in metaphyton of slow running streams, lakes, pond, and spring.

Table 1. Habitats of *Nostoc* and *Desmonostoc* species (aerophytic/hydrophytic species are underlined)

Habitats	Taxa	Taxa number
Lentic ecosystems	*Nostoc caeruleum*, *N. carneum*, *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. entophytum*, *N.* cf. *humifusum*, *N. kihlmanii*, *N. linckia*, *N. paludosum*, *N. passerinianum*, *N. piscinale*, *N. pruniforme*, *N. punctiforme*, *N.* cf. *sphaeroides*, *N. spongiaeforme*, *N. verrucosum*, *N.* cf. *wollnyanum*, *Nostoc* sp. 2, *Nostoc* sp. 3, *Nostoc* sp. 6, *Nostoc* sp. 7, *Nostoc* sp. 8, *Nostoc* sp. 9, *Nostoc* sp. 11, *Nostoc* sp. 12, *Nostoc* sp. 13, *Nostoc* sp. 14, *Nostoc* sp. 15, *Nostoc* sp. 18, *Desmonostoc* sp.	30
Lotic ecosystems	*N. spongiaeforme*, *N. verrucosum*, *Nostoc* sp. 10	3
Coastlines of water bodies	*N. caeruleum*, *N. commune, N. ellipsosporum*, *N. entophytum*, *N.* cf. *foliaceum, N.* cf. *sphaericum*, *Nostoc* sp. 5, *Nostoc* sp. 16, *D. muscorum*	9
Roadsides, meadows	*N. commune, N. edaphicum, N. ellipsosporum*, *N. passerinianum*, *Nostoc* sp. 1, *Nostoc* sp. 4, *Nostoc* sp. 17	7

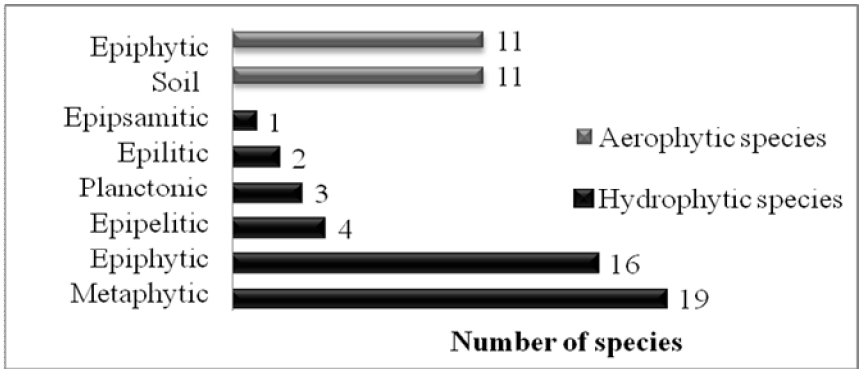


Fig. 7. *Nostoc* and *Desmonostoc* species number in aquatic and terrestrial habitats

Nostoc species were found in water bodies with pH ranging from 6.0 to 8.7, while there were no cyanobacteria found in waters with pH 3.8–5.9 (Fig. 8). According to

many scientific publications (MOLLENHAUER et al., 1999; DOUTELERO et al., 2004; PEREIRA et al., 2005, 2009; PERONA & MATEO, 2006; SHERWOOD, 2006; MUTHUKUMAR et al., 2007; ABOSEDE & IKEGWU, 2010; DEGTEVA et al., 2010), *Nostoc* cyanobacteria usually grow in waters with pH varying from 5.5 to 9.1. The highest diversity of species (95.2 % of species, which the determined values of factors) was recorded in alkaline Lithuanian water bodies (pH 7.1–8.7). *N. punctiforme* (pH 6.0–7.5) and *N. caeruleum* (pH 6.5–8.4) were growing in both acidic and alkaline waters. *N. verrucosum* were only detected in alkaline waters (pH 7.1–8.7), which corresponds to MOLLENHAUER et al. (1999) and SKINNER & ENTWISLE (2001) data. *Desmonostoc* sp. was found in alkaline water body (pH 8.0).

During the study, which was usually carried out in the period of June–August, the highest diversity of *Nostoc* species and *Desmonostoc* sp. (95.2 % of species, which the determined values of factors) were detected in water bodies with warm water (18.1–26.0 ^{o}C) and only five *Nostoc* species (23.8 %) were developed in cooler waters (10.0–18.0 ^{o}C) (Fig. 9.). We presume that water temperature has no influence to the occurence of most *Nostoc* species. *N. spongiaeforme* (10.0–21.0 ^{o}C), *N. caeruleum* (16.0–26.0 ^{o}C) and *N. verrucosum* (13.0–23.0 ^{o}C) species were found in waters with various temperature. *N. spongiaeforme* was reported in the rice fields, where water temperature reaches 20.0–25.0 ^{o}C (PEREIRA et al., 2005, 2009), while in Lithuania macroscopic colonies of *N. spongiaeforme* were found in spring water in winter time. *N. piscinale* species was found in lentic waters with temperature of 21.4–25.0 ^{o}C in Lithuania. This cyanobacterium also grows in sorghum fields with water temperature of 40 ^{o}C (SMITH, 2008) and in Spanish rivers at temperature 13.0–22.4 ^{o}C (DOUTELERO et al., 2004). Consequently, it could be stated that the influence of water temperature to distribution of *Nostoc* species is insufficiently investigated at present and thus further ecological studies could reveal valuable information.

The tolerance to fluctuations of water conductivity was detected among *Nostoc* species. *Nostoc* cyanobacteria were found in waters, which have conductivity varying from 78 to 580 µS/cm, and *Desmonostoc* sp. – 314 µS/cm (Fig. 10). However, cyanobacterial species were not found in water bodies with conductivity of 5–77 µS/cm. The highest species diversity of *Nostoc* species (76.2% of the total number of taxa) and *Desmonostoc* sp. were detected in waters with conductivity of 201–400 µS/cm. As values of water conductivity increases or decreases the diversity of *Nostoc* species is lower in the investigated water ecosystems. According to previous studies (MOLLENHAUER et al., 1999; DOUTELERO et al., 2004; PERONA & MATEO, 2006; SHERWOOD, 2006; ABOSEDE & IKEGWU, 2010; DEGTEVA et al., 2010), the interval of water conductivity, which is suitable to development of *Nostoc* cyanobacteria, is wider – 18–1138 µS/cm.

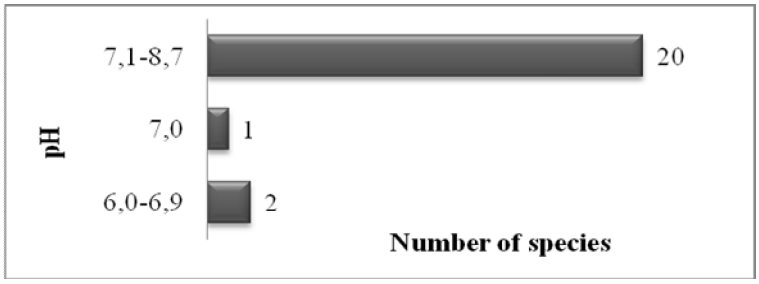


Fig. 8. Number of *Nostoc* and *Desmonostoc* species depending on water pH values

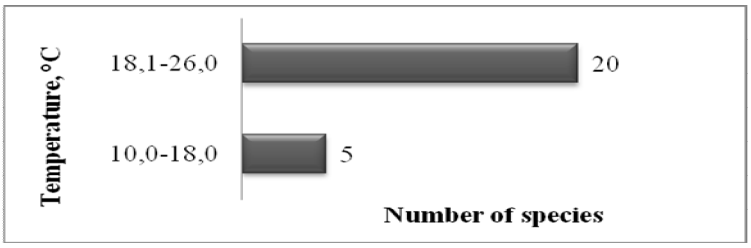


Fig. 9. Number of *Nostoc* and *Desmonostoc* species depending on water temperature

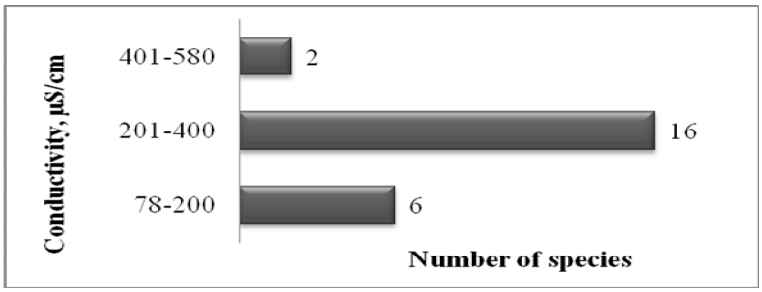


Fig. 10. Number of *Nostoc* and *Desmonostoc* species depending on water conductivity

Principal components analysis of environmental factors of *Nostoc* and *Desmonostoc* habitats established that the most varied factor is water conductivity (Fig. 11). The limits of tolerance to changes of water conductivity of *N. caeruleum* (150–580 µS/cm), *N. cuticulare* f. *polymorphum* (78–290 µS/cm), *N. piscinale* (78–329 µS/cm) and *N. spongiaeforme* (200–350 µS/cm) populations were determined. According to DOUTELERO et al. (2004), *N. piscinale* species was found in rivers with water conductivity of 92–768 µm/cm. *N. carneum* and *N. verrucosum* were found in Lithuanian waters with less varied water conductivity values (respectively 320–390 µS/cm and 260–300 µS/cm). Water conductivity of *N. verrucosum* habitats in Lithuania were determined higher than refered in SHERWOOD (2006) study (102±79 µS/cm). Based on the present study it could be assumed that *N. caeruleum* and *N. punctiforme* species are tolerant to variation of water pH concentration (respectively pH 6.5–8.4 and 6.0–7.5), while *N. spongiaeforme* and *N. verrucosum* – to variation of water temperature (respectively 10.0–21.0 ^{o}C and 13.0–23.0 ^{o}C). *N. carneum* species was determined in alkaline (pH 8.1–8.4), at warm water (23.0–25.0 ^{o}C) and conductivity 320–390 µS/cm Lithuanian water bodies. However, according to literature data this species also grow in acidic (pH 6.5–7.46) (MUTHUKUMAR et al., 2007; ABOSEDE & IKEGWU, 2010), at higher

water conductivity (251–782 µS/cm) waters (DOUTELERO et al., 2004). MOLLENHAUER et al. (1999) states that *N. caeruleum* develops in water bodies, in which water pH is about 7, and water conductivity are not higher than 240 µS/cm. Heterogenity of *N. caeruleum* habitats was determined in Lithuania: water pH 6.5–8.4, temperature – 16.0–26.0 °C and conductivity – 150–580 µS/cm. According to study data it can be stated that *N. caeruleum* is a species of extensive ecological amplitude and most possibly it develop not merely in oligotrophic water bodies, as stated by KAŠTOVSKÝ et al. (2010), but also in mesotrophic or eutrophic water bodies.

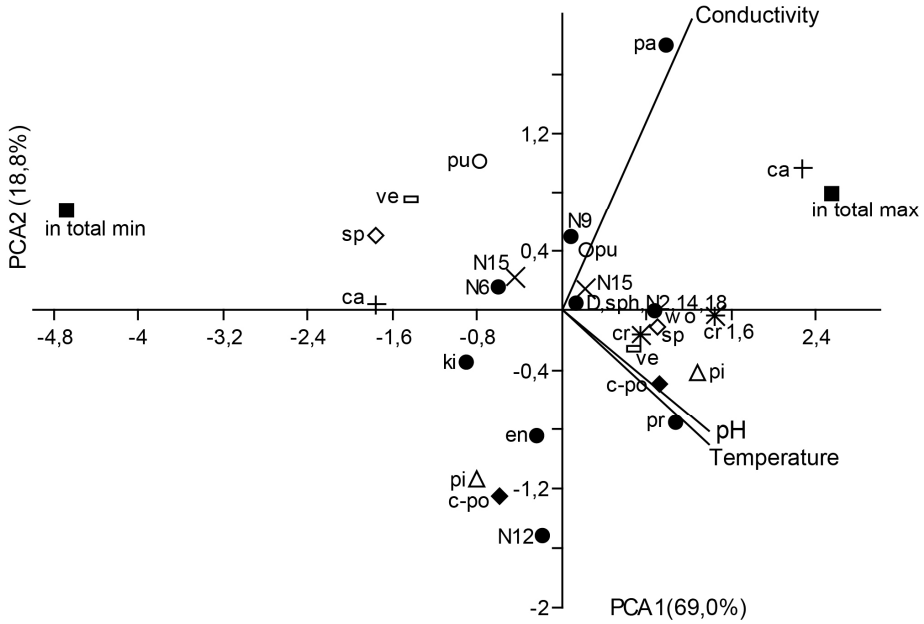


Fig. 11. Principal component analysis of some water habitats' pH, temperature, and conductivity of *Nostoc* and *Desmonostoc* species (ca+ – *Nostoc caeruleum*, cr✳ – *N. carneum*, c-po◆ – *N. cuticulare* f. *polymorphum*, D● – *Desmonostoc* sp., en● – *N. entophytum*, ki● – *N. kihlmanii*, pa● – *N. paludosum*, pi△ – *N. piscinale*, pr● – *N. pruniforme*, puO – *N. punctiforme*, sph● – *N.* cf. *sphaeroides*, sp◇ – *N. spongiaeforme*, ve▭ – *N. verrucosum*, wo● – *N.* cf. *wollnyanum*, N2● – *Nostoc* sp. 2, N6● – *Nostoc* sp. 6, 14● – *Nostoc* sp. 14, 15✕ – *Nostoc* sp. 15, 18● – *Nostoc* sp. 18, in total min, in total max ■ – the minimum and maximum values of all investigated water bodies)

Fingerprint TGGE analysis

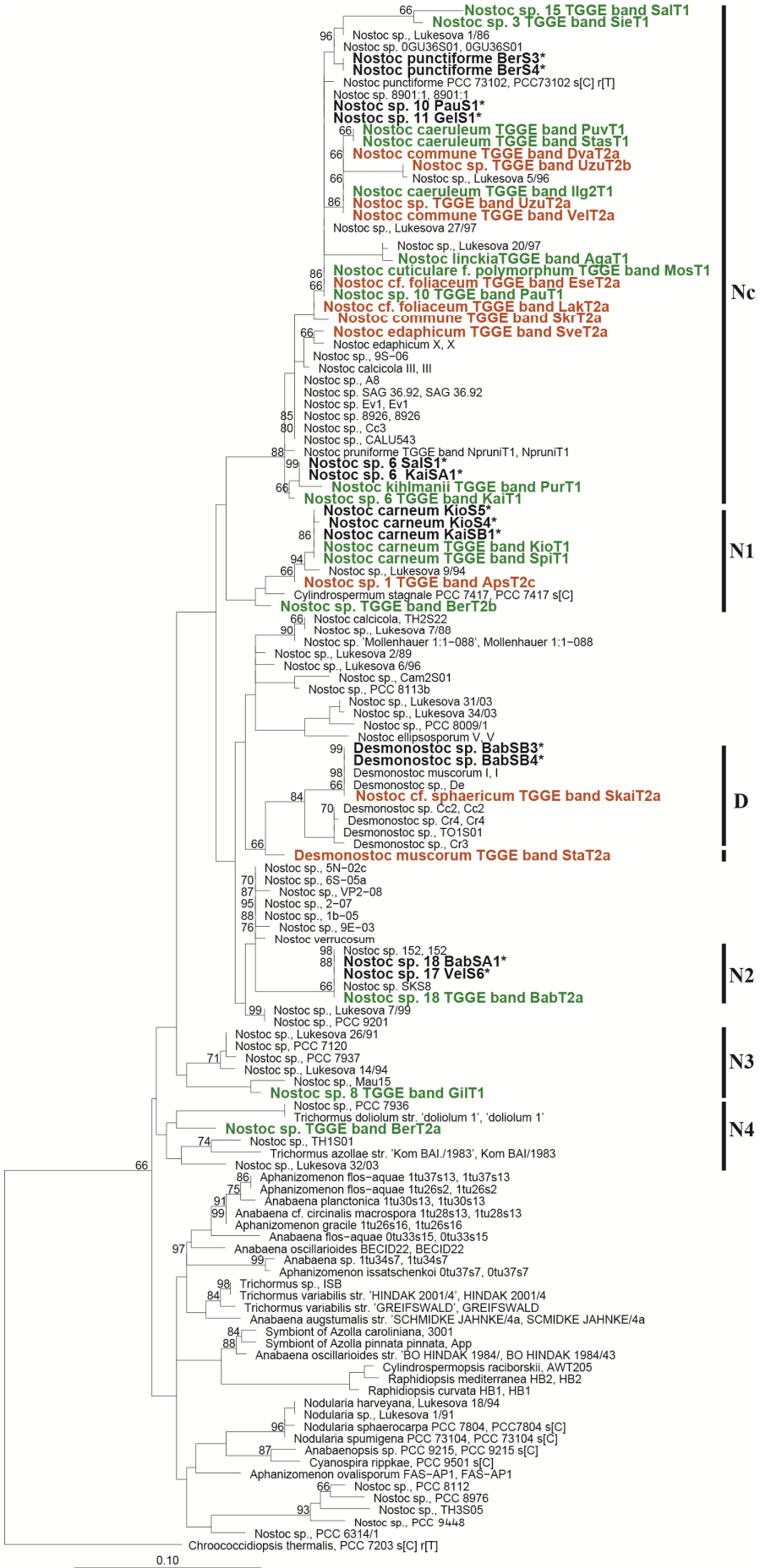
Molecular fingerprint TGGE or DGGE (*Denaturing gradient gel electrophoresis*) methods are significant for identification of a diversity of microorganisms and for ecologic studies of various ecosystems. Molecular research data is of great relevance when analysed together with the morphologic analysis data of natural populations (MUYEZ, 1999; SIGLER et al., 2003; TURICCHIA et al., 2005; AKOIJAM et al., 2012; LOZA et al., 2013). The molecular fingerprint TGGE method was applied to identify the natural populations of Lithuanian *Nostoc* and *Desmonostoc* species in aquatic and terrestrial samples and also, the morphological analysis of the populations was done in parallel. A

wide genetic and morphological diversity of the Lithuanian *Nostoc* and *Desmonostoc* species was identified during the studies. TGGE band sequences belonged to different 27 genotypes. During the morphological analysis different *Nostoc* and *Desmonostoc* taxa were identified within the range of the species or genus: *Desmonostoc muscorum*, *Nostoc caeruleum*, *N. carneum*, *N. commune*, *N. cuticulare* f. *polymorphum*, *N. edaphicum*, *N.* cf. *foliaceum*, *N. kihlmanii*, *N. linckia*, *N.* cf. *sphaericum* and *Nostoc* spp.. The morphological analysis of *Nostoc* and *Desmonostoc* species revealed morphologic differences of the species, i.e. the species belonged to different clusters of dendrogram of morphological data (Fig. 4). The phylogenetic analysis showed that the TGGE bands sequences located in *Nostoc* s. l. cluster where several different clusters are distinguished with TGGE bands (Fig. 12). The TGGE bands belonging to *Nostoc* species fell into *Nostoc commune* (*Nostoc sensu stricto*, Nc) cluster. Several TGGE bands identified as *Nostoc* species fell into phylogenetically separated clusters of *Desmonostoc* genus (D) and *Nostoc* species (N1, N2, N3, N4), which are remote from *Nostoc commune* cluster. The TGGE StaT2a band, identified as *Desmonostoc muscorum*, formed a separate sister branch to *Desmonostoc* genus cluster. The phylogenetic analysis of 16S RNA sequences of TGGE bands and strains isolated from Lithuanian aquatic and terrestrial habitats showed that some of the distinguished strains correspond to natural populations. *N. carneum* TGGE KioT1 and SpiT1 bands clustered with *N. carneum* KioS4 and KioS5 strains to one group. *Nostoc* sp. 6 TGGE KaiT1 band and *Nostoc* sp. 6 KaiSA1 strain, *Nostoc* sp. 18 TGGE BabT2a band and *Nostoc* sp. 18 BabSA1 strain are similar from the genotypic point of view, and were placed in to common clusters.

Phylogenetic analysis (16S rRNA) of *Nostoc* and *Desmonostoc* strains

The phylogenetic analysis covered 16S rRNA sequences of Lithuanian sixteen hydrophytic and aerophytic *Nostoc* and *Desmonostoc* strains. In the phylogenetic tree (Fig. 13) the strains fell into four *Nostoc commune* (*Nostoc sensu stricto*; Nc), *Desmonostoc* (D), *Nostoc* (N1) and *Anabaena*/*Nostoc* (A/N) clusters, which are different from the phylogenetic perspective. The phylogenetic and morphological analyses of the distinguished strains belonging to genera *Nostoc* and *Desmonostoc* provide an informative supplementation to the previous research data (RAJANIEMI et al., 2005; HROUZEK et al., 2005; SVENNING et al., 2005; ŘEHÁKOVÁ et al., 2007; PAPAEFTHIMIOU, 2008; HROUZEK et al., 2013).

Fig. 12. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of cyanobacteria. TGGE band sequences obtained in this study are marked in green (aquatic habitats) and brown (terrestrial habitats), while sequences from Lithuanian *Nostoc* and *Desmonostoc* strains are marked in bold and with asterisk. Numbers near nodes indicate bootstrap values ≥65%. *Nostoc commune* (*Nostoc sensu stricto*, **Nc**), *Desmonostoc* (**D**) and different *Nostoc* (**N1, N2, N3, N4**) clusters


Nostoc sp. 15 TGGE band SalT1
Nostoc sp. 3 TGGE band SieT1
Nostoc sp., Lukesova 1/86
Nostoc sp. 0GU36S01, 0GU36S01
Nostoc punctiforme BerS3*
Nostoc punctiforme BerS4*
Nostoc punctiforme PCC 73102, PCC73102 s[C] r[T]
Nostoc sp. 8901:1, 8901:1
Nostoc sp. 10 PauS1*
Nostoc sp. 11 GelS1*
Nostoc caeruleum TGGE band PuvT1
Nostoc caeruleum TGGE band StasT1
Nostoc commune TGGE band DvaT2a
Nostoc sp. TGGE band UzuT2b
Nostoc sp., Lukesova 5/96
Nostoc caeruleum TGGE band Ilg2T1
Nostoc sp. TGGE band UzuT2a
Nostoc commune TGGE band VelT2a
Nostoc sp., Lukesova 27/97
Nostoc sp., Lukesova 20/97
Nostoc linckiaTGGE band AgaT1
Nostoc cuticulare f. polymorphum TGGE band MosT1
Nostoc cf. foliaceum TGGE band EseT2a
Nostoc sp. 10 TGGE band PauT1
Nostoc cf. foliaceum TGGE band LakT2a
Nostoc commune TGGE band SkrT2a
Nostoc edaphicum TGGE band SveT2a
Nostoc edaphicum X, X
Nostoc sp., 9S−06
Nostoc calcicola III, III
Nostoc sp., A8
Nostoc sp. SAG 36.92, SAG 36.92
Nostoc sp. Ev1, Ev1
Nostoc sp. 8926, 8926
Nostoc sp., Cc3
Nostoc sp., CALU543
Nostoc pruniforme TGGE band NpruniT1, NpruniT1
Nostoc sp. 6 SalS1*
Nostoc sp. 6 KaiSA1*
Nostoc kihlmanii TGGE band PurT1
Nostoc sp. 6 TGGE band KaiT1
Nostoc carneum KioS5*
Nostoc carneum KioS4*
Nostoc carneum KaiSB1*
Nostoc carneum TGGE band KioT1
Nostoc carneum TGGE band SpiT1
Nostoc sp., Lukesova 9/94
Nostoc sp. 1 TGGE band ApsT2c
Cylindrospermum stagnale PCC 7417, PCC 7417 s[C]
Nostoc sp. TGGE band BerT2b
Nostoc calcicola, TH2S22
Nostoc sp., Lukesova 7/88
Nostoc sp. 'Mollenhauer 1:1−088', Mollenhauer 1:1−088
Nostoc sp., Lukesova 2/89
Nostoc sp., Lukesova 6/96
Nostoc sp., Cam2S01
Nostoc sp., PCC 8113b
Nostoc sp., Lukesova 31/03
Nostoc sp., Lukesova 34/03
Nostoc sp., PCC 8009/1
Nostoc ellipsosporum V, V
Desmonostoc sp. BabSB3*
Desmonostoc sp. BabSB4*
Desmonostoc muscorum I, I
Desmonostoc sp., De
Nostoc cf. sphaericum TGGE band SkaiT2a
Desmonostoc sp. Cc2, Cc2
Desmonostoc sp. Cr4, Cr4
Desmonostoc sp., TO1S01
Desmonostoc sp., Cr3
Desmonostoc muscorum TGGE band StaT2a
Nostoc sp., 5N−02c
Nostoc sp., 6S−05a
Nostoc sp., VP2−08
Nostoc sp., 2−07
Nostoc sp., 1b−05
Nostoc sp., 9E−03
Nostoc verrucosum
Nostoc sp. 152, 152
Nostoc sp. 18 BabSA1*
Nostoc sp. 17 VelS6*
Nostoc sp. SKS8
Nostoc sp. 18 TGGE band BabT2a
Nostoc sp., Lukesova 7/99
Nostoc sp., PCC 9201
Nostoc sp., Lukesova 26/91
Nostoc sp, PCC 7120
Nostoc sp., PCC 7937
Nostoc sp., Lukesova 14/94
Nostoc sp., Mau15
Nostoc sp. 8 TGGE band GilT1
Nostoc sp., PCC 7936
Trichormus doliolum str. 'doliolum 1', 'doliolum 1'
Nostoc sp. TGGE band BerT2a
Nostoc sp., TH1S01
Trichormus azollae str. 'Kom BAI./1983', Kom BAI/1983
Nostoc sp., Lukesova 32/03
Aphanizomenon flos−aquae 1tu37s13, 1tu37s13
Aphanizomenon flos−aquae 1tu26s2, 1tu26s2
Anabaena planctonica 1tu30s13, 1tu30s13
Anabaena cf. circinalis macrospora 1tu28s13, 1tu28s13
Aphanizomenon gracile 1tu26s16, 1tu26s16
Anabaena flos−aquae 0tu33s15, 0tu33s15
Anabaena oscillarioides BECID22, BECID22
Anabaena sp. 1tu34s7, 1tu34s7
Aphanizomenon issatschenkoi 0tu37s7, 0tu37s7
Trichormus sp., ISB
Trichormus variabilis str. 'HINDAK 2001/4', HINDAK 2001/4
Trichormus variabilis str. 'GREIFSWALD', GREIFSWALD
Anabaena augstumalis str. 'SCHMIDKE JAHNKE/4a, SCMIDKE JAHNKE/4a
Symbiont of Azolla caroliniana, 3001
Symbiont of Azolla pinnata pinnata, App
Anabaena oscillarioides str. 'BO HINDAK 1984/, BO HINDAK 1984/43
Cylindrospermopsis raciborskii, AWT205
Raphidiopsis mediterranea HB2, HB2
Raphidiopsis curvata HB1, HB1
Nodularia harveyana, Lukesova 18/94
Nodularia sp., Lukesova 1/91
Nodularia sphaerocarpa PCC 7804, PCC7804 s[C]
Nodularia spumigena PCC 73104, PCC 73104 s[C]
Anabaenopsis sp. PCC 9215, PCC 9215 s[C]
Cyanospira rippkae, PCC 9501 s[C]
Aphanizomenon ovalisporum FAS−AP1, FAS−AP1
Nostoc sp., PCC 8112
Nostoc sp., PCC 8976
Nostoc sp., TH3S05
Nostoc sp., PCC 9448
Nostoc sp., PCC 6314/1
Chroococcidiopsis thermalis, PCC 7203 s[C] r[T]
0.10
Nc
N1
D
N2
N3
N4

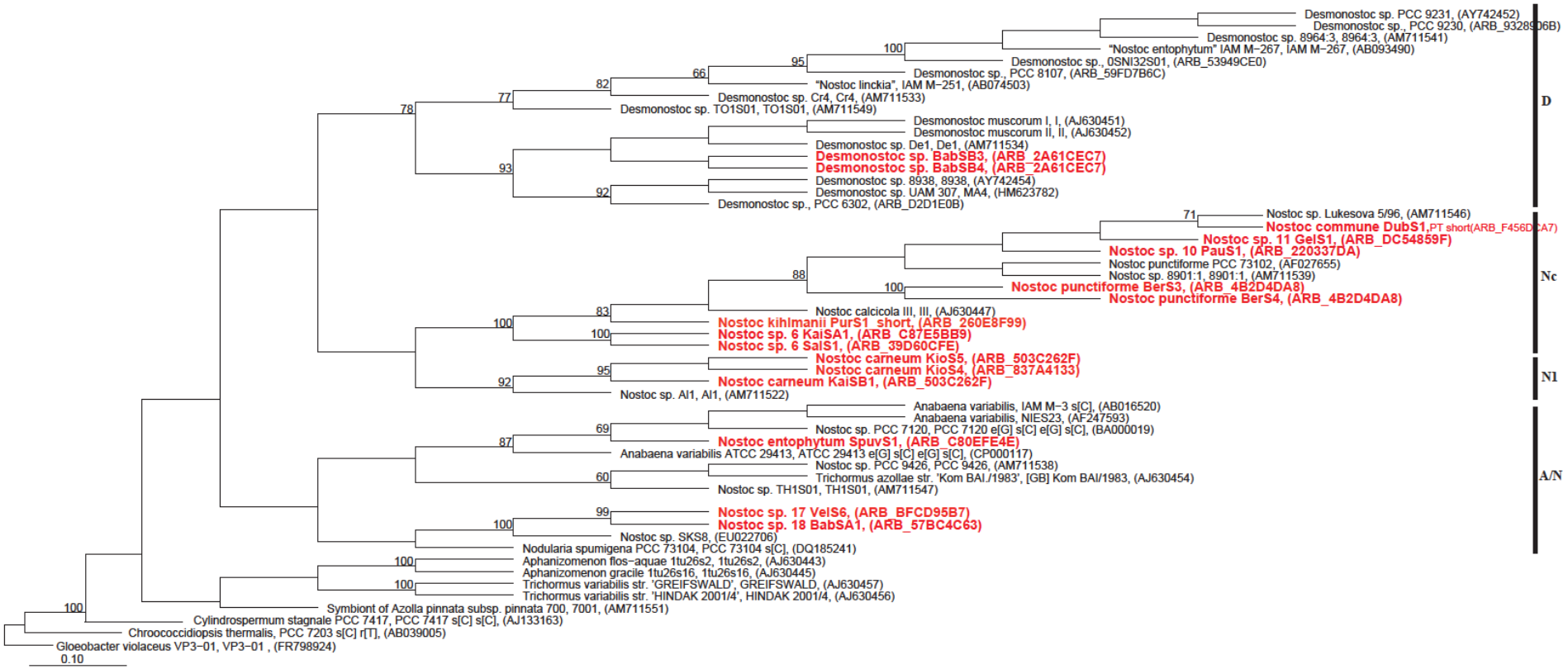


Fig. 13. Maximum parsimony phylogenetic tree based on 16S rRNA gene sequences of cyanobacteria. Sequences obtained in this study are marked in red. Numbers near nodes indicate bootstrap values ≥65%. *Desmonostoc* (**D**), *Nostoc commune* (*Nostoc sensu stricto*; **Nc**), *Nostoc* (**N1**) and *Anabaena*/*Nostoc* species (**A/N**) clusters

Morphologically and phylogenetically different strains that confirm the polyphyletic origin of *Nostoc* genus and the distinction of *Desmonostoc* genus were determined in the present study. Terrestrial *N. commune* DubS1 strain and *N. kihlmanii* PurS1, *N. punctiforme* BerS3, BerS4, *Nostoc* sp. 6 SalS1, KaiSA1, *Nostoc* sp. 10 PauS1 and *Nostoc* sp. 11 GelS1 strains, which were isolated from different water bodies, fell into *Nostoc commune* cluster (Fig. 13). *N. commune* DubS1 strain linked to *Nostoc* sp. Lukesova 5/96 strain, which was isolated from soil (HROUZEK et al. 2005). *Nostoc* sp. 10 PauS1 and *Nostoc* sp. 11 GelS1 strains are placed close to strains that are last mentioned above. *Nostoc* sp. 10 PauS1 strain was isolated from benthos of river, while *Nostoc* sp. 11 GelS1 strain was isolated from metaphyton of lake. *Nostoc* sp. 6 SalS1 and KaiSA1 strains clustered in a separate stable group (bootstrap values100 %) within *Nostoc commune* cluster. *N. kihlmanii* PurS1 strain formed a relatively separate branch within *Nostoc commune* cluster, while *N. punctiforme* BerS3 and BerS4 strains, which are almost morphologically identical, contained a separate reliable group (100 %). *Nostoc* species belonging to *Nostoc commune* cluster are also different by their morphological features (Fig. 4, 14).

Desmonostoc sp. BabSB3 and BabSB4 strains, isolated from a freshwater body, formed group together with *Desmonostoc* sp. De1, isolated from *Dioon edule* (VAGNOLI et al., 1992), soil *D. muscorum* I and *D. muscorum* II strains (HROUZEK et al., 2005; 2013) within *Desmonostoc* genus cluster (Fig. 13). *Desmonostoc* sp. fell into I cluster of morphological analysis of studied species together with *D. muscorum*, *N. ellipsosporum*, *Nostoc* sp. 5, and other species (Fig. 4).

N. carneum KaiSB1, KioS4 and KioS5 strains together with *Nostoc* sp. Al1 strain, isolated from *Anthoceros laevis* (VAGNOLI et al., 1991), composed a new cluster of *Nostoc* species (N1), a sister to *Nostoc commune* cluster (Fig. 13). *Nostoc* sp. Al1 strain formed a separate brach in the large cluster *Nostoc* species, according to the phylogenetic analysis of the symbiotic *Nostocales* cyanobacteria performed by PAPAEFTHIMIOU et al. (2008). Following the data provided by the authors, *Nostoc* sp. Al1 strain is morphologically different from strains of *Nostoc* (*Nostoc* s. str.) cluster, and is similar to the present *Desmonostoc* strains (HROUZEK et al., 2013). The morphological analysis of Lithuanian species revealed that *N. carneum* KaiSB1, KioS4 and KioS5 strains are morphologically more similar to *Desmonostoc* BabSB3 and BabSB4 strains than to strains of *Nostoc commune* cluster (Fig. 13, 14).

Nostoc sp. 17 VelS6, *Nostoc* sp. 18 BabSA1 and *N. entophytum* SpuvS1 strains, morphologically identified as *Nostoc*, fell in to *Anabaena*/*Nostoc* cluster (A/N; Fig. 13). The terrestrial *Nostoc* sp. 17 VelS6 and hydrophytic *Nostoc* sp. 18 BabSA1 strains, which morphologically are very similar (Fig. 14), linked to *Nostoc* sp. SKS8, isolated from *Blasia pusilla*, in the phylogenetic tree (Fig. 13). The phylogenetic analysis revealed that *N. entophytum* SpuvS1, *N. carneum* KaiSB1, KioS4, KioS5, *Nostoc* sp. 17

VelS6 and *Nostoc* sp. 18 BabSA1 strains do not belong to *Nostoc* s. str. genus, and therefore in the future they could be identified as species of new genera.

Morphology of *Nostoc* and *Desmonostoc* strains

The results of the morphological and molecular studies of Lithuanian *Nostoc* and *Desmonostoc* species (Fig. 13, 14) confirms the importance of combinated methods in the taxonomy of cyanobacteria, which is highlighted in many works (HROUZEK et al., 2005; RAJANIEMI et al. 2005; ŘEHÁKOVÁ et al., 2007; PAPAEFTHIMIOU, 2008; KOMÁREK, 2010 a; 2011). The morphological analysis of strains comprehensively complements the phylogenetic analysis of strains that are identified as *Nostoc* and *Desmonostoc* (Fig. 13, 14). *N. caeruleum* ObeS1, SpuvSA1 and *N. carneum* SpiS1 strains were not used in the phylogenetic analysis. The strains that belong to the *Nostoc commune* (*Nostoc sensu stricto*) cluster are distinguished in two groups (I and III) in the morphological analysis of strains, while the strains included into *Desmonostoc*, *Nostoc* and *Nostoc*/*Anabaena* clusters compound a separate group (II) (Fig. 13, 14). Non-parametric ANOSIM test showed that differences between morphological groups are statistically significant ($r = 0.9258$, $p < 0.05$). Test results are given in Table 2. The morphological and phylogenetic analyses of strains showed that the *Nostoc* s. str. species (*Nostoc commune* cluster) are characterized by a morphological and genetic diversity and morphologically varies from the phylogenetically different strains that are identified as *Nostoc* and *Desmonostoc* (group II) (Fig. 13, 14). *Desmonostoc* strains, which distinguishe morphological features from all of the investigated *Nostoc* s. l. strains (Fig. 14), were isolated during the study.

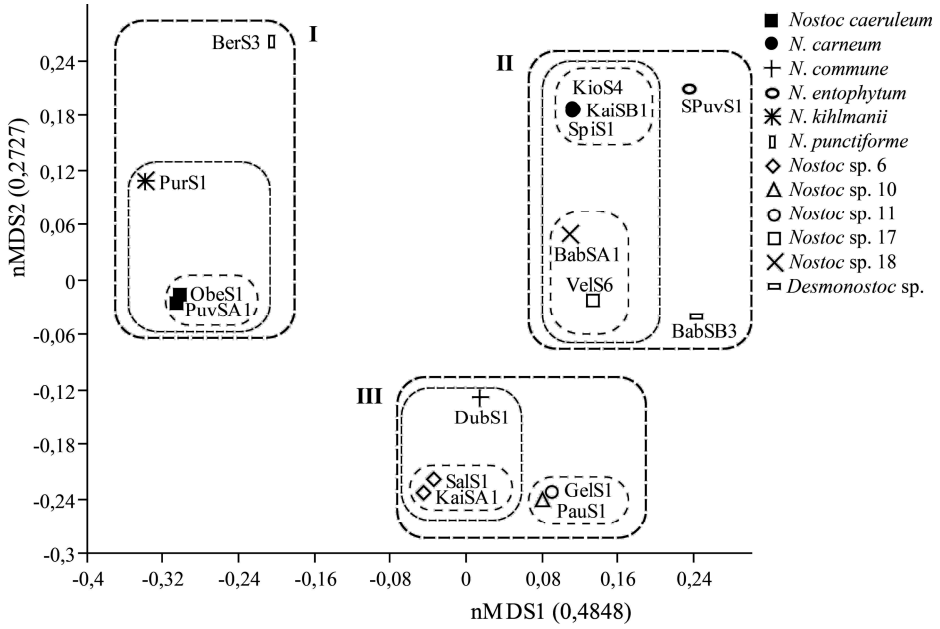


Fig. 14. Non-metric multidimensional scaling (NMDS) of *Nostoc* and *Desmonostoc* strains based on morphological data (Gower's index; ANOSIM test – R=0.9258, p< 0.05)

Table 2. Results of one-way ANOSIM test (based on the Gower's index) for differences in groups of *Nostoc* and *Desmonostoc* strains (see Fig. 17). R – ANOSIM statistics, p – probability

	R	p
I and II	0.0039	0.8378
I and III	0.0114	0.9788
II and III	0.0288	1

According to MATEO et al. (2011), 16S rRNA gene sequences are an important criterion in describing *Nostoc* strains, especially when phylogenetic data are consistent with stable morphological characteristics. Our research has shown that morphologically similar strains are phylogenetically close as well (*N. carneum* KaiSB1, KioS4 and KioS5, *Nostoc* sp. 17 VelS6 and *Nostoc* sp. 18 BabSA1, *Nostoc* sp. 6 SalS1 and KaiSA1, *Nostoc* sp. 10 PauS1 and *Nostoc* sp. 11 GelS1) (Fig. 13, 14). Other morphologically different strains are included in different clusters (*Desmonostoc* sp. BabSB3, BabSB4, *N. entophytum* SpuvS1) or are distant in the same cluster (*N. kihlmanii* PurS1, *N. punctiforme* BerS3) in a phylogenetic tree. According to the morphological analysis of strains (Fig. 14), specific morphological features of phylogenetically different strains or their groups (Fig. 13) are presented. The morphology of *Nostoc commune* cluster strains varies. The colonies of *N. kihlmanii* PurS1 and *N. punctiforme* BerS3 (also *N. caeruleum* ObeS1, SpuvSA1) strains are of clear shapes (spherical, oval, oblong), solid consistency, with firm surface layer, the trichomes are usually highly curved and densely entangled (Fig. 15 a, d-f, h, i). While the colonies of *N. commune* DubS1, *Nostoc* sp. 6 KaiSA1 and SalS1, *Nostoc* sp. 10 PauS1 and *Nostoc* sp. 11 GelS1 strains are mucilaginous, with a diffuse surface layer, the trichomes are flexuous and loosely entangled (Fig. 15 k-m, 16 a, d, f). These strains formed hormogonia (Fig., 15 j, m, o, 16 a, c, f), solitary cells (*N. punctiforme* BerS3, *Nostoc* sp. 6 KaiSA1 and SalS1, *Nostoc* sp. 10 PauS1; Fig 15 b, n, p), whereas akinetes were not observed or some of the strains were formed sparsely and solitary (*N. kihlmanii* PurS1, *N. commune* DubS1; Fig. 15 g, l). Aseriate stages (Fig. 15 b, r, s, 16 b, c) were observed for most of *Nostoc commune* cluster strains (except *N. commune* DubS1, *Nostoc* sp. 6 KaiSA1 and SalS1). Unique stages of developmental cycle were observed in *Nostoc* sp. 10 PauS1 strain. Each of the increasing cells of uniaseriate structures develop pluriseriate structures or young colonies consisting of curved and densely entangled trichomes within firmer surface layer (Fig. 16 b, c).

Desmonostoc sp. BabSB3 and BabSB4 strains are characterized by the following morphological features: colonies are irregularly expanded, soft, gelatinous, smooth-surfaced, with a diffuse surface layer; trichomes are long, flexuous and loosely entangled; akinetes are oval to cylindrical, differentiated in long chains (Fig. 17 e, g-l). The aseriate stage or any young colonies with a firm surface layer were not determined in *Desmonostoc* sp. BabSB3 and BabSB4 strains.

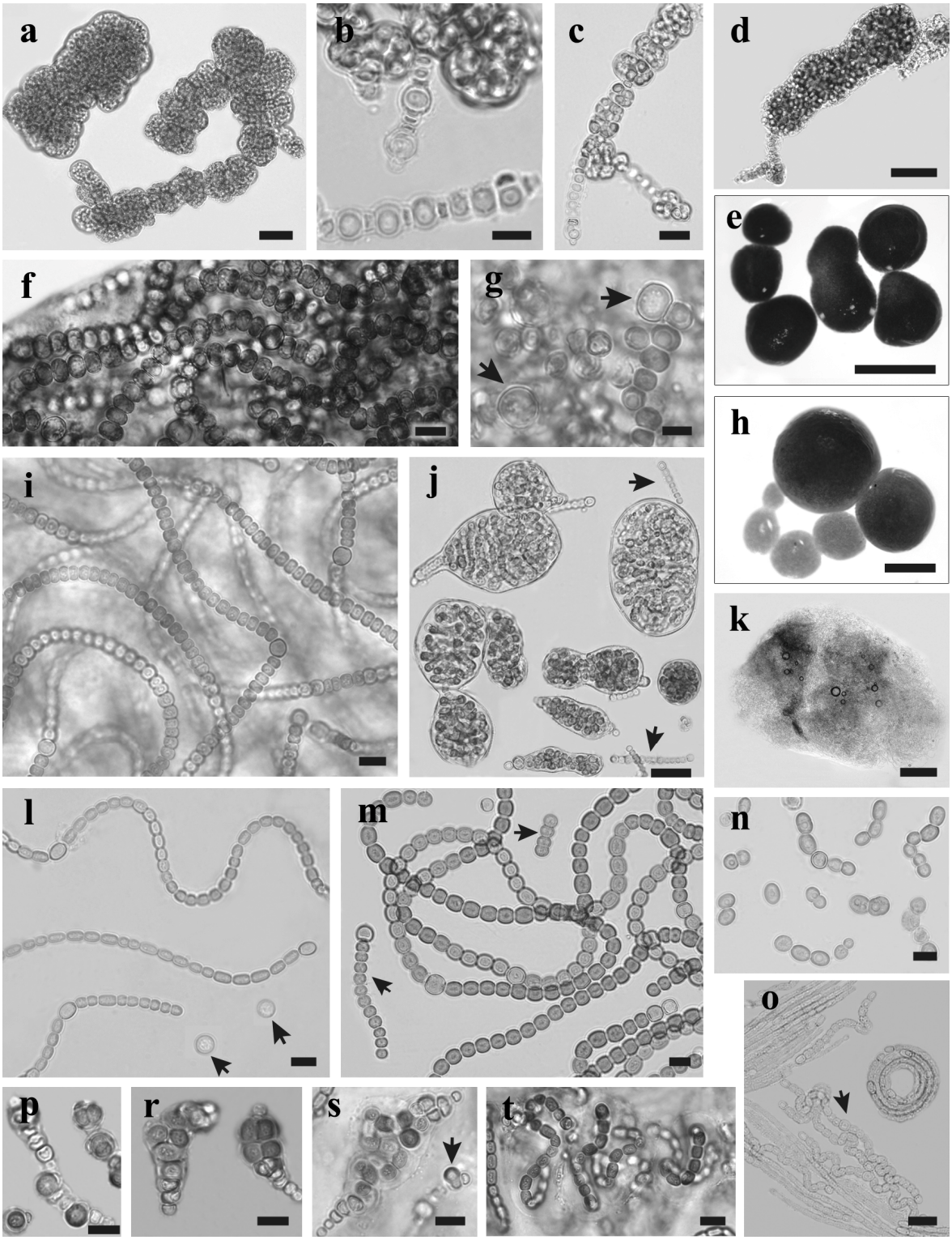


Fig. 15. *Nostoc punctiforme* BerS3 (a–c), *N. kihlmanii* Purs1 (d–g), *N. caeruleum* ObeS1 (h, i) and PuvSA1 (j), *Nostoc* sp. 6 KaiSA1 (k, m, n), *N. commune* DubS1 (l), *Nostoc* sp. 11 GelS1 (o), *Nostoc* sp. 10 PauS1 (p–t) strains; a – colonies, c, d, j – young colonies of various stages, hormogonia (j, ➔), b, n, p – solitary cells, e, h, k – macroscopic colonies, g – akinetes (➔), f, i, l, m, t – vegetative trichomes, akinetes (l,➔) and hormogonia (m, ➔), r, s – aseriate structures, solitary cell (s, ➔), o – hormogonia and their development stage (➔) (scale bars: b, c, f, g, i, l–o – 10 µm, j – 20 µm, a, d – 40 µm, e, h – 1 mm, k – 1 mm)

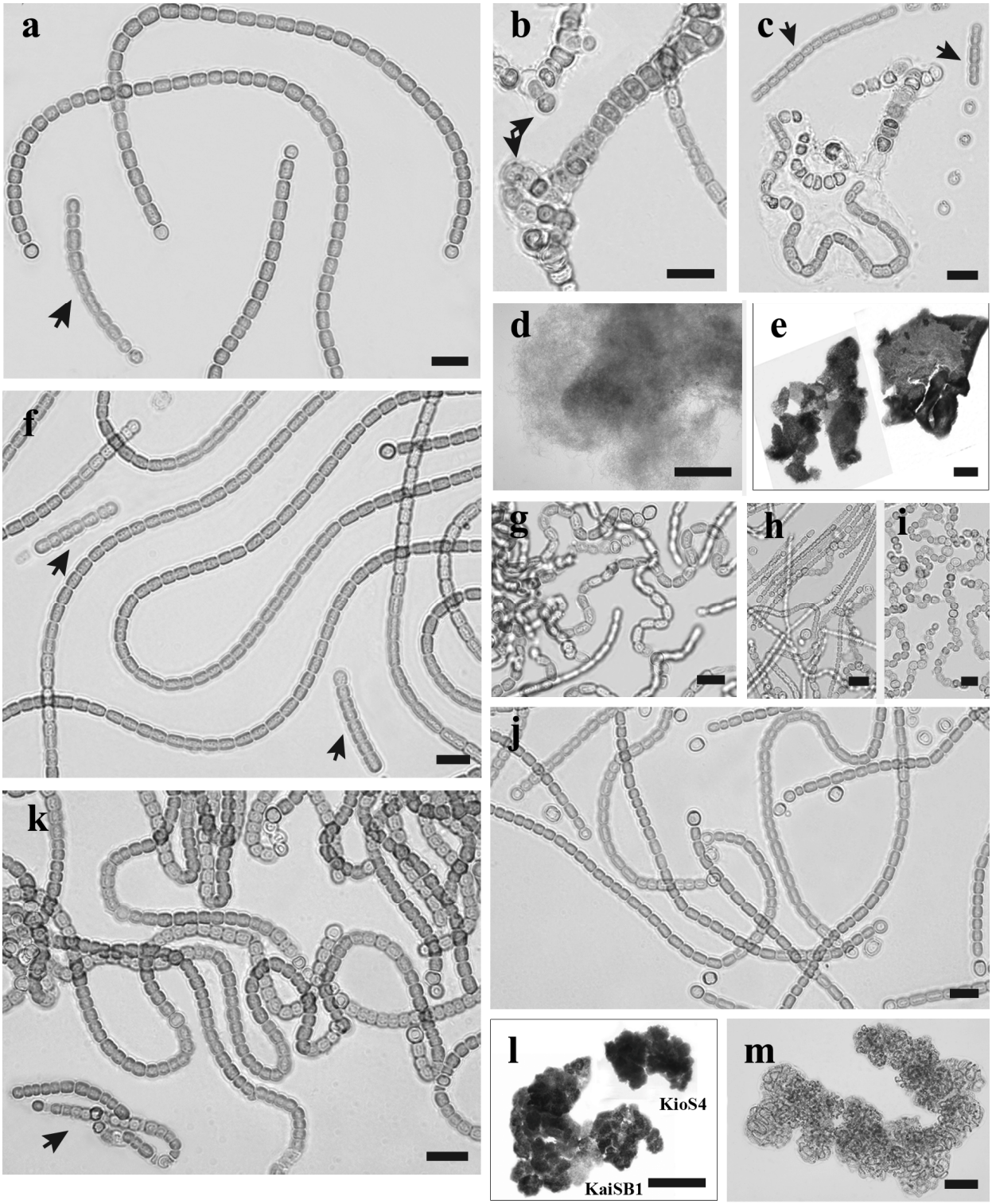


Fig. 16. *Nostoc* sp. 10 PauS1 (a–c), *Nostoc* sp. 11 GelS1 (d, f, g), *Nostoc* sp. 17 VelS6 (e, h–j), *Nostoc* sp. 18 BabSA1 (k), *N. carneum* KaiSB1 (m), KioS4 (l, m) strains; a, f, j, k – vegetative trichomes, hormogonia (➔), b – development of new trichome from cells of aseriate structures, c – trichome arising from cells of aseriate structure, hormogonia (➔), d, e, m – macroscopic colonies, g, i – akinetes-like cells, h – hormogonia, l – colonies (scale bars: a–c, f–k – 10 μm, d, e, l – 2 mm)

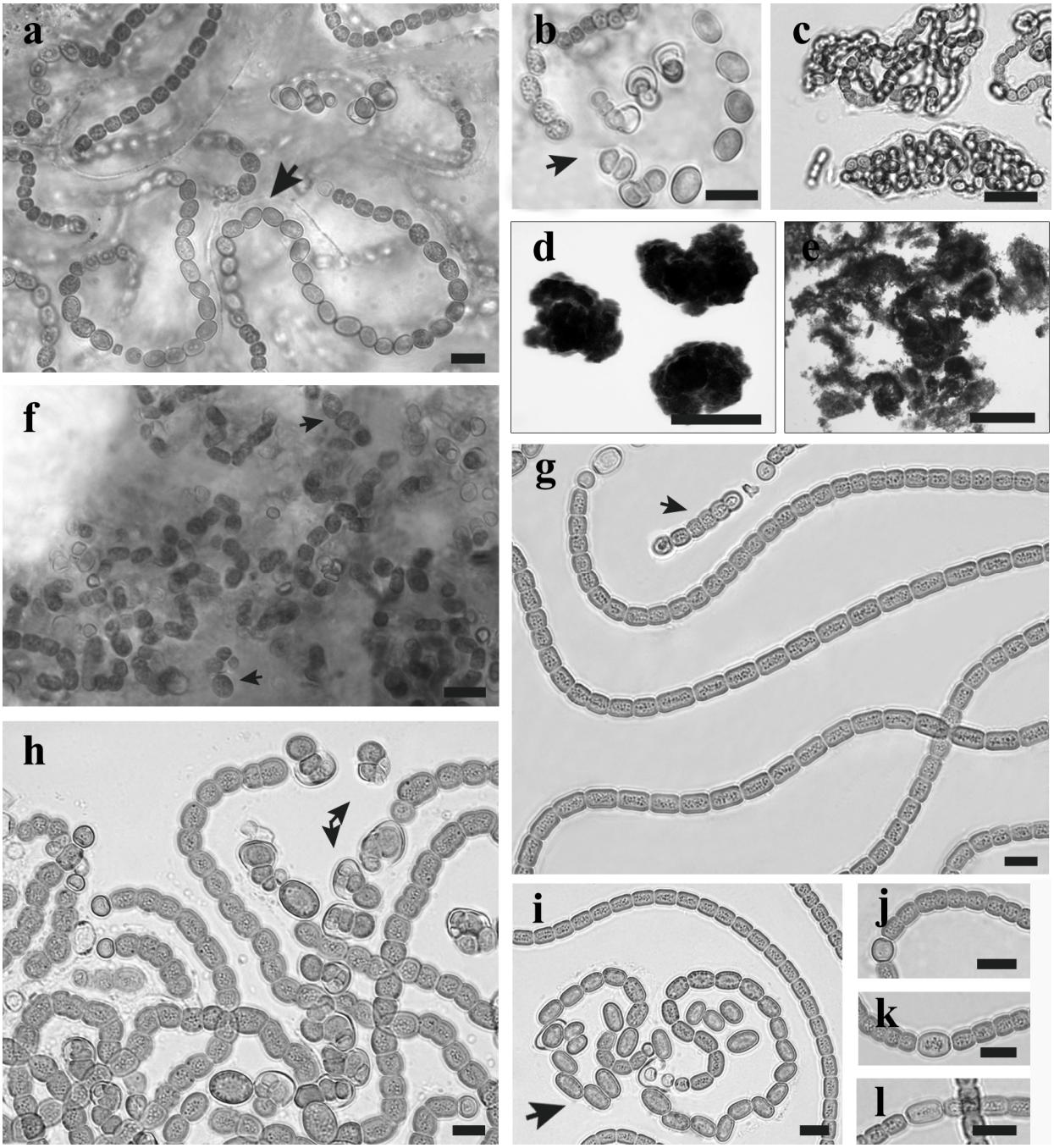


Fig. 17. *Nostoc carneum* KaiSB1 (a, c), KioS4 (b), *N. entophytum* SpuvS1 (d, f) and *Desmonostoc* sp. BabSB3 (e, g–l) strains; a, i – trichomes with akinetes in chains (➔), b – akinetes, germinated akinetes (➔), c – colonies of young stage, d, e – macroscopic colonies, f – arrangement of trichomes in the colony, akinetes (➔), g – trichomes, hormogonium (➔), h – trichomes, germinated akinetes (➔), j–l – trichomes fragments with intercalar heterocytes (scale bars: a, b, f–l – 10 µm, c – 20 µm, d – 1 mm, e – 2 mm)

The colonies of *N. carneum* KaiSB1, KioS4 and KioS5 strains are soft, gelatinous, with a firm surface layer, the trichomes usually are closely entangled, oval akinetes differentiated in long chains (Fig. 16 l, m, 17 a-c). Both *Desmonostoc* BabSB3, BabSB4 and *N. carneum* KaiSB1, KioS4, KioS5 strains are characterized by long chains of

akinetes, however some morphological differences of colonies and trichomes were established between these strains and the cells were different in measumerent ($p<0.05$). *N. entophytum* SpuvS1 strain is characterized by the following morphological features: the colonies are soft, gelatinous, irregularly expanded, roughly-surfaced, the trichomes are highly curved and densely entangled, uniquely curved in an acute angle (V letter shape), the akinetes are oval (Fig. 17 d, f). The colonies of *Nostoc* sp. 17 VelS6 and *Nostoc* sp. 18 BabSA1 strains are soft, gelatinous, irregularly expanded, and smooth-surfaced; the trichomes are slightly curved, flexuous and closely entangled (Fig. 16 e, j, k). These strains germinated only by hormogonia (Fig. 16 h, j), whereas akinetes were found in the natural population of *Nostoc* sp. 17 and *Nostoc* sp. 18 species.

The morphological analysis of *Nostoc* (and *Desmonostoc*) strains by HROUZEK et al. (2005; 2013) showed that the length of trichomes, their arrangement in the colony, shape of mucilaginous sheaths (and surface layers) and terminal heterocytes, and the morphology of hormogonia are significant to the species identification. The study of Lithuanian strains showed that the morphological features of colonies (shape, consistency, surface layer, mucilaginous sheaths), the arrangements of trichomes in the colony, the type of reproductive structures (hormogonia, akinetes, solitary cells), the abundance of akinetes within trichomes (solitary or in long chains, <10 akinetes) and the morphometric data of cells are important to the separation of *Nostoc* and *Desmonostoc* strains. However, unlike in HROUZEK et al. (2005) studies, no differences in the trichome length (trichomes of all strains are long) and shape of terminal heterocytes were determined in the Lithuanian study. Oval/rounded terminal heterocytes were observed in the investigated strains (Fig 15 l, m, 1, a, f, j, k, 17 g), and occasionally conical terminal heterocytes were found in the *Desmonostoc* BabSB3, *Nostoc* sp. 10 PauS1, *Nostoc* sp. 11 GelS1 and *Nostoc* sp. 17 VelS6 strains. The same pattern was observed in the natural populations as well. The shape of terminal heterocytes had no significance to the identification in case of the Lithuanian species. A firm surface layer of colonies or individual sheaths are characteristic to *Nostoc* species (*N. edaphicum*, *N. punctiforme*, *N. lichenoides*, *N. desertorum* and *N. indistinguendum*) (ŘEHÁKOVÁ et al., 2007; HROUZEK et al., 2005; 2013). This feature was also determined in some of the Lithuanian strains belonging to *Nostoc* s. str. genus (*N. kihlmanii* PurS1, *N. punctiforme* BerS3; Fig. 15 a, d, j). However, other strains that belong to *Nostoc commune* cluster (*N. commune* DubS1, *Nostoc* sp. 6 SalS1 and KaiSA1, *Nostoc* sp. 10 PauS1, *Nostoc* sp. 11 GelS1) formed diffused mucilaginous sheaths as well (Fig, 15, 16). Based on the Lithuanian study of the *Nostoc* and *Desmonostoc* species, it can be stated that firm sheaths or firm surface layer of colonies is characteristic only to some of the *Nostoc* species. During the studies, diffused colourless mucilaginous sheaths were mostly observed. In rare cases, mainly in the formation of new structures or in younger colonies, mucilaginous sheaths of trichomes are distinct. Therefore, the shape of individual sheaths had no significance to the identification of *Nostoc* and *Desmonostoc* strains as well (see more in 18–19 p.).

CONCLUSIONS

1. 20 *Nostoc* species and two intraspecific taxa, and 18 taxa to the *Nostoc* genus level were identified in Lithuania. Twelve *Nostoc* species and intraspecific taxa (*N. carneum*, *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. edaphicum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. passerinianum*, *N. piscinale*, *N.* cf. *sphaericum*, *N. spongiaeforme*, *N.* cf. *wollnyanum*), *Desmonostoc* genus including two taxa (*D. muscorum* and *Desmonostoc* sp.) were recorded for the first time in Lithuania. Nineteen strains belonging to *Nostoc* and *Desmonostoc* genera were isolated. *Nostoc commune*, *N. caeruleum*, *N. piscinale*, *N. spongiaeforme*, *N. verrucosum* and *N. cuticulare* f. *polymorphum* were found most often in the present study.
2. Morphological heterogeneity is characteristic to Lithuanian *Nostoc* species. Classical morphological descriptions are sufficient to identify only some *Nostoc* and *Desmonostoc* species (*Nostoc edaphicum*, *N. caeruleum*, *N. commune* (?), *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. ellipsosporum*, *N. kihlmanii*, *N. passerinianum*, *N. pruniforme*, *N. punctiforme*, *N. verrucosum,* and *Desmonostoc muscorum*), while morphological identification criteria for *Nostoc carneum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. linckia*, *N. paludosum*, *N. piscinale*, *N. spongiaeforme*, *N.* cf. *sphaericum*, *N.* cf. *sphaeroides* and *N.* cf. *wollnyanum* must be revised.
3. An applied research by different types of *Nostoc* and *Desmonostoc* cyanobacteria samples was valuable in morphological analysis – species were characterized precisely, suitability for identification and stability of diagnostic morphological features in species was identified. A stability of morphological features was determined for *Nostoc caeruleum*, *N. carneum*, *N. cuticulare* f. *polymorphum*, *N. entophytum*, *N. punctiforme*, *N.* cf. *wollnyanum* and *Desmonostoc muscorum* species, while an individual variability of morphological features was determined in *N. commune*, *N.* cf. *foliaceum*, *N. kihlmanii*, *N. linckia*, *N. piscinale* and *N.* cf. *sphaericum* species.
4. Morphological features of *Nostoc* species colony, shape and arrangements of trichomes, type of reproductive structures and abundance of akinetes within trichomes are significant for identification of species belonging to genus *Nostoc* sensu lato.
Shape of mucilaginous individual sheaths and granulation of akinetes had no significance to the identification of *Nostoc* species.
5. Reproduction by proliferation was newly described for *Nostoc edaphicum*, *N. kihlmanii*, *N. paludosum*, *N.* cf. *sphaericum*, *N. spongiaeforme* and *N. verrucosum*, while formation of akinetes – for *N. caeruleum*, *N. commune* and *N. kihlmanii*.
6. The highest diversity of *Nostoc* and *Desmonostoc* species (30 species) were recorded in lentic ecosystems and only three *Nostoc* species were found in lotic ecosystems. Most of *Nostoc* species were found in periphyton (23 species) and metaphyton (19 species) of water bodies, and only three *Nostoc* species – in plankton. In terrestrial habitats, fourteen

epiphytic and soil *Nostoc* and *Desmonostoc* species were discovered. *N. passerinianum* and *N. wollnyanum*, that are terrestrial species according to literature sources, were also found in epiphyton of water bodies in Lithuania.

7. The highest diversity of *Nostoc* and *Desmonostoc* species was recorded in water bodies with water temperature of 18.1–26.0 ^{o}C, pH of 7.1–8.7 and conductivity of 201–400 µS/cm, and were not found in water bodies with water pH of 3.8–5.9 and conductivity of 5–77 µS/cm. *Nostoc caeruleum*, *N. cuticulare* f. *polymorphum*, *N. piscinale*, *N. spongiaeforme* and *N. verrucosum* species are tolerant to variation of environmental factors mentioned above.

8. A wide genetic and morphological diversity of Lithuanian *Nostoc* and *Desmonostoc* species was identified while performing fingerprint TGGE and morphological analyses of cyanobacterial natural populations. TGGE bands fell into phylogenetically separated *Nostoc commune*, *Desmonostoc* genus and *Nostoc* species clusters in phylogenetic tree. The TGGE StaT2a band, identified as *Desmonostoc muscorum*, formed a separate sister branch to *Desmonostoc* cluster.

9. Lithuanian strains belonging to *Nostoc* and *Desmonostoc* genera are placed in different *Nostoc commune*, *Nostoc*, *Nostoc*/*Anabaena* and *Desmonostoc* clusters of phylogenetic tree. Lithuanian *N. carneum* KaiSB1, KioS4 and KioS5 strains together with *Nostoc* sp. Al1 strain, isolated from *Anthoceros laevis*, composed a new *Nostoc* cluster. Some of cyanobacterial taxa of genera *Nostoc* and *Desmonostoc* could be described as new species to Lithuania or new taxa for sciences in the future.

LIST OF PUBLICATIONS

KOSTKEVIČIENĖ J., ŠPAKAITĖ I., 2009: Diversity and distribution of the genus *Nostoc* in Lithuania. – Botanica Lithuanica, **15(1)**: 31–40.

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2009: *Nostoc* genties melsvabakterių įvairovė ir jos apsauga Lietuvoje. – Mokslas – Lietuvos ateitis (Science – Future of Lithuania), **1(4)**: 21–25.

Abstracts of scientific conferences

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., VENTURA S., MASCALCHI C., SILI C., HROUZEK P., 2010: Diversity of freshwater and terrestrial populations of *Nostoc* spp. in Lithuania. – 18th Symposium of the International Association for Cyanophyte Research (České Budějovice, Czech Republic): 36

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2010: Freshwater *Nostoc carneum* and *N. spongiaeforme*: variety of morphology and ecology. – 18th Symposium of the International Association for Cyanophyte Research, České Budějovice, Čekijos

Respublika. – 18th Symposium of the International Association for Cyanophyte Research (České Budějovice, Czech Republic): 92.

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2010: The morphology and life cycle of terrestrial Cyanobacteria *Nostoc ellipsosporum*. – XXIX International conference of Polish phycological society "Taxonomy the Queen of Science – the Beauty of Algae" (Krakow, Poland): 162–163.

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2009: The life cycle of *Nostoc ellipsosporum* (Cyanobacteria). – XI Young Systematists' Forum (London, United Kingdom).

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2009: *Nostoc* genus diversity, distribution and ecology in Lithuania. – 5th International Conference „Research and Conservation of Biological Diversity in Baltic Region" (Daugavpils, Latvia): 143.

ŠPAKAITĖ I., KOSTKEVIČIENĖ J., 2009: *Nostoc* genties melsvabakterių įvairovė ir jos apsauga Lietuvoje. – 12-oji Lietuvos jaunųjų mokslininkų konferencija „Mokslas – Lietuvos ateitis"; sekcija – Aplinkos inžinerija. – Vilniaus Gedimino Technikos Universitetas (Vilnius, Lithuania).

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Scientific Interest	Diversity, taxonomy end ecology of cyanobacteria, *Nostoc*

SANTRAUKA

Melsvabakterės – vieni seniausių pagal kilmę fotosintetinių prokariotinių pasaulio organizmų, turinčių svarbią ekologinę reikšmę įvairiose sausumos bei vandens ekosistemose (WHITTON & POTTS, 2000). Dabartiniu metu viena pagrindinių melsvabakterių mokslinių tyrimų problemų yra šių organizmų įvairovės pažinimas ir jų taksonominė klasifikacija (KOMÁREK, 2011). Šis darbas skirtas *Nostoc* Vaucher ex Bornet et Flahault 1886 ir *Desmonostoc* Hrouzek et Ventura genčių melsvabakterių tyrimams. Gleivėčiai (*Nostoc* genties melsvabakterės) paplitę visuose Žemės kontinentuose, sausumos ir vandens ekosistemose dažnai auga labai gausiai, gali netgi vyrauti organizmams atšiauriose ir skurdžiose maistmedžiagėmis buveinėse (GEITLER,

1932; POTTS, 2000). *Desmonostoc* genties melsvabakterės, matyt, taip pat plačiai paplitusios pasaulyje, dažniausiai tarpsta įvairiose sausumos buveinėse, nebūdingos poliarinių ar karštųjų dykumų regionams (HROUZEK et al., 2013). *Nostoc* ir *Desmonostoc* genčių melsvabakterės geba fiksuoti oro molekulinį azotą, todėl šių genčių atstovai gali vystytis neturtingoje azotu aplinkoje ir gali būti azoto junginių šaltinis augalams bei dumbliams sausumos ir vandens ekosistemose. Melsvabakterės taip pat sudaro įvairius simbiontinius ryšius su kai kuriais augalais augalais (*Gunnera*, cikainiai, samanos), o *Nostoc* genties melsvabakterės – ir su grybais (*Geosiphon pyriformis*), yra kerpių fikobiontai (DODDS et al., 1995; HROUZEK et al., 2013).

Šiuolaikinių kompleksinių (filogenetiniais ir morfologiniais) tyrimų metodų panaudojimas parodė, kad *Nostoc* gentis yra heterogeniška, sudaryta iš kelių skirtingų monofiletinių grupių, kurios netgi galėtų būti atskirtos tarpusavyje kaip naujos gentys (LACHANCE, 1981; HROUZEK et al., 2005; KOMÁREK, 2010 a). Tokie procesa vyksta – ŘEHÁKOVÁ ir kt. (2007) išskyrė *Mojavia* Řeháková et Johansen gentį. Remiantis ilgamečiais melsvabakterių *Nostoc* genties tyrimais, 2013 metais išskirta dar viena melsvabakterių *Desmonostoc* gentis (HROUZEK et al., 2013). *Mojavia* ir *Desmonostoc* genčių melsvabakterės morfologiškai labai panašios į *Nostoc*, tačiau skiriasi filogenetiškai. *Nostoc* genties rūšių monofiletinė grupė filogenetinėje analizėje dažniausiai nurodoma kaip *Nostoc commune* ar *Nostoc sensu stricto* (ŘEHÁKOVÁ et al., 2007; CUZMAN et al., 2010; HROUZEK et al., 2013). KOMÁREK (2010 a) duomenimis, šiuo metu yra žinoma apie 60 morfologinių *Nostoc* genties rūšių ir padermių, kurių dauguma nėra tiksliai identifikuotos. *Nostoc* genties rūšių identifikavimas vis dar išlieka problematiškas dėl genties heterogeniškumo, nepakankamų šios genties rūšis charakterizuojančių požymių, sudėtingo gyvenimo ciklo, kuris klasikiniuose darbuose labai retai analizuojamas. Dauguma *Nostoc* genties rūšių yra kritinės, todėl toks jų statusas reikalauja detalesnių taksonominių tyrimų (GEITLER, 1932; ELENKIN 1938; KONDRATYEVA & KISLOVA, 2002; KAŠTOVSKÝ et al., 2010). Daugelio melsvabakteres tiriančių mokslininkų pagrindinis tikslas yra šių organizmų įvairovės ir taksonomijos tyrimai, kuriems atlikti gali būti taikomi morfologiniai, molekuliniai bei ekologiniai tyrimo metodai.

Lietuvoje iki šiol nebuvo atlikti detalūs *Nostoc* genties melsvabakterių tyrimai, todėl apskritai mūsų šalyje yra labai mažai sukaupta duomenų apie šių organizmų rūšių įvairovę, biologiją ir ekologiją. Šiek tiek įvairaus pobūdžio informacijos apie *Nostoc* genties rūšių įvairovę ir ekologiją galima rasti istoriniuose Lietuvoje tyrimus atlikusių botanikų darbuose (GILIBERT, 1781; S. B. JUNDZIŁŁ, 1791; 1811; J. JUNDZIŁŁ, 1830; PABRIEŽA, 1900; SOKOŁOWSKA-RUTKOWSKA, 1932; MATUSZKIEWICZÓWNA, 1934). Duomenų apie sausumos *Nostoc* rūšis yra POCIENĖS (1960; 1961; 1981), POCIENĖS ir INDRAŠYTĖS (1977) darbuose, o JANKAVIČIŪTĖS (1996) bei KAROSIENĖS ir KASPEROVIČIENĖS (2009) darbuose – apie kelias gėlų vandenų *Nostoc* rūšis. Taip pat iki

šiol nėra atlikti *Nostoc* ar morfologiškai į šios genties melsvabateres panašių ir filogenetiškai artimų genčių filogenetiniai tyrimai.

Darbo tikslas – atlikti Lietuvos *Nostoc* ir *Desmonostoc* genčių melsvabakterių morfologijos, ekologijos ir filogenijos tyrimus.

Darbo uždaviniai:

1) surinkti *Nostoc* ir *Desmonostoc* genčių rūšių mėginius skirtinguose Lietuvos regionuose;
2) išanalizuoti ir identifikuoti surinktus ir Vilniaus universiteto herbariuminėse kolekcijose saugomus *Nostoc* ir *Desmonostoc* genčių melsvabakterių mėginius;
3) atlikti *Nostoc* ir *Desmonostoc* genčių rūšių požymių morfologinę analizę, įvertant morfologinių požymių pastovumą ir jų diagnostinį tinkamumą;
4) išanalizuoti *Nostoc* ir *Desmonostoc* genčių rūšių dauginimosi struktūras ir jų vystymosi ypatybes;
5) išskirti *Nostoc* ir *Desmonostoc* genčių rūšių padermes ir atlikti jų požymių morfologinę analizę;
6) sudaryti išplėstinį *Nostoc* ir *Desmonostoc* genčių rūšių konspektą su autentiškais melsvabakterių morfometriniais duomenimis ir originaliomis nuotraukomis;
7) pritaikyti molekulinių tyrimų metodus *Nostoc* ir *Desmonostoc* gečių rūšių įvairovės tyrimams;
8) ištirti *Nostoc* ir *Desmonostoc* genčių rūšių ekologiją *in situ*.

Ginami teiginiai:

1. Lietuvos *Nostoc* genties rūšių įvairovė yra kur kas didesnė nei buvo žinoma iki šių tyrimų.
2. Gamtinių populiacijų ir kultūrų naudojimas *Nostoc* ir *Desmonostoc* genčių rūšių morfologiniuose tyrimuose yra svarbus rūšių identifikavimui bei diagnostinių morfologinių požymių tinkamumo vertinimui. *Nostoc* genties rūšių diagnostiniai morfologiniai požymiai yra pakankami tik dalies melsvabakterių rūšių identifikavimui.
3. Lietuvos *Nostoc* genties rūšys ne tik morfologiškai, bet ir filogenetiškai yra heterogeniški organizmai.

Darbo mokslinis naujumas. Pirmą kartą atlikti detalūs gėlųjų vandenų ir sausumos Lietuvos melsvabakterių *Nostoc* ir *Desmonostoc* genčių rūšių įvairovės, taksonomijos, biologijos ir ekologijos tyrimai. Melsvabakterių rūšių diagnostikai pritaikytas skirtingo tipo mėginių analizės metodas. Sudarytas Lietuvos 40 *Nostoc* genties ir 2 *Desmonostoc* genties taksonų išplėstinis, autentiškomis nuotraukomis iliustruotas konspektas, kuriame pateikiami originalūs melsvabakterių rūšių aprašymai ir jų ekologijos duomenys. Dešimt *Nostoc* genties rūšių ir du vidurūšiniai taksonai bei dvi *Desmonostoc* genties rūšys identifikuotos pirmą kartą Lietuvoje. Išskirta septyniolika *Nostoc* genties ir dvi *Desmonostoc* genties padermės. Pirmą kartą Lietuvoje melsvabakterių *Nostoc* ir *Desmonostoc* genčių įvairovės, taksonomijos ir filogenijos tyrimuose taikyti molekuliniai duomenų analizės metodai.

Darbo reikšmė. Atlikti *Nostoc* ir *Desmonostoc* genčių melsvabakterių tyrimai papildo žinias apie jų rūšių įvairovę Lietuvoje ir suteikia naujos informacijos apie šių organizmų biologiją ir ekologiją. Išplėstinis *Nostoc* ir *Desmonostoc* genčių rūšių konspektas gali būti panaudotas ruošiant Lietuvos dumblių florą. *Nostoc* ir *Desmonostoc* genčių rūšių išsamūs morfologijos ir biologijos aprašymai, iliustruoti autentiškomis nuotraukomis, ir ekologiniai duomenys yra vertingi šių organizmų rūšių identifikavimo procese. *Nostoc* ir *Desmonostoc* genčių rūšių morfologinių ir molekulinių-filogenetinių tyrimų rezultatai gali būti panaudoti tolesniuose *Nostocales* eilės melsvabakterių taksonomijos tyrimuose. Išskirtos *Nostoc* ir *Desmonostoc* rūšių padermės gali būti naudojamos melsvabakterių chemotaksonominiuose ir biocheminiuose tyrimuose.

Išvados

Lietuvoje identifikuota 22 *Nostoc* genties rūšys ir vidurūšiniai taksonai bei 18 *Nostoc* taksonų identifikuota iki genties rango. Pirmą kartą Lietuvoje nustatyta 12 *Nostoc* genties rūšių ir vidurūšinių taksonų (*N. carneum*, *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. edaphicum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. passerinianum*, *N. piscinale*, *N.* cf. *sphaericum*, *N. spongiaeforme* ir *N.* cf. *wollnyanum*) bei dvi *Desmonostoc* genties melsvabakterės (*D. muscorum* ir *Desmonostoc* sp.). Išskirta 19 *Nostoc* ir *Desmonostoc* genčių padermių. Dažniausios Lietuvoje melsvabakterių rūšys yra *Nostoc commune*, *N. caeruleum*, *N. piscinale*, *N. spongiaeforme*, *N. verrucosum* ir *N. cuticulare* f. *polymorphum*.

Lietuvos *Nostoc* genties rūšys pasižymi morfologiniu heterogeniškumu. *Nostoc* ir *Desmonostoc* genčių rūšių klasikiniuose aprašymuose pateikiami morfologiniai požymiai yra pakankami tik dalies rūšių (*Nostoc edaphicum*, *N. caeruleum*, *N. commune* (?), *N. cuticulare* f. *ligericum*, *N. cuticulare* f. *polymorphum*, *N. ellipsosporum*, *N. kihlmanii*, *N. passerinianum*, *N. pruniforme*, *N. punctiforme*, *N. verrucosum* ir *Desmonostoc muscorum*) atpažinimui. Kitų *Nostoc* genties rūšių – *N. carneum*, *N. entophytum*, *N.* cf. *foliaceum*, *N.* cf. *humifusum*, *N. linckia*, *N. paludosum*, *N. piscinale*, *N. spongiaeforme*, *N.* cf. *sphaericum*, *N.* cf. *sphaeroides* ir *N.* cf. *wollnyanum* – morfologiniai identifikavimo kriterijai dar turi būti tikslinami.

Nostoc ir *Desmonostoc* genčių rūšių morfologinėje analizėje taikytas skirtingo tipo pavyzdžių tyrimas pasitvirtino – tiksliai apibūdintos rūšys, įvertintas rūšių diagnostinių morfologinių požymių stabilumas ir identifikacinis tinkamumas. Morfologinių požymių stabilumas nustatytas *Nostoc caeruleum*, *N. carneum*, *N. cuticulare* f. *polymorphum*, *N. entophytum*, *N. punctiforme*, *N.* cf. *wollnyanum* ir *Desmonostoc muscorum* rūšims, o tokio tipo požymių kintamumas – *N. commune*, *N.* cf. *foliaceum*, *N. kihlmanii*, *N. linckia*, *N. piscinale* ir *N.* cf. *sphaericum*.

Nostoc genties rūšių analizė atskleidė svarbius *Nostoc* sensu lato genties rūšių diagnostinius požymius, tokius kaip brandžių kolonijų struktūra, trichomų vingiuotumas ir išsidėstymas kolonijoje, dauginimosi struktūrų tipas bei akinečių susidarymo

dėsningumas. Nepasitvirtino iki šiol tyrimuose naudotų požymių – akinečių granuliuotumas ir trichomų gleivinės makštys – svarba *Nostoc* rūšių melsvabakterių diagnostikoje.

Septynioms *Nostoc* genties rūšims (*N. edaphicum*, *N. kihlmanii*, *N. paludosum*, *N.* cf. *sphaericum*, *N. spongiaeforme* ir *N. verrucosum*) nustatytas iki šiol nežinomas dauginimasis pumpuravimo būdu, trims (*N. caeruleum*, *N. commune* ir *N. kihlmanii*) – akinetėmis.

Lietuvoje didžiausia *Nostoc* ir *Desmonostoc* genčių rūšių įvairovė (30 rūšių) nustatyta lentinėse ekosistemose; lotinėse ekosistemose aptiktos tik 3 *Nostoc* genties rūšys. Daugiausia *Nostoc* genties rūšių aptikta perifitone (23 rūšys) ir metafitone (19), mažiausiai – planktone (3). Sausumos buveinėse konstatuota 14 epifitinių ir epigėjinių *Nostoc* ir *Desmonostoc* genčių rūšių. Literatūros šaltiniuose *N. passerinianum* ir *N. wollnyanum* nurodomos kaip sausumos rūšys, Lietuvoje aptiktos ir vandens telkiniuose.

Didžiausia *Nostoc* ir *Desmonostoc* genčių rūšių įvairovė nustatyta vandens telkiniuose, kurių temperatūra buvo 18,1–26,0 ^{o}C, pH 7,1–8,7, o savitojo elektrinio laidžio reikšmės svyravo 201–400 µS/cm ribose. *Nostoc* ir *Desmonostoc* genčių rūšių neaptikta vandens telkiniuose, kurių pH 3,8–5,9 ir savitasis elektrinis laidis – 5–77 µS/cm. *Nostoc caeruleum*, *N. cuticulare* f. *polymorphum*, *N. piscinale*, *N. spongiaeforme* ir *N. verrucosum* rūšims būdingas ekologinis plastiškumas minėtiems aplinkos veiksniams.

Melsvabakterių gamtinių populiacijų molekulinių žymenų TGGE ir morfologinės analizių metu nustatyta gana didelė Lietuvos *Nostoc* ir *Desmonostoc* genčių rūšių genetinė ir morfologinė įvairovė. Filogenetiniame medyje TGGE juostos patenka į *Nostoc commune*, *Desmonostoc* genties ir kitus skirtingus *Nostoc* genties rūšių klasterius, o *Desmonostoc muscorum* TGGE juosta sudaro seserinę šaką *Desmonostoc* genties klasteriui.

Lietuvos *Nostoc* ir *Desmonostoc* genčių padermės filogenetiniame medyje patenka į skirtingus *Nostoc commune*, *Nostoc*, *Nostoc*/*Anabaena* ir *Desmonostoc* klasterius. Lietuvos *N. carneum* KaiSB1, KioS4 ir KioS5 padermės bei *Anthoceros laevis* kerpsamanės simbiontinė *Nostoc* sp. Al1 padermė sudarė naują *Nostoc* genties rūšių klasterį. Dalis *Nostoc* ir *Desmonostoc* genčių melsvabakterių ateityje gali būti pripažintos kaip naujos Lietuvai šių organizmų rūšys ar net kaip mokslui nauji taksonai.