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## FOREWORD OF THE EDITOR-IN-CHIEF

In 2014, after 10 years of interruption, Book 2 – Botany of the Annual of Sofia University “St Kliment Ohridski”, comes to life again. The volumes of the Annual of Sofia University are more than a century old and have been recognized and acknowledged by audience not only as a pure scientific collection of papers, but also as a vital historical documentation, which reflects the activities of University teachers. This, together with the fact, that the Annual is issued just once in a year, makes it an unique periodical publication, with a totally different fate from other pure scientific journals. It was a long discussion among the members of the newly established Editorial Board how to proceed in future, if we decide to continue at all in the circumstances of the new requirements for scientists and new scientific style, where the impact peer reviewed journals are appreciated as the most valuable ones. In spite of this, it was our common decision to take the risk of *revival* of this periodical on the highest possible scientific level, keeping in the same time the best of its traditions. We strongly believe that these efforts are worthy, especially now, when biodiversity is a focal point of all nature conservational activities but “classical studies, characterizing different categories of biodiversity on all levels, are no longer appreciated by many younger (and also older) biologists” (MOLLENHAUER 2014). Therefore we accept as our scientific and national duty to keep this special periodical issue, in which could be published valuable scientific data on local and regional biodiversity of Bulgaria, based on the solid fundamental knowledge of classical botany but strongly linked with modern systematics methods and paradigms. In addition, other papers, orientated towards different aspects of plant sciences are and will continue to be more than welcome in our specialized volume. Honoring the best traditions, Book 2–Botany will keep its readers informed about the research carried-out by the youngest botanists and mycologists – bachelors, master students and PhD students of the Faculty of Biology of Sofia University, scientific conferences reports and book reviews. In spite of being created and declared as departmental periodical, Book 2–Botany of the Annual of Sofia University, as a peer-reviewed periodical is permanently open for all botanists and mycologists, who would like to share their knowledge and recent findings.

Maya P. Stoyneva. Editor-in-Chief

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CONGRATULATION NOTE TO PROF. DRSC DOBRINA  
N. TEMNISKOVA-TOPALOVA  
ON OCCASION OF HER 80TH BIRTHDAY



It is really difficult in a brief way and in a concise style, as it has to be done in a scientific journal, to describe the various activities of Prof. DrSc Dobrina N. Temniskova-Topalova and her role in Bulgarian algology and botany. This is even more difficult to be done after the detailed and very emotional, touching paper, dedicated to her in the honour of her 70<sup>th</sup> birthday. This article, published in a special “Festschrift in honour of Prof. Dobrina Temniskova-Topalova” (2004, Pensoft & St. Kl. Ohridski University Publishing House) and co-written by her supervisor and respected teacher Prof. D. Vodenicharov with two of her

students-successors in diatomology – K. Manoylov and N. Ognjanova, with the subtitle “Life dedicated to students” is strongly recommended to all readers, who want to follow in detail the professional and life-time of Prof. Temniskova.

Prof. Dobrina Temniskova was borne on November 12, 1934 in the renowned historical Bulgarian town Veliko Turnovo. There she made her first steps in elementary school and there she graduated from gymnasium. In 1952, she started her biological studies in Sofia State University, Faculty of Biology, Geology and

Geography. As a student, she specialized in the Department of Plant Systematics and Plant Geography (nowadays named Department of Botany), from which she graduated in 1957. During the university educational period raised her interest to algae and their magnificent microscopical world. In 1962 she started work as external assistant in the same department, and in 1964 occupied there a position of a regular assistant. Since 1962 till her retirement in 2004, she spent all her lifetime in the Department of Botany, growing step by step in her career. In 1972 she defend her PhD Thesis on euglenophytes and other flagellate algae in small temporary water basins of Bulgaria, in 1982 she became Associated Professor, in 1994 she defend a second thesis on fossil diatom in Bulgaria and obtained the degree “Doctor of Biological Science”, and in 1995 became a Professor of Botany. Extremely helpful for her scientific development was the possibility to specialize in the Diatom lab of Sankt Petersburg University and in the Botanical Institute of the Academy of Sciences of USSR (1968–1969).

The scientific and teaching professional development of Prof. Temniskova ran parallel with different administrative duties in the Department of Botany, Faculty of Biology and Sofia University. She was a Head of the Lab of Diatom analysis in the Department of Botany (1982–2002), Head of the Department of Botany (1996–2002), member of the Faculty Council of the Faculty of Biology (1987–2002), as well as of the Academic Council of the Sofia University (1999–2003) and a member of the Editorial Boards of the Book 2–Botany of the Annual of Sofia University and of the Volume 1 – Plants of the Red Data Book of R. Bulgaria. She was elected as the first Ombudsman of Sofia University (2004–2012). Noteworthy in her administrative activities was the support for the Botanical Gardens of Sofia University, which had to overcome extremely hard circumstances and for the Herbarium of the Department of Botany.

Prof. D. Temniskova was a deeply respected member of many scientific councils and boards in Bulgaria: Commission on Medico-Biological Sciences in the frame of the High Attestation Commission at the Ministry Council in Bulgaria, Specialized Council in Botany and Mycology in the frame of the High Attestation Commission, Scientific Council of the Central Laboratory of Ecology at the Bulgarian Academy of Sciences, Scientific Council of the Institute of Biodiversity and Ecosystems at the Bulgarian Academy of Sciences, Scientific Coordination Center for Global Projects at the Bulgarian Academy of Sciences, *etc.* In addition, she is a member of many scientific societies, among which have to be noted the International Society for Diatom Research, International Phycological Society, Union of Bulgarian Scientists and especially – the Bulgarian Botanical Society. In 1999 she became a Chair of this eminent Bulgarian scientific organization, founded in 1923. She plays an important role in its recent establishment, scientific and public awareness, keeping strongly its traditions and scientific grounds developed by its first Chair (the first Bulgarian algologist Prof. S. Petkov) and

other prominent Bulgarian botanists among its founders – Acad. N. Stoyanov and Acad. D. Yordanov. Leading Bulgarian Botanical Society, she organized and chaired 6<sup>th</sup> and 7<sup>th</sup> National Conferences of Botany, the proceedings of which were published in special issues.

Very special note has to be given in this tribute to the efforts of Prof. Temniskova, made from the position of already respected professor, to unify and consolidate Bulgarian algologists, who belong to different scientific schools and traditions. It is to hope that these efforts, which already showed many positive results, shall be not forgotten by future generations.

Prof. D. Temniskova was a favourite interesting and attractive lecturer, teaching mainly in bachelor and master compulsory courses in the Department of Botany, related with systematics of algae and fungi, in which she was a reputable tutor. She created the course of Diatom analysis for students, who specialized in Botany, and later on in the more narrow specialization of Algology. Diatoms became the “great love” in the scientific studies of Prof. Temniskova, which she hand down to her students and successors (N. Ognyanova, M. Vuleva, S. Passy, K. Manoylov, P. Ivanov, R. Stancheva and R. Zidarova). Some of her Diploma paper students left the field of algology, but continued successfully their work in other fields of botany and kept their narrow connections with the Department of Botany and Faculty of Biology as external or regular teachers there. Among them are Assoc. Prof. Dr K. Uzunova, Assoc. Prof. Dr A. Uzunova and Prof. DrSc D. Ivanov.

The scientific heritage of Prof. Temniskova, written separately or in co-authorship, consists of scientific papers, presentations on prestigious scientific meetings, reports on scientific projects, bibliographic papers, Handbook for exercises in Systematics of algae and fungi (in two editions), manual on Algology (in two volumes) and a book, compiled on the life and scientific legacy of the first Bulgarian algologist Prof. S. Petkov. In all these works clearly can be seen her respect to the traditions of the world best algological schools, of the so-called “old masters”, her personal scientific conviction and her wish to raise the level of Bulgarian algological publications. This scientific heritage was evaluated in peer reviews, some of which were published by eminent scientists like Academician Prof. DrSc T. Nikolov, Academician Prof. DrSc. V. Golemansky, Prof. DrSc G. Gärtner, and therefore has not be appraised in more detail here. The scientific works of Prof. Temniskova represent the stable ground for future studies of fossil and recent biodiversity of algae in Bulgaria, and for its conservation.

For her various teaching and administrative activities and scientific contributions Prof. D. Temniskova-Topalova was awarded by the prestigious signs of Sofia University – by the Honorary Sign of Sofia University (level 1) and Honorary Sign of Sofia University with Blue Ribbon, and additionally, especially from the Rectorate of Sofia University, she obtained the Icon of Saint Kliment Ohridski.

Obviously, the small tribute, presented here, can not compete with these respectful awards, but it is wealthy due to its warmest cordial and grateful acknowledgment of her personality and scientific trace, which leaves memorable part in everyone, who was in contact with her. The best expression for this is the Old Indian wise sentence: “King is honoured only in his own country, to the Scientist homage is paid everywhere”.

Prof. Maya P. Stoyneva, PhD, DrSc  
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*CALOTHRIX CONFERVICOLA* AGARDH EX BORNET ET  
FLAHAULT (CYANOPROKARYOTA) – A NEW POSSIBLE  
CAUSATIVE AGENT OF *SEEWEED DERMATITIS*?

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** The present paper is intended to serve as *alarm* in order to sharpen the attention of scientists to the benthic heterocytous cyanoprokaryote *Calothrix confervicola*, which seems to be the most probable potential causative agent for a human skin irritation (*seeweed dermatitis*, or *swimmer's itch*), detected recently in one site on the southern Black Sea coast of Bulgaria.

**Key words:** benthic toxic algae, Black Sea, cyanotoxins, coastal harmful cyanoprokaryotes, swimmer's itch

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Scientific and public awareness of toxic cyanoprokaryotic (cyanobacterial) blooms in freshwaters, and of health hazards which they can cause, is constantly increasing. Many data on distribution of toxic producers, history of animals and fish deaths, as well as outbreaks of human illness and poisonings are available since years all over the world (e.g. CARMICHAEL 1994, 2001; VASCONCELOS 1994; CODD 1995; CHORUS & BARTRAM 1999; CODD ET AL. 1999, 2005; MARŠÁLEK ET AL. 2000; CARMICHAEL ET AL. 2001; PAVLOVA & BRATANOVA 2005; AGRAWAL ET AL. 2012 among the many others and citations there-in). Nowadays much more attention is paid to these events also in the countries of south-eastern Europe, in spite of the fact that, as a rule, they are not controlled by national legislations (e.g. PAVLOVA ET AL. 2006, 2007, 2013, 2014; JUKOVIĆ ET AL. 2008, TENEVA ET AL. 2010; PANTELIĆ ET AL. 2013, SVIRČEV ET AL. 2013 and citations therein). The most frequently found and almost everywhere mentioned toxin producers belong to the genera *Microcystis* Kützing ex Lemmermann, *Dolichospermum* (Ralfs ex Bornet et Flahault) Wacklin, Hoffmann et Komárek (Syn. *Anabaena* p.p.), *Nodularia* Mertens ex Bornet et Flahault, *Planktothrix* Anagnostidis et Komárek, *Aphanizomenon* Morren ex Bornet et Flahault, *Cylindrospermopsis* Seenaya et Subba Raju in Desikachary, etc. A detailed description of the toxicological properties of cyanotoxins known so far is out of the scope of this study and can be found in extensive publications on the topic (e.g. CHORUS & BARTRAM 1999; MERILUOTO & CODD 2005; FUNARI & TESTAI 2008).

However, much less attention has been paid to the freshwater benthic or soil mat-forming genera and their effects on human health or animals, and to the brackish and marine cyanoprokaryotes as well (e.g. CODD 1994; MATERN ET AL. 2001; HROUZEK ET AL. 2005; TENEVA ET AL. 2005, 2013; BECHER & JUTTNER 2006; CARVALHO ET AL. 2013; QUIBLIER ET AL. 2013 and citations there in). As summarised by CODD 1994 and later by SELLNER 1997, there are at least three toxic producing genera: *Lyngbya* C. Agardh ex Gomont, *Schizothrix* Kützing ex Gomont and *Oscillatoria* Vaucher ex Gomont. The most popular species among them is the benthic coastal filamentous *Moorea producens* Engene et al. 2012 (Syn. *Lyngbya majuscula* Harvey et Gomont 1892). According to WENDY GUIRY (2014) this recent taxonomic transformation was supported by WYNNE (2013). The species is well known for the production of a variety of biologically active components. Among them are the cyanotoxins from lyngbyatoxin group (A, B, C), aplysiatoxin and their brominated derivatives, used as a defensive secretion to protect the species itself from predation by fish, being potent irritants and vesicants, as well as carcinogens (FUJIKI ET AL. 1981; AIMI ET AL. 1990; KOZIKOWSKI ET AL. 1991; OSBORNE ET AL. 2001; ITO ET AL. 2002; EDWARDS & GERWICK 2004; JIANG ET AL. 2014, etc.). *Moorea* became negatively popular mostly as a causative agent of human skin irritation (*seaweed dermatitis*, or *swimmer's itch*) – CARDELLINA ET AL. (1979), BURJA ET AL. (2001). In spite of the increasing knowledge on the coastal hazardous cyanoprokaryotes and their toxins, the relevant risk assessment of other widespread coastal benthic

cyanoprokaryote species (from the genera *Calothrix* Agardh ex Bornet et Flahault, *Rivularia* Agardh ex Bornet et Flahault, *etc.*) still is insufficient and can be outlined as “needed research”. Nevertheless of the relatively scattered character of the investigations, it has to be mentioned that in some strains of these genera strong biologically active compounds (calophycin, calothrixins A and B, *etc.*) with antialgal, antifungal, antibacterial and/or other allelopathic activities, have already been detected (e.g. FLORES & WOLK, 1986; MOON ET AL. 1992; ABARZUA ET AL. 1999; RICKARDS ET AL. 1999; SCHLEGAL ET AL. 1999; DOAN ET AL. 2000, 2001; BERRY ET AL. 2008; TUET 2010).

The aim of the present paper is to serve as *alarm* in order to sharpen the attention of scientists to the benthic heterocytous cyanoprokaryote *Calothrix confervicola*, which seems to be the most probable potential causative agent for a human skin irritation, detected recently in one site on the southern Black Sea coast of Bulgaria.

A 36-year old woman was referred to a dermatologist on the occasion of itching skin rash, which appeared after a contact with the sea-rocks, overgrown by algal mats in the place “Mekite Skali” (which means in Bulgarian language “soft rocks”) near the Varvara village on the Black Sea coast (Fig. 1). The dermatological examination revealed an erythematous papular rash, localized on the skin of the abdomen, glutei, outer surface of the elbows and thighs (Fig. 2). The histopathological investigation of the irritated skin showed moderate and irregular acanthosis, edema in the papilar derma and significant inflammation infiltrate in the middle derma. The diagnosis made was *allergic dermatitis* and treatment with oral antihistamine and topical corticosteroid cream was applied. A complete disappearance of the skin rash in three weeks was observed.

The pointed rocky places (Fig. 1) were visited and living material was collected from them (by scratching of the algal mats, which covered the rock surface in the pseudolittoral zone) almost immediately after the event, in spite of the fact that the patient visited the dermatologist 10 days after the first symptoms appear.

The investigation of the collected algal material revealed a dominating heterocytous cyanoprokaryote with heteropolar trichomes in yellow-brown sheaths (10–12 µm wide trichomes, 14–16 µm wide filaments) and one basal heterocyte per trichome (Fig. 3), and typical numerous hormogonia. Young trichomes were ensheathed in colourless mucilage. The morphological features of the specimens, observed by light microscopy (under immersion 100 objective on Motic 400 microscope, equipped with digital microphotocamera Moticam 2000 and supplied with photoprocessing program Motic Images Plus 2,0) coincided with the description of the marine coastal species *Calothrix confervicola* Agardh ex Bornet et Flahault 1886, given by KOMÁREK (2014). The species is considered to be cosmopolitan, marine, widespread in coastal regions, known as quite common in Mediterranean region and Black Sea (KOMÁREK 2014).



Fig. 1. Map of Bulgaria with the location of the sampling point.



Figs. 2–3. Skin rash and *Calothrix confervicola*. Fig. 2: Skin rash (seaweed dermatitis) on the patient body (detailed description is in the text). Fig. 3: *Calothrix confervicola* – heteropolar trichome with a terminal heterocyst, ensheathed in a well-developed yellow-brownish mucilage sheath.

Discussing the possible causative agent of the observed skin rash, we have not to ignore the fact that many cyanoprokaryotic crusts and mats are formed mainly by filamentous species (including those of *Calothrix*), able to excrete extracellular polymeric substances. This polysaccharide-rich matrix not only confers desiccation and freeze tolerance to the given mat, but serves as a trap for many sediment particles and forms a new habitat with a new physical medium for a great variety of other organisms, including viruses, heterotrophic bacteria, protists and microinvertebrates, and has different chemical properties in comparison to the overlying water or air (e.g. VINCENT 2009). In addition, it has to be taken into account that the pigment composition generally shows differences in the mat profile: the surface layer is rich in photoprotective pigments, especially carotenoids (orange and red) but also sometimes scytonemin (black or brown), overlying a deeper blue-green layer rich in light-harvesting phycobiliproteins and chlorophyll *a* (VINCENT 2009). Therefore we would like boldly to underline that, in spite of the fact, that the output of biologically active compounds (incl. hepatotoxin) of different strains of *Calothrix* was proved and in some regions its freshwater and soil species were mentioned among the toxic producers (e.g. MOHAMED ET AL. 2006; AL-ARAJY & SULTAN 2008; MOHAMED 2008), in the present paper we could only suggest the *probability* for *Calothrix confervicola* mats to induce human skin rash. Rendering an account to its abundant development in the studied site, as well as its wide distribution in the coastal regions, we would like to propose further detailed investigations of this species and its compounds.

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## AEROPHYTIC GREEN ALGAE, EPIMYCOTIC ON *FOMES FOMENTARIUS* (L. EX FR.) KICKX.

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*The paper is dedicated to Prof. D. Temnsikova  
on the occasion of her 80th jubilee*

**Abstract:** Four species of green algae were found on the upper surface of *Fomes fomentarius* (L. ex Fr.) Kickx. basidiome. *Desmococcus vulgaris* (represented by both vegetative cells and aplanosporangia) dominated among them. *Trebouxia arboricola* (represented by free-living vegetative cells and autosporangia), and two species of *Stichococcus* (*S. bacillaris*, *S. minutus*) were the other identified algae. It is proposed to use the term “epimycotic” (from the greek “epi”- over and “mykes” – fungus) for algae and/or other organisms capable of growing and developing on the upper surface of fungal fruiting bodies.

**Key words:** basidiocarp, basidiome, Bulgaria, coccal algae, Danube island, filamentous algae, fungal host

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## INTRODUCTION

Aerophytic algae are well known colonists on different substrates as bark, wood, rocks, buildings, *etc.* and occur also as epiphytes on living organisms (*e.g.* on leaves of trees and shrubs, coniferous needles, lichens, *etc.* – Ettl & Gärtner 1995, 2014). Fruiting bodies of fungi have very rarely been reported to host algae on their surface and among them only a few species were identified (Burdshall *et al.* 1996; Zavada & Simoes 2001). Moreover, it was underlined that the occurrence of “epiphytes” on *Trametes versicolor* (L.) Lloyd is common, but not universal (Zavada *et al.* 2004). The recent investigation of an old, intermediate between bracket- and hoof-like, fruit body of *Fomes fomentarius* (L. ex Fr.) Kickx., collected in a floodplain Danube forest, revealed different green algae growing abundantly on its upper surface. Obviously, the air moisture of the site favors the development of the aerophytic algae found. For the algae and other organisms capable of growing and developing on such substrate, the term “epimycotic” is advocated.

## MATERIAL AND METHODS

*Fomes fomentarius* from a *Populus* sp. trunk was collected in the middle of July 2014, in a floodplain forest at the Bulgarian Danube island Tsibur (=Ibisha). The island is situated between 716 and 719 river kilometers (Montana district, Bulgaria – Fig. 1.) and covers area of 0,9 km<sup>2</sup>. In spite of this small territory, it is very interesting from nature conservational point of view and contains two protected territories: the protected area “Ostrov Tsibur” (situated in north-western part of the island with area of 101,48 ha, declared by State Order RD-292/10.04.2007) and managed reserve “Ibisha” (situated in the south-eastern part of the island with area of 34,3 ha, declared by Order RD-394/15.10.1999 of the Bulgarian Ministry of Environment and Waters). The “Ibisha” reserve is periodically inundated and contains floodplain forest. The *Fomes* fruiting body (also basidiocarp, basidiome, basidioma, fruit-body – after Kirk *et al.* 2008) was collected by Assoc. Prof. Dr. P. Mitov during his work on the Management Plan of the managed reserve “Ibisha” and on the next day transported to the Botany Department of Sofia University “St. Kliment Ohridski”.

The basidiome was about 55 cm in diameter and up to 22 cm thick (measured on the highest part from top to basis) – Figs. 2a-b. The upper surface was tough, bumpy, hard and woody, mainly greyish. It was covered with many spots – light to dark-green, rounded or irregular in shape, 3 mm to 3 cm, and even in bigger size (Figs. 2a-b). Samples from six spots (marked with different coloured mini-flags and numbers – Fig. 2b) were taken with sterile needles and transferred directly to slides for investigation in light microscope. Microscopy was done with a Motic 400 microscope (40x and 100x immersion objectives). Photomicrographs were taken with a Motic Cam 2,0 and processed with software Motic Images Plus 2,0. Cell walls were stained with Methylene Blue and starch was coloured with Lugol’s

solution (ETTL & GÄRTNER 1995, 2014). The taxonomic identification followed ETTL & GÄRTNER (2014).



Fig. 1. Map of Bulgaria with the location of the managed reserve “Ibisha”, and *Fomes fomentarius*, respectively.

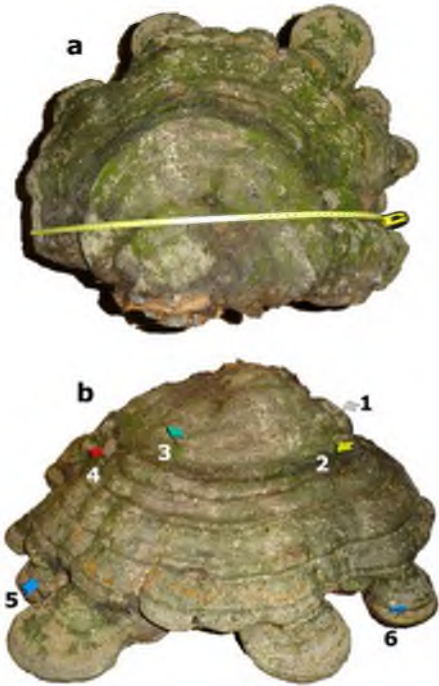


Fig. 2a-b: a) Basidiome of *Fomes fomentarius* with dark-green spots of epimycotic green algae; b) Locations of sampling points, marked with small coloured flags and numbers (1–6).

## RESULTS AND DISCUSSION

Different live stages of the most common aerophytic green alga *Desmococcus olivaceus* (Pers. ex Ach.) Laundon (Trebouxiophyceae, Prasiolales) were found in all investigated samples from the upper surface of the basidiome. The alga formed 2–4-celled cuboidal packets with short unbranched filaments of 3–4 cells (Figs. 3a-d, 4a-c). The cells were rounded, relatively compressed in filamentous stages (Fig. 3a, 4c), with a well-developed, even massive, wall. The parietal chloroplast (one per cell) with irregularly lobed margin contained a small pyrenoid, covered with a fine starch sheath (visible when stained with Lugol's solution). Additionally, aplanosporangia with thick irregular cell walls, were recorded in one of the samples (Fig. 3d). Dimensions of vegetative cells in the few-celled filaments varied from 7 to 12  $\mu\text{m}$ . *D. olivaceus* was firstly reported for Bulgaria as a distinct taxon (from cultures) by STOYNEVA & GÄRTNER (2009), who recorded it from moist rock surfaces of the tunnel cave “Prohodna” and from an old glass piece, found in the entrance of the same cave. Most probably, it was found earlier in Bulgaria by PETKOFF (1925) on tree barks in Pirin Mts. and included in the algal flora of VODENICHAROV ET AL. (1971) under the unclear name *Protococcus viridis* Agardh, which led to taxonomic confusions (UZUNOV et al. 2008).

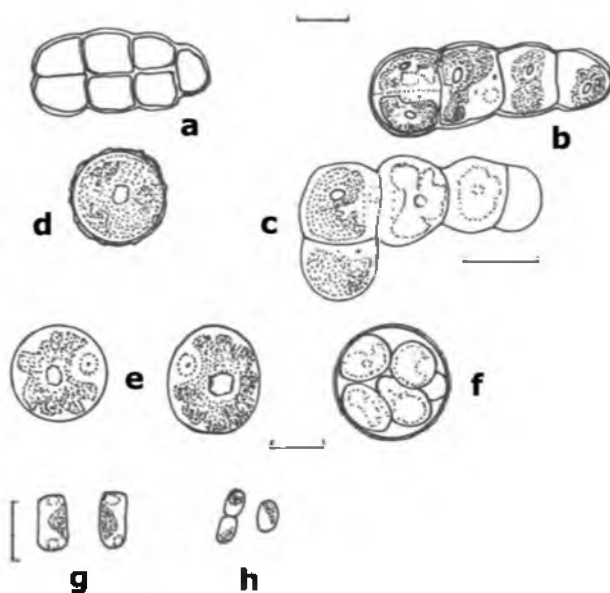


Fig. 3a-f. Epimycotic algae on *Fomes fomentarius*. 3a-d: *Desmococcus olivaceus*: a-c) Vegetative stages of cuboidal cell packets and short filaments; d) Aplanosporangium of *Desmococcus olivaceus* with thick cell wall. 3e-f: *Trebouxia arboricola*: e) vegetative cells with nucleus in the sinus of chloroplast; f) autosporangium with 8 autospores of different size. 3g-h: *Stichococcus*: g) vegetative cells of *St. bacillaris*; h) vegetative cells of *St. minutus*. Scale bars on the figs. – 10  $\mu\text{m}$ .

*Trebouxia arboricola* Puymaly (Trebouxiophyceae, Trebouxiales) was detected in two of the surface samples (green and light-blue flags /= sites 3 and 6 on Fig. 2b). It was non-lichenized, free-living and appeared in both stages of vegetative cells and autosporangia (Figs. 3e-f, 4c-d). The vegetative cells possessed all typical diagnostic features of *Trebouxia arboricola*: they were globular, sometimes slightly ellipsoidal, with a parietal massive, lobed to incised chloroplast with a naked pyrenoid, which lacks a distinct starch sheath. The nucleus was eccentric in the cell lumen, situated in an expressed sinus of the chloroplast (Fig. 3e, 4e). The cells were 13–15 µm in diameter and coincided with the authentic strain (or “type culture”) investigated by GÄRTNER (1975). The observed autosporangia contained mainly 8 autospores of slightly different size (Fig. 4f). *T. arboricola* was reported for Bulgaria by VODENICHAROV ET AL. (1971) as found on tree bark in Rodopi Mts, without mentioning of cultures. Later on, it was collected in a free-living stage and cultivated from granite stone monuments (GÄRTNER & STOYNEVA 2003), from the walls of the tunnel cave “Prohodna” (STOYNEVA & GÄRTNER 2009) and from the surface of sandstones of the rock phenomenon “Belogradshishki Skali” (MANCHEVA 2013).

Two species of the very common aerophytic green filamentous genus *Stichococcus* Nägeli (Trebouxiophyceae, Prasiolales) were found in one of the studied samples (green flag/=site 3 on Fig. 2b): *S. bacillaris* Nägeli and *S. minutus* Grintzesco & Péterfi. Both algae differ in size and morphology of chloroplasts in their vegetative cells (HINDÁK 1996). The cells of *S. bacillaris* were ± cylindrical, 3µm broad and 7 (10) µm long, each with a median inserted chloroplast (Fig. 3g, 4d). By contrast, the vegetative cells of *S. minutus* were more rounded, 2–3µm broad and 4µm long, with a polar chloroplast within the cell lumen (Fig. 3h, 4d). *Stichococcus bacillaris* was recorded in many sites of Bulgaria (VODENICHAROV ET AL. 1971; UZUNOV ET AL. 2007, 2008), whereas *S. minutus* was found only by UZUNOV (2009) in soils of Pirin Mts.

The recent findings are the first documented observations of *Desmococcus olivaceus*, *Trebouxia arboricola*, *Stichococcus bacillaris* and *S. minutus* as “epiphytic” algae on a basidiome surface in Bulgaria. According to our knowledge, this is also their first documentation on *Fomes fomentarius*, whereas “*Characium* sp. and *Coccomyxa* sp.” were found on *Bridgeoporus nobilissimus* (W.B. Cooke) Volk, Burdsall & Ammirati (BURDSALL et al. 1996), “*Hormidium* sp., *Stichococcus bacillaris*, *Chlorococcum* sp., and *Trebouxia* sp.” were determined on *Trametes versicolor* and it was suggested that the basidiocarps of *T. versicolor* have the potential to be lichenized (ZAVADA et SIMOES 2001). Later on, ZAVADA et al. (2004) proved the capacity for *T. versicolor* to exploit algae as a carbon source. We believe that many algologists, mycologists and ecologists know the possibility algae (and particularly green algae) to develop on the surface of different fungi. However, it is obvious that this so-well-known knowledge remained very less documented, at least in the literature available and checked for this study. In any case, we found illogical to continue to use the term “epiphytes” for the organisms from such surfaces, since long-ago it is clear that fungi do not belong to the Plant

Kingdom. Therefore, we plead to use for algae and/or other organisms growing on the upper surface of fungal fruiting bodies, the term “epimycotic” (from the greek “*epi*”- over and “*mykes*” – fungus), which occasionally and without discussions has been used (e.g. REYNOLDS 1978; REDBERG et al. 2003).

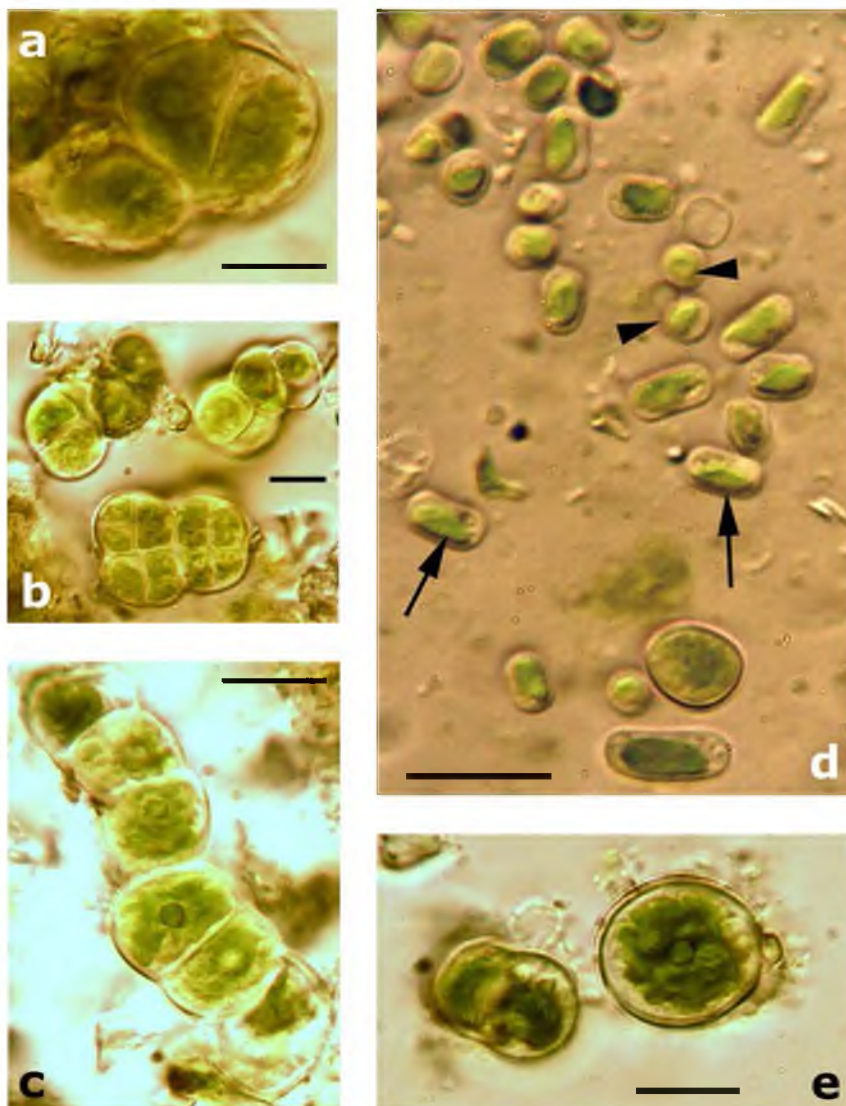


Fig. 4a-f. Photomicrographs of epimycotic algae on *Fomes fomentarius*. 4a-c: *Desmococcus olivaceus*: a) Single cells in binary division; b) Cuboidal packages of vegetative cells; c) Short filaments. 4d: *Stichococcus*: vegetative cells of *S. bacillaris* (arrow heads) and of *S. minutus* (arrows). 4e: *Trebouxia arboricola* – vegetative cells. Scale bars on the figs. – 10  $\mu$ m.

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## DIATOM DIVERSITY OF SPRINGS AND SPRING-FED STREAMS IN VITOSHA NATURE PARK, BULGARIA

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** The paper presents the first contemporary study of the diatom diversity of the springs, spring-fed streams, peat bogs and mires forming the headwaters of three rivers (Bistrishka River, Boyanska River and Strouma River) in Vitosha Nature Park. Diatom samples were collected from all natural substrata at each sampling site – epilithon, epiphyton and epipelon. A total of 353 taxa (298 species, 53 varieties and 2 forms) belonging to 70 genera were found. The highest number of species was observed in the genera *Navicula* s.l., *Achnanthes* s.l., *Pinnularia*, *Gomphonema* and *Eunotia*. Fifty-nine taxa are new to the Bulgarian algal flora, and 305 taxa are reported for first time for Vitosha Mountain. The dominant structure of the diatom communities was identified and the number of rare and threatened species was assessed. One hundred and seven taxa (30% of all taxa found in the study) are included in the Red List of limnic diatoms of Central Europe. Notes on some interesting taxa are provided.

**Key words:** Bacillariophyceae, Bulgaria, diatoms, headwaters, mountains, springs.

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## INTRODUCTION

High altitude aquatic habitats are one of the most sensitive ecosystems, sustaining specific and rich biodiversity (CANTONATI ET AL. 2006; KILROY ET AL. 2006). These habitats determine the water quality, biodiversity and ecological health of the lower stretches of rivers, which depend on the functions provided by the most upper headwater streams (LOWE & LIKENS 2005). Moreover, high altitude habitats serve as a 'refugia' or 'habitat islands' for many species (CANTONATI ET AL. 2006; ROTT ET AL. 2006).

In Europe, remote high altitude habitats are one of the very few left, which are not fully affected by humans, however they are highly fragile and sensitive to disturbances (CANTONATI ET AL. 2006). In Bulgaria they are extremely endangered ecosystems, threatened by direct anthropogenic impacts, e.g. water abstraction, destruction and fragmentation of habitats, primarily due to forest management activities, development of winter tourism activities and pollution from wastewater discharges.

In general, diatom communities from oligotrophic habitats in mountain areas are understudied and much less known in comparison to those inhabiting anthropogenically affected waters (LANGE-BERTALOT & METZELTIN 1996). Habitat destruction and eutrophication threaten many diatom species with extinction, especially those which occur in restricted habitats and are found in low frequencies (WOJTAŁ 2009).

The first studies of diatoms in mountain springs, streams and peat bogs in Bulgaria were made in the beginning of the 20<sup>th</sup> century by PETKOFF (1900, 1904, 1905, 1922, 1925) from Vitosha, Pirin and Rila mountains. Later on KAWECKA (1974, 1976, 1980a, 1980b, 1981) published diatoms from Malyovitsa stream in Rila Mt. Diatom communities from mountain springs, spring-fed streams and peat bogs of Ograjden Mt. were studied by TEMNISKOVA-TOPALOVA & MISALEVA (1982). There are also data on the algal flora of the upper catchment area of the Beli and Cherni Osum rivers in Stara Planina Mt. (KIRJAKOV & VODENICHAROV 1986; VODENICHAROV & KIRJAKOV 1987) and from the upper catchment of Mesta River in Rila Mt. (PASSY-TOLAR ET AL. 1999). Recently, diatoms from Bulgarian high altitude lakes have been investigated (OGNJANOVA-RUMENOVA et al. 2006, 2009, 2011; OGNJANOVA-RUMENOVA 2012). This brief enlistment of studies shows the general lack of contemporary data on diatoms from diverse aquatic mountain habitats of Bulgaria. The most recent data on diatoms in the region of Vitosha Mt. are from Strouma River between the villages Bosnek and Chuipetlovo (IVANOV ET AL. 2006, 2007).

Due to its proximity to the capital, Vitosha Mountain is the most visited tourist site in Bulgaria, on average by more than 2,5 mln tourists per year (GUSSEV ET AL. 2005). This results in a high risk of destruction and fragmentation of habitats, which is one of the main threats for diatom diversity in mountain areas. Investigations of oligotrophic habitats in mountain areas in Europe confirm the presense of a rich diatom diversity (LEVKOV ET AL. 2005), as well as a high number of endangered and rare species and

the existence of new and potentially new taxa (LANGE-BERTALOT & METZELTIN 1996). Therefore, the main aim of the study was to investigate the diatom diversity in aquatic habitats located in the protected area Vitosha Nature Park, to assess the taxonomic richness, dominant structure and rare and threatened taxa as well.

## STUDIED SITES

Vitosha Mountain (311 km<sup>2</sup> total area, highest peak: Cherni Vrah 2290 m a.s.l.), situated in the middle of Western Bulgaria between Stara Planina Mountain and Rila-Rhodopi massif, is the first National Park in Bulgaria and on the Balkan Peninsula, declared in October 1934. At present, since year 2000, 270,1 km<sup>2</sup> of the area of Vitosha Mt. were declared as protected area “Vitosha Nature Park”. The mountain is rich in waters – from the high plateaus and peat bogs originate many springs, which form the headwaters of the catchments of several rivers (e.g. Palakaria, Zheleznishka, Bistrishka, Dragalevska, Vladaiska, Boyanska and Strouma).

The investigated sites (springs, streams, peat bogs, mires) form the headwaters of three main rivers – Strouma, Boyanska and Bistrishka, located in the boundaries of Vitosha Nature Park, between 995 m and 2068 m a.s.l. (Fig. 1).



Fig. 1. Map of the investigated region. The dotted line shows the boundaries of Vitosha Nature Park; dots mark the sampling sites (St1-St7: sampling sites from the catchment of Strouma River; Bo1-Bo6: sampling sites from the catchment of Boyanska River catchment; Bi1-Bi5: sampling sites from the catchment of Bistrishka River catchment).

Table 1. List of investigated river catchments, sampling sites, type and number of samples from each site, GPS coordinates, altitude and sampled substrata.

<b>Sampling site</b>	<b>River catchment</b>	<b>Location (gg mm ss.s)</b>	<b>Altitude [m]</b>	<b>Number and type of samples</b>
<b>St1</b>	Strouma	N 42 29 59,5 E 23 12 20,1	995	4 samples (epilithon, epiphython)
<b>St2</b>	Strouma	N 42 31 41,9 E 23 15 05,3	1275	3 samples (epilithon, epiphython)
<b>St3</b>	Strouma	N 42 31 41,9 E 23 15 05,3	1425	3 samples (epilithon, epiphython)
<b>St4</b>	Strouma	N 42 32 17,9 E 23 15 31,1	1485	4 samples (epilithon, epiphython, epipelon)
<b>St5</b>	Strouma	N 42 32 36,7 E 23 15 37,8	1700	4 samples (epilithon, epiphython)
<b>St6</b>	Strouma	N 42 33 04,6 E 23 16 03,9	1988	3 samples (epilithon, epiphython)
<b>St7</b>	Strouma	N 42 30 35,3 E 23 12 52,7	1075	3 samples (epilithon, epiphython)
<b>Bi1</b>	Bistrishka	N 42 34 16,2 E 23 19 47,6	1210	3 samples (epilithon, epiphython)
<b>Bi2</b>	Bistrishka	N 42 34 01,8 E 23 19 06,9	1455	4 samples (epilithon, epiphython)
<b>Bi3</b>	Bistrishka	N 42 33 51,2 E 23 18 52,6	1650	4 samples (epilithon, epiphython)
<b>Bi4</b>	Bistrishka	N 42 33 26,9 E 23 17 49,3	1983	4 samples (epilithon, epiphython, epipelon)
<b>Bi5</b>	Bistrishka	N 42 33 02,5 E 23 18 12,5	2031	4 samples (epilithon, epiphython)
<b>Bo1</b>	Boyanska	N 46 06 40,9 E 26 44 37,6	1060	4 samples (epilithon, epiphython)
<b>Bo2</b>	Boyanska	N 42 37 80,3 E 23 15 30,8	1300	3 samples (epilithon, epiphython)
<b>Bo3</b>	Boyanska	N 42 37 48,2 E 23 15 18,5	1425	4 samples (epilithon, epiphython)
<b>Bo4</b>	Boyanska	N 42 36 51,0 E 23 15 26,6	1650	4 samples (epilithon, epiphython)
<b>Bo5</b>	Boyanska	N 42 33 24,2 E 23 17 52,0	1834	4 samples (epilithon, epiphython)
<b>Bo6</b>	Boyanska	N 42 34 22,6 E 23 16 08,8	2068	4 samples (epilithon, epiphython, epipelon)

## MATERIAL AND METHODS

During the period March-November 2008, a total of 66 periphytic diatom samples (54 from lotic habitats – springs, spring-fed streams and 12 from lentic habitats – mires and peat bogs) were collected from 18 sites (Fig. 1, Table 1):

Boyanska River springs and headwaters – 23 samples from six sampling sites, Bistrishka River springs and headwaters – 19 samples from five sampling sites and Strouma River springs and headwaters – 24 samples from seven sampling sites. The samples were collected from all available natural substrata at each sampling site: stones, cobbles and pebbles (epilithic samples), plants (epiphytic samples: from lotic habitats and from surrounding peat bogs and mires) and epipelithic samples from the sediments' surface. The epiphytic samples were taken from water mosses (*Fontinalis* spp.); peat mosses (*Sphagnum* spp.) and filamentous green algae (*Ulothrix* spp., *Cladophora* spp., *Zygnema* spp.). Sampling was done according to European Standard EN 13946/2003 (*Water quality. Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*). The samples were fixed *in situ* with 4% formaldehyde. In the laboratory, pretreatment of samples was done according to A.5.2 method of EN 13946/2003 with cold sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and potassium permanganate (KMnO<sub>4</sub>). The cleaned material was mounted on permanent slides with Naphrax®. From each sample two permanent slides were prepared, therefore a total of 132 slides were processed. The materials are stored in the Algal Collection of the Department of Botany, Faculty of Biology, Sofia University "St. Kliment Ohridski".

Light microscopy (LM) was performed on Amplival Carl Zeiss Jena, Nikon Eclipse and Olympus BX51, with 100x oil-immersion objectives, the latter two equipped with digital cameras for light micrographs. Scanning electron microscopy (SEM) was performed with JOEL JSM-5510 operating at 20 kV at the Faculty of Chemistry and Pharmacy (Sofia University "St. Kliment Ohridski").

Diatoms were identified according to KRAMMER & LANGE-BERTALOT (1986–1991), LANGE-BERTALOT & KRAMMER (1989), LANGE-BERTALOT (1993, 2001), LANGE-BERTALOT & METZELTIN (1996), KRAMMER (1997a, 1997b, 2000, 2002, 2003), REICHARDT (1999, 2004), HÅKANSSON (2002), HOUK (2003), NAGUMO (2003), WERUM & LANGE-BERTALOT (2004) and BUKHTIYAROVA & ROUND (1996).

Four hundred valves per slide were counted. The abundance of each taxon in the samples was estimated based on the following categories: rare (one to 5 valves per slide), common (six to 15 valves per slide), subdominant (sixteen to 50 valves per slide) and dominant taxa (above fifty-one valves per slide or the taxon/taxa with the highest relative abundance). The Red List of limnic diatoms (LANGE-BERTALOT 1996) was used to assess the rare and endangered diatoms.

## RESULTS AND DISCUSSION

### *Taxonomic richness*

A total of 353 species, varieties and forms from 70 genera were identified (Table 2). Some of the taxa found are shown on Fig. 2. The diatom flora was predominantly composed of raphid pennate diatoms – 305 taxa (85,3% of all) from 49 genera.

The following genera had the highest number of species: *Navicula* s.l. (including *Naviculadicta* and *Eolimna*) presented with 38 taxa (10,8% of all), *Achnanthes* s.l. (including *Achnanthidium*, *Planothidium*, *Psammothidium* and *Rossithidium*) with 36 taxa (10,2%), *Pinnularia* – 33 taxa (9,4%), *Gomphonema* – 25 taxa (7,1%) and *Eunotia* – 23 taxa (6,5%).

Table 2. List of diatom taxa found on Vitosha Mt. with their distribution (St = Strouma River; Bo = Boyanska River; Bi = Bistrishka River), abundance (1 = rare, 2 = common, 3 = subdominant, 4 = dominant taxa); BG = new taxa to Bulgarian algal flora; RL = Red List (1 = almost extinct, 2 = strongly endangered, 3 = endangered, V = not endangered but in regression, G= presumably endangered, R =extremely rare, D = data insufficient).

№	Taxa	St	Bi	Bo	BG	RL
1	<i>Achnanthes conspicua</i> Mayer	2	1	1		
2	<i>Achnanthes exigua</i> Grun.	1				
3	<i>Achnanthes ingratiiformis</i> L-B	1			+	
4	<i>Achnanthes rricula</i> Hohn et Hellerman	1				
5	<i>Achnanthes rupestris</i> Krasske	1				1
6	<i>Achnanthes saccula</i> Carter		1			
7	<i>Achnanthes silvahercynia</i> L-B		1	1	+	R
8	<i>Achnanthes subsalsa</i> Petersen			1	+	R
9	<i>Achnanthidium</i> aff. <i>atomus</i> (Hust.) Monnier, L-B et Ector			1		
10	<i>Achnanthidium kranzii</i> (L-B) Round et Bukht.	1			+	G
11	<i>Achnanthidium kryophila</i> (Petersen) Bukht.		1	1		3
12	<i>Achnanthidium laevis</i> var. <i>austriaca</i> (Hust.) L-B	1		1	+	
13	<i>Achnanthidium lineare</i> Smith	1	1	1		
14	<i>Achnanthidium minutissimum</i> (Kütz.) Czarnecki	4	4	4		
15	<i>Achnanthidium minutissimum</i> var. <i>affinis</i> (Grun.) L-B	1				
16	<i>Achnanthidium minutissimum</i> var. <i>macrocephala</i> Hust.	2			+	
17	<i>Achnanthidium pyrenaicum</i> (Hust.) Kobayasi		1	1		
18	<i>Achnanthidium subatomus</i> (Hust.) L-B	4	4	4		
19	<i>Adlafia bryophila</i> (Petersen) Moser, L-B et Metzeltin	2	2	1	+	V
20	<i>Adlafia minuscula</i> (Grun.) L-B	1	1	1		
21	<i>Adlafia suchlandtii</i> (Hust.) L-B			1		
22	<i>Amphipleura pellucida</i> (Kütz.) Kütz.	2	1			
23	<i>Amphora inariensis</i> Krammer			1		3
24	<i>Amphora normanii</i> Rabenhorst	1	1	1		V
25	<i>Amphora ovalis</i> (Kütz.) Kütz.		1			
26	<i>Amphora pediculus</i> (Kütz.) Van Heurck	1	1	1		
27	<i>Asterionella formosa</i> Hassall	1	1	1		
28	<i>Aulacoseira alpigena</i> (Grun.) Krammer		4	1		G
29	<i>Aulacoseira distans</i> (Ehr.) Simonsen		2	1		G
30	<i>Aulacoseira distans</i> var. <i>nivalis</i> (Smith) Haworth		1			
31	<i>Aulacoseira granulata</i> (Ehr.) Simonsen	1	1	1		

№	Taxa	St	Bi	Bo	BG	RL
32	<i>Aulacoseira subarctica</i> (Müller) Haworth	1	1	1		G
33	<i>Boreozonacola hustedtii</i> L-B, Kulikovskiy et Witkowski		1			
34	<i>Brachysira brebissonii</i> Ross Morph. I	1	1			
35	<i>Brachysira brebissonii</i> Ross Morph. II		1	1		
36	<i>Brachysira intermedia</i> (Østrup) L-B		1		+	
37	<i>Brachysira styriaca</i> (Grun.) Ross		1		+	3
38	<i>Caloneis</i> aff. <i>branderii</i> (Hust.) Krammer		1			
39	<i>Caloneis bacillum</i> (Grun.) Cleve	1	1			
40	<i>Caloneis branderii</i> (Hust.) Krammer	1			+	
41	<i>Caloneis fontinalis</i> (Grun.) Cleve-Euler	1	1	1	+	
42	<i>Caloneis pulchra</i> Messikommer		1	1		
43	<i>Caloneis sublinearis</i> (Grun.) Krammer			1	+	D
44	<i>Caloneis tenuis</i> (Greg.) Krammer	1	1	1		G
45	<i>Campylodiscus</i> sp.1 Ehr.	1				
46	<i>Cavinula cocconeiformis</i> (Greg. ex Grev.) Mann et Stickle		1	1		G
47	<i>Cavinula lapidosa</i> (Krasske) L-B		1	1		G
48	<i>Cavinula pseudoscutiformis</i> (Hust.) Mann et Stickle			1		
49	<i>Cavinula variostrata</i> (Krasske) Mann et Stickle	1	1	1		3
50	<i>Chamaepinnularia schaupiana</i> L-B et Metzeltin		1		+	
51	<i>Chamaepinnularia soehrensensis</i> var. <i>hassica</i> (Krasske) L-B		1			V
52	<i>Cocconeis disculus</i> (Schumann) Cleve		1			R
53	<i>Cocconeis neodiminuta</i> Krammer	1	1			R
54	<i>Cocconeis pediculus</i> Ehr.	1	1	1		
55	<i>Cocconeis placentula</i> var. <i>euglypta</i> (Ehr.) Grun.	1	1	2		
56	<i>Cocconeis placentula</i> var. <i>klinoraphis</i> Geitler	1	2	3		
57	<i>Cocconeis placentula</i> var. <i>lineata</i> (Ehr.) van Heurck	2	3	2		
58	<i>Cocconeis placentula</i> var. <i>pseudolineata</i> Geitler	2	2	4		
59	<i>Cocconeis</i> sp. 1		1			
60	<i>Coscinodiscus</i> sp. 1		1			
61	<i>Cyclotella meneghiniana</i> Kütz.	1				
62	<i>Cyclotella ocellata</i> Pantocsek	1	1	1		
63	<i>Cyclotella radiosa</i> (Grun.) Lemmermann			1		
64	<i>Cyclotella tripartita</i> Håkansson		1			
65	<i>Cymbella affinis</i> Kütz.		1			
66	<i>Cymbella aspera</i> s.l. (Ehr.) Cleve		1	1		V
67	<i>Cymbella compacta</i> Østrup	4	1	1		
68	<i>Cymbella helvetica</i> Kütz.		1	1		V
69	<i>Cymbopleura</i> aff. <i>subaequalis</i> (Grun.) Krammer	1		1		G
70	<i>Cymbopleura</i> aff. <i>subcuspidata</i> Krammer	1		1		
71	<i>Cymbopleura cuspidata</i> (Kütz.) Krammer		1			
72	<i>Cymbopleura naviculiformis</i> (Auerswald) Krammer	1	1	1		
73	<i>Cymbopleura</i> sp.1	1	1	1		

№	Taxa	St	Bi	Bo	BG	RL
74	<i>Cymboplectura subaequalis</i> var. <i>alpestris</i> Krammer		1		+	
75	<i>Decussata hexagona</i> (Torka) L-B	1	1	1		
76	<i>Denticula tenuis</i> Kütz.	2	1			
77	<i>Diadasmus biceps</i> Arnott		1	1	+	
78	<i>Diadasmus contenta</i> (Grun.) Mann	1	1	1		
79	<i>Diadasmus contenta</i> var. <i>parallela</i> (Petersen) Aboal			1	+	
80	<i>Diatoma anceps</i> (Ehr.) Kirchner		1			
81	<i>Diatoma hyemalis</i> (Roth) Heiberg	3	4	1		
82	<i>Diatoma mesodon</i> (Ehr.) Kütz.	4	4	4		
83	<i>Diatoma vulgare</i> Bory		1			
84	<i>Diatomella balfouriana</i> Grev.	1	1	1		
85	<i>Diploneis</i> aff. <i>puella</i> (Schumann) Cleve		1			V
86	<i>Diploneis boldtiana</i> Cleve		1		+	
87	<i>Diploneis fontanella</i> L-B		1		+	
88	<i>Diploneis fontium</i> Reichardt et L-B	1		1	+	
89	<i>Diploneis oblongella</i> (Nägeli ex Kütz.) Cleve-Euler			1		V
90	<i>Diploneis ovalis</i> (Hilse) Cleve	1				V
91	<i>Diploneis peterseni</i> Hust.		1	1		3
92	<i>Diploneis pseudoovalis</i> Hust.	1	1	2		R
93	<i>Diploneis separanda</i> L-B			1		
94	<i>Diploneis subovalis</i> Cleve		1			
95	<i>Discotella nana</i> Hust.	1	1	1		
96	<i>Encyonema gracile</i> Rabenhorst	1	4	1		3
97	<i>Encyonema minutum</i> (Hilse) Mann	2	1	2		
98	<i>Encyonema perpusilla</i> (Cleve) Mann	1	1	1		G
99	<i>Encyonema silesiacum</i> (Bleisch) Mann	4	2	2		
100	<i>Encyonema ventricosum</i> (Ag.) Grun.	3	1	1		
101	<i>Encyonopsis cesatii</i> Krammer		1		+	
102	<i>Encyonopsis falaisensis</i> Krammer et L-B		1			G
103	<i>Encyonopsis</i> sp. 1		1			
104	<i>Eolimna minima</i> (Grun.) L-B et Schiller	2	1	1		
105	<i>Eolimna tantula</i> (Hust.) L-B	2	1	1	+	
106	<i>Epithemia adnata</i> (Kütz.) Bréb.	2	4			
107	<i>Epithemia turgida</i> var. <i>granulata</i> (Ehr.) Brun	1				
108	<i>Eunotia arculus</i> (Grun.) L-B et Nörpel		1			2
109	<i>Eunotia bilunaris</i> (Ehr.) Schaarschmidt	1	1	1		
110	<i>Eunotia boreoalpina</i> L-B et Nörpel-Schempp	1	2	4		
111	<i>Eunotia diodon</i> Ehr.		1			
112	<i>Eunotia exigua</i> var. <i>exigua</i> (Bréb. ex Kütz.) Rabenhorst		2	1		
113	<i>Eunotia flexuosa</i> Kütz.			1		2
114	<i>Eunotia glacialis</i> Meister		1			G
115	<i>Eunotia groenlandica</i> (Grun.) Nörpel-Schempp et L-B		2		+	

№	Taxa	St	Bi	Bo	BG	RL
116	<i>Eunotia implicata</i> Nörpel, L-B et Alles		1			G
117	<i>Eunotia incisa</i> Smith ex Greg.		3			
118	<i>Eunotia inflata</i> (Grun.) Nörpel-Schempp et L-B		1		+	
119	<i>Eunotia minor</i> (Kütz.) Grun.	1	1			
120	<i>Eunotia monodon</i> var. <i>monodon</i> Ehr.		1	1		2
121	<i>Eunotia paludosa</i> Grun.		4	1		V
122	<i>Eunotia praerupta</i> var. <i>praerupta</i> Ehr.	1	1			3
123	<i>Eunotia pseudopectinalis</i> Hust.		1			1
124	<i>Eunotia rhomboidea</i> Hust.		1		+	V
125	<i>Eunotia serra</i> (s.l.) Ehr.	1	1	1		1
126	<i>Eunotia soleirolii</i> (Kütz.) Rabenhorst	2	2	1		G
127	<i>Eunotia subarcuatoides</i> Alles, Nörpel et L-B		1	1		
128	<i>Eunotia tetraodon</i> Ehr.		1			2
129	<i>Eunotia triodon</i> Ehr.		1			1
130	<i>Eunotia valida</i> Hust.		2	1		
131	<i>Fallacia insociabilis</i> (Krasske) Mann			1		
132	<i>Fallacia pygmaea</i> (Kütz.) Stickle et Mann	1				
133	<i>Fragilaria alpestris</i> Krasske ex Hust.	2				V
134	<i>Fragilaria austriaca</i> (Grun.) L-B	3		1		
135	<i>Fragilaria bicapitata</i> Mayer		2	1		
136	<i>Fragilaria capensis</i> Grun.	1			+	
137	<i>Fragilaria capucina</i> (s.l.) Desmazières		3	2		
138	<i>Fragilaria capucina</i> var. <i>capitellata</i> (Grun.) L-B	1				
139	<i>Fragilaria capucina</i> var. <i>vaucheriae</i> (Kütz.) L-B	2	2	1		
140	<i>Fragilaria construens</i> f. <i>binodis</i> (Ehr.) Hust.	1				
141	<i>Fragilaria crotonensis</i> Kitton		1			
142	<i>Fragilaria fasciculata</i> (Ag.) L-B		1			
143	<i>Fragilaria gracilis</i> Østrup	2	2	1		
144	<i>Fragilaria leptostauron</i> (Ehr.) Hust.		1			
145	<i>Fragilaria perminuta</i> (Grun.) L-B	1				
146	<i>Fragilaria polonica</i> Witak et L-B		2		+	
147	<i>Fragilaria rumpens</i> (Kütz.) Carlson	2	2	2		
148	<i>Fragilariforma virescens</i> (Ralfs) Williams et Round	1	1	3		V
149	<i>Frustulia crassinervia</i> (Bréb.) L-B et Krammer	1	3			V
150	<i>Frustulia rhomboides</i> (Ehr.) De Toni		1			
151	<i>Frustulia saxonica</i> Rabenhorst		3	1		V
152	<i>Frustulia vulgaris</i> (Thwaites) De Toni	1	2	1		
153	<i>Geissleria decussis</i> (Østrup) L-B et Metzeltin	1				
154	<i>Geissleria ignota</i> var. <i>palustris</i> (Hust.) L-B et Metzeltin			1	+	3
155	<i>Geissleria similis</i> (Krasske) L-B et Metzeltin	1				V
156	<i>Gomphonema acidoclinatum</i> L-B et Reichardt		1	1		
157	<i>Gomphonema acuminatum</i> Ehr.	1		1		



№	Taxa	St	Bi	Bo	BG	RL
158	<i>Gomphonema</i> aff. <i>amoenum</i> L-B	1				
159	<i>Gomphonema</i> aff. <i>anjae</i> L-B et Reichardt	1		1		
160	<i>Gomphonema</i> aff. <i>lagerheimii</i> Cleve		1			2
161	<i>Gomphonema</i> aff. <i>variscohercynicum</i> L-B et Reichardt		1			
162	<i>Gomphonema</i> <i>amoenum</i> L-B	1				3
163	<i>Gomphonema</i> <i>clavatum</i> Ehr.	2	2	1		
164	<i>Gomphonema</i> <i>exilissimum</i> (Grun.) L-B et Reichardt	1	2	1		V
165	<i>Gomphonema</i> <i>gracile</i> Ehr.		1	1		
166	<i>Gomphonema</i> <i>hebridense</i> Greg.	1	1			V
167	<i>Gomphonema</i> <i>intricatum</i> Kütz.	1				
168	<i>Gomphonema</i> <i>micropus</i> Kütz.	2	1	3		
169	<i>Gomphonema</i> <i>minutum</i> (Ag.) Ag.		1	1		
170	<i>Gomphonema</i> <i>olivaceoides</i> Hust.	2	2	2		
171	<i>Gomphonema</i> <i>parvulum</i> (Kütz.) Kütz.	1		1		
172	<i>Gomphonema</i> <i>productum</i> (Grun.) L-B et Reichardt	1	1	1		
173	<i>Gomphonema</i> <i>pumilum</i> var. <i>elegans</i> Reichardt et L-B	4	4	4		
174	<i>Gomphonema</i> <i>rhombicum</i> Fricke	4	2	4		V
175	<i>Gomphonema</i> <i>sarcophagus</i> Greg.		1			V
176	<i>Gomphonema</i> sp.	1	2	1	+	
177	<i>Gomphonema</i> <i>subclavatum</i> (Grun.) Grun.	1	1	1		
178	<i>Gomphonema</i> <i>tergestinum</i> (Grun.) Fricke	3	4	2		G
179	<i>Gomphonema</i> <i>truncatum</i> Ehr.	1				
180	<i>Gomphonema</i> <i>utae</i> L-B et Reichardt	1	1	1	+	
181	<i>Hannea</i> <i>arcus</i> (Ehr.) Patrick	4	3	4		
182	<i>Hantzschia</i> <i>amphioxys</i> (Ehr.) Grun.	1				
183	<i>Hippodonta</i> <i>capitata</i> (Ehr.) L-B, Metzeltin et Witkowski			1		
184	<i>Humidophila</i> <i>perpusilla</i> (Grun.) Lowe et al.	1	2	4		
185	<i>Hygropetra</i> <i>balfouriana</i> (Grun.ex Cleve) Krammer et L-B	1	1		+	
186	<i>Karayevia</i> <i>amoena</i> (Hust.) Bukht.			1	+	
187	<i>Karayevia</i> <i>laterostrata</i> (Hust.) Round et Bukht.	1	1	1		3
188	<i>Kobayasiella</i> <i>parasubtilissima</i> (Kobayasi et Nagumo) L-B		3		+	
189	<i>Kobayasiella</i> <i>subtillissima</i> (Cleve) L-B		1	1		V
190	<i>Lemnicola</i> <i>hungarica</i> (Grun.) Round et Basson	1				
191	<i>Luticola</i> <i>acidoclinata</i> L-B	1	1	1		
192	<i>Luticola</i> aff. <i>charlatii</i> (Peragallo) Metzeltin et L-B	1				R
193	<i>Luticola</i> <i>goeppertiana</i> (Bleisch) Mann, Crawford et Mann		1			
194	<i>Luticola</i> <i>goeppertiana</i> var. <i>peguana</i> (Bleisch) Mann			1	+	
195	<i>Luticola</i> <i>mutica</i> (Kütz.) Mann	1	1	1		
196	<i>Luticola</i> <i>saxophila</i> (Bock ex Hust.) Crawford et Mann		1		+	R
197	<i>Mayamaea</i> <i>atomus</i> var. <i>permitis</i> (Hust.) L-B	1	1			
198	<i>Melosira</i> <i>varians</i> Ag.	4		1		
199	<i>Meridion</i> <i>circulare</i> (Grev. ) Ag.	3	3	2		

№	Taxa	St	Bi	Bo	BG	RL
200	<i>Meridion constrictum</i> (Ralfs) Van Heurck	1	2	1		
201	<i>Navicula</i> aff. <i>lacunolaciniata</i> L-B et Bonik	1				
202	<i>Navicula</i> aff. <i>riparia</i> Hust.		1	1		R
203	<i>Navicula angusta</i> Grun.		1			3
204	<i>Navicula bremensis</i> Hust.		1			R
205	<i>Navicula clementis</i> Grun.	1				
206	<i>Navicula cryptocephala</i> Kütz.	1	2	2		
207	<i>Navicula cryptotenella</i> L-B	2	1			
208	<i>Navicula disjuncta</i> Hust.			1		
209	<i>Navicula exilis</i> Kütz.	1	1	1		G
210	<i>Navicula germainii</i> Wallace			1		
211	<i>Navicula gregaria</i> Donkin	2		1		
212	<i>Navicula hambergii</i> Hust.		1			
213	<i>Navicula harderii</i> Hust.	2		2		
214	<i>Navicula jaagii</i> Meister		1		+	3
215	<i>Navicula joubaudii</i> Germain	1	1	1		
216	<i>Navicula laevisissima</i> var. <i>perhibita</i> (Hust.) L-B		1			
217	<i>Navicula lanceolata</i> Ehr.	1	2	1		
218	<i>Navicula lundii</i> Reichardt			1	+	
219	<i>Navicula menisculus</i> Schumann	1				V
220	<i>Navicula meniscus</i> Schumann	1				V
221	<i>Navicula porifera</i> var. <i>opportuna</i> (Hust.) L-B		1			2
222	<i>Navicula radiosa</i> Kütz.	2	2			
223	<i>Navicula recens</i> (L-B) L-B	1				1
224	<i>Navicula reichardtiana</i> L-B			1		
225	<i>Navicula rhynchocephala</i> Kütz.	1	1	2		
226	<i>Navicula</i> sp.1			1		
227	<i>Navicula striolata</i> (Grun.) L-B			1		3
228	<i>Navicula tripunctata</i> (Müller) Bory de Saint-Vincent	1	2	1		
229	<i>Navicula weinzierlii</i> Schimanski		1	1		R
230	<i>Naviculadicta difficilima</i> Hust.		1			G
231	<i>Naviculadicta digituloides</i> L-B	1	1	1		3
232	<i>Naviculadicta multiconifusa</i> L-B			1	+	R
233	<i>Naviculadicta seminulum</i> Grun.	1	1	1		
234	<i>Naviculadicta suchlandtii</i> Hust.	1	1		+	V
235	<i>Naviculadicta tridentula</i> (Krasske) L-B		1			R
236	<i>Neidium ampliatus</i> (Ehr.) Krammer			1		V
237	<i>Neidium bisulcatum</i> (Lagerstedt) Cleve		1	1		3
238	<i>Neidium bisulcatum</i> var. <i>subampliatum</i> Krammer			1		3
239	<i>Neidium dubium</i> (Ehenberg) Cleve		1			
240	<i>Neidium hercynicum</i> Mayer		1		+	R
241	<i>Nitzschia acidoclinata</i> L-B		1	1		

№	Taxa	St	Bi	Bo	BG	RL
242	<i>Nitzschia agnita</i> Hust.	1				
243	<i>Nitzschia amphibia</i> Grun.	1	1	1		
244	<i>Nitzschia dissipata</i> var. <i>dissipata</i> (Kütz.) Rabenhorst	1	2	1		
245	<i>Nitzschia dissipata</i> var. <i>media</i> (Hantzsch) Grun.			1		
246	<i>Nitzschia fonticola</i> (Grun.) Grun.	1	1	3		
247	<i>Nitzschia frustulum</i> (Kütz.) Grun.					
248	<i>Nitzschia gracilis</i> Hantzsch		2	3		
249	<i>Nitzschia hantzschiana</i> Rabenhorst	1	2			
250	<i>Nitzschia inconspicua</i> Grun.	1				
251	<i>Nitzschia linearis</i> var. <i>linearis</i> Smith	1	1	1		
252	<i>Nitzschia linearis</i> var. <i>subtilis</i> (Grun.) Hust.	1				
253	<i>Nitzschia palea</i> (Kütz.) Smith	1				
254	<i>Nitzschia palea</i> var. <i>debilis</i> (Kütz.) Grun.	1				
255	<i>Nitzschia paleacea</i> Grun.	1				
256	<i>Nitzschia perminuta</i> (Grun.) Peragallo	1	1	1		
257	<i>Nitzschia pura</i> Hust.	1		1		
258	<i>Nitzschia recta</i> Hantzsch ex Rabenhorst	1	1	1		
259	<i>Nupela lapidosa</i> (Krasske) L-B	1	1	3		V
260	<i>Opephora mutabilis</i> (Grun.) Sabbe et Wyverman	1	1	1		
261	<i>Orthoseira roeseana</i> (Rabenhorst) O'Meara	1	1	1		V
262	<i>Pinnularia acrosphaeria</i> Smith			1		
263	<i>Pinnularia appendiculata</i> (Ag.) Schaarschmidt		1			
264	<i>Pinnularia borealis</i> aff. var. <i>scalaris</i> (Ehr.) Rabenhorst		1			
265	<i>Pinnularia borealis</i> var. <i>borealis</i> Ehr.		1	1		
266	<i>Pinnularia borealis</i> var. <i>scalaris</i> (Ehr.) Rabenhorst	1	1			R
267	<i>Pinnularia borealis</i> var. <i>sublinearis</i> Krammer		1	1		
268	<i>Pinnularia brevicostata</i> Cleve	1				R
269	<i>Pinnularia divergentissima</i> (Grun.) Cleve		1			G
270	<i>Pinnularia diversa</i> Østrup			1	+	
271	<i>Pinnularia eifelana</i> (Krammer) Krammer		1		+	
272	<i>Pinnularia esoxiformis</i> Krammer	1				G
273	<i>Pinnularia gibba</i> Ehr.	1	1	1		
274	<i>Pinnularia intermedia</i> (Lagerstedt) Cleve			1		V
275	<i>Pinnularia microstauron</i> (Ehr.) Cleve		1	1		V
276	<i>Pinnularia microstauron</i> var. <i>nonfasciata</i> Krammer		1		+	
277	<i>Pinnularia neomajor</i> var. <i>inflata</i> Krammer		2		+	G
278	<i>Pinnularia rabenhorstii</i> (Grun.) Krammer			1	+	
279	<i>Pinnularia rhombarea</i> Krammer		1		+	
280	<i>Pinnularia rupestris</i> Hantzsch		1			G
281	<i>Pinnularia schoenfelderi</i> Krammer			1	+	G
282	<i>Pinnularia schroederii</i> (Hust.) Krammer			1		
283	<i>Pinnularia schwabei</i> Krasske		1	1	+	

№	Taxa	St	Bi	Bo	BG	RL
284	<i>Pinnularia scotica</i> Krammer		2	1	+	
285	<i>Pinnularia silvatica</i> Petersen			1	+	
286	<i>Pinnularia sinistra</i> Krammer		1			
287	<i>Pinnularia</i> sp. 1		1			
288	<i>Pinnularia</i> sp. 2			1		
289	<i>Pinnularia stomatophora</i> var. <i>irregularis</i> Krammer		1		+	
290	<i>Pinnularia stomatophora</i> (Grun.) Cleve		1	1		G
291	<i>Pinnularia subcapitata</i> Greg.	1	2	2		
292	<i>Pinnularia subrostrata</i> (Cleve) Cleve-Euler		1			
293	<i>Pinnularia viridiformis</i> morph. II Krammer		1	2		G
294	<i>Pinnularia viridiformis</i> var. <i>viridiformis</i> Krammer		1			G
295	<i>Pinnularia viridis</i> var. <i>viridis</i> (Nitzsch) Ehr.	1	2	1		
296	<i>Placoneis abiskoensis</i> Hust.		1	1		
297	<i>Placoneis elginensis</i> (Greg.) Ralfs	1	1	1		
298	<i>Placoneis elginensis</i> var. <i>cuneata</i> (Møller ex Foged) L-B	1	1			3
299	<i>Placoneis ignorata</i> (Schimanski) L-B		1			
300	<i>Placoneis paraelginensis</i> L-B	1	1	1	+	
301	<i>Planothidium biporomum</i> (Hohn et Hellerman) L-B	1	1	1	+	
302	<i>Planothidium ellipticum</i> (Cleve) Round et Bukht.			1		
303	<i>Planothidium frequentissimum</i> (L-B) L-B	2	1	1		
304	<i>Planothidium lanceolatum</i> (Bréb.) Round et Bukht.	4	3	2		
305	<i>Planothidium lanceolatum</i> aff. var. <i>boyei</i> (Østrup) L-B	1				
306	<i>Planothidium rostratum</i> (Østrup) L-B	4	2	1		
307	<i>Psammothidium bioretii</i> (Germain) Bukht. et Round	1	1	3		V
308	<i>Psammothidium chlidanos</i> Hohn et Hellerman	1	1	2		3
309	<i>Psammothidium curtissimum</i> (Carter) Aboal		1	2		R
310	<i>Psammothidium grischunum</i> (Wuthrich) Bukht. et Round	1	1	1	+	
311	<i>Psammothidium daonense</i> (L-B) L-B	1	1	1		G
312	<i>Psammothidium helvetica</i> var. <i>minor</i> Flower et Jones		1		+	
313	<i>Psammothidium helveticum</i> (Hust.) Bukht. et Round	1	1	1		
314	<i>Psammothidium rechtensis</i> (Leclercq) L-B			1		
315	<i>Psammothidium subatomoides</i> (Hust.) L-B et Archibald	2	1	3		V
316	<i>Pseudostaurosira</i> aff. <i>brevistriata</i> (Grun.) Williams et Round		1			
317	<i>Reimeria sinuata</i> (Greg.) Kociolek et Stoermer	2	1	1		
318	<i>Rhoicosphenia abbreviata</i> (Ag.) L-B	1	2	2		
319	<i>Rhopalodia gibba</i> var. <i>gibba</i> (Ehr.) Müller	3	2			
320	<i>Rhopalodia gibba</i> var. <i>paraella</i> (Grun.) Holmboe	4				
321	<i>Rossithidium petersenii</i> (Hust.) Aboal et al.	1				3
322	<i>Sellaphora laevisissima</i> (Kütz.) Mann	1		1		
323	<i>Sellaphora laevisissima</i> var. <i>perhibita</i> (Kütz.) Mann			1		R
324	<i>Sellaphora pupula</i> var. <i>pupula</i> (Kütz.) Mereschkovsky		1	1		

№	Taxa	St	Bi	Bo	BG	RL
325	<i>Sellaphora rectangularis</i> (Greg.) L-B et Metzeltin		1			
326	<i>Stauroneis acidoclinata</i> L-B et Werum		1	1	+	
327	<i>Stauroneis</i> aff. <i>separanda</i> L-B et Werum			1		
328	<i>Stauroneis gracilis</i> Ehr.			1		V
329	<i>Stauroneis intricans</i> van de Vijver et L-B		1		+	
330	<i>Stauroneis kriegerei</i> Patrick		1		+	
331	<i>Stauroneis siberica</i> (Grun.) L-B et Krammer	1				
332	<i>Stauroneis smithii</i> Grun.	1	1			
333	<i>Stauroneis tackei</i> (Hust.) Krammer et L-B		1		+	R
334	<i>Stauroneis thermicola</i> (Petersen) Lund	1	1			
335	<i>Stauroneis</i> sp. 1		1			
336	<i>Staurosira construens</i> Ehr.			1		
337	<i>Staurosira pinnata</i> var. <i>intercedens</i> (Grun.) Hamilton			1		
338	<i>Staurosira venter</i> (Ehr.) Hamilton		4	1		
339	<i>Staurosirella pinnata</i> (Ehr.) Williams et Round	1	1	2		
340	<i>Staurosirella pinnata</i> var. <i>acuminata</i> Mayer			1	+	
341	<i>Stephanodiscus hantzschii</i> Grun.			1		
342	<i>Stephanodiscus medius</i> Håkansson		1			
343	<i>Stephanodiscus parvus</i> Stoermer et Håkansson	1		1		
344	<i>Surirella angusta</i> Kütz.	1	1	1		
345	<i>Surirella biseriata</i> Bréb.		1			
346	<i>Surirella brebissonii</i> Krammer et L-B	1				
347	<i>Surirella brebissonii</i> var. <i>kuetzingii</i> Krammer et L-B	1				
348	<i>Surirella helvetica</i> Brun	1				
349	<i>Surirella linearis</i> Smith		1			
350	<i>Surirella spiralis</i> Kütz.		1			V
351	<i>Surirella minuta</i> Bréb.	1	1			
352	<i>Synedra ulna</i> (Nitzsch) Ehr.	3	3	4		
353	<i>Tabellaria flocculosa</i> (Roth) Kütz.	2	2	1		

Twelve taxa were found in more than 50% of the samples: *Achnantheidium minutissimum* (Kütz.) Czarnecki (in 78,8% of all samples), *Planothidium lanceolatum* (Bréb.) Round et Bukht. (75,8%), *Diatoma mesodon* (Ehr.) Kütz. (69,7%), *Encyonema silesiacum* (Bleisch ex Rabenhorst) Mann (65,2%), *Gomphonema pumilum* var. *elegans* Reichardt et L-B (62,1%), *Humidophila perpusilla* (Grun.) Lowe, Kociolek, Johansen, Van de Vijver, L-B et Kopalová (59,1%), *Gomphonema rhombicum* Fricke (57,6%), *Hannaea arcus* (Ehr.) Kütz. (57,6%), *Achnantheidium subatomus* (Hustedt) L-B (57,6%), *Cocconeis placentula* var. *pseudolineata* Geitler (56,6%), *C. placentula* var. *lineata* (Ehr.) Van Heurck (53%) and *Meridion circulare* (Grev.) Ag. (53%).

The investigated sites forming the headwaters of Bistrishka River had the highest diatom diversity – 244 taxa, followed by the headwaters of Boyanska River with 188 taxa and those of Strouma River with 162 taxa.

Some of the taxa are found in most of the samples, however in low abundance, e.g. *Cavinula lapidosa* (Krasske) L-B, *C. pseudoscutiformis* (Hustedt) Mann et Stickle, *Chamaepinnularia schaupiana* L-B et Metzeltin, *C. soehrensensis* var. *hassica* (Krasske) L-B, *Eunotia tetraodon* Ehr., *Gomphonema acidoclinatum* L-B et Reichardt, *G. hebridense* Greg., *Hygropetra balfouriana* (Grunow ex Cleve) Krammer et Lange-Bertalot, *Karayevia laterostrata* (Hust.) Round et Bukht., *Navicula joubaudii* Germain, *Orthoseira roeseana* (Rabenhorst) O'Meara, *Placoneis paraelginensis* L-B and other, which is a typical characteristic of high altitude aquatic environments (WOJTAL 2009, LANGE-BERTALOT & METZELTIN 1996).

#### *Dominant structure*

*Achnantheidium minutissimum* was the most frequent and abundant species in the samples, dominant in 15% of the samples and subdominant also in 15% of the samples. Other abundant (dominant and subdominant) taxa were: *Achnantheidium subatomus*, *Cocconeis placentula* var. *pseudolineata*, *Humidophila perpusilla*, *Diatoma hyemalis* (Roth) Heiberg, *D. mesodon*, *Encyonema gracile* Rabenhorst, *E. silesiacum* (Bleisch) Mann, *Gomphonema pumilum* var. *elegans*, *G. rhombicum*, *G. tergestinum* (Grun.) Fricke, *Hannaea arcus*, *Melosira varians* Ag., *Meridion circulare*, *Planothidium lanceolatum* and *Synedra ulna* (Nitzsch) Ehr.

The most frequent and abundant species in the headwaters of Strouma River was *Hannaea arcus*; in the headwaters of Boyanska River such species was *Achnantheidium minutissimum*, whereas in the headwaters of Bistrishka river it was *Gomphonema pumilum* var. *elegans*.

Due to understandable reasons, it is difficult, if possible at all, at present state-of-art to make an objective and detailed comparison between our results and earlier data published on few common diatom taxa from Vitosha Mt. (PETKOFF 1922). In general, the taxonomic and dominant structure of the diatom communities of Vitosha Mt. resemble those of Malyovitsa stream in Rila Mt. (KAWECKA 1974), where the studied sites were situated at the same altitude range (1000 m – 2000 m a.s.l.). Common taxa for both Rila Mt. and Vitosha Mt. were *Achnantheidium minutissimum*, *Hannaea arcus*, *Diatoma mesodon*, *Meridion circulare* and *Gomphonema pumilum*.

#### *New species to Bulgarian diatom flora and for Vitosha Mt.*

Fifty-nine species, varieties and forms from 28 genera were recorded for the first time for the Bulgarian recent diatom flora, which is 17% of all identified taxa (Table 2.). New to Vitosha's algal flora are 305 taxa (Table 2). The high number of new species for Bulgaria and for Vitosha Mt. found in this study, on one hand reflects the lack of sufficient data about recent diatoms in mountain habitats in this region and, on the other hand, results from the high number of new combinations and newly described taxa in the last two decades (e.g. *Gomphonema utae* and *Luticola acidoclinata* L-B).

### *Red List of limnic diatoms*

One hundred and seven taxa (30% of all) found during this study are included in the Red List of limnic diatoms in Germany, respectively Central Europe (LANGE-BERTALOT 1996). Four of them have a status of ‘almost extinct’: *Achnanthes rupestris* Krasske, *Eunotia pseudopectinalis* Hust., *E. serra* (s.l.) Ehr., *E. triodon* Ehr. Six taxa are ‘strongly endangered’: *Eunotia arculus* (Grun.) L-B et Nörpel, *E. flexuosa* Kütz., *E. monodon* Ehr., *E. tetraodon* (Ehr.) Nörpel et L-B, *Gomphonema lagerheimii* (Cleve) Krasske, *Navicula porifera* var. *opportuna* (Hust.) L-B. Nineteen taxa are ‘endangered’, 25 taxa – ‘presumably endangered’, 20 taxa – ‘extremely rare’ and 31 taxa are ‘not endangered but in regression’ (Table 2).

Some of the rare taxa found were: *Achnanthes silvaheer cynia* L-B, *Cocconeis disculus* (Schumann) Cleve, *Luticola* aff. *charlatii* (Peragallo) Metzeltin & L-B, *Navicula bremensis* Hust., *Naviculadicta tridentula* (Krasske) L-B and other (Table 2).

### *Notes on some interesting species*

*Decussata hexagona* (Torka) L-B (Fig. 2: 24) was found in the mires and peat bogs surrounding all of the investigated river catchments, but in low abundance. Until now, the species has been reported for Bulgaria only from Holocene sediments of two mountain peat bogs in the central Sredna Gora Mountains (STANCHEVA & TEMNISKOVA 2006). According to LANGE-BERTALOT (2001) *D. hexagona* is a rare species, so far known only from Europe, with few localities. The insufficient records of the species in the literature may be due to the limited number of investigations made in mountain habitats. For instance, the species has been found in peat bog and mire samples (Ivanov & Isheva unpubl.) from Osogovo Mountain (Southwestern Bulgaria).

*Boreozonacola hustedtii* L-B, Kulikovskiy et Witkowski (Fig. 3), previously reported as *Naviculadicta pseudosilicula* (by Lange-Bertalot et Metzeltin in 1996, by Lange-Bertalot et Genkal in 1999 and as *Navicula pseudosilicula* by Hustedt in 1942) has been found in isolated habitats in alpine and boreal regions throughout the world – Northern Europe, North America, Alaska, Asia (Mongolia). In Central Europe, *B. hustedtii* occurs dispersed in oligotrophic water bodies of the Alps with low electrolyte content (SPAULDING ET AL. 2010). So far, for the Balkans, *B. hustedtii* has been reported from Shara Mountain, Macedonia (LEVKOV ET AL. 2005) and from few Romanian mountains (CARAUS 2012).

*Gomphonema* aff. *amoenum* L-B (Fig. 4) was recorded only in one site (St4, Strouma River), in an epiphytic sample, as subdominant species, at water temperature of 1,5 C° and altitude of 1485 m a.s.l. The size and shape of the valve in our material differ from the species diagnosis: in *Gomphonema amoenum* valve length is 30–65 µm, valve width is 9–16 µm and striae density is 10–11/10 µm (KRAMMER & LANGE-BERTALOT 1991), whereas in *Gomphonema* aff. *amoenum* valve length is 36–66 µm, valve width is 8–12 µm and striae density is 9–12/10 µm.

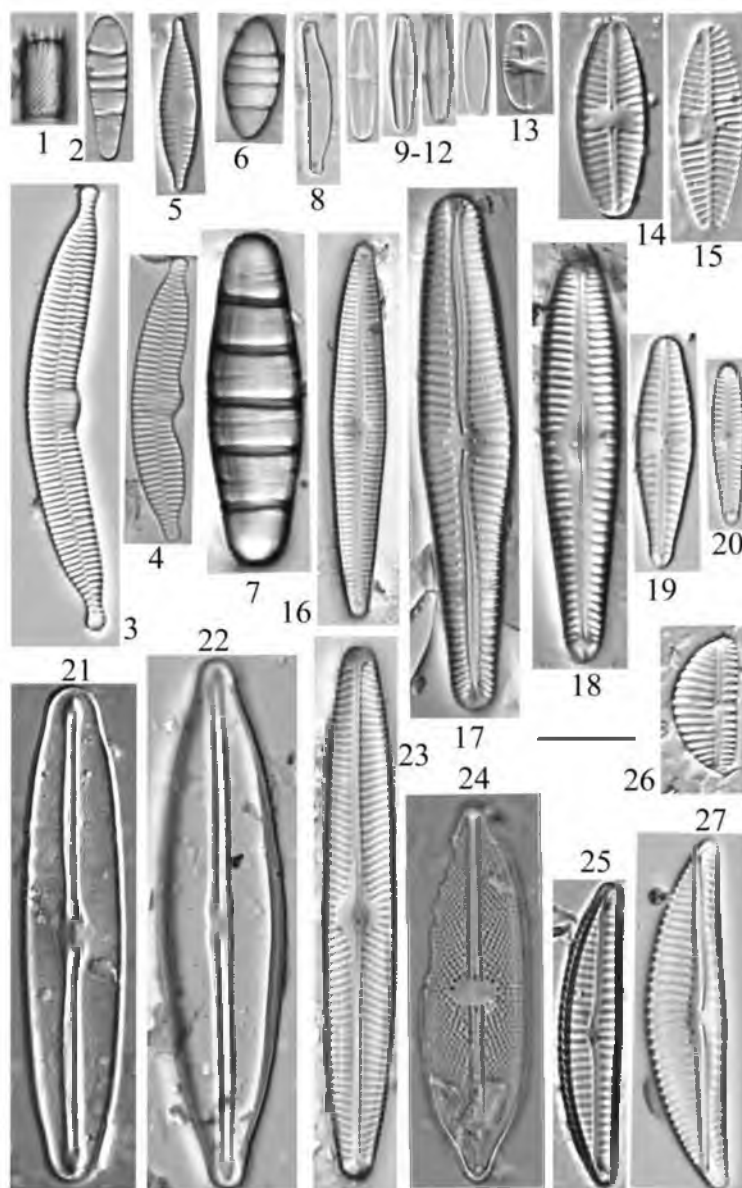


Fig. 2:1-27. Light microscopic micrographs of some diatoms found on Vitosha Mt.: 1 – *Aulacoseira subarctica*, 2 – *Meridion circulare*, 3-4 – *Hannaea arcus*, 5 – *Fragilaria capucina*, 6 – *Diatoma mesodon*, 7 – *D. hyemalis*, 8 – *Eunotia paludosa*, 9-12 – *Achnanthidium minutissimum*, 13 – *Psammothidium subatomoides*, 14, 15 – *Planothidium lanceolatum*, 16 – *Gomphonema acidoclinatum* 17 – *G. clavatum*, 18 – *G. rhombicum*, 19 – *G. tergestinum*, 20 – *G. pumilum* var. *elegans*, 21 – *Frustulia vulgaris*, 22 – *F. rhomboides*, 30 – *Navicula angusta*, 24 – *Decussata hexagona*, 25 – *Encyonema gracile*, 26 – *E. minutum*, 27 – *E. silesiacum*. Scale bar = 10µm.





Fig. 3. SEM micrograph of *Boreozonacola hustedtii* L-B, Kulikovskiy et Witkowski.

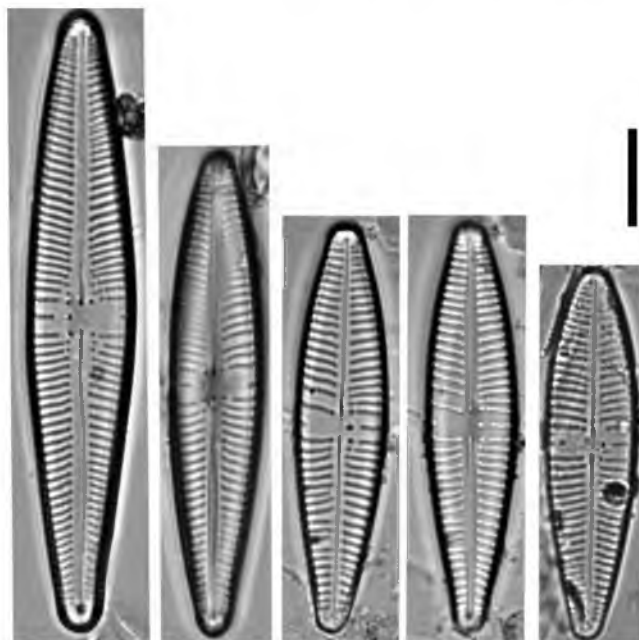


Fig. 4. LM micrographs of *Gomphonema* aff. *amoenum* L-B. Scale = 10 μm.

One species from genus *Gomphonema* (*Gomphonema* sp., Fig. 5) was found in all investigated rivers at 5 sampling sites (epilithic samples, rare and common). It was not possible to identify the species, according to the currently available literature. It was characterized by valve length of 15–35/10 μm, width of 5,0–7,0 μm, striae density of 9–13/10 μm and valves in girdle view 2,0–3,0 μm wide. Observation in SEM showed that the striae are composed of double rows of areolae, with density of 50/10 μm. Only two other species with biseriate areolae are known in the Bulgarian diatom flora: *Gomphonema minutum* (Ag.) Ag. and *G. rhombicum* Fricke. With high probability, *Gomphonema* sp. is a new species to science, which will be further investigated and described elsewhere.

In conclusion, it is possible to state that the diatom flora found in Vitosha Nature Park is characterized by high biodiversity and includes species of high conservation importance. The results obtained during this study could serve as comparative basis for

future studies and doubtless show that future more detailed investigations in different regions of Bulgaria will contribute to our knowledge on recent Bulgarian diatom flora.

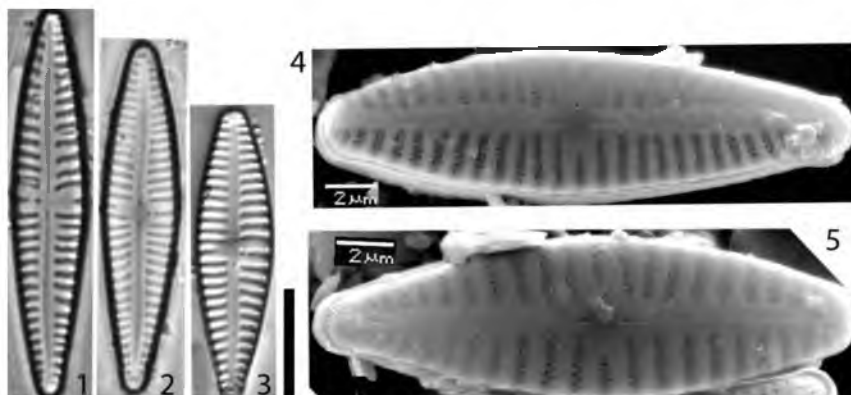


Fig. 5. LM (1–3) and SEM micrographs (4–5) of *Gomphonema* sp. Scale = 10  $\mu$ m.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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PHYTOPLANKTON OF THE RESERVOIR “DOSPAT”  
(RODOPI MTS, BULGARIA) AS INDICATOR OF NEGATIVE  
TREND IN RESERVOIR DEVELOPMENT DUE  
TO LONG-TERM CAGE FISH FARMING

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** The paper presents the results of the recent (2011) investigation of phytoplankton composition and abundance, with its seasonal and spatial dynamics in the reservoir “Dospat” (the first in Bulgaria used for cage fish farming) and shows the changes in its waters after 30 years of exploitation. Totally 55 taxa from 7 divisions have been identified: Cyanoprokaryota (8), Pyrrophyta (3), Euglenophyta (4), Cryptophyta (1), Ochrophyta (Bacillariophyceae – 26; Synurophyceae – 2), Chlorophyta (9) and Streptophyta (2). Cyanoprokaryotes have been recorded for first time in the reservoir and their harmful species *Planktothrix rubescens* and *Aphanizomenon flos-aquae* were found among the dominants and subdominants. The highest number of species was detected in June (42), the phytoplankton communities near to the dam were more species-rich and with higher abundance in comparison with those in the tailwaters, most probably due to the effect of the fish cages situated in the uppermost part of the reservoir.

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According to the average phytoplankton biomass ( $1.39 \text{ mg l}^{-1}$ ) in 2011 the reservoir “Dospat” could be classified as mesotrophic. However, it has to be noted that this value is twofold higher in comparison with 1972–1975 period, when the biomass was  $0.7 \text{ mg l}^{-1}$ . Most probably, the changes in the species composition, the increase in the phytoplankton biomass and of the reservoir trophic status are related with the effects from the cage fish farming.

**Key words:** cage fish farming, cyanoprokaryotes, harmful algae, mesotrophic status, anthropogenic impact

## INTRODUCTION

“Worldwide use in the increase of water bodies and anthropogenic pressures on them alter their health, which is of significant importance for maintaining of the water quality, biodiversity and fisheries” (ANNEVILLE ET AL. 2008, p. 1122). This is especially valid for the reservoir ecosystems, which have key role in human life. Therefore accumulation of data on their planktonic and benthic communities and natural or anthropogenically speeded-up successional changes with their possible causative factors and driving forces, is a permanent task for limnologists, and reservoir protection, based on good ecological evaluation, is among the cornerstone conservation activities. Phytoplankton is commonly used for water quality and ecological state assessments (*e.g.* HANPONGKITTIKUL 2005, DOMINGUES ET AL. 2008, JAKHAR 2013). Long-term data sets or comparison of phytoplankton structure during different periods are considered to be reliable indicators of environmental changes and trends (*e.g.* NASELLI-FLORES 2013 among the many others).

The reservoir “Dospat” was created by impoundment of a former large peat-bog. Its exploitation started in 1968 and since then it is used for aquaculture (cage farm for rainbow trout *Oncorhynchus mykiss*), as electric power source and for irrigation (STOYNEVA & MICHEV 2007). The first data on the phytoplankton, its structure and dynamics (seasonal and annual), as well as on some abiotic parameters of the newly formed reservoir, were published by NAIDENOV & SAIZ (1977) for the period 1972–1975. These authors explained the relatively low number of taxa found (45) with the short insolation period for this long and narrow reservoir, situated in a deep mountain valley.

The aim of the present paper is to provide recent data (2011) on the phytoplankton of this important Bulgarian reservoir with an attempt to outline the changes in the aquatic system, caused by long-term cage fish farming and 30-years long reservoir succession.

## MATERIAL AND METHODS

The reservoir “Dospat” (N  $41^{\circ}41'54''$  E  $24^{\circ}05'10''$ ) is situated in the Western Rodopi Mts, at 1 200 m a.s.l. and is oriented in NW-SE direction. It is the first water

body of the largest Bulgarian hydro-energetic water cascade “Dospat-Vucha” and is one of the largest reservoirs in Bulgaria – 18 km long, ca. 2 km broad with a total area > 2005,6 ha, maximum volume of 446,4 mln. m<sup>3</sup> and minimum – 20 mln. m<sup>3</sup>, with an average depth of 32 m, which varies from ca. 40 m at the dam to 20–30 m in the other parts of the water body (STOYNEVA & MICHEV 2007).

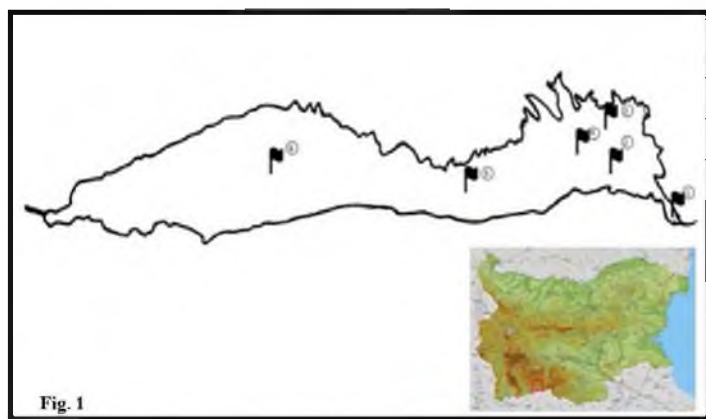


Fig. 1. Map of Bulgaria with the position of the reservoir “Dospat” and its scheme with the location of the sampling sites.

Table 1. Chemical and physical characteristics of the reservoir “Dospat” in the studied sites (1–6) from April (IV) till October (X) 2011.

Station №	Month	Coordinates	Temperature, °C	Transparency (Secchi), m	O <sup>2</sup> , mg/l	Saturation O <sup>2</sup> , %	pH
	IV		9,8	2,2	11	112,4	6,96
1	VI	N 41°38'69"; E 24°09'13"	18,9	4,8	8,74	109	7,05
	VIII		20,5	4,7	7,96	101,7	7,23
	X		11,7	3,3	9,24	97,5	6,38
	IV		9,8	2,15	10,95	112,3	7,28
2	VI	N 41°39'45"; E 24°09'06"	19,1	5	8,7	108,9	8,15
	VIII		21,9	4	8,78	115,3	7,17
	X		11,5	3,3	8,44	88,9	6,79
	IV		9,7	2,2	10,35	105,8	7,41
3	VI	N 41°39'80"; E 24°09'18"	18,3	4,2	7,63	84,2	7
	VIII		22,2	4,8	7,44	98,4	7,05

	X		11,6	3,3	8,44	88,6	6,62
	IV		8,9	2,1	11,37	113,7	7,17
4	VI	N41°39'72``; E 24°8'89``	18,7	5,3	8,8	109,1	7,7
	VIII		21,7	4,8	7,8	101,9	7,29
	X		11,4	3,4	8,28	86,9	6,83
	IV		8,7	2,3	11,45	114	7,44
5	VI	N 41°40'41``; E 24°07'46``	19	5,2	8,9	110,7	7,59
	VIII		21,3	5,6	7,58	98,3	7,47
	X		11,3	3,5	8,85	92,8	5,71
	IV		8	2,15	11,58	113,5	7,67
6	VI	N 41°42'45``; E 24°04'55``	18,6	5,3	8,85	109,8	7,13
	VIII		21,2	5,6	7,44	96	7,28
	X		11,1	3,1	8,85	92	6,76

In total, 24 phytoplankton samples were collected at 6 sites of the reservoir “Dospat” (Fig. 1) in the period from April till November 2011. It has to be noted especially that sites 1–4 were in the main reservoir bed, where site 1 was situated near to the dam and site 2 – near to the cage fish farm, while sites 5 and 6 were more near to the “tail” of the reservoir. The samples were taken from depth 0–0,5 m by batometer of „Danish” type and were 1 200 ml in volume. They were fixed in formaldehyde (2–4% final concentration) and stored in glasses with volume of 1 l. Additional living samples were collected from the same sites. The quantitative analysis was done on Bürker blood-counting chamber (LAUGASTE 1974). The species composition was determined in parallel way on fixed and living samples by light microscope “Carl Zeiss Axioscope 2” with magnification 200x and 400x, and diatoms were identified after Cox (1996). The main counting unit was the cell and biomass was estimated by the method of stereometrical approximations (ROTT 1981; DEISINGER 1984). The following standard parameters were measured: water temperature (T°C), dissolved oxygen (O<sub>2</sub> mg l<sup>-1</sup>), saturation (O<sub>2</sub>%), pH and transparency (as Secchi depth) – Table 1. Their values in 2011 were similar to those registered in 2010 (HADJINKOLOVA & ILIEV 2011).

## RESULTS

### *Taxonomic structure of the phytoplankton*

Totally 55 taxa from 7 divisions were established in the reservoir phytoplankton: Ochrophyta (28: Bacillariophyceae – 26; Synurophyceae – 2), Chlorophyta (9),



Cyanoprokaryota (8), Euglenophyta (4), Streptophyta (2), Pyrrhophyta (3) and Cryptophyta (1). Their percentage representation was as follows: Ochrophyta (50,9%: Bacillariophyceae – 47,3%; Synurophyceae – 3,6%), Chlorophyta (16,4%), Cyanoprokaryota (14,5%), Euglenophyta (7,3%), Streptophyta (3,6%), Pyrrhophyta (5,5%) and Cryptophyta (1,8%) – Fig. 2.

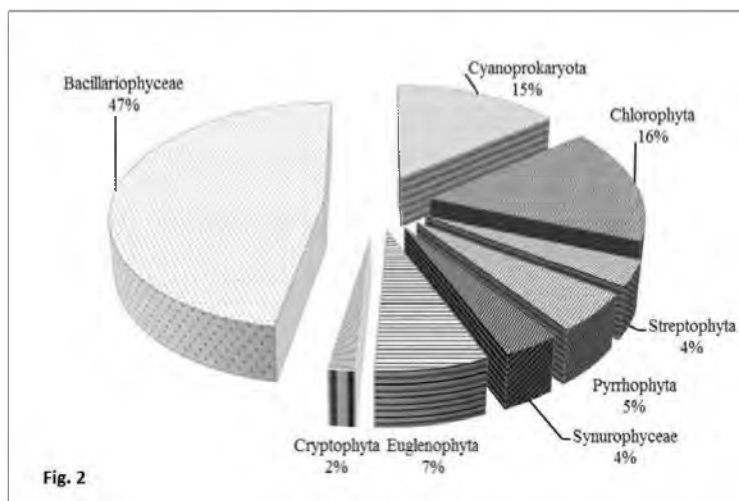


Fig. 2. Taxonomic structure of the phytoplankton of the reservoir “Dospat” (2011).

In April, 21 taxa from 6 divisions were identified (Table 2). Most of the species belong to Bacillariophyceae (6, or 52,3%) and Cyanoprokaryota (5, or 19 %). From each of the other taxonomic groups (Synurophyceae, Euglenophyta, Cryptophyta, Chlorophyta and Streptophyta,) only 1 taxon was found (4,7%) – Fig. 3A. *Asterionella formosa* Hassall (66,8%), *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek (23%) and *Tabellaria fenestrata* (Lyngbye) Kützing (10,2%) dominated the phytoplankton all over the reservoir aquatory, while the diatoms *Hannea arcus* (Ehrbg.) Patrick, *Meridion circulare* (Grev.) Ag. and *Diatoma vulgare* Bory were found only more near to the “tail” of the reservoir. The number of taxa per site was low and almost similar: in each of the sites 1–4, situated in the reservoir’s main bed, 8 taxa were found, while in the sites 5 and 6, situated more near to the tail, 6 and 7 taxa, respectively, were found (Table 2).

Table 2. Species composition of the phytoplankton in the reservoir “Dospat” by sites and months during the studied period (2011).

Taxa	Site №																									
	IV						VI						VIII						X							
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6		
Cyanoprokaryota																										
Anabaena sphaerica Bornet et Flahault							+ +						+ +						+ +							
Anabaena flos-aquae G.S. West							+ +																			
Aphanocapsa sp.							+												+							
Aphanizomenon flos-aquae Ralfs ex Born. et Flahault	+	+	+				+ + + +						+ + + +						+ + + +							
Chroococcus limneticus Lemmermann							+						+ + + +						+ + + +							
Microcystis aeruginosa (Kützing) Kützing																			+							
Planktothrix rubescens (De Candolle ex Gomont)	+	+	+	+	+	+	+	+		+		+		+							+ +					
Anagnostidis et Komárek																										
Planktothrix agardhii (Gomont) Anagnostidis et Komárek	+ +						+ + +																			
Chlorophyta																										
Coelastrum sphaericum Nägeli							+																			
Elakatothrix gelatinosa Wille																			+ + +							
Oocystidium ovale O.Korshikov							+						+													
Oocystis borgeri J.Snow													+													
Pandorina morum (O. F. Müller) Bory de Saint-Vincent													+						+							
Pediastrum duplex Meyen							+ +												+							
Scenedesmus bijugatus Kutzing							+												+							
Scenedesmus communis (Breb.) Hegewald							+ + + +												+							
Tetrastrum glabrum (Y. V. Roll) Ahlstrom et Tiffany													+						+							

<b>Streptophyta</b>				
<i>Cosmarium sp.</i>	+	+	+	+
<i>Staurastrum planctonicum</i> Teiling		+	+	+
<b>Pyrrhophyta</b>				
<i>Ceratium hirundinella</i> (O. F. Müller) Dujardin		+	+	+
<i>Peridinium sp.</i>		+	+	+
<i>Gymnodinium uberrimum</i> (G. J. Allman) Kofoid et Swezy	+	+	+	+
<b>Euglenophyta</b>				
<i>Euglena acus</i> (O. F. Müller) Ehrenberg		+		+
<i>Phacus orbicularis</i> K. Hübner		+	+	
<i>Strombomonas sp.</i>			+	
<i>Trachelomonas volvocina</i> (Ehrenberg) Ehrenberg	+	+	+	
<b>Cryptophyta</b>				
<i>Cryptomonas sp.</i>	+	+		
<b>Ochromophyta</b>				
<b>Synurophyceae</b>				
<i>Mallomonas elongata</i> Reverdin	+		+	+
<i>Mallomonas caudata</i> Iwanoff			+	+
<b>Bacillariophyceae</b>				
<i>Asterionella formosa</i> Hassall	+	+	+	+
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen		+	+	+
<i>Aulacoseira islandica</i> O. Mull.		+		
<i>Cocconeis placentula</i> Ehrenberg		+		+
<i>Cocconeis pediculus</i> Ehrenberg			+	

<i>Caloneis amphisbaena</i> (Bory de Saint Vincent) Cleve		+	+	
<i>Cymbella cymbiformis</i> C. Agardh	+			+
<i>Cyclotella meneghiniana</i> Kützing		+	+	+
<i>Diatoma vulgare</i> Bory		+	+	
<i>Diatoma elongatum</i> (Lyngbye) C. Agardh			+	
<i>Diploneis ovalis</i> (Hilse) Cleve	+		+	
<i>Fragilaria crotonensis</i> Kitton	+	+	+	+
<i>Meridion circulare</i> (Greville) C. Agardh		+		
<i>Hannea arcus</i> (Ehrenberg) Patrick		+		+
<i>Gomphonema constrictum</i> Ehrenberg				+
<i>Gomphonema acuminatum</i> Ehrenberg			+	
<i>Gomphonema truncatum</i> Ehrenberg		+	+	
<i>Melosira varians</i> C. Agardh				+
<i>Navicula rynchocephala</i> Kützing				+
<i>Navicula radiosa</i> Kützing				+
<i>Navicula</i> sp.		+	+	+
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	+		+	+
<i>Synedra acus</i> Kützing			+	+
<i>Tabellaria fenestrata</i> (Lyngbye) Kützing	+	+	+	+
<i>Tabellaria floccuosa</i> (Roth) Kützing		+		
<i>Stephanodiscus hantzschii</i> Grunow	+	+	+	+

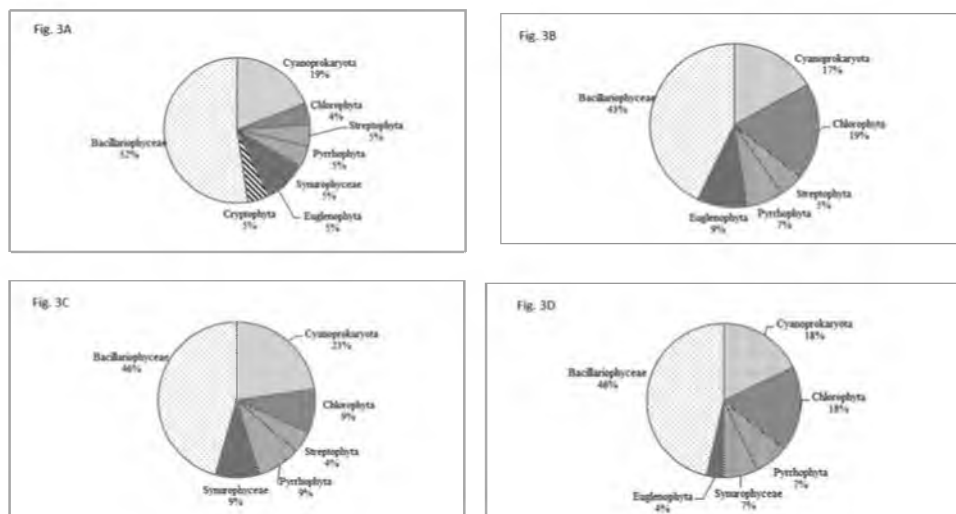


Fig. 3. Taxonomic structure of the phytoplankton of the reservoir "Dospat" during the studied period: A – in April 2011; B – in June 2011; C – in August 2011; D – in October 2011.

In June, 42 taxa from 6 divisions were found: Bacillariophyceae (26, or 42,8%), Chlorophyta (11, or 19%), Cyanoprokaryota (10, or 16,7%), Euglenophyta (9, or 9,5%), Pyrrhophyta (3, or 7,14%), Synurophyceae (2, or 4,76%) and Streptophyta (1, or 2,38%) – Fig. 3 B. In the same time the number of species of Bacillariophyceae decreased, but those of Chlorophyta and Euglenophyta increased. Dominants by numbers and biomass were *Fragilaria crotonensis* Kitton (31,5%), *Gymnodinium uberrimum* (G. J. Allman) Kofoed et Swezy (39%) and *Planktothrix rubescens* (22%), while *Aulacoseira granulata* (Ehrenberg) Simonsen was subdominant in almost all sites. Frequently occurring, but in small numbers, were the green algae *Scenedesmus communis* (Breb.) Hegewald, *Pediastrum duplex* Meyen and *Oocystidium ovale* Korshikov. The number of taxa per site was low, but showed more pronounced differences in comparison with the previous period: the richest in taxa was site 4 (25 taxa) in the reservoir bed, and the smallest number of taxa (7) was found in site 6, situated more near to the tail of the reservoir (Table 2).

In August, 22 taxa from 5 divisions were registered: Bacillariophyceae (10, or 45,5%), Cyanoprokaryota (5, or 22,7%), Chlorophyta (2, or 9%), Pyrrhophyta (2, or 9%), Synurophyceae (2, or 9%) and Streptophyta (1, or 0,5%) – Fig. 3C. Dominants by both numbers and biomass were *Cyclotella meneghiniana* Kützinger (35,7%), *Chroococcus limneticus* Lemmermann (37,7%), *Stephanodiscus hantzschii* Grunow (14,1%) and *Tabellaria fenestrata* (9,2 %), while *Asterionella formosa* and *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault were subdominants. The number of taxa per site was the highest detected, and was relatively similar in all studied sites. It was the biggest in sites 2 (11) and 1 (10) and was more or less the

same in sites 5 (9) and 6 (10), while the smallest number of taxa (7) was found in site 3 (Table 2).

In the end of October, 28 taxa from 5 divisions were identified: Bacillariophyceae (13, or 46,4%), Cyanoprokaryota and Chlorophyta (each of them with 5 taxa, or 17,85%), Synurophyceae (2, or 7,14%), Pyrrophyta (2, or 17,85%) and Euglenophyta (1, or 3,57%) – Fig. 3D. The most abundant was the group of diatoms, from which *Tabellaria fenestrata* (85,2%) and *Asterionella formosa* (11,1%) dominated. Subdominants were the pyrrhophytes *Gymnodinium uberrimum* and *Ceratium hirundinella* (O. F. Müller) Dujardin. The number of taxa per site increased significantly and its maximum for the studied period was detected. The maximum number of taxa was found in site 6 (19), thus being almost twice higher in comparison with the previous periods, while the minimum was in site 5 (7) – Table 2.

#### *Phytoplankton abundance (numbers and biomass)*

In 2011, total phytoplankton numbers varied from  $2,35 \times 10^6$  cells/l<sup>-1</sup> (August) to  $98,3 \times 10^6$  cells/l (April), being  $42,11 \times 10^6$  cells l<sup>-1</sup> in average, and total biomass varied from 0,036 mg l<sup>-1</sup> to 3,100 mg l<sup>-1</sup>, being 1,39 mg l<sup>-1</sup> in average (Fig. 4). These values indicated the general mesotrophic status of reservoir waters. In the text below seasonal and spatial changes of phytoplankton abundance are briefly described.

In April, during the mass bloom of diatoms, phytoplankton numbers varied from  $13,7 \times 10^6$  cells l<sup>-1</sup> (site 3) to  $340 \times 10^6$  cells l<sup>-1</sup> (site 2) – Fig. 5A. Phytoplankton abundance was the biggest at sites 2 and 1 ( $340 \times 10^6$  cells l<sup>-1</sup> and  $71,7 \times 10^6$  cells l<sup>-1</sup>, respectively), situated near the cage farm and near to the dam. The lowest abundance was found at site 3 ( $13,7 \times 10^6$  cells l<sup>-1</sup>). More near to the tail of the reservoir, at sites 5 and 6, the numbers were  $36,4 \times 10^6$  cells l<sup>-1</sup> and  $65,25 \times 10^6$  cells l<sup>-1</sup>, respectively. The biomass values varied from 0,457 mg l<sup>-1</sup> (site 3) to 10,652 mg l<sup>-1</sup> (site 2) – Fig. 5A. The month average value of the biomass was 3,103 mg l<sup>-1</sup> and indicated the eutrophic state of the reservoir waters for this period.

In June the abundance of cyanoprokaryotes and green algae increased. The phytoplankton numbers were 10 times lower than in April and varied from  $0,4 \times 10^6$  cells l<sup>-1</sup> (site 5) to  $36,4 \times 10^6$  cells l<sup>-1</sup> (site 1) – Fig. 5B. Minimum numbers were registered at sites 5 and 6 –  $0,4 \times 10^6$  cells l<sup>-1</sup> and  $0,76 \times 10^6$  cells l<sup>-1</sup>, respectively. Phytoplankton biomass varied from 0,013 mg l<sup>-1</sup> (site 6) to 1,115 mg l<sup>-1</sup> (site 1). Maximum values of both numbers and biomass were registered at sites 1 and 2, situated near to the dam and cage farm, respectively. The month average value of the phytoplankton biomass was 0,511 mg l<sup>-1</sup>, indicating the oligotrophic state of the reservoir waters.

In August, during the summer stratification, the phytoplankton numbers were the lowest and varied from  $0,44 \times 10^6$  cells l<sup>-1</sup> (site 6, in the tailwaters and in the vicinity of the inflow from the rivulet “Dospatska Reka”) to  $5,33 \times 10^6$  cells l<sup>-1</sup> (site 1, near to the dam) – Fig. 5C. Biomass values ranged from 0,017 mg l<sup>-1</sup> (site 5)

to 0,091 mg l<sup>-1</sup> (site 1), also decreasing from the dam and cage farm region to the tailwaters. The average phytoplankton biomass was the lowest detected – 0,090 mg l<sup>-1</sup> and indicated oligo- to ultraoligotrophic state of the reservoir waters.

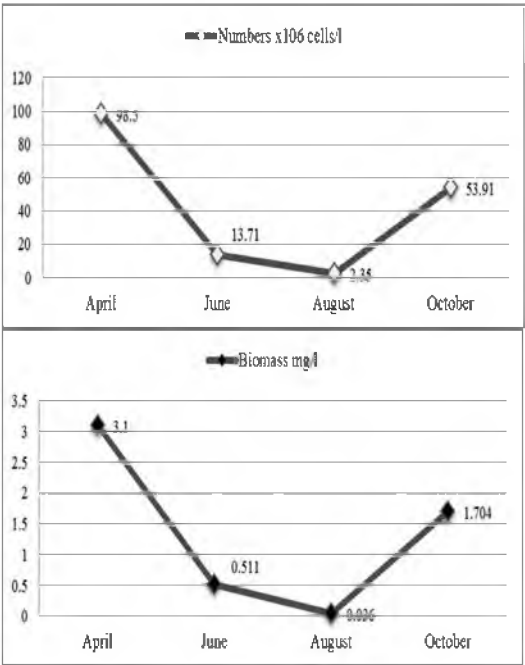


Fig. 4. Annual average values of phytoplankton numbers and biomass (B) in the reservoir “Dospat” in 2011.

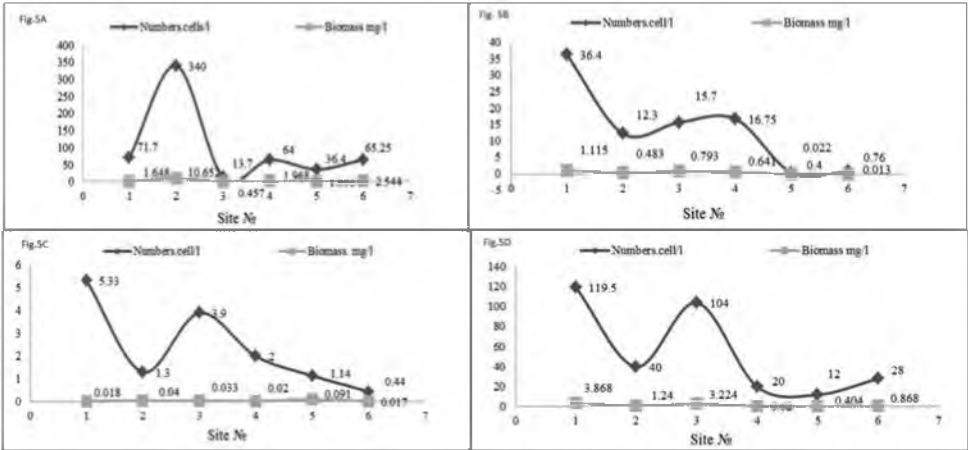


Fig. 5. Phytoplankton numbers and biomass in the reservoir “Dospat” during the studied period: A – in April 2011; B – in June 2011; C – in August 2011; D – in October 2011

In October, during the autumn homothermic conditions, diatoms dominated again. Phytoplankton numbers varied from  $12 \times 10^6$  cells  $l^{-1}$  (site 5) to  $119,5 \times 10^6$  cells  $l^{-1}$  (site 1), and the biomass ranged from 0,404 mg  $l^{-1}$  (site 5) to 3,868 mg  $l^{-1}$  (site 1) – Fig. 5D. The phytoplankton abundance decreased significantly from the dam region to the tailwaters. The month average value of the biomass (1,702 mg  $l^{-1}$ ) indicated the meso- to eutrophic status of the reservoir waters.

## DISCUSSION

The results obtained during this study show that temporal and spatial distribution of the phytoplankton in the reservoir “Dospat” was heterogeneous in both quantitative and qualitative aspect. The phytoplankton abundance was with two pronounced maxima (highest spring peak in April and lower autumn peak in October) and a low summer peak in June, with the relevant quantitative representation of taxonomic groups – leading role of diatoms in colder periods with increase of the participation of cyanoprokaryotes and green algae during summer stratification. These data are on general conformity with the classical PEG-model for seasonal phytoplankton succession (SOMMER ET AL. 1986). They completely coincide with the data on the general seasonal phytoplankton dynamics, published on the same reservoir by NAIDENOW & SAIZ (1977), who also indicated the lowest phytoplankton abundance for August and highest – for April. However, the results obtained by us on the species composition show pronounced difference with those published by NAIDENOW & SAIZ (1977). The highest total number of taxa detected by us was in June (42), being almost twice higher in comparison with number of species found in all other months (21–28), while in the 1972–1975 the highest number of taxa was in the summer periods (35), being almost twice higher in comparison with the spring periods (19).

The average biomass value in 2011 was 1,39 mg  $l^{-1}$ , while according to NAIDENOW & SAIZ (1977) for the period 1972–1975 it was 0,7 mg  $l^{-1}$ . In spite of the fact, that according to these values the reservoir yet has to be classified as mesotrophic (UZUNOV & KOVACHEV 2002; STOYNEVA & MICHEV 2007), this twofold increase in 30 years period has to be outlined, since, in our opinion, it is due mainly to the influence of cage fish farming on reservoir waters. NAIDENOW & SAIZ (1977) did not discuss the horizontal phytoplankton distribution and therefore no comparison is possible, but we would like to discuss the data obtained during this study. The strange, at first look, were the results on the spatial distribution of the species composition, according to which more taxa were generally found in the deeper sites near to the dam (1–2) in comparison with the more shallow sites situated more near to the tail (5–6), and on the more abundant phytoplankton development near the dam, as well. It has to be taken into account that near to site 6 is the inflow of the rivulet Dospatska Reka, which could cause a dilution of the reservoir waters. However,



the amount entering from this rivulet is not high enough to explain alone such a significant decrease of phytoplankton quantity. It is possible to suppose that one of the most probable reasons for the higher phytoplankton amounts in the uppermost part of the reservoir is the influence from the cage farm, situated there.

Our suggestion finds support in the data and conclusions published much earlier by NAUMOVA & ZHIVKOV (1988). They were the first authors, who noted the significant concentration of biogenes (ammonium and nitrites in particular) in the aquatory of several square kilometers near to the reservoir dam and explained it with the effects of cage farming. According to them, enormous amount of organic matter (1 500–2 500 tons) had been spread annually in and near to the cages. A big part of it remains unutilized and sinks on the bottom. Its degradation results in enrichment of the waters with biogenes, followed by nuisance algal blooms combined with night oxygen depletions and even short-termed fish-kills. The effect is most strong in spring, when the homothermy is combined with the typical for the region south-eastern wind, and the accumulated biodegradation products rise to the surface water levels. The highest nutrient concentrations were registered by NAUMOVA & ZHIVKOV (1988) in the dam region, and were triple in amounts in comparison with the values of nitrites, and twofold higher for nitrates, iron and magnesium in comparison with the values, recorded for these ions earlier by NAIDENOV & SAIZ (1977).

Additional prove for the statement on the strong negative effect of cage farms on the reservoir could be found in the recent detection of cyanoprocaryotes with notable quantities and 8 taxa in the phytoplankton. This taxonomic group was not found during the studies by NAIDENOV & SAIZ (1977) and its occurrence is commonly accepted as related with higher trophic status of the inhabited waters. Among the cyanoprocaryotes, the abundant development of both harmful species *Planktothrix rubescens* and *Aphanizomenon flos-aquae* (GUIRY & GUIRY 2014) has to be outlined. These species are more typical for lowland eutrophic waters, and have been rare found in deep mountain waters (*e.g.* D'ALLELIO & SALMASO 2011). Therefore, taking into account the detected changes in phytoplankton quantitative and qualitative structure and its reverse (in comparison with standard horizontal distribution in reservoirs) spatial distribution with more abundant and species-rich development near to the dam, we can outline the negative trend in reservoir development due to long-term cage fish farming.

#### ACKNOWLEDGEMENTS

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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## FLORA, MYCOTA AND VEGETATION OF DUPKATA RESERVE (RODOPI MTS, BULGARIA)

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** The study represents a pilot scientific research of flora, mycota and vegetation within the Dupkata Reserve. Twenty three species, referred to 2 divisions, 3 classes and 16 families are recorded for the bryoflora. One of them is listed in the Habitat Directive. The vascular flora is presented by 103 species from 38 families. Forty eight vascular plants are considered as medicinal plants. Forty two species of larger ascomycetes and basidiomycetes are found in the reserve. Three of them are of high conservation value. The vegetation cover is consisted mostly of floristically poor communities of Spruce forest and mixed Spruce and Scots pine forests. Habitats within the reserve are presented by six types according to the Habitat Directive classification.

**Key words:** bryophytes, conservation, habitats, larger fungi, medicinal plants, plant communities.

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## INTRODUCTION

Dupkata was designated as a strict reserve in 1951 with an area of 65,2 ha. It is located in Western Rodopi Mts, southern slopes of Batashka Mt and includes the Valley of Devinska River. Nowadays this protected area is extended to 1210,8 ha and spread between 600 and 1300 m altitude. The reserve is declared for protection of wild flora and fauna (especially century-old Scots pine forests and the Red deer) and is listed in the UNESCO's Man and the Biosphere Programme (Executive Environment Agency).

Any data about mycota, vascular flora and bryoflora for Dupkata Reserve hasn't been published so far. The paper represents results from a pilot scientific research in the reserve and thus sets a base for further detailed studies.

## MATERIAL AND METHODS

The study has been done during the vegetation period of 2014. The route method has been used to describe the biodiversity of bryophytes, larger fungi, vascular plants and habitats. In the process of field studies the taxa found have been recorded in lists, and in case of difficulties to identify the species on the field samples were gathered for further identification. Identification of taxa and nomenclature for vascular plants and bryophytes were according the main taxonomic sources for Bulgaria (KOZHUHAROV 1992; DELIPAVLOV & CHESHMEDZHIEV 2003; JORDANOV 1963, 1964, 1966, 1970, 1973, 1976, 1979; VELCHEV 1982, 1989; KOZHUHAROV 1995; KOZHUHAROV & ANCHEV 2012; PETROV 1975). Author's names of fungal taxa are abbreviated according to KIRK & ANSELL (2004) and Index Fungorum. The list of medicinal plants followed Appendix 1 of Bulgarian Medicinal Plants Act (2000). Special attention has been paid to taxa of high conservation value. To evaluate the conservation status, the lists of established taxa were checked for endemics – Bulgarian and Balkan (PETROVA & VLADIMIROV 2010), protected species (Appendix 3 of Bulgarian Biological Diversity Act 2002), rare and endangered species according to Bulgarian Red lists and Red Data books (PETROVA & VLADIMIROV 2009; PEEV ET AL. 2011), Red List of the Bryophytes in Bulgaria (NATCHEVA ET AL. 2006), Red List of Fungi in Bulgaria (GYOSHEVA ET AL. 2006), as well as European and international documents (*e.g.* Bern convention, Directive 92/43/EEC, IUCN Red List, CITES). The syntaxonomy follows the methodological school of BRAUN-BLANQUET (1965). Habitats are defined according to the Habitat Directive (Council Directive 92/43/EEC).

## RESULTS AND DISCUSSION

### *Bryophytes*

Data on the distribution of bryophytes in Bulgaria show that 8% (of 754 species) of the species found in Bulgaria so far have localities in Western Rodopi

Mts. Bryophyte flora in the reserve comprises 23 species, referred to 2 divisions (liverworts and mosses), 3 classes and 16 families and occupies various substrata as decaying wood, rocks, bare soil (Appendix 1). Bryophytes found in the reserve are typical for such kind of areas as dense coniferous forests, well preserved and not affected by human activities. Bryophyte species *Buxbaumia viridis* (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl. is listed in the Habitat Directive and the Bulgarian Biological Diversity Act (Table 1). It grows on coniferous decaying wood (mainly spruce logs) in shady spruce, pine-spruce and fir-spruce forests with high humidity. The species was subjected to monitoring observations in the frame of the National Biodiversity Monitoring System. The localities of *Buxbaumia viridis* in the Dupkata Reserve could be included in monitoring programs as referent sites.

### *Vascular flora*

A total list of 103 species of vascular plants has been established (see Appendix 1). This number represents some 10% of diversity of the vascular flora of Batashka planina, which counts 1024 taxa according to the last inventory study (MESHINEV 2002). The plant diversity in Dupkata Reserve is presented by 38 families. Ferns (Polypodiophyta) are represented by 2 species, 4 species are conifers (Pinophyta) and others (97 species) are flowering plants (Magnoliophyta), including 17 monocots and 80 dicots. The most species rich families are Asteraceae (11 species), Poaceae (10 species), Lamiaceae (7 species), Fabaceae and Caryophyllaceae (each with 6 species). The families with the highest number of genera are Asteraceae (11 genera) followed by Poaceae (9 genera), Lamiaceae (6 genera) and Rosaceae (5 genera). The most species-rich genera are *Luzula*, *Campanula*, *Hypericum* and *Trifolium* – with 3 species each one. Seven species of conservation significance are found in the reserve (see Table 1).

### *Medicinal plants*

Among the established vascular plants 48 species are considered as medicinal plants (Appendix 1). The richest families are Asteraceae (6 species), Lamiaceae (5 species) and Rosaceae (4 species). According to their protection status and possibilities to collect them from wild areas, medicinal plants in Bulgaria have been grouped into 5 groups as follows:

I – protected species according to Appendix 3 of the Bulgarian Biological Diversity Act: not found within this study;

II – species forbidden for collection from nature for commercial purposes but with possibility to be collected for personal purposes, according to Order RD-83/3.02.2014 of the Minister of Environment and Waters: not found;

III – species with limited permission for collection from nature for commercial purposes with annually defined regions and quantities, according to Order RD-

83/3.02.2014 of the Minister of Environment and Waters: 4 species – *Primula veris* L.; *Betonica officinalis* L.; *Carlina acanthifolia* All.; *Sedum acre* L.;

IV – species object of preservation and regulated use from nature, according to Appendix 4 of the Bulgarian Biological Diversity Act: 2 species – *Dryopteris filix-mas* (L.) Schott.; *Primula veris* L.;

V – widespread medicinal plants: 43 species. In this category as common species with abundant populations in Dupkata Reserve *Vaccinium myrtillus* L., *V. vitis-idaea* L., *Veronica officinalis* L., *Euphorbia amygdaloides* L. as well as some dominants in coniferous forests like *Pinus sylvestris* L. and *Picea abies* (L.) Karst. have to be mentioned.

### *Larger fungi*

In total 42 species of larger fungi were registered on the territory of the reserve during the field investigation – 1 species from Ascomycota and 41 species from Basidiomycota. The species belong to 2 classes, 8 orders, 23 families and 35 genera (Appendix 1). Three species are of high conservation value and are included in the Red List of Fungi in Bulgaria (GYOSHEVA ET AL. 2006) under different threat categories: *Agaricus macrocarpus* (F. H. Møller) F. H. Møller and *Auriscalpium vulgare* Gray – both estimated as Endangered (EN), and *Macrotyphula fistilosa* (Holmsk. : Fr.) R. H. Peterson – Vulnerable (VU) (Fig. 1). *A. macrocarpus* and *A. vulgare* are included also in the Red Data Book of Republic of Bulgaria (PEEV ET AL. 2011) – All taxa of larger fungi were registered in forest habitats – coniferous (*Picea abies*, *Pinus sylvestris*, *Abies alba* Mill.) and mixed with *Fagus sylvatica* L. woods. The prevailing number of species are saprothrophs and parasites on wood and mycorrhizal fungi.



Fig. 1. *Macrotyphula fistilosa* (Holmsk. : Fr.) R. H. Peterson – a Vulnerable (VU) species listed in the Red List of Fungi in Bulgaria.

Table 1. Species of conservation significance.

Abbreviations used: Bal – Balkan, Bul – Bulgarian, CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora, IUCN – International Union for Conservation of Nature, LC – Least concerned, NT – Near threatened, VU – Vulnerable

Species	Bulgarian Biological Diversity Act (App. 3)	Habitat Directive	IUCN	CITES	Endemics	Red Books of Bulgaria	Red lists of Bulgaria
<b>Bryophytes</b>							
<i>Buxbaumia viridis</i> (Lam. & DC.) Moug. & Nestl.	+	+					NT
<b>Vascular flora</b>							
<i>Crocus veluchensis</i> Herb.					Bal		
<i>Lathraea rhodopea</i> Dingler	+				Bal		NT
<i>Neottia nidus-avis</i> (L.) L.C. Richard			LC	+			
<i>Seseli rhodopaeum</i> Vel.							NT
<i>Silene roemerii</i> Friv.					Bal		
<i>Soldanella rhodopaea</i> F.K. Meyer					Bul		VU
<i>Viscaria vulgaris</i> Rohl. ssp. <i>arthropurpurea</i> (Griseb.) Stoj.					Bal		
<b>Larger fungi</b>							
<i>Agaricus macrocarpus</i> (F. H. Møller) F. H. Møller						EN	EN
<i>Auriscalpium vulgare</i> Gray						EN	EN
<i>Macrotyphula fistilosa</i> (Holmsk. : Fr.) R. H. Peterson							VU

### *Vegetation and habitats*

The reserve territory is situated in the coniferous belt (VELCHEV 2002). Spruce forests prevail and they are well preserved, almost wholly covering all possible exposures. Their age is different but varies between 40 and 100 years. Similar vegetation is to be found not only in the Rodopi Mts (BONDEV ET AL. 1985; NIKOLOV & VALCHEV 1998), but in other Bulgarian mountains also – Rila Mt (BONDEV ET AL. 1981; NIKOLOV & VULCHEV 2001), Vitosha Mt (BONDEV ET AL. 1983), western part of Balkan Mt (BONDEV ET AL. 1995), Osogovska Mt (LAZAROV 1995). Mixed Spruce and Scots pine forests, where *Pinus sylvestris* shares more than 20% of the

tree layer, are developed mostly on southern slopes. The stands with dominance of *Pinus sylvestris* occupy very limited area. Usually, they are on the top of the ridges or southern slopes, where the radiation is higher and sufficient for the light demanding Scots pine. Rarely *Abies alba* takes part in the forests.

Syntaxonomically the vegetation belongs to Vaccinio-Piceetea class. It is characteristic for coniferous forests in the continental and boreal zones. For the region these forests are zonal and climax vegetation which tends to sustain for long period of time. Studied communities have well developed vertical structure of tree, herbaceous and moss layers. The herbaceous stratum is species poor, while ground moss cover represents high biodiversity and significant coverage. Most common are *Vaccinium myrtillus*, *V. vitis-idaea*, *Luzula sylvatica* (Hudson) Gaudin, *L. luzuloides* (Lam.) Dandy, *Calamagrostis arundinacea* (L.) Roth, *Dicranum scoparium* Hedw., *Hylocomium splendens* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw. and *Eurhynchium angustirette* (Broth.) T.J.Kop. The species composition of mixed forests and the monodominant spruce forests does not differ. Monodominant *Pinus sylvestris* forests are more biodiversity rich as a result of better lightening. There the young trees and seedlings are mostly *Picea abies* and this indicates the successional trend toward climax vegetation. Vaccinio myrtilli-Pinetum sylvestris and Calamagrostio arundinaceae-Pinetum sylvestris associations within Dicrano-Pinion alliance, and Vaccinio myrtilli-Piceetum abietis association within Piceion abietis are established. Alnetum incanae community occupies restricted area along a small river. It is related to Alnion incanae alliance of Carpino-Fagetea class.

Five habitat types of Council Directive 92/43 EEC are found in Dupkata Reserve: 9410 Acidophilous *Picea* forests of the montane to alpine levels (Vaccinio-Piceetea), 91CA Rhodopide and Balkan Range Scots pine forests, 91E0 \* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae), 3260 Water courses of plain to montane levels with *Ranunculus fluitans* and *Callitriche-Batrachion* vegetation and 8220 Siliceous rocky slopes with chasmophytic vegetation.

It could be stated that the reserve territory has high natural value. It preserves an example of natural primary coniferous forests at the southern distribution boundary. Currently forests develop completely naturally and we observed places with high abundance of fallen trees which slowly will be replaced by new generation of spruce. The studied vegetation could be used as a standard for conservation measures in other part of the Rodopi Mts as a model for potential natural vegetation, for education and scientific purposes, or as a source of genetic material.



## Appendix 1. List of established taxa in the Dupkata Reserve

### **Bryophytes**

#### **Marchantiophyta (Liverworts)**

##### **Jungermanniopsida**

**Plagiochilaceae:** *Plagiochila porelloides* (Torrey ex Nees) Lindenb.,

**Geocalyceae:** *Lophocolea heterophylla*

**Cephaloziaceae:** *Nowellia curvifolia*

#### **Bryophyta (Mosses)**

##### **Polytrichopsida**

**Buxbaumiaceae:** *Buxbaumia viridis* (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl.

**Polytrichaceae:** *Polytrichum piliferum* Hedw., *P. formosum* Hedw.

##### **Bryopsida**

**Grimmiaceae:** *Racomitrium canescens* (Hedw.) Brid.

**Dicranaceae:** *Dicranum scoparium* Hedw., *D. tauricum* Sapjegin

**Hedwigiaceae:** *Hedwigia stellata* Hedenäs

**Rhabdoweisiaceae:** *Dicranoweisia crispula* (Hedw.) Milde

**Orthotrichaceae:** *Orthotrichum rupestre* Schleich. ex Schwägr.

**Bryaceae:** *Bryum moravicum* Podp.

**Mniaceae:** *Plagiomnium affine* (Blandow ex Funck) T.J.Kop.

**Campyliaceae:** *Sanionia uncinata* (Hedw.) Loeske

**Hylocomiaceae:** *Hylocomium splendens* (Hedw.) Schimp., *Pleurozium schreberi* (Willd. ex Brid.) Mitt.

**Pterigynandraceae:** *Pterigynandrum filiforme* Hedw.

**Brachytheciaceae:** *Brachytheciastrum velutinum* (Hedw.) Ignatov & Huttunen, *Eurhynchium angustirete* (Broth.) T.J.Kop., *Isothecium alopecuroides* (Lam. ex Dubois) Isov.

**Hypnaceae:** *Hypnum cupressiforme* Hedw., *Herzogiella seligeri* (Brid.) Z.Iwats.

**Vascular plants** [medicinal plants are marked by asterix (\*):]

##### **Polypodiophyta:**

**Athyriaceae:** *Athyrium filix-femina* (L.) Roth; **Aspidiaceae:** *\*Dryopteris filix-mas* (L.) Schott.;

##### **Pinophyta:**

**Cupressaceae:** *Juniperus communis* L.; **Pinaceae:** *\*Abies alba* Mill.; *\*Picea abies* (L.) Karst.; *\*Pinus sylvestris* L

##### **Magnoliophyta:**

**Magnoliopsida:** **Aceraceae:** *Acer campestre* L.; *Acer pseudoplatanus* L.; **Apiaceae:** *\*Heracleum verticillatum* Pančić; *\*Sanicula europaea* L.; *Seseli rhodopaeum* Vel.; **Asteraceae:** *\*Carlina acantifolia* All.; *Centaurea nervosa* Willd.; *Cirsium appendiculatum* Griseb.; *\*Doronicum columnae* Ten.; *Hieracium murorum* gr.; *Leontodon autumnalis* L.; *Mycelis muralis* (L.) Dumort.; *\*Petasites albus* (L.) Gaertn.; *\*Senecio nemorensis* L.; *\*Taraxacum officinale* F.H. Wigg; *\*Tussilago farfara* L.; **Betulaceae:** *\*Carpinus betulus* L.; **Boraginaceae:** *Myosotis arvensis* (L.) Hill; *Myosotis sylvatica* Ehrh. ex Hoffm.; *Pulmonaria rubra* Schott; *Symphytum tuberosum* L.; **Brassicaceae:** *Rorippa sylvestris* (L.) Besser; **Campanulaceae:** *Campanula patula* L.; *Campanula persicifolia* L.; *Campanula*

*rapunculoides* L.; **Caryophyllaceae:** *Moehringia pendula* (Waldst. & Kit.) Fenzl; *Moehringia trinervia* (L.) Clairv.; *Silene roemerii* Friv.; *Silene vulgaris* (Moench) Garcke; \**Stellaria media* (L.) Vill.; \**Viscaria vulgaris* Röhl; **Chenopodiaceae:** \**Chenopodium bonus-henricus* L.; **Crassulaceae:** \**Sedum acre* L.; **Ericaceae:** *Bruckenthalia spiculifolia* (Salisb.) Rchb.; \**Vaccinium myrtillus* L.; \**Vaccinium vitis-idaea* L.; **Euphorbiaceae:** \**Euphorbia amygdaloides* L.; **Fabaceae:** \**Chamaespartium sagittale* (L.) Gibbs; *Genista carinalis* Griseb.; *Trifolium aureum* Poll.; *Trifolium medium* L.; \**Trifolium pratense* L.; *Vicia cassubica* L.; **Fagaceae:** \**Fagus sylvatica* L.; *Quercus dalechampii* Ten.; **Geraniaceae:** \**Geranium macrorrhizum* L.; \**Geranium robertianum* L.; **Hypericaceae:** \**Hypericum maculatum* Crantz; *Hypericum olympicum* L.; \**Hypericum perforatum* L.; **Lamiaceae:** *Ajuga genevensis* L.; *Ajuga reptans* L.; \**Betonica officinalis* L.; \**Clinopodium vulgare* L.; \**Lamium purpureum* L.; \**Mentha arvensis* L.; \**Prunella vulgaris* L.; **Oxalidaceae:** \**Oxalis acetosella* L.; **Plantaginaceae:** \**Plantago major* L.; **Primulaceae:** \**Primula veris* L.; *Soldanella rhodopaea* F.K. Meyer; **Pyrolaceae:** *Pyrola minor* L.; **Ranunculaceae:** \**Caltha palustris* L.; \**Ranunculus polyanthemus* L.; **Rosaceae:** \**Arenaria agrimonoides* (L.) DC.; \**Fragaria vesca* L.; \**Potentilla erecta* (L.) Raeusch.; *Rosa tomentosa* Sm.; \**Rubus idaeus* L.; **Rubiaceae:** *Cruciata glabra* (L.) Ehrend.; \**Cruciata laevipes* Opiz; **Salicaceae:** \**Populus tremula* L.; **Saxifragaceae:** \**Chrysosplenium alternifolium* L.; **Scrophulariaceae:** *Lathraea rodopaea* Dingler; *Melampyrum sylvaticum* L.; *Verbascum longifolium* Ten.; \**Veronica chamaedrys* L.; \**Veronica officinalis* L.; **Urticaceae:** *Urtica dioica* L.; **Violaceae:** \**Viola tricolor* L.;

**Liliopsida:** **Cyperaceae:** *Scirpus sylvaticus* L.; **Juncaceae:** *Juncus conglomeratus* L.; *Luzula forsteri* (Sm.) DC.; *Luzula luzuloides* (Lam.) Dandy; *Luzula sylvatica* (Hudson) Gaudin; **Iridaceae:** *Crocus veluchensis* Herbert; **Orchidaceae:** *Neottia nidus-avis* (L.) Rich.; **Poaceae:** *Agrostis capillaris* L.; *Brachypodium pinnatum* (L.) Beauv.; *Brachypodium sylvaticum* (Hudson) Beauv.; \**Briza media* L.; *Calamagrostis arundinacea* (L.) Roth; *Dactylis glomerata* L.; *Deschampsia caespitosa* (L.) Beauv.; *Festuca drymeja* Mert. et Koch.; *Lerchenfeldia flexuosa* (L.) Schur; *Poa nemoralis* L.

### **Larger fungi**

#### **Ascomycota:**

**Leotiomycetes:** *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C.S. Ramamurthi, Korf & L. R. Batra.

#### **Basidiomycota:**

**Agaricomycetes:** *Agaricus arvensis* Schaeff.; *A. macrocarpus* (F. H. Møller) F. H. Møller; *Amanita battarrae* (Buod.) Bon; *A. gemmata* (Fr.) Bertill.; *A. rubescens* Pers. : Fr.; *A. vaginata* (Bull. : Fr.) Lam.; *Auriscalpium vulgare* Gray; *Boletus chrysenteron* Bull.; *B. edulis* Bull. : Fr.; *Bovista plumbea* Pers. : Pers.; *Cerrena unicolor* (Bull. : Fr.) Murril var. *unicolor*; *Chroogomphus helveticus* (Singer) M. M. Moser; *Clitocybe gibba* (Pers. : Fr.) P. Kumm.; *Coltricia perennis* (L. : Fr.) Murril var. *perennis*; *Fomes fomentarius* (L. : Fr.) J. J. Kickx; *Fomitopsis pinicola* (Sw. : Fr.) P. Karst.; *Galerina hypnorum* (Schränk : Fr.) Kühner; *Gomphidius glutinosus* (Schaeff. : Fr.) Fr.; *Hydnum repandum* L. : Fr.; *Inocybe lacera* (Fr. : Fr.) P. Kumm.; *Kuchneromyces mutabilis* (Schaeff. Fr.) Singer & A. H. Sm.; *Lactarius aurantiacus* (Pers. : Fr.) Gray; *Lepiota clypeolaria* (Bull. : Fr.) P. Kumm.; *L. cristata* (Bolton. : Fr.) P. Kumm.; *Lycoperdon perlatum* Pers. : Pers.; *Macrotyphula fistulosa* (Holmsk. : Fr.) R. H. Peterson; *Megacollybia platyphylla* (Pers. : Fr.) Kotl. & Pouzar;

*Mycena epipterygia* (Scop. : Fr.) Gray var. *viscosa*; *Pluteus cervinus* (Schaeff.) P. Kumm.; *Polyporus leptcephalus* (Jacq. : Fr.) Fr.; *Ramaria formosa* (Pers. : Fr.) Quél.; *Rhizopogon roseolus* (Corda) Th. Fr.; *Rhodocollybia butyracea* (Bull. : Fr.) Lennox f. *butyracea*; *Russula queletii* Fr.; *Setulipes andrasaceus* (L. : Fr.) Antonín; *Stereum hirsutum* (Willd. : Fr.) Gray; *S. subtomentosus* Pouzar; *Suillus luteus* (L. : Fr.) Roussel; *Trametes versicolor* (L. : Fr.) Lloyd; *Trichaptum abietinum* (Pers. ex J. F. Gmel. : Fr.) Ryvarden; *Xerula radicata* (Rehhan & Fr.) Dörfelt.

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## NEW LOCALITIES OF *BATTARREA PHALLOIDES* (BASIDIOMYCOTA) IN BULGARIA

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** *Battarrea phalloides*, a rarely found basidiomycete which is included in the Red List of fungi in Bulgaria and also in the Red Data Book of the Republic of Bulgaria with threat status EN B2a(i,ii,iv), has been recorded in two new localities in Eastern Bulgaria near to the Black Sea coastal region. The paper provides data on macro- and microscopic description of the basidiomata, coordinates, and map of distribution of the fungus in Bulgaria.

**Key words:** basidiome, endangered species, fungi

The increasing interest in conservation of fungal diversity in Bulgaria (DRUMIEVA-DIMCHEVA & GYOSHEVA-BOGOEVA 1993; DENCHEV 2005; GYOSHEVA ET AL. 2000, 2006; PEEV 2011) logically provokes the need for accumulation of new data on distribution and habitat requirements of fungi. *Battarrea phalloides* (Dicks.) Pers. is a basidiomycete which is included in the Red List of fungi in Bulgaria

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(GYOSHEVA ET AL. 2006) and also in the Red Data Book of the Republic of Bulgaria (GYOSHEVA & STOICHEV 2011) with threat status EN B2a(i,ii,iv). Most of the previous findings of *Battarreia phalloides* in Bulgaria are concentrated in the Thracian Lowland, one is in the Srednd Gora Mts and all data published on the site characteristics and fungus description are quite scarce (GYOSHEVA & STOICHEV 2011; LACHEVA 2012A, B, DINEV 2013). Therefore, the purpose of this paper is to supplement data on this endangered species with its more detailed morphological descriptions and to provide information on its recent findings in a new region of the country.

During this study, 25 basidiomata of *Battarreia phalloides* were found. Two of them were discovered in the close vicinity of the guard house and the Dam of the Reservoir Tsonevo in the Eastern Stara Planina Mountains (Varna District) on 31 July 2012. The altitude of this site is 66 m a.s.l. and its coordinates are 43°01,835' N 27°24,331' E (taken by GPS Garmin Montana 600). The other 23 fruiting bodies were observed on 12 July 2013 at 5 m a.s.l on the path to the Specialized Hospital for Rehabilitation 'Tuzlata', very close to the hyperhaline wetland Balchishka Tuzla and 'Tuzlata' beach (the Black Sea, Dobrich District). The coordinates are 43°24,100' N 28°13,453' E. Map template is after PEEV (2011).

One basidioma from the first locality and two basidiomata from the second one were taken for further microscopic investigations. The collected specimens are kept in the Mycological Collection of Sofia University "St. Kliment Ohridski".

Basidiospores and elaters were observed in the lab on non-permanent slides by light microscopy (LM) on Olympus BX53 microscope. The photos were taken by Olympus DP72 camera.

According to macroscopic observations (Fig. 1) the height of basidiomata observed in the both localities ranged from 13 to 30 cm including the spore sac. Stipe was 11–27 cm long, 0,5–2 cm broad, rusty-brown, woody and hollow. Its surface was longitudinally striated and covered with fibers that peel or split to form fine to very coarse needle-like, ribbon-like, or shaggy scales. Volva at the stipe base was up to 4x5 cm (when dry), saclike, free, fragile, and two-layered: the inner layer was similar to the scales of the stipe; the outer layer was thick, membranous, dirty white in colour. Spore sac was sub-globose, 1,5–6 cm broad and 1–3 cm high.

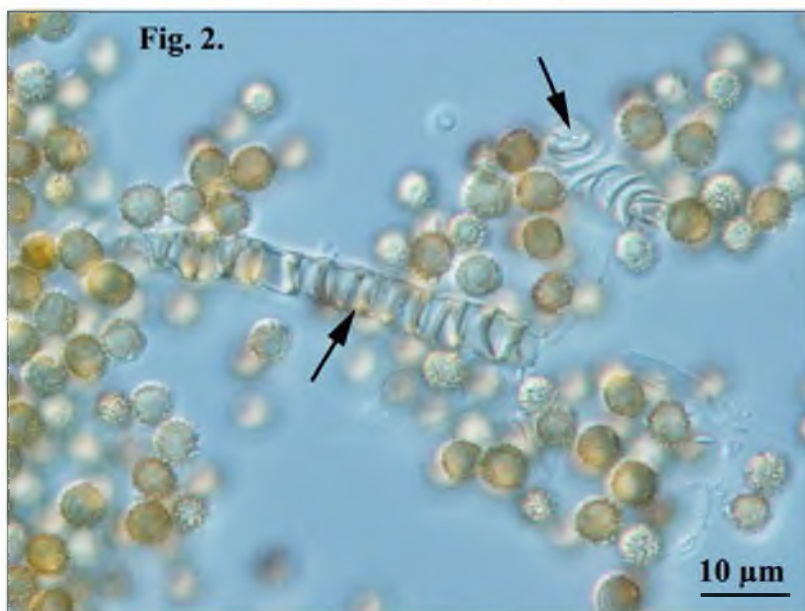
Microscopic study (Fig. 2) showed that elaters were 4–5,5 µm wide and 20–68 µm long, cylindrical, tapered, with irregular spiral thickening along the length, whitish to pale yellow in colour, not branched. Basidiospores were round to globose, 4–5,5 µm in diameter, with short, broad pedicel, verrucose or warty ornamentation, and yellow-ochre in colour.

The endangered species *Battarreia phalloides* was found in two distant sites, characterized by similar features: warm, free-draining sandy soils, situation near to the trunk bases of *Cupressus sempervirens* L. and near to asphalt road. According to GYOSHEVA & STOICHEV (2011, Fig. 3) in Bulgaria the fungus was found for first time on sandy soil with decaying wood of poplar (*Populus canescens*) in the city park 'Ostrova' near the Maritsa River (Plovdiv town). In the same Plovdiv District

**Fig. 1.**



**Fig. 2.**



Figs. 1–2: 1 – Basidiomata of *Battarrea phalloides* (measure size is 20 cm);  
2 – Basidiospores and elaters (arrows) of the fungus.



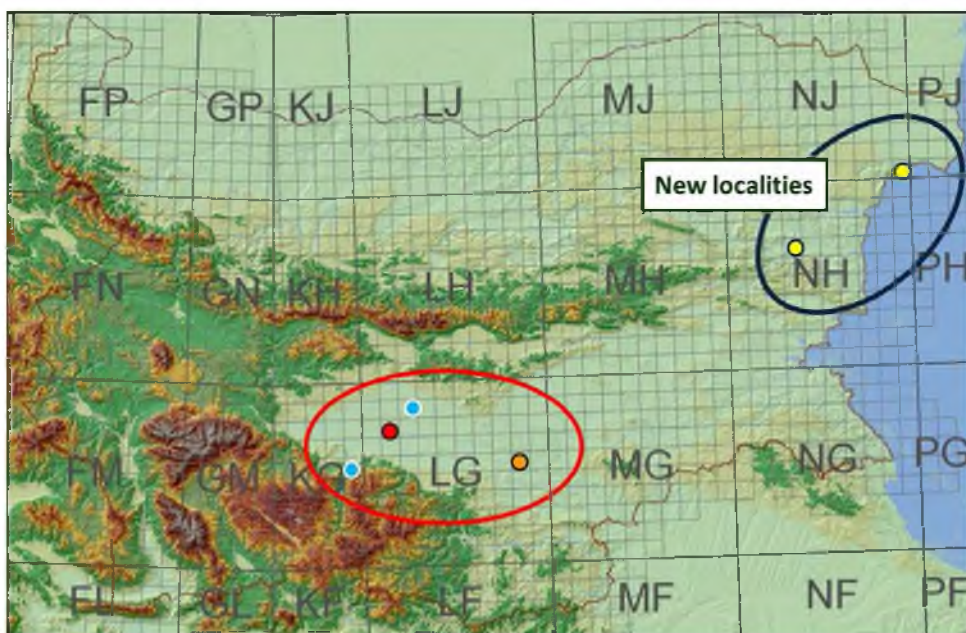


Fig. 3. Map of distribution of *Battarrea phalloides* in Bulgaria.

LACHEVA (2012 a, b, 2014) recorded *Battarrea phalloides* on soil with decaying wood in Bogdan village and in the parkland of the Palace ‘Krichim’, and also in a meadow situated on the left site of the bridge over the Stryama River near to Stryama village. DINEV (2013) notified in an electronic newspaper that Mr Nikolay Apostolov found the species in the park ‘Penyo Penev’ in Dimitrovgrad town Park, but did not mention the type of the substrate. All these findings are concentrated in the Thracian Lowland and Sredna Gora Mts (Central South Bulgaria), but no data on their coordinates have been provided. The new localities of *Battarrea phalloides* reported in this paper with their coordinates are situated in Eastern Bulgaria near to the Black Sea. Additionally, map of the species distribution in Bulgaria is provided (Fig. 3) in order to facilitate its future monitoring.

#### ACKNOWLEDGEMENTS

The study was carried on the microscope of the Algal Collection of Sofia University (ACUS). The author is thankful also to the anonymous reviewer for the suggestions and improvement of the text of the paper.



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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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## NEW LOCALITIES OF *CLATHRUS RUBER* (BASIDIOMYCOTA) IN BULGARIA

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** In Bulgaria the basidiomycete *Clathrus ruber* is known generally from the Black Sea coastal region and has been sporadically recorded in Sofia city, Vitosha Mt and Rodopi Mts. In the last edition of the Red List of fungi in Bulgaria it has been declared as “near threatened”. The paper provides data on three new localities of the species in south-western part of Bulgaria and presents a chorological map for this fungus in the country.

**Key words:** Belasitsa Mt, fungi, Maleshevska Mt, Tisata Nature Reserve, Struma River

According to the data published so far it could be stated that in the Northern Hemisphere the basidiomycete *Clathrus ruber* P. Micheli ex Pers. is spread erratically throughout warmer areas of temperate zone. In Europe the fungus appears mainly in the Mediterranean region, but not everywhere and rarely (KUTHAN

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& KOTLABA 1981). In Bulgaria this species is known generally from the Black Sea coastal region (including localities in the regions of eastern Stara planina Mts and Strandzha Mt) and has been sporadically recorded also in Sofia city, Vitosha Mt and Rodopi Mts (HINKOVA 1961; KUTHAN & KOTLABA 1981, 1988; GYOSHEVA ET AL. 2000; ASSYOV ET AL. 2010; DENCHEV & ASSYOV 2010; LACHEVA 2012). The present paper provides new data on the distribution of *Clathrus ruber* in a new region of Bulgaria and presents a chorological map of the fungus in the country.

The first locality of *Clathrus ruber* is in Belasitsa Mt near to village Samuilovo (41°22'08,0"N 23°05'30,8"E) at 371 m a. s. l. There twice (on 19 May 2008 and 16 September 2009) were found single basidiomata in the same *Platanus orientalis* L. and *Castanea sativa* Mill. forest (Figs. 1–2, leg. P. Mitov). Second locality of the fungus was found in Tisata Nature Reserve (41°45'03,50"N 23°08'57,25"E) at 200 m a. s. l. in Maleshevska Mt (leg. B. Zlatkov & O. Sivilov). On 01 May 2014 in this place also only one fruit body (Fig. 3) was determined in sparse *Platanus orientalis* forest. The third finding of the species was on the left Struma River bank (41°23'12,5"N 23°20'39,7"E) at 77 m a. s. l. near to the village Kulata. There were found two basidiomata of *Clathrus ruber* and one of them was still in egg stage (Fig. 4). These two fruiting bodies were discovered on 15 November 2014 among decaying plant materials (leg. P. Mitov). Soil temperature in all new localities ranged between 16,6°C and 28°C.

The all new findings of *Clathrus ruber* in SW Bulgaria were related with *Platanus orientalis* and *Castanea sativa* forests and decaying plant materials on the river bank. According to HINKOVA (1961) the fungus was found for the first time in Bulgaria in 1936 by Acad. Nikolay Stoyanov in flower-beds of the Botanical Garden of Sofia. KUTHAN & KOTLABA (1981, 1988) reported the species from the ground between *Ruscus acuteatus* L. and also the ground below *Acer campestre* L., *Carpinus orientalis* Mill., *Carpinus* sp., *Fraxinus angustifolia* Vahl, *Fraxinus ornus* L., *Fraxinus* sp., *Pinus nigra* J. F. Arnold, *Quercus* sp. and *Ulmus* sp. Later on ASSYOV ET AL. (2010) recorded the fungus in woodland strips of *Quercus cerris* L. and on the soil under small group of oaks (*Quercus* sp.). Likewise LACHEVA (2012) found *Clathrus ruber* in deciduous forests of oak (*Quercus cerris* and *Quercus* sp.). Comparison of all data on the species findings in the country proved that it is a soil saprotroph and shows no strong dependence on the dominant tree species. However, it is possible to suggest that the species distribution in Bulgaria (Fig. 5) is generally related to the regions climatically influenced by the Black Sea and the Mediterranean Sea. Basidiomata of the fungus have been found at different altitudes in the country – between 10 and 600 m a. s. l. According to KUTHAN & KOTLABA (1981, p. 104) “outside Mediterranean area *Clathrus ruber* appears from time to time in Central and Eastern Europe, especially in gardens, cemeteries, parks and more rarely in the wild nature”. This could explain the findings of basidiomata of the species in Sofia city gardens by HINKOVA (1961) and ASSYOV ET AL. (2010) and in Vitosha Mt (GYOSHEVA ET AL. 2000; DENCHEV & ASSYOV 2010).



Figs. 1–4. Basidiomata of *Clathrus ruber*: 1, 2 – the region of village Samuilovo (19.V.2008 and 16.IX.2009); 3 – Tisata Nature Reserve (01.V.2014); 4 – Struma River bank (15.XI.2014). Photos: P. Mitov (1, 2, 4) and O. Sivilov (3).

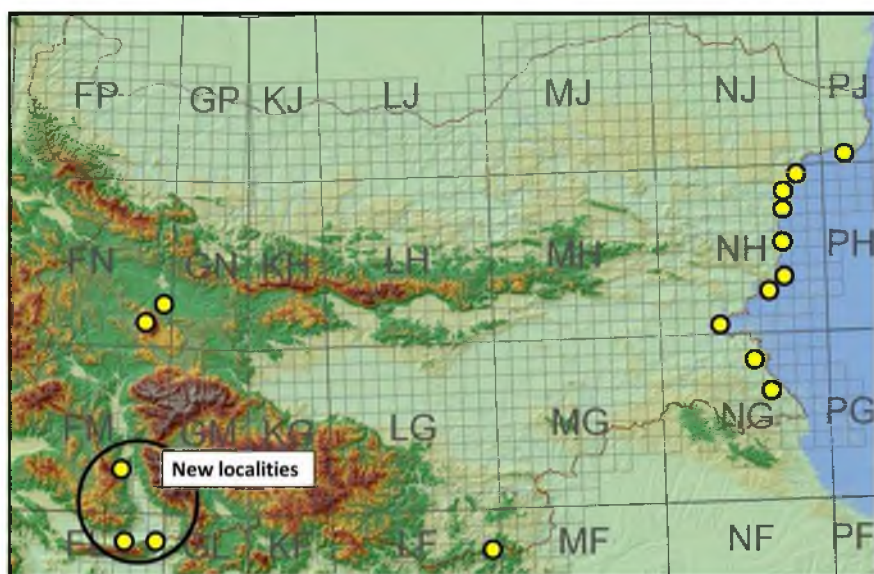


Fig. 5. Map of distribution of *Clathrus ruber* in Bulgaria. Map template is after PEEV (2011).

In the preliminary checklist of the Bulgarian threatened macromycetes GYOSHEVA ET AL. (2000) included *Clathrus ruber* in the threat category “Rare” in accordance with IUCN Red Data List Categories. Later on, in the Red List of fungi in Bulgaria, in conformity with new established and adopted version of the IUCN Red Data List, GYOSHEVA ET AL. (2006) changed the threat status of the fungus to “Near Threatened” but did not include species in the Red data Book of the Republic of Bulgaria (PEEV 2011). The new findings of the species in SW Bulgaria and a large period of fruiting bodies producing – between May and November correspond with the opinion of HINKOVA (1961, p. 257) that *Clathrus ruber* is “not rare” in the country and its “near threatened” conservation status, proposed by GYOSHEVA ET AL. (2006).

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## NEW RECORDS OF LARGER FUNGI IN BULGARIA

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*We dedicate this paper  
to Professor Dobrina Temniskova  
with respect and gratitude on the  
occasion of her eighty birthday*

**Abstract:** The paper reports 12 rare larger fungi from Bulgaria. All taxa belong to basidiomycetes. Three of them – *Climacodon septentrionalis*, *Limacella delicata* var. *vinosorubescens* and *Mycena flos-nivium* are new records for Bulgaria. Five species are of high conservation value, included in the Red List of fungi in Bulgaria. Most of the species are uncommon and endangered in Europe.

**Key words:** basidiomycetes, Bulgarian mycota, fungal conservation

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## INTRODUCTION

During the last years data about rare and threatened larger fungi from Bulgaria were published by ALEXOV ET AL. (2012), ASSYOV ET AL., (2010, 2012), GYOSHEVA ET AL. (2012) and LACHEVA (2012a,b). They are important contribution to the study of the fungal diversity and its protection in the country, and especially to the more precise determination of threat status of species of high conservation value. However, more investigations in the country are still necessary. Therefore the aim of present paper is to enrich the information about rare and threatened larger fungi in Bulgaria with new data.

New data about the distribution of twelve uncommon and less known larger basidiomycetes are present as a result of author's investigations of the fungal diversity within the framework of several scientific projects in different regions in Bulgaria. Among them, three species are newly recorded for the country and five species are of high conservation value, included in the Red List of Bulgarian fungi (GYOSHEVA ET AL. 2006): *Calocybe ionides* (Bull.: Fr.) Donk., *Clavicornia pyxidata* (Pers.: Fr.) Doty, *Geastrum triplex* Jungh., *Grifolia frondosa* (Dicks.: Fr.) Gray and *Sarcodon leucopus* (Pers.) Maas Geest. & Nannf. Three of them are enlisted also in the Red Data Book of Republic of Bulgaria (PEEV ET AL. 2011).

## MATERIAL AND METHODS

Fungal species were registered during the 2013–2014 period, mostly by the authors. The studied specimens are kept in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF). Microscopic features were observed in water and 3% KOH under Amplival LM and in Lactophenol Cotton Blue stain under Leitz Laborlux S LM.

Author's name of fungal taxa are abbreviated according to KIRK & ANSELL (2004) and Index Fungorum. The threat status follows the Red List of fungi in Bulgaria (GYOSHEVA ET AL. 2006).

## RESULTS

*New larger basidiomycetes for Bulgaria.*

*Climacodon septentrionalis* (Fr.) P. Karst., Rev. Mycol. Toulouse 3 (9): 20 (1881) (Plate I, Fig.1).

**Basidiomata** annual, pileate, often in abundant numbers, imbricate, forming large clusters. **Pileus** 5–10 (12) cm wide, 1,5–2 cm thick at the base, kidney or fan shaped, convex to flat, upper surface roughened, whitish, creamy to brownish in older specimens, margin enrolled when dry. Under surface with spines, 0,5–1 cm long, cylindrical, acute, whitish to brownish in age. **Context** whitish-cream, fibrous, smell not distinctive, unpleasant in dry specimens. **Hyphal system** monomitic.

**Basidia** 4-spored, slender. **Basidiospores** 3,5–5 x 1,5–2 µm, ellipsoid, smooth, hyaline. **Cystidia** 35–45 x 12–15 µm, cylindrical, fusoid, mucronate at the apex, thick-walled (Plate I, Fig. 2).

**Specimen examined.** Mt Strandzha, 1 km north of Kondolovo village at 350 m a.s.l., on living stem of *Fagus orientalis* LIPSKI, 22.11.2013, leg. T. NEDELIN, det. M. GYOSHEVA (SOMF 29643).

*Climacodon septentrionalis* is parasite on trunks of living deciduous trees (mostly from genera *Acer* L., *Alnus* Mill., *Betula* L., *Fagus* L., *Fraxinus* L., *Populus* L., etc.). The species is distributed in Europe, Asia, North America. In Europe it occurs exceptionally in the northernmost regions.

*C. septentrionalis* is rare and very rare in the Central and Southern Europe (NIKOLAEVA 1961; KOSKI-KOTIRANTA & NIEMALÄ 1987).

*Limacella delicata* var. *vinosorubescens* (Furrer-Ziogas) Gminder, Z. Mykol. 60 (2): 386 (1994) (Plate I, Fig. 3).

**Pileus** up to 7 cm in diameter, convex to flat, or slightly depressed in the center, umbonate, slimy, moist, later finely scaly, wine-red, discoloured to cream-pink. Margin initially incurved. **Stipe** 5–8 x 1–2 cm, cylindrical, with whitish annular zone, soon fugaceous, surface smooth, whitish to pinkish to the apex, fine pink-fibrous at the base. **Context** creamy to pink-reddish. Smell farinaceous. **Gills** free, broad, whitish, cream-pink, vinaceous pink spotted in old specimens. **Basidia** 4-spored, cylindrical. **Basidiospores** 3,5–5 x 3–4,5 µm, subglobose, smooth, hyaline. **Cystidia** absent.

**Specimen examined.** Northern Pirin Mts, near Gotse Delchev chalet, above Dobrinishte town, at 1500 m a.s.l., in mixed wood (*Fagus sylvatica* L., *Picea abies* (L.) Karst., *Abies alba* Mill.), 31.07.2014, leg. & det. M. GYOSHEVA (SOMF 29645).

*Limacella delicata* var. *vinosorubescens* is an uncommon fungus with high conservation value (COURTECUISE & DUHEM 1994; PHILLIPS 2006). It occurs in deciduous (oak, beech) and mixed woods (beech, spruce), July–November. In Europe the species is distributed exceptionally in northern, western and central regions (GMINDER 1994; COURTECUISE & DUHEM 1995; KRIEGLSTEINER 2003).

*Mycena flos-nivium* Kühner, Bull. Soc. Nat. Oyonnax 6: 71 (1952) (Plate I, Fig. 4).

**Pileus** up to 2 cm in diameter, convex to almost flat, surface smooth, hygrophanous, radially striate up to halfway, dark grey-brown to pale-brown. **Stipe** 3–4 x 0,2–0,25 cm, cylindrical, smooth, hollow, grey-brownish, white to the apex, base white-strigose. **Context** thin, white. Smell not distinctive. **Gills** broad, ventricose, some with anastomoses, whitish to whitish-grey. **Basidia** 4-spored, clavate. **Basidiospores** 7–11 (11,5) x 4–5 µm, cylindrical ellipsoid, smooth, hyaline, guttulate. **Cheilocystidia** clavate. **Pileipellis** consisting of distinctly branched, gnarled hyphae, 2–4 µm thick.





Plate I: (Figs. 1–6): 1 – *Climacodon septentionalis* – cluster of fruit bodies; 2 – *Climacodon septentionalis* – hymenium with cystidia; 3 – *Limacella delicata* var. *vinosorubescens* – fruit body; 4 – *Mycena flos-nivium* – fruit body; 5 – *Sarcodon leucopus* – fruit bodies; 6 – *Sarcodon leucopus* – basidiospores and hymenium.

**Specimen examined:** Northern Pirin Mts, near Peyo K. Yavorov chalet, above Razlog town, 1815 m a.s.l., in mixed coniferous forest (*Picea abies* (L.) Karst.,

*Pinus peuce* Griseb., *P. heldreichii* Christ.), on rotten stump of spruce, 10.05.2014, leg & det. M. GYOSHEVA (SOMF 29644).

*Mycena flos-nivium* is an uncommon species. It is saprotrophic fungus on dead wood and in forest litter under conifers in mountains, March-May. The species is distributed in Europe and North Asia (KRIEGLSTEINER 2001).

### **New localities of rare larger basidiomycetes to Bulgaria**

#### ***Agaricus bresadolianus* Bohus**

**Specimen examined.** Northern Black Sea Coast, Botanical Garden, Balchik town, on soil among grasses, 15.10.2013, leg. V. VLADIMIROV, det. M. GYOSHEVA (SOMF 29654).

The species was reported so far only from Thracian Lowland (DENCHEV & ASSYOV 2010).

#### ***Calocybe ionides* (Bull. : Fr.) Donk.**

**Specimen examined.** Central Rodopi Mts, Chervenata Stena Reserve, above Bachkovo monastery, in beech forest, in the litter, 11.04.2014, leg. A. GANEVA, det. M. GYOSHEVA (SOMF 29650).

The species is included in the Red List of fungi in Bulgaria under category *Data Deficient* (DD), known from Southern Black Sea coast – Ropotamo Reserve and Central Rodopi Mts – near Dedovo village (Kuthan & Kotlaba 1981; Stoichev 1982).

#### ***Cantharellus amethysteus* (Quél.) Sacc.**

**Specimen examined.** Northern Pirin Mts, near Gotse Delchev chalet, above Dobrinishte town, at 1500 m a.s.l., in mixed wood of *Fagus sylvatica* and *Picea abies*, 31.07.2014, leg. & det. M. Gyosheva (SOMF 29655).

Rarely recorded species, so far known from Western and Central Stara Planina Mts (ASSYOV *ET AL.* 2012; DENCHEV & ASSYOV 2010).

#### ***Clavicornia pyxidata* (Pers. : Fr.) Doty**

**Specimen examined.** Tundzha Hilly Country, in Dolna Topchiya managed Reserve, north of Elhovo town, in riparian forest, on dead deciduous wood, 21.05.2014, leg. T. MESHINEV, det. M. GYOSHEVA (SOMF 29649).

*Critically Endangered* (CR) species, so far known from the Central and Eastern Stara Planina Mts, Vitosha Mt, Rila Mts and Western Rodopi Mts (DENCHEV & ASSYOV 2010; PEEV *ET AL.* 2011).

#### ***Geastrum triplex* Jungh.**

**Specimen examined.** Northern Pirin Mts, 250 m southwest from Banderitsa chalet, near Baykusheva mura, 1900 m a.s.l., under *Pinus heldreichii*, 22.09.2014, leg. T. NEDELIN, det. M. GYOSHEVA and T. NEDELIN (SOMF 29651).

The species is included in the Red List of Bulgarian fungi under category *Vulnerable* (VU). It is known from Black Sea coast, Sofia region – Lyulin Mt, Vitosha Mt, Central Rodopi Mts (ASSYOV ET AL. 2010; DENCHEV & ASSYOV 2010).

***Grifola frondosa* (Dicks. : Fr.) Gray**

**Specimen examined.** Sofia region – Sofia city, Vrana park, 565 m a.s.l., at the base of a living trunk of *Quercus robur* L., 04.11.2014, leg. A. PENCHEVA, det. T. NEDELIN (SOMF 29647).

*Endangered* (EN) species. In Bulgaria known from the Western and Central Stara Planina Mts and Znepole region – Golo Bardo Mt (DENCHEV & ASSYOV 2010; PEEV ET AL. 2011).

***Rhodotus palmatus* (Bull. : Fr.) Maire**

**Specimen examined.** – Tundzha Hilly Country, in Dolna Topchiya managed Reserve, north of Elhovo town, on the bank of Tundzha River, on rotten stump of *Ulmus minor* MILL., 21.05.2014, leg. T. MESHINEV, det. M. GYOSHEVA (SOMF 29648).

The species is rare to very rare in Europe, with high conservation value. It occurs exclusively on dead wood of *Ulmus* spp. (COURTECUISSE & DUHEM 1994; KRIEGLSTEINER 2001; PHILLIPS 2006; SHNITTLER 1996). This species has been reported only once in Bulgaria, from Vitosha Mt – Zlatnite mostove locality, on dead beech wood (BARSAKOV 1926).

***Sarcodon leucopus* (Pers.) Maas Geest. & Nannf. (Plate I, Figs. 5–6; Fig. 7).**

**Specimen examined.** Western Rodopi Mts. – 1,3 km. southeast under Yundola village, along the road to Velingrad town, 1320 m a.s.l., under *Pinus sylvestris*, 11.09.2014, leg. and det. T. NEDELIN (SOMF 29646).

*Critically Endangered* (CR) species. Reported so far from Pirin Mts, above Razlog town and Western Rodopi Mts, above Dospat town (DENCHEV & ASSYOV 2010; PEEV ET AL. 2011).

***Suillus lakei* (Murrill) A. H. Sm. & Thiers**

**Specimen examined.** Northern Black Sea Coast, Botanical Garden, Balchik town, under *Pseudotsuga menziesii* (Mirb.) Franco, 15.10. 2013, leg V. VLADIMIROV, det. M. GYOSHEVA (SOMF 29652).

The species is so far known from Western Stara Planina Mts, West Frontier Mts – Ograzhden Mt and Central Rodopi Mts (ASSYOV ET AL. 2010; ALEXOV ET AL. 2012; LACHEVA 2012A).

*Suillus lakei* is mycorrhizal fungus with *Pseudotsuga* spp. It was reported from Bulgaria only in *Pseudotsuga menziesii* cultures.



Fig 7. *Sarcodon leucopus* – Map of locality in Western Rodopi Mts.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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## CHECKLIST OF MACROMYCETES, OBSERVED DURING THE LAST 20 YEARS (1994–2014) IN THE SOFIA CITY PARK BORISOVA GRADINA (BULGARIA)

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*The paper is dedicated to Prof. D. Temnsikova  
on the occasion of her 80th jubilee*

**Abstract.** A checklist of 115 species of asco- and basidiomycetes, recorded in the south-eastern part of the city park of Sofia Borisova Gradina between 1994 and 2014 is presented and briefly discussed in the paper.

**Key words:** mycorrhizal fungi, parasitic fungi, park, saprotrophic fungi, threatened species, urban area

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## INTRODUCTION

Assessing, understanding, and improving urban biodiversity is of great importance from both conservation and social point of views (KOWARIK 2011). Urban green areas are a vital part of the urban landscape, providing contact with wildlife and environmental services with additional socio-ecological benefits to the overall quality of life (BARRICO ET AL. 2012). However, the value of their biota is often underestimated and socio-ecological functions have not been comprehensively studied (EEA 2010). In spite of the fact that, as far as it is known, there are no specifically evolved fungi with adaptation to man-made habitats (SPOONER & ROBERTS 2005), fungi comprise important biological and ecological component in the ecosystems of urban green areas. Additionally there is evidence that land management practices can affect fungal diversity (MOORE ET AL. 2011). Therefore during the last decades they, and especially macromycetes, incl. wood-decay fungi, start to attract the special attention of mycologists and have been discussed from different points of view (e.g. SLATER 1993; SEDE & LOPEZ 1999; SZCZEPKOWSKI A. 2007; TERHO ET AL. 2007; BABENKO & TKACHENKO 2008; BARRICO ET AL. 2012 and citations therein).

Studies of Bulgarian mycota started more than century ago (DRUMEVA-DIMCHEVA & GYOSHEVA-BOGOEVA 1993; DENCHEV ET AL. 2005) and increase rapidly during the last decade, but data on urban habitats are more than meagre. BARZAKOW (1926A, B, C) and BARZAKOFF (1929, 1933, 1936) reported 54 fungal species from the city park Borisova Gradina<sup>1</sup> and from the pine and oak forests situated near the Ecclesiastical school in Sofia<sup>2</sup>. DIMITROV (1926) noted one parasite (*Rhytisma acerinum* (Pers.) Fr.) and HINKOVA (1950, 1955, 1961), in a frame of purposive study, published 81 fungal species from the same park. Years later YURUKOVA (1994) mentioned five edible species from “eastern and southern part” of the city of Sofia, studied for heavy metal contamination and DIMITROVA ET AL. (2007) reported 40 saprotrophic and parasitic fungal species from the central part of the city park Borisova Gradina. Later on, ASSYOV ET AL. (2010) added one more species (*Clathrus ruber* P. Micheli ex Pers.) for the same place. LACHEVA (2010) found 92 species lignicolous macromycetes in the city of Plovdiv. Nowadays, ALEXOV ET AL. (2012) discover *Hericium erinaceus* (Bull.) Pers. in another Sofia city park Zapaden Park.

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<sup>1</sup> Constructed in the late 19<sup>th</sup> century (ca. 1884–1892) and named after Bulgarian Tsar Boris III, with older names “Razsadnika” (=Nursery-garden), “Pipinierata”, “Tsarigradska Gradina” and renamed as “Park na Svobodata” (=Freedom Park) during the socialist period in the development of the country, until its fall in 1989, when it reverted to its most used name “Borisova Gradina” (=Boris Garden), or Knyaz Borisova Gradina (=Prince Boris Garden).

<sup>2</sup> Recently the whole region around this school is implicitly included in the city park Borisova Gradina. Obviously, the author distinguished between the more cultivated part, situated more near to the centre of Sofia and most popular as “Borisova Gradina” and more “wild” part at its edge.



The present paper is based on 20-years observations in the south-eastern part of the oldest and best known park of Sofia (Borisova Gradina). The results presented here are mainly qualitative, based on presence–absence of given species, with some minor comments on obvious decrease of abundance of certain species, related with human impact or natural forest aging.

## MATERIAL AND METHODS

The observations, presented in this study, were made incessantly by the first author during the last 20 years (1994–2014) in the middle south-eastern part of the Sofia city park Borisova Gradina by tracing the same route twice per day during the obligatory dog walks (Fig. 1). Additional frequent, but occasional zigzag traces in the square, closed by the main route, were followed. Exception was made in the years 2003 and 2004, but since it concerns the non-vegetational period between the end of November and April, we believe that this interruption can be taken as insignificant. The route follows the forest paths through mixed coniferous and deciduous forests (plantations), containing mainly *Quercus rubra* L., *Quercus robur* L., *Pinus sylvestris* L. and *Pinus nigra* Arn., intermittent with *Hedera helix* L., *Crataegus monogyna* Sacq., *Corylus avellana* L., *Sambucus nigra* L., *Sambucus racemosa* L., *Prunus cerasifera* Ehrh., *Cornus sanguinea* L., *Sorbus aucuparia* L., *Acer tataricum* L., *Acer campestre* L., *Acer heldreichii* Orph., *Acer platanoides* L., *Acer pseudoplatanus* L., *Ulmus minor* Mill., *Fagus sylvatica* L., *Carpinus betulus* L., *Fraxinus excelsior* L., *Fraxinus oxycarpa* Willd., *Tilia parvifolia* Ehr., *Amorpha fruticosa* L., *Ailanthus altissima* (Mill.) Swingle, *Gleditsia triacanthos* L., *Periploca graeca* L., *Rubus caesius* L., rarely *Betula pendula* Roth. and *Syringa vulgaris* L., and meadow-like open areas bordered with *Vinca minor* L., *Spiraea douglasii* Hook., *Symphoricarpos albus* (L.) S. F. Blake, *Forsythia intermedia* Zabel, *Rosa canina* L., etc. According to PETKOV & ANTONOV (1994), who studied the soil moisture content in 1992–1993 in the same region of the park, especially under *Quercus*-trees, the soils were well supplied and stocked with available moisture.

The observations were focused preliminary on the macromycete community, based on fruiting bodies. Although fruit bodies' production is unlikely to reflect the belowground fungal community (LILLESKOV & BRUNS 2001), their surveys can be particularly valuable as indicators for assessing the impacts of different land use types on macromycete populations, as observed by AZUL ET AL. (2009). Some parasite species (e.g. *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam., *Rhytisma acerinum* (Pers.) Fr.) were included in the list according to description for macromycetes in KIRK ET AL. (2008).

Identification of fungi was done according to ROMAGNESI (1970, 1971), JORDANOV ET AL. (1978), HANSEN & KNUDSEN (1992, 1997, 2000) and BON (2005). Author names for each taxon are abbreviated according to INDEX FUNGORUM. Data



on species occurrence and distribution in Bulgaria were compared with the lists in DENCHEV & ASSYOV (2013), and conservation status was checked according to GYOSHEVA ET AL. (2006) and PEEV (2011). Trophic status is provided according to BUROVA (1986), PECORARO ET AL. (2007), SZCZEPKOWSKI (2007), BABEKO & TKACHENKO (2008) and authors' observations. The potential uses are after BOA (2004), BON (2005), BAI ET AL (2013), RUTHES ET AL. (2013), PETROVIC ET AL. (2014) and YOO & CHOI (2014).



Fig. 1. Map of Bulgaria (red asterisk indicates the capital) and of part of Sofia with the studied park (square line) Borisova Gradina and Ecclesiastical school (arrow). The last map is provided after [http://poseti.guide-bulgaria.com/a/807/park\\_borisova\\_gradina.htm](http://poseti.guide-bulgaria.com/a/807/park_borisova_gradina.htm).

## RESULTS AND DISCUSSION

In total, 115 species of ascomycetes and basidiomycetes have been observed in the nearest vicinity of the pathways of the route, shown on Fig. 1. They are enlisted bellow in alphabetical order in the frames of the phyla, in order to facilitate the reader (Table 1).

**Table 1.** Checklist of macromycetes, found in the city park Borisova Gradina (Sofia) during the last 20 years (1994–2014). The abbreviations are as follows: **BG** – species reported for the park by BARZAKOW (1926<sub>A,B,C</sub>, 1928), DIMITROV (1926), BARSAKOFF (1929, 1933, 1936), HINKOVA (1950, 1955, 1961), DIMITROVA ET AL. (2007); **SR** – species reported for the Sofia region by DENCHEV & ASSYOV (2010); and **Ts** – trophic status of the fungal species, where **Ls** = litter saprotroph, **Xy** = xylotroph, **P** = parasite, **Hs** = humic saprotroph and **M** = mycorrhizal; \* – species included in the Red List of fungi in Bulgaria; \*\* – species included in the Red List and in the Red Data Book of the Republic of Bulgaria.

№	Taxon	BG	SR	Ts	Potential uses
	<b>Ascomycota</b>				
1.	<i>Aleuria aurantia</i> (Pers.) Fuckel	+		Ls	Edible
2.	<i>Ascocoryne cylichnium</i> (Tul.) Korf.			Xy	Medicinal, non edible
3.	<i>Erysiphe alphitoides</i> (Griffon & Maubl.) U. Braun & S. Takam.	+		P	Non edible
4.	<i>Helvella lacunosa</i> Afzel.	+		Hs	Non edible
5.	<i>Nectria cinnabarina</i> (Tode) Fr.			Xy	Non edible
6.	<i>Rhytisma acerinum</i> (Pers.) Fr.	+		P	Non edible
7.	<i>Sarcoscypha coccinea</i> (Jacq.) Boud.			Xy	Edible
	<b>Basidiomycota</b>				
8.	<i>Agaricus arvensis</i> Schaeff.	+	+	Hs	Medicinal, edible
9.	<i>Agaricus campestris</i> L.		+	Hs	Medicinal, edible
10.	<i>Agaricus comptulus</i> Fr.		+	Hs	Edible
11.	<i>Agaricus sylvaticus</i> Schaeff.	+	+	Hs	Edible
12.	<i>Agaricus sylvicola</i> (Vittad.) Peck			Hs	Edible
13.	<i>Agaricus xanthodermus</i> Genev.			Hs	Non edible
14.	<i>Agrocybe praecox</i> (Pers.) Fayod		+	Hs	Edible
15.	<i>Amanita citrina</i> (Schaeff.) Pers.			M	Non edible
16.	<i>Amanita muscaria</i> (L.) Lam.		+	M	Medicinal
17.	<i>Amanita phalloides</i> (Vaill. ex Fr.) Link	+	+	M	Poisonous
18.	<i>Amanita rubescens</i> Pers.			M	Edible
19.	<i>Amanita virosa</i> Bertill.			M	Poisonous
20.	<i>Armillaria mellea</i> (Vahl) P. Kumm.	+	+	Xy	Medicinal, edible
21.	<i>Auricularia auricula-judae</i> (Bull.) Quél.	+	+	Xy	Medicinal, edible
22.	<i>Boletus calopus</i> Pers.		+	M	Non edible

№	Taxon	BG	SR	Ts	Potential uses
23.	<i>Boletus edulis</i> Bull.		+	M	Medicinal, edible
24.	<i>Boletus impolitus</i> Fr.			M	Edible
25.	<i>Boletus pinophilus</i> Pilát & Dermek			M	Edible
26.	<i>Boletus queletii</i> Schulzer			M	Edible
27.	<i>Boletus subtomentosus</i> L.	+	+	M	Edible
28.	<i>Bovista plumbea</i> Pers.		+	Hs	Medicinal, edible
29.	* <i>Caloboletus radicans</i> (Pers.) Vizzini			M	Non edible
30.	<i>Calocybe gambosa</i> (Fr.) Donk	+	+	Hs	Medicinal, edible
31.	<i>Cantharellus cibarius</i> Fr.	+	+	M	Medicinal, edible
32.	<i>Chalciporus piperatus</i> (Bull.) Bataille			M	Edible
33.	<i>Chroogomphus rutilus</i> (Schaeff.) O.K. Mill.			M	Edible
34.	<i>Clitocybe geotropa</i> (Bull. ex DC.) Quél.			Ls	Edible
35.	<i>Clitocybe gibba</i> (Pers.) P. Kumm.			Ls	Edible
36.	<i>Clitocybe nebularis</i> (Batsch) P. Kumm.	+	+	Ls	Edible
37.	<i>Clitocybe phyllophila</i> (Pers.) P. Kumm.			Ls	Poisonous
38.	<i>Clitopilus prunulus</i> (Scop.)P. Kumm.			Ls	Edible
39.	<i>Coprinellus congregates</i> (Bull.) P. Karst.		+	Hs	Medicinal, non edible
40.	<i>Coprinopsis atramentaria</i> (Bull.) Redhead, Vilgalys & Moncalvo	+	+	Hs	Medicinal, edible
41.	<i>Coprinus comatus</i> (O.F. Müll.) Pers.		+	Hs	Edible
42.	** <i>Cortinarius caperatus</i> (Pers.) Fr.			M	Edible
43.	** <i>Cortinarius praestans</i> (Cordier) Gillet			M	Edible
44.	<i>Cortinarius purpurascens</i> Fr.			M	Medicinal, edible
45.	<i>Craterellus cornucopioides</i> (L.) Pers.			M	Medicinal, edible
46.	<i>Cyathus striatus</i> (Huds.) Willd.			Ls	Non edible
47.	<i>Entoloma clypeatum</i> (L.)P. Kumm.			M	Edible
48.	<i>Entoloma rhodopolium</i> (Fr.) P. Kumm.		+	M	Poisonous
49.	<i>Exidia glandulosa</i> (Bull.) Fr.		+	Xy	Non edible
50.	<i>Fistulina hepatica</i> (Schaeff.) With.			Xy	Medicinal, edible
51.	<i>Flammulina velutipes</i> (Curtis) Singer	+	+	Xy	Medicinal, edible
52.	<i>Fomes fomentarius</i> (L.) Fr.			Xy	Medicinal, non edible
53.	<i>Fomitiporia robusta</i> (P. Karsten) Fiasson & Niemelä			Xy	Non edible
54.	<i>Fomitopsis pinicola</i> (Sw.) P. Karst.	+	+	Xy	Medicinal, non edible
55.	<i>Ganoderma lucidum</i> (Curtis) P. Karst.	+	+	Xy	Medicinal, non edible
56.	<i>Gymnopus fusipes</i> (Bull.) Gray		+	Ls	Non edible
57.	<i>Gymnopus perforans</i> (Hoffm.) Antonín & Noordel.	+	+	Ls	Non edible

№	Taxon	BG	SR	Ts	Potential uses
58.	<i>Hygrophorus russula</i> (Schaeff.) Kauffman			M	Edible
59.	<i>Hypholoma capnoides</i> (Fr.) P. Kumm.			Xy	Edible
60.	<i>Hypholoma fasciculare</i> (Huds.) P. Kumm.	+	+	Xy	Poisonous
61.	<i>Imleria badia</i> (Fr.) Vizzini		+	M	Edible
62.	<i>Inocybe erubescens</i> A. Blytt			M	Poisonous
63.	<i>Kuehneromyces mutabilis</i> (Schaeff.) Singer & A.H. Sm.		+	Xy	Edible
64.	<i>Laccaria laccata</i> (Scop.) Cooke	+	+	Hs	Edible
65.	<i>Lactarius deliciosus</i> (L.) Gray	+	+	M	Edible
66.	<i>Lactarius piperatus</i> (L.) Pers.		+	M	Edible
67.	<i>Lactarius rufus</i> (Scop.) Fr.			M	Medicinal, non edible
68.	<i>Lactarius vellereus</i> (Fr.) Fr.			M	Edible
69.	<i>Laetiporus sulphureus</i> (Bull.) Murrill		+	Xy	Edible
70.	<i>Lepiota cristata</i> (Bolton) P. Kumm.			Hs	Non edible
71.	<i>Lepista nuda</i> (Bull.) Cooke	+	+	Hs	Medicinal, edible
72.	<i>Leucoagaricus leucothites</i> (Vittad.)Wasser		+	Hs	Non edible
73.	<i>Leucopaxillus giganteus</i> (Sowerby) Singer			Hs	Edible
74.	<i>Lycoperdon echinatum</i> Pers.			Ls	Edible
75.	<i>Lycoperdon perlatum</i> Pers.	+	+	Ls	Medicinal, edible
76.	<i>Lycoperdon pyriforme</i> Schaeff.			Xy	Medicinal, edible
77.	<i>Macrolepiota procera</i> (Scop.) Singer		+	Hs	Edible
78.	<i>Marasmius alliatus</i> (Schaeff.) J. Schröt.		+	Ls	Non edible
79.	<i>Megacollybia platyphylla</i> (Pers.) Kotl. & Pouzar			Xy	Non edible
80.	<i>Melanoleuca grammopodia</i> (Bull.) Murrill			Hs	Edible
81.	<i>Meripilus giganteus</i> (Pers.) P. Karst.			Xy	Medicinal, non edible
82.	<i>Mycena galericulata</i> (Scop.) Gray			Xy	Non edible
83.	<i>Omphalotus olearius</i> (DC.) Singer			Xy	Poisonous
84.	<i>Paxillus involutus</i> (Batsch) Fr.	+	+	M	Poisonous
85.	<i>Peniophora quercina</i> (Pers.) Cooke			Xy	Non edible
86.	<i>Pleurotus cornucopiae</i> (Paulet) Rolland	+		Xy	Edible
87.	<i>Pluteus cervinus</i> (Schaeff.) P. Kumm.	+	+	Xy	Edible
88.	<i>Polyporus squamosus</i> (Huds.) Fr.	+	+	Xy	Edible
89.	<i>Psathyrella spadicea</i> (P. Kumm.) Singer		+	Xy	Edible
90.	<i>Russula aeruginea</i> Lindbl. ex Fr.		+	M	Edible
91.	<i>Russula alutacea</i> (Fr.) Fr.			M	Non edible
92.	<i>Russula cyanoxantha</i> (Schaeff.) Fr.	+	+	M	Edible
93.	<i>Russula delica</i> Fr.			M	Edible
94.	<i>Russula emetica</i> (Schaeff.) Pers.	+	+	M	Poisonous?
95.	<i>Russula nigricans</i> Fr.		+	M	Edible

№	Taxon	BG	SR	Ts	Potential uses
96.	<i>Russula olivacea</i> (Schaeff.) Fr.			M	Non edible
97.	<i>Russula rosea</i> Pers.		+	M	Edible
98.	<i>Russula vesca</i> Fr.			M	Edible
99.	<i>Russula xerampelina</i> (Schaeff.) Fr.		+	M	Edible
100.	<i>Schizophyllum commune</i> Fr.	+	+	Xy	Medicinal, non edible
101.	<i>Scleroderma citrinum</i> Pers.		+	M	Poisonous
102.	<i>Stereum hirsutum</i> (Willd.) Pers.	+	+	Xy	Medicinal, non edible
103.	<i>Stropharia hornemannii</i> (Fr.) S. Lundell & Nannf.			Hs	Poisonous
104.	<i>Suillus granulatus</i> (L.) Roussel	+	+	M	Edible
105.	<i>Suillus luteus</i> (L.) Roussel		+	M	Medicinal, edible
106.	<i>Tapinella atrotomentosa</i> (Batsch) Šutara	+	+	Xy	Non edible
107.	<i>Trametes hirsuta</i> (Wulfen) Lloyd		+	Xy	Medicinal, non edible
108.	<i>Trametes versicolor</i> (L.) Lloyd	+	+	Xy	Medicinal, non edible
109.	<i>Tremella mesenterica</i> Retz.		+	Xy	Medicinal, edible
110.	<i>Tricholoma imbricatum</i> (Fr.) P. Kumm.			M	Non edible
111.	<i>Tricholoma portentosum</i> (Fr.) Quél.		+	M	Edible
112.	<i>Tricholoma saponaceum</i> (Fr.) P. Kumm.			M	Poisonous
113.	<i>Tricholoma virgatum</i> (Fr.) P. Kumm.			M	Non edible
114.	<i>Xerocomellus chrysenteron</i> (Bull.) Šutara	+	+	M	Edible
115.	<i>Xerocomellus rubellus</i> (Krombh.) Šutara			M	Edible

In spite of the fact that comparisons with older data are practically impossible due to lack of detailed descriptions of the visited localities, it is possible to outline that only 35 species from our list coincide with the species, published by BARZAKOV (1926A, B, C, 1928), DIMITROV (1926), BARZAKOFF (1929, 1933, 1936), HINKOVA (1950, 1955, 1961) and DIMITROVA ET AL. (2007), and 59 of them have already been reported for Sofia region by DENCHEV & ASSYOV (2013).

As it could be seen from Table 1, the fungi observed by us, belong mostly to the major ecological groups of saprotrophs (65), mycorrhizal species (48) and parasites (2). Xylotrophs predominated (32) in the first group and were followed by humic saprotrophs (21) and litter saprotrophs (12). Three of the found species are known to be of conservational importance with different threat status in the Red List of fungi in Bulgaria (GYOSHEVA ET AL. 2006) – *Caloboletus radicans* VU, *Cortinarius caperatus* EN and *Cortinarius praestans* CR. Two of them (*Cortinarius praestans* CR and *Cortinarius caperatus* EN) are included also in the Red Data Book of the Republic of Bulgaria (GYOSHEVA 2011).

In spite of the fact that the abundance was not estimated, it could be noted that the most numerous (except trunk inhabitants like *Collybia fusipes*, *Flammulina velutipes*, etc. and xylophyllous *Trametes*, *Fomes*, etc.) in almost all years were different representatives of the genus *Russula*, while abundant development of *Macrolepiota procera*, *Clitocybe geotropa*, *Amanita rubescens*, *Laetiporus sulphureus* and representatives of *Boletus-Xerocomus* group, was detected only periodically, with interruptions of 2–4(5) years. Macromycetes like *Ascocoryne cylichnium*, *Amanita muscaria*, *Amanita phalloides*, *Boletus queletii*, *Chroogomphus rutilus*, *Cortinarius praestans*, *Cortinarius purpurascens*, *Craterellus cornucopioides* and *Leucoagaricus leucothites* were rarely found, in singular specimens or groups/clumps. The abundance of *Auricularia auricula* decreased obviously with falling down and taking out of the old decaying *Sambucus* stems. The abundance, and even the occurrence, of *Ganoderma lucidum* decreased strongly due to its intensive uncontrolled collection for decorative and medicinal purposes.

## CONCLUSION

The results obtained in this work provide pilot data on the recent mycota in urban park area of Sofia and, most probably, are the first data published from the south-eastern part of the city park Borisova Gradina. Nevertheless of their preliminary character, they reveal the presence of considerable fungal biodiversity with the 115 species of asco – and basidiomycetes recorded non-purposively, only during dog walks. Therefore we claim that they could serve as a good contemporary basis for future studies of biodiversity in Sofia and other Bulgarian towns. Moreover, 70% of the macromycetes observed are of multiple potential interest (e.g. food, medicinal properties, wood status, soil protection), which, according to BARRICA ET AL. (2012), demonstrates that we should take into account not only the biological and ecological perspectives of the landscapes but also their potential products and environmental services with regard to future land use and urban life.

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## REVIEW ON THE MEDICINAL PLANTS OF THE NORTH BLACK SEA COAST (BULGARIA)

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** This is the first review of the studies of medicinal plants found in the region of North Black Sea Coast. It shows the significant diversity of the medicinal plants in the northern part of Black Sea Coast floristic region: 593 species of vascular plants from 357 genera and 96 families. The majority of the families and genera are represented by a small number of species, which ranges from 1 to 4. The analysis of life forms indicates that the hemipterophytes dominate (43.17%). The biological types are represented mainly by perennial herbaceous plants (54.81%). The highest percentage of species belongs to the European type (55.48%), followed by species of the Mediterranean type (17.54%). Among the medicinal plants one Balkan endemic, one Bulgarian endemic and 32 relicts are found. The number of species with conservation status is 55 (9.27%). 18 species are protected by a law prohibiting the herb collection within their natural habitats and collecting of 9 species is restricted by the law regulating the collection of herbs from their natural habitats. The number of angiosperms is relatively high 374 (63.07%).

**Key words:** floristic analysis, vascular plants

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The vascular plants in Bulgaria are subject of a considerable number of publications. During the 20th Century, ca. 10,000 articles on Bulgarian flora were published. About 70% of them focus on the vascular plants in the country. To date, several handbooks for determining plants and floras were issued, all of which include chorological information but none focuses specifically on the medicinal plants in the region of North Black Sea Coast.

The known chorological data were organised for the first time as a standalone publication in *Conspectus of the Bulgarian Vascular Flora* (KOZHUHAROV & ANDREEV 1980). This information has later been updated in the last three editions of *Conspectus of the Bulgarian Vascular Flora: Distribution Maps and Floristic Elements* (ASSYOV et al. 2002, 2006, 2012). According to latest data, in Bulgaria are found 4, 102 species of vascular plants that belong to 913 genera and 155 families.

The number of medicinal plant species in Bulgaria totals 844 and they belong to 444 genera and 118 families. 730 species of vascular plants are distributed naturally and are included in the Medicinal Plants Act (2000). The remaining 114 species are also distributed naturally and are described in the literature on medicinal plants in Bulgaria (STOYANOV & KITANOV 1960; STEFANOV 1972, 1973; IVANOV ET AL. 1973; KITANOV 1987; PAMUKOV & AHTARDZHIEV 1989; NIKOLOV ET AL. 2006).

Special attention to the distribution of most commonly used medicinal plants in the country is given in *Chorological Atlas of Medicinal Plants in Bulgaria* (BONDEV 1995) and several other documents. For instance, such information can be found in the management plans of national and natural parks in the country that include lists of medicinal plants established on their territory. But only some of the most widely used medicinal plants are included in the municipal development plans and forest management plans of state forests and arboretums.

As evident from the scholarly review, to date there are no known specialized publications focusing on the distribution of medicinal plants in specific Bulgarian regions. Furthermore, there is a problem with outlining the boundaries of a region. The municipal division in regions is not appropriate for this purpose because of periodic changes of the given administrative units. It is, therefore, more appropriate to use phytogeographic zoning, even though alterations can be made over time here as well. The most widely accepted phytogeographic zoning was first published in 1966 in *Flora of PR Bulgaria*, vol. 3 (JORDANOV 1966) and has been ever since widely used in Bulgarian botanical literature. According to this research, the country is divided into 20 floristic regions. One of these areas is the Black Sea Coast, which is divided into two sub-regions: North and South Black Sea Coast. Our publication focuses on the medicinal plants that are naturally distributed in the North Black Sea Coast. This region covers the coastline of the Black Sea from the Bulgarian-Romanian border in the north to the northern slopes of the eastern part of the Balkan Mountain to the south, with a width ranging from 1 to 20 km (Fig. 1).

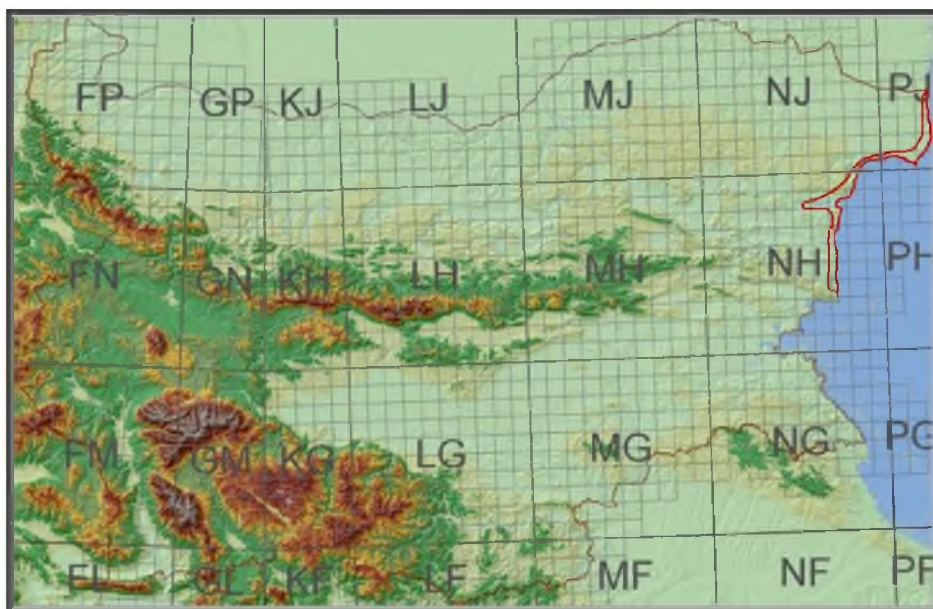


Fig. 1. UTM grid map of Bulgaria and study area: Northern Black Sea Coast floristic region.

The North Black Sea Coast is located in two climatic regions: Dobrudzha Black Sea Coast and Varna Black Sea Coast that belong to the Continental-Mediterranean climatic area (VELEV 2002). There are several lakes on the territory of the region in question, the largest among them are: Durankulashko, Shablensko, Ezeretsko, Varnensko, and Beloslavsko lakes and there are also several rivers, of which the largest are Batova and Kamchiya.

The soil consists of the following soil types and subtypes (indicated in parentheses) presented according to the classification of FAO: fluvisols (calcaric), gleysols (calcic), leptosols (lithic and rendzic), chernozems (haplic), phaeozems (luvic), luvisols (albic), planosols (dystric), nitisols (haplic) and hystosols (fibric) (NINOV 2002).

The vegetation of the Northern Black Sea Coast is very diverse. BONDEV (1991), for instance, has recorded 30 plant communities: 13 tree, 3 shrubby, 3 grassy, 2 water communities, 8 agricultural areas and 1 forestry culture.

To date, several authors have studied The North Black Sea Coast flora (DAVIDOV 1905, 1909, 1914; DELIPAVLOV ET AL. 1997; DIMITROV ET AL. 2000, 2005; KOZHUHAROV ET AL. 1997; KOCHEV 1976; MARINOVA-FILIPOVA 2000, 2002; PENEV 1981; STOYANOV 1928; TZONEV ET AL. 2005; VELCHEV 2002; VICHEREK 1971). However, there is no comprehensive study specifically of the medicinal plants in the North Black Sea Coast.

Our review is based on most recent data on the vascular plants in Bulgaria (ASSYOV ET AL. 2012). In our list of medicinal plants, distributed in the North Black

Sea Coast, we included three new species not listed by ASSYOV ET AL. (2012) as present in this region: *Phytolacca americana* L., recorded first by IVANOV et al. (2002), *Viburnum opulus* L., documented first by DIMITROV ET AL. (2000) and *Crocus pallasii* Goldb., established by the authors of the present study within the premises of the University Botanic Garden in Varna.

All taxonomic position and names of the taxa are used in accordance to *Conspectus of the Bulgarian Vascular Flora: Distribution Maps and Floristic Elements* (ASSYOV et al. 2012).

The life forms are represented according to the system of RAUNKIAER (1934). For their determination we used *Flora of PR Bulgaria*, Volumes 1 to 9 (JORDANOV 1963, 1964, 1966, 1970, 1973, 1976, 1979; VELCHEV 1982, 1989) and *Flora of the Republic of Bulgaria*, Volumes 10 and 11 (KOZHUHAROV 1995; PEEV 2013). The biological types are determined according to *Handbook for Plants in Bulgaria* (DELIPAVLOV ET AL. 2011). The floristic elements and the endemics are determined according to *Conspectus of the Bulgarian Vascular Flora: Distribution Maps and Floristic Elements* (ASSYOV ET AL. 2012). The relics are determined according to GRUEV & KUZMANOV (1994), PEEV ET AL. (1998), PEEV (2001), BOŽA ET AL. (2005).

The conservation status is recognized using the following documents: Annex II and Annex V to Council Directive 92/43/EEC of the European Community to Protect Natural Habitats of Wild Fauna and Flora, Annex I to Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention), Annex II to Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Red Data Book of the Republic of Bulgaria, Vol. 1. Plants and Fungi (PEEV et al. 2011), IUCN Red List for Bulgaria (PETROVA & VLADIMIROV 2009), Annex III and Annex IV to Biodiversity Act (2002). Recorded species are included in Bulgarian Order for Special Arrangements for the Conservation and Use of the Medicinal Plants (Nr. RD/83 from March 2, 2014).

The anthropophytes are determined according to STEFANOV & KITANOV (1962).

The results from the literature analysis show a significant diversity of medicinal plants in the northern part of the Black Sea Coast floristic region: 593 species of vascular plants from 357 genera and 96 families. They represent 70,26% of the species, 80,41% of the genera and 81,36% of the families of medicinal plants in Bulgaria. The number of identified taxa is very similar to this in the Northeastern Bulgarian floristic region: 600 species of vascular plants from 357 genera and 101 families (ZAHARIEV & IVANOV 2014). A systematic list of identified species is presented in Appendix 1.

Most of the families and genera are presented with smaller number of lower taxa – 1 to 4. The majority of families, 78 (81,25%) are presented with 1–4 genera. Only 18 (18,75%) of the families include 5 or more species. Most genera are found in the families: Asteraceae (39), Lamiaceae (26), Apiaceae (25), Brassicaceae (23) and Fabaceae (22). These are some of the families with the highest number of genera in Bulgarian flora.

Most families, 67 (69,79%), have 1–4 species. Only 29 (30,21%) of the families are represented by 5 or more species. Most species belong to the following families: Asteraceae (71), Lamiaceae (58), Fabaceae (41) and Brassicaceae (35). This correlation is reinforced by the genera: only 17 genera (4,76%) include 5 or more species. Most species belong to the following genera: *Artemisia* (8), *Rumex* (8), *Veronica* (8), *Centaurea* (7), *Salvia* (7) and *Ranunculus* (7). These genera are among the medicinal plants in Bulgaria with the largest number of species.

The analysis of life forms shows a dominance of the hemicryptophytes: 256 species (43,17%), followed by the therophytes: 100 species (16,86%) and the phanerophytes: 95 species (16,02%). The prevalence of hemicryptophytes is typical for the flora in temperate climatic zones, which is confirmed in our study of a particular group of plants, in this case, the medicinal plants. The distribution of the life forms is observed in the same order among non-medicinal and medicinal plants in the Northeastern Bulgarian floristic region.

Perennial herbaceous plants are dominant among the biological types: 325 species (54,81%). Relatively large are in number the annual herbaceous plants: 100 species (16,86%). The distribution of medicinal plant species is similar to that in the Northeastern Bulgarian floristic region, which can be explained by the geographical proximity of the two floristic regions, similar geographical location, as well as the climatic and soil conditions.

In regard to the phytogeographical structure, the highest percentage of the species represents the European type (55,48%), followed by species of Mediterranean type (17,54%) and Boreal type (11,13%). This distribution corresponds to the geographical location of the study area.

Among the medicinal plants in North Black Sea Coast there are only two endemic taxa (0,34%): one Balkan endemic (*Achillea clypeolata* Sm.) and one Bulgarian endemic (*Opopanax chironium* subsp. *bulgaricum* (Velen.) N. Andr.). The relicts are 32 species (5,40%). 31 of these, are Tertiary relicts: *Acer campestre* L., *Cotinus coggygria* Scop., *Hedera helix* L., *Ruscus aculeatus* L., *Ruta graveolens* L., *Salix alba* L., *Viscum album* L. and other. One species is a Quaternary relict: *Galanthus nivalis* L. In the study region, the number of endemic and relict species is comparable to that in the Northeastern Bulgarian floristic region, where there are 3 endemic and 35 relict species.

The species with conservation status are 55 (9,27%). Four of them are included in Directive 92/43/EEC. Two species are included in Annex II: Plant and Animal Species of Value to the Community Which Requires their Conservation in Designated Areas: *Echium russicum* J. F. Gmel. and *Himantoglossum caprinum* (M. Bieb.) Spreng. Two further species are under the protection of Annex V: Plant and Animal Species of Value to the Community Interest Which Requires Regulation of their Removal or Exploitation Under Penalty: *Galanthus nivalis* L. and *Ruscus aculeatus* L.

In the Annex I of the Bern Convention are included 3 species: *Cyclamen coum* Mill., *Himantoglossum caprinum* (M. Bieb.) Spreng. and *Paeonia tenuifolia* L.

In the Annex II of CITES Convention are included 15 species: *Adonis vernalis* L., *Anacamptis pyramidalis* (L.) Rich., *Cyclamen coum* Mill., *Cyclamen hederifolium* Aiton, *Galanthus nivalis* L., *Himantoglossum caprinum* (M. Bieb.) Spreng., *Ophrys cornuta* Steven, *Orchis coriophora* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* Lam., *Orchis tridentata* Scop., *Orchis ustulata* L., *Platanthera bifolia* (L.) Rich. and *Platanthera chlorantha* (Custer) Rchb.

In the IUCN Red List for Bulgaria are included 30 species. Two species are included in the category Critically endangered: *Astragalus dasyanthus* Pall. and *Hippophae rhamnoides* L. 8 species in the category Endangered are: *Anethum graveolens* L., *Dianthus pontederiae* A. Kern. subsp. *kladovanus* (Degen) Stoj. & Stef., *Eringium maritimum* L., *Galanthus nivalis* L., *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Paeonia tenuifolia* L. and *Ruta graveolens* L. 12 species are in the category Vulnerable: *Anacamptis pyramidalis* (L.) Rich., *Echium russicum* J. F. Gmel., *Ephedra distachya* L., *Euphorbia peplis* L., *Himantoglossum caprinum* (M. Bieb.) Spreng., *Leucojum aestivum* L., *Limonium vulgare* Mill., *Ophrys cornuta* Steven., *Opopanax chironium* (L.) Koch subsp. *bulgaricum* (Velen.) Andreev, *Orchis ustulata* L., *Primula acaulis* (L.) L. *rubra* (Sm.) Greuter & Burdet and *Ranunculus lingua* L. 4 species are in the category Near threatened: *Anemone sylvestris* L., *Artemisia lerchiana* Waber., *Artemisia pontica* L. and *Cercis siliquastrum* L. 4 species are in the category Least concern: *Cyclamen coum* Mill., *Ficus carica* L., *Samolus valerandi* L. and *Tilia rubra* DC.

In the *Red Book of Bulgaria* are included 13 species. 2 species are included in the category Critically Endangered: *Astragalus dasyanthus* Pall. and *Hippophae rhamnoides* L. 8 species appear in the category Endangered: *Anethum graveolens* L., *Dianthus pontederiae* A. Kern. subsp. *kladovanus* (Degen) Stoj. & Stef., *Eringium maritimum* L., *Galanthus nivalis* L., *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Paeonia tenuifolia* L. and *Ruta graveolens* L. 3 species are in the category Vulnerable: *Echium russicum* J. F. Gmel., *Himantoglossum caprinum* (M. Bieb.) Spreng. and *Opopanax chironium* (L.) Koch subsp. *bulgaricum* (Velen.) Andreev.

In the Biodiversity Act are included 44 species. 20 species are included in Annex III, Protected Species: *Anacamptis pyramidalis* (L.) Rich., *Anemone sylvestris* L., *Artemisia lerchiana* Waber., *Astragalus dasyanthus* Pall., *Cyclamen coum* Mill., *Dianthus pontederiae* A. Kern. subsp. *kladovanus* (Degen) Stoj. & Stef., *Echium russicum* J. F. Gmel., *Ephedra distachya* L., *Eringium maritimum* L., *Euphorbia peplis* L., *Galanthus nivalis* L., *Himantoglossum caprinum* (M. Bieb.) Spreng., *Hippophae rhamnoides* L., *Limonium vulgare* Mill., *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Opopanax chironium* (L.) Koch subsp. *bulgaricum* (Velen.) Andreev, *Ophrys cornuta* Steven, *Paeonia tenuifolia* L. and *Ruta graveolens* L. In Annex IV: Under the conservation and regulated use of the nature are included 24 species: *Asparagus officinalis* L., *Bupleurum rotundifolium* L.,

*Crocus chrysanthus* (Herbert) Herbert, *Cyclamen hederifolium* Aiton, *Dryopteris filix-mas* (L.) Schott, *Echinops ritro* L., *Echinops sphaerocephalus* L., *Gladiolus communis* L., *Gladiolus imbricatus* L., *Helichrysum arenarium* (L.) Moench, *Leucojum aestivum* L., *Orchis coriophora* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* Lam., *Orchis tridentata* Scop., *Orchis ustulata* L., *Paeonia peregrina* Mill., *Polygonatum odoratum* (Mill.) Druce, *Primula acaulis* (L.) L., *Primula veris* L., *Ruscus aculeatus* L., *Salix caprea* L. and *Scilla bifolia* L.

Among the medicinal plants with conservation status with the highest status are as follows: *Himantoglossum caprinum* (M. Bieb.) Spreng. (included in 6 documents); *Galanthus nivalis* L. (included in 5 documents); *Cyclamen coum* Mill., *Echium russicum* J. F. Gmel. and *Paeonia tenuifolia* L. (included in 4 documents).

The number of medicinal plants with conservation status in North Black Sea Coast is close to this in floristic region in Northeastern Bulgaria, where 60 species have been identified.

In Order №RD-83 of 03.02.2014 on Special Arrangements for Conservation and Use of Medicinal Plants from 2014, 27 species are included. 18 species are protected from collection from their natural habitats: *Adonis vernalis* L., *Althaea officinalis* L., *Artemisia santonicum* L. subsp. *patens* (Neilr.) K. Pers., *Asarum europaeum* L., *Asplenium trichomanes* L., *Convallaria majalis* L., *Glaucium flavum* Crantz, *Helichrysum arenarium* (L.) Moench, *Inula helenium* L., *Orchis coriophora* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* L., *Orchis tridentata* Scop., *Orchis ustulata* L., *Ruscus aculeatus* L., *Salvia tomentosa* Mill. and *Valeriana officinalis* L. Under restricted collection of herbs from their natural habitats are 9 species: *Artemisia alba* L., *Berberis vulgaris* L., *Betonica officinalis* L., *Carlina acanthifolia* All., *Frangula alnus* Mill., *Galium odoratum* (L.) Scop., *Paeonia peregrina* Mill., *Primula veris* L. and *Sedum acre* L.

The number of anthropophytes among the medicinal plants is high – 374 species (63,07%). Many of them are distributed as weeds in the arable land or as ruderal plants: *Amaranthus retroflexus* L., *Artemisia absinthium* L., *Capsella bursa-pastoris* Moench., *Chenopodium hybridum* L., *Conium maculatum* L., *Elymus repens* (L.) Gould., *Galium aparine* L., *Melilotus officinalis* (L.) Pall., *Nigella arvensis* L., *Plantago major* L., *Sambucus ebulus* L., *Taraxacum officinale* L., *Urtica dioica* L. and more.

The inventory of medicinal plants on the territory of North Black Sea Coast shows significant taxonomic diversity. It is comparable to studies on the neighboring floristic region of Northeast Bulgaria, which is significantly larger in size. The floristic analysis showed also similar results for both regions. It is necessary to conduct a number of further studies in the future: the medicinal plants' distribution needs to be mapped out; their status needs to be studied in more detail, the threats and prospects for the population of species of great economic importance needs to be accounted for, as well as for those with a conservation status; and resource characteristics of the deposits of industrial stocks can be established. After the



preparation of such inventories for other floristic regions, the resulting data can be compared and will be possible to identify eventual regularities in the distribution of medicinal plants in Bulgaria.

Appendix 1. Systematic list of species of vascular medicinal plants, established in Northern Black Sea coast

### **Equisetophyta**

**Equisetaceae:** *Equisetum arvense* L., *Equisetum palustre* L., *Equisetum telmateia* Ehrh.

### **Polypodiophyta**

**Aspidiaceae:** *Dryopteris filix-mas* (L.) Schott; **Aspleniaceae:** *Asplenium adiantum-nigrum* L., *Asplenium ruta-muraria* L., *Asplenium septentrionale* (L.) Hoffm., *Asplenium trichomanes* L., *Ceterach officinarum* DC; **Hypolepidaceae:** *Pteridium aquilinum* (L.) Kuhn; **Polypodiaceae:** *Polypodium vulgare* L.

### **Magnoliophyta**

#### **Pinopsida**

**Cupressaceae:** *Juniperus communis* L.

#### **Gnetopsida**

**Ephedraceae:** *Ephedra distachya* L.

#### **Magnoliopsida**

**Aceraceae:** *Acer campestre* L., *Acer negundo* L., *Acer platanoides* L., *Acer pseudoplatanus* L., *Acer tataricum* L.; **Amaranthaceae:** *Amaranthus retroflexus* L.; **Anacardiaceae:** *Cotinus coggygria* Scop., *Rhus coriaria* L.; **Apiaceae:** *Aegopodium podagraria* L., *Aethusa cynapium* L., *Anethum graveolens* L., *Angelica sylvestris* L., *Anthriscus cerefolium* (L.) Hoffm., *Apium graveolens* L., *Bifora radians* M. Bieb., *Bupleurum rotundifolium* L., *Chaerophyllum bulbosum* L., *Chaerophyllum temulentum* L., *Conium maculatum* L., *Daucus carota* L., *Eryngium campestre* L., *Eryngium maritimum* L., *Ferulago sylvatica* (Besser) Rchb., *Foeniculum vulgare* Mill., *Heracleum sibiricum* L., *Laser trilobum* (L.) Borkh., *Oenanthe aquatica* (L.) Poir., *Opopanax chironium* (L.) Koch, *Pastinaca sativa* L., *Peucedanum arenarium* Waldst. & Kit., *Sanicula europaea* L., *Scandix pecten-veneris* L., *Seseli rigidum* Waldst. & Kit., *Seseli tortuosum* L., *Tordylium maximum* L., *Torilis arvensis* (Hudson) Link; **Apocynaceae:** *Vinca herbacea* Waldst., *Vinca minor* L.; **Araliaceae:** *Hedera helix* L.; **Aristolochiaceae:** *Aristolochia clematitis* L., *Asarum europaeum* L.; **Asclepiadaceae:** *Cionura erecta* (L.) Griseb., *Periploca graeca* L., *Vincetoxicum hirundinaria* Medicus; **Asteraceae:** *Achillea clypeolata* Sm., *Achillea millefolium* L., *Achillea nobilis* L., *Anthemis arvensis* L., *Anthemis cotula* L., *Anthemis tinctoria* L., *Arctium lappa* L., *Arctium minus* Bernh., *Arctium tomentosum* Mill., *Artemisia absinthium* L., *Artemisia alba* L., *Artemisia annua* L., *Artemisia campestris* L., *Artemisia lerchiana* Waber., *Artemisia pontica* L., *Artemisia santonicum* L. ssp. *patens* (Neilr.) K. Pers., *Artemisia vulgaris* L., *Bellis perennis* L., *Bidens tripartita* L., *Carduus acanthoides* L., *Carlina acanthifolia* All., *Carlina vulgaris* L., *Carthamus lanatus* L., *Centaurea calcitrapa* L., *Centaurea cyanus* L., *Centaurea diffusa* Lam., *Centaurea pannonica* (Heuffel) Simonk., *Centaurea rocheliana* (Heuffel) Dostál, *Centaurea stoebe* L., *Centaurea solstitialis*

*L.*, *Chamomilla recutita* (L.) Rauscher, *Cichorium intybus* L., *Cirsium arvense* (L.) Scop., *Cirsium vulgare* (Savi) Ten., *Conyza canadensis* (L.) Cronquist, *Echinops ritro* L., *Echinops sphaerocephalus* L., *Eupatorium cannabinum* L., *Filago lutescens* Jord., *Filago vulgaris* Lam., *Galinsoga parviflora* Cav., *Gnaphalium uliginosum* L., *Helichrysum arenarium* (L.) Moench, *Hieracium pilosella* L., *Inula aschersoniana* Janka, *Inula britannica* L., *Inula ensifolia* L., *Inula germanica* L., *Inula helenium* L., *Lactuca serriola* L., *Leucanthemum vulgare* Lam., *Logfia arvensis* (L.) Holub., *Matricaria trichophylla* (Boiss.) Boiss., *Onopordum acanthium* L., *Onopordum tauricum* Wilid., *Petasites hybridus* (L.) Gaertn., *Pulicaria dysenterica* (L.) Bernh., *Pulicaria vulgaris* Gaertn., *Scorzonera hispanica* L., *Senecio jacobaea* L., *Senecio viscosus* L., *Senecio vulgaris* L., *Silybum marianum* (L.) Gaertn., *Solidago virgaurea* L., *Tanacetum vulgare* L., *Taraxacum officinale* Weber, *Tragopogon pratensis* L., *Tussilago farfara* L., *Xanthium spinosum* L., *Xanthium strumarium* L., *Xeranthemum annuum* L.; **Berberidaceae:** *Berberis vulgaris* L.; **Betulaceae:** *Alnus glutinosa* (L.) Gaertn., *Betula pendula* Roth, *Carpinus betulus* L., *Corylus avellana* L., *Corylus colurna* L.; **Boraginaceae:** *Anchusa officinalis* L., *Buglossoides arvensis* (L.) I. M. Johnst., *Buglossoides purpureocaerulea* (L.) I. M. Johnst., *Cerinthe minor* L., *Cynoglossum officinale* L., *Echium italicum* L., *Echium russicum* J. F. Gmel., *Echium vulgare* L., *Heliotropium europaeum* L., *Lithospermum officinale* L., *Myosotis arvensis* (L.) Hill., *Pulmonaria officinalis* L., *Symphytum officinale* L.; **Brassicaceae:** *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Alyssum alyssoides* (L.) L., *Armoracia rusticana* G. Gaertn., B. Mey. & Scherb., *Barbarea vulgaris* R.Br., *Beta vulgaris* L., *Brassica nigra* (L.) Koch, *Brassica juncea* (L.) Czern. & Coss., *Bunias orientalis* L., *Capsella bursa-pastoris* (L.) Medicus, *Cardamine bulbifera* (L.) Crantz, *Cardamine pratensis* L., *Cardaria draba* (L.) Desv., *Coronopus squamatus* (Forssk.) Asch., *Descurainia sophia* (L.) Webb ex Prantl, *Diplotaxis tenuifolia* (L.) DC., *Erysimum crepidifolium* Rchb., *Erysimum diffusum* Ehrh., *Erysimum repandum* L., *Eruca vasicaea* (L.) Cav. ssp. *sativa* (Miller) Thell., *Euclidium syriacum* (L.) R. Br., *Hesperis matronalis* L., *Lepidium campestre* (L.) R. Br., *Lepidium graminifolium* L., *Lepidium latifolium* L., *Lepidium perfoliatum* L., *Lepidium ruderae* L., *Myagrum perfoliatum* L., *Nasturtium officinale* R. Br., *Raphanus raphanistrum* L., *Rorippa austriaca* (Crantz) Besser, *Rorippa pyrenaica* (L.) Rchb., *Rorippa sylvestris* (L.) Besser, *Sisymbrium loeselii* L., *Sisymbrium officinale* (L.) Scop., *Thlaspi alliaceum* L., *Thlaspi arvense* L.; **Campanulaceae:** *Campanula persicifolia* L.; **Cannabaceae:** *Cannabis sativa* L., *Humulus lupulus* L.; **Caprifoliaceae:** *Lonicera xylosteum* L., *Sambucus ebulus* L., *Sambucus nigra* L., *Viburnum opulus* L.; **Caryophyllaceae:** *Agrostemma githago* L., *Dianthus pottederae* A. Kern., *Herniaria glabra* L., *Herniaria hirsuta* L., *Herniaria incana* Lam., *Lychnis coronaria* (L.) Desr., *Lychnis flos-cuculi* L., *Minuartia setacea* (Thuill.) Hayek, *Saponaria officinalis* L., *Scleranthus annuus* L., *Scleranthus perennis* L., *Silene otites* (L.) Wibel., *Spergularia rubra* (L.) J. & C. Presl, *Stellaria graminea* L., *Stellaria media* (L.) Vill., *Viscaria vulgaris* Röhl.; **Celastraceae:** *Euonymus europaeus* L., *Euonymus verrucosus* Scop.; **Chenopodiaceae:** *Atriplex rosea* L., *Camphorosma monspeliaca* L., *Chenopodium album* L., *Chenopodium botrys* L., *Chenopodium hybridum* L., *Chenopodium polyspermum* L., *Chenopodium rubrum* L., *Salsola ruthenica* Iljin; **Convolvulaceae:** *Calystegia sepium* (L.) R. Br., *Convolvulus arvensis* L.; **Cornaceae:** *Cornus mas* L.; **Crassulaceae:** *Sedum acre* L., *Sedum album* L., *Sedum maximum* (L.) Suter; **Cucurbitaceae:** *Bryonia alba* L., *Echium elaterium* (L.) A. Rich.; **Cuscutaceae:** *Cuscuta europaea* L.; **Dioscoreaceae:** *Tamus communis* L.; **Dipsacaceae:** *Dipsacus fullonum* L., *Dipsacus*

*laciniatus* L., *Knautia arvensis* (L.) Coult., *Scabiosa ochroleuca* L.; **Elaeagnaceae:** *Elaeagnus angustifolia* L., *Hippophae rhamnoides* L.; **Euphorbiaceae:** *Euphorbia amygdaloides* L., *Euphorbia cyparissias* L., *Euphorbia myrsinites* L., *Euphorbia peplis* L., *Euphorbia peplus* L., *Mercurialis annua* L.; **Fabaceae:** *Amorpha fruticosa* L., *Anthyllis vulneraria* L., *Astragalus dasyanthus* Pall., *Astragalus glycyphyllos* L., *Bituminaria bituminosa* (L.) Stirt., *Cercis siliquastrum* L., *Chamaecytisus hirsutus* (L.) Link, *Chamaecytisus lejocarpus* (A.Kern) *Chamaespartium sagittale* (L.) Gibbs., *Colutea arborescens* L., *Coronilla scorpioides* (L.) C. Koch., *Coronilla varia* L., *Galega officinalis* L., *Genista ovata* Waldst. & Kit., *Genista tinctoria* L., *Gleditsia triacanthos* L., *Lathyrus niger* (L.) Bernh., *Lathyrus pratensis* L., *Lathyrus sativus* L., *Lathyrus sylvestris* L., *Lathyrus tuberosus* L., *Lathyrus vernus* (L.) Bernh., *Lotus corniculatus* L., *Medicago sativa* L., *Melilotus alba* Medicus, *Melilotus officinalis* (L.) Pall., *Ononis arvensis* L., *Ononis spinosa* L., *Robinia pseudoacacia* L., *Spartium junceum* L., *Trifolium alpestre* L., *Trifolium arvense* L., *Trifolium pannonicum* Jacq., *Trifolium pratense* L., *Trifolium repens* L., *Trigonella coerulea* (L.) Ser., *Trigonella foenum-graecum* L., *Trigonella procumbens* (Besser) Rchb., *Vicia cracca* L., *Vicia grandiflora* Scop., *Vicia sativa* L.; **Fagaceae:** *Fagus orientalis* Lipsky, *Fagus sylvatica* L., *Quercus dalechampii* Ten., *Quercus frainetto* Ten., *Quercus robur* L.; **Gentianaceae:** *Centaurium erythraea* Raf., *Centaurium pulchellum* (Sw.) Druce, *Gentiana cruciata* L.; **Geraniaceae:** *Erodium cicutarium* (L.) L'Her., *Geranium dissectum* L., *Geranium pyrenaicum* Burm. f., *Geranium robertianum* L., *Geranium sanguineum* L.; **Globulariaceae:** *Globularia aphyllanthes* Crantz; **Haloragaceae:** *Myriophyllum spicatum* L.; **Hypericaceae:** *Hypericum maculatum* Crantz., *Hypericum perforatum* L.; **Juglandaceae:** *Juglans regia* L.; **Lamiaceae:** *Acinos arvensis* (Lam.) Dandy, *Acinos suaveolens* (Sm.) Don, *Ajuga chamaepitys* (L.) Schreb., *Ajuga laxmanii* (L.) Benth., *Ajuga reptans* L., *Ballota nigra* L., *Betonica officinalis* L., *Calamintha nepeta* (L.) Savi, *Calamintha sylvatica* Bromf., *Clinopodium vulgare* L., *Galeopsis ladanum* L., *Galeopsis speciosa* Mill., *Galeopsis tetrachit* L., *Glechoma hederacea* L., *Glechoma hirsuta* Waldst. & Kit., *Lamium maculatum* L., *Lamium purpureum* L., *Leonurus cardiaca* L., *Lycopus europaeus* L., *Marrubium parviflorum* Fisch. & C. A. Mey., *Marrubium peregrinum* L., *Marrubium vulgare* L., *Melissa officinalis* L., *Melittis melissophyllum* L., *Mentha aquatica* L., *Mentha arvensis* L., *Mentha longifolia* (L.) Huds., *Mentha pulegium* L., *Mentha spicata* L., *Nepeta cataria* L., *Origanum vulgare* L., *Phlomis tuberosa* L., *Prunella vulgaris* L., *Salvia aethiops* L., *Salvia glutinosa* L., *Salvia nemorosa* L., *Salvia pratensis* L., *Salvia sclarea* L., *Salvia tomentosa* Mill., *Salvia verticillata* L., *Satureja montana* L., *Scutellaria altissima* L., *Scutellaria galericulata* L., *Scutellaria hastifolia* L., *Sideritis montana* L., *Stachys annua* L., *Stachys germanica* L., *Stachys recta* L., *Stachys sylvatica* L., *Teucrium chamaedrys* L., *Teucrium montanum* L., *Teucrium polium* L., *Teucrium scordium* L., *Thymus callieri* Borbás ex Velen., *Thymus glabrescens* Willd., *Thymus pulegioides* L., *Thymus sibthorpii* Benth., *Thymus striatus* Vahl.; **Lemnaceae:** *Lemna minor* L., *Spirodela polyrhiza* (L.) Schleid.; **Loranthaceae:** *Loranthus europaeus* Jacq., *Viscum album* L.; **Lythraceae:** *Lythrum salicaria* L., *Lythrum virgatum* L.; **Malvaceae:** *Alcea pallida* (Waldst. & Kit. ex Willd.) Waldst. & Kit., *Alcea rosea* L., *Althaea officinalis* L., *Lavatera thuringiaca* L., *Malva neglecta* Wallr., *Malva pusilla* Sm., *Malva sylvestris* L.; **Moraceae:** *Ficus carica* L., *Morus alba* L.; **Nymphaeaceae:** *Nuphar lutea* (L.) Sm., *Nymphaea alba* L.; **Oleaceae:** *Fraxinus ornus* L., *Fraxinus oxycarpa* M. Bieb. ex Willd., *Jasminum fruticans* L., *Ligustrum vulgare* L., *Phillyrea latifolia* L., *Syringa vulgaris*

L.; **Onagraceae**: *Epilobium angustifolium* Vill., *Epilobium parviflorum* Schreb., *Oenothera biennis* L.; **Paeoniaceae**: *Paeonia peregrina* Mill., *Paeonia tenuifolia* L.; **Papaveraceae**: *Chelidonium majus* L., *Corydalis bulbosa* (L.) DC., *Corydalis solida* (L.) Schwarz, *Fumaria officinalis* L., *Fumaria vaillantii* Loisel., *Glaucium flavum* Crantz, *Papaver rhoeas* L.; **Phytolacaceae**: *Phytolacca americana* L.; **Plantaginaceae**: *Plantago lanceolata* L., *Plantago coronarius* L., *Plantago major* L., *Plantago media* L., *Plantago scabra* Moench; **Plumbaginaceae**: *Limonium vulgare* Mill., *Plumbago europaea* L.; **Polygalaceae**: *Polygala major* Jacq.; **Polygonaceae**: *Bilderdykia dumetorum* (L.) Dumort., *Persicaria hydropiper* (L.) Opiz, *Persicaria lapathifolia* (L.) Gray, *Persicaria maculata* (Raf.) Gray, *Persicaria mitis* (Schrank) Opiz, *Polygonum arenastrum* Boreau, *Polygonum aviculare* L., *Rumex acetosa* L., *Rumex acetosella* L., *Rumex crispus* L., *Rumex hydrolapathum* Huds., *Rumex obtusifolius* L., *Rumex palustris* Sm., *Rumex patientia* L., *Rumex pulcher* L.; **Portulacaceae**: *Portulaca oleracea* L.; **Primulaceae**: *Anagallis arvensis* L., *Cyclamen coum* Mill., *Cyclamen hederifolium* Aiton, *Lysimachia nummularia* L., *Primula acaulis* (L.) L., *Primula veris* L., *Samolus valerandi* L.; **Ranunculaceae**: *Actaea spicata* L., *Adonis aestivalis* L., *Adonis vernalis* L., *Anemone ranunculoides* L., *Anemone sylvestris* L., *Clematis vitalba* L., *Consolida hispanica* (Costa) Greuter & Burdet, *Consolida regalis* Gray, *Helleborus odoratus* Waldst. & Kit., *Isopyrum thalictroides* L., *Nigella arvensis* L., *Nigella damascena* L., *Pulsatilla pratensis* (L.) Mill., *Ranunculus acris* L., *Ranunculus ficaria* L., *Ranunculus flammula* L., *Ranunculus lingua* L., *Ranunculus polyanthemus* L., *Ranunculus repens* L., *Ranunculus sceleratus* L., *Thalictrum aquilegifolium* L., *Thalictrum minus* L.; **Resedaceae**: *Reseda lutea* L., *Reseda luteola* L.; **Rhamnaceae**: *Frangula alnus* Mill., *Paliurus spina-christi* Mill., *Rhamnus catharticus* L.; **Rosaceae**: *Agrimonia eupatoria* L., *Crataegus monogyna* Jacq., *Crataegus pentagyna* Waldst. & Kit., *Filipendula vulgaris* Moench, *Fragaria vesca* L., *Geum urbanum* L., *Malus sylvestris* Mill., *Potentilla argentea* L., *Potentilla cinerea* Chaix ex Vill., *Potentilla erecta* (L.) Roesch., *Potentilla reptans* L., *Prunus avium* L., *Prunus cerasifera* Ehrh., *Prunus fruticosa* Pall., *Prunus mahaleb* L., *Prunus spinosa* L., *Pyrus pyraeaster* Burgsd., *Rosa canina* L., *Rosa corymbifera* Borkh., *Rosa gallica* L., *Rubus caesius* L., *Rubus idaeus* L., *Sanguisorba minor* Scop., *Sorbus aucuparia* L., *Sorbus domestica* L., *Sorbus torminalis* (L.) Crantz; **Rubiaceae**: *Cruciata glabra* (L.) Ehrend., *Cruciata laevipes* Opiz, *Galium aparine* L., *Galium odoratum* (L.) Scop., *Galium verum* L., *Rubia tinctorum* L.; **Rutaceae**: *Dictamnus albus* L., *Ruta graveolens* L.; **Salicaceae**: *Populus alba* L., *Populus nigra* L., *Populus tremula* L., *Salix alba* L., *Salix caprea* L., *Salix fragilis* L., *Salix purpurea* L.; **Saxifragaceae**: *Saxifraga rotundifolia* L.; **Scrophulariaceae**: *Digitalis lanata* Ehrh., *Euphrasia rostkoviana* Hayne, *Euphrasia stricta* D. Wolff., *Gratiola officinalis* L., *Kickxia elatine* (L.) Dumort., *Kickxia spuria* (L.) Dumort., *Lathraea squamaria* L., *Linaria vulgaris* Mill., *Scrophularia canina* L., *Scrophularia nodosa* L., *Scrophularia umbrosa* Dumort., *Verbascum densiflorum* Bertol., *Verbascum nigrum* L., *Verbascum phlomoides* L., *Verbascum phoeniceum* L., *Veronica anagallis-aquatica* L., *Veronica arvensis* L., *Veronica austriaca* L., *Veronica beccabunga* L., *Veronica chamaedrys* L., *Veronica officinalis* L., *Veronica prostrata* L., *Veronica spicata* L. subsp. *orchidea* (Crantz) Hayek; **Simaroubaceae**: *Ailanthus altissima* (Mill.) Swingle; **Solanaceae**: *Datura stramonium* L., *Hyoscyamus niger* L., *Lycium barbarum* L., *Nicandra physaloides* (L.) Gaertn., *Physalis alkekengi* L., *Solanum dulcamara* L., *Solanum nigrum* L.; **Staphyleaceae**: *Staphylea pinnata* L.; **Tamaricaceae**: *Tamarix ramosissima* Ledeb., *Tamarix tetrandia* Pall. ex M. Bieb.; **Tiliaceae**: *Tilia cordata* Mill., *Tilia platyphyllos* Scop.,

*Tilia rubra* DC., *Tilia tomentosa* Moench; **Ulmaceae**: *Celtis australis* L., *Ulmus glabra* Huds., *Ulmus minor* Mill.; **Urticaceae**: *Parietaria lusitanica* L., *Parietaria officinalis* L., *Urtica dioica* L., *Urtica urens* L.; **Valerianaceae**: *Valeriana officinalis* L., *Valerianella coronata* (L.) DC.; **Verbenaceae**: *Verbena officinalis* L.; **Violaceae**: *Viola hirta* L., *Viola odorata* L., *Viola tricolor* L.; **Zygophyllaceae**: *Peganum harmala* L., *Tribulus terrestris* L., *Zygophyllum fabago* L.

### Liliopsida

**Alismataceae**: *Alisma plantago-aquatica* L.; **Amaryllidaceae**: *Galanthus nivalis* L., *Leucojum aestivum* L.; **Araceae**: *Arum italicum* Mill., *Arum maculatum* L.; **Butomaceae**: *Butomus umbellatus* L.; **Cyperaceae**: *Carex ligerica* J. Gay, *Carex riparia* Curtis; **Hydrocharitaceae**: *Hydrocharis morsus-ranae* L.; **Iridaceae**: *Crocus chrysanthus* (Herbert) Herbert, *Crocus pallasii* Goldb., *Gladiolus communis* L., *Gladiolus imbricatus* L., *Iris graminea* L., *Iris pseudacorus* L., *Iris pumila* L.; **Juncaceae**: *Juncus inflexus* L.; **Liliaceae**: *Allium rotundum* L., *Allium scorodoprasum* L., *Asparagus officinalis* L., *Colchicum autumnale* L., *Convallaria majalis* L., *Nectaroscordum siculum* (Ucria) Lindl., *Polygonatum multiflorum* (L.) All., *Polygonatum odoratum* (Mill.) Druce, *Ruscus aculeatus* L., *Scilla bifolia* L., *Veratrum nigrum* L.; **Najadaceae**: *Najas marina* L.; **Orchidaceae**: *Anacamptis pyramidalis* (L.) Rich., *Himantoglossum caprinum* (M. Bieb.) Spreng., *Ophrys cornuta* Steven, *Orchis coriophora* L., *Orchis morio* L., *Orchis purpurea* Huds., *Orchis simia* Lam., *Orchis tridentata* Scop., *Orchis ustulata* L., *Platanthera bifolia* (L.) Rich., *Platanthera chlorantha* (Custer) Rchb.; **Poaceae**: *Anthoxanthum odoratum* L., *Briza media* L., *Cynodon dactylon* (L.) Pers., *Elymus repens* (L.) Gould., *Lolium temulentum* L., *Sclerochloa dura* (L.) P. Beauv., *Sorghum halepense* (L.) Pers.; **Smilacaceae**: *Smilax excelsa* L.; **Sparganiaceae**: *Sparganium erectum* L.; **Typhaceae**: *Typha angustifolia* L., *Typha latifolia* L.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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## CREATING E-LEARNING COURSE ON BIODIVERSITY IN THE EUROPEAN PROJECT KEY TO NATURE

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*The paper is dedicated to Prof. D. Temniskova  
on the occasion of her 80th jubilee*

**Abstract:** An e-learning course in biodiversity was conducted for the first time among universities in Bulgaria in Shumen University. The aim was to use the opportunities provided by the project Key to Nature (sponsored by the EC Programme eContentPlus) and the e-learning platform Moodle to develop and support the delivery of specialised training courses in Higher Education. In order to realise this goal the authors have developed a sample course in Botany. The course was conducted in the period September 2010 – February 2011. Thirty six students studying biology participated in the course. The results show that it provides an accessible option for remote collection, publication and verification of information of different nature and sources. The course proved the effectiveness of e-learning as a form of self-study that complements traditional forms. In the future, we expect an increase of the role of e-learning in Bulgarian universities, and even to replace some (parts) of the conventional study forms.

**Key words:** botany, electronic key, trees, shrubs, Moodle

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## INTRODUCTION

Key to Nature was a project under the European Commission (EC) programme *eContentPlus*. The project duration was from 2007 to 2010. It involved 14 partners from 11 European countries. The aim was to achieve a common European approach to teaching topics in biodiversity at all levels of education (<http://www.keytonature.eu>). The main objectives of KeyToNature were:

- Increasing the access and simplifying the use of e-Learning tools for identifying organisms.
- Addressing the issue of interoperability among the many educational tools devoted to biodiversity across Europe.
- Optimizing their educational efficiency and increasing their quality for educational purposes.
- Adding value to them by providing multilingual access.
- Suggesting best practices against barriers that prevent use, production, exposure, discovery and acquisition of educational tools in the biodiversity field.

To achieve the objectives of the project partners presented their electronic databases that are related to biodiversity. One part of the partners who have taken this step was:

- Faculty of Life Sciences, The University of Trieste, Italia (coordinator of the project)
- British Natural History Museum, London.
- "ETI-Bioinformatics" – Dutch organization funded by UNESCO.
- German Federal Research Centre for Biology.
- Real Jardín Botánico (of the Spanish National Research Council).
- Slovenian Museum of Natural History.
- German and Estonian universities with rich electronic databases for biodiversity.

The project worked in two main areas: software-technological and educational.

The software-technological direction was aimed at reconciliation of existing partner electronic databases for biodiversity and providing uniform access to them.

The educational direction was associated with the development of best practices and pilot testing for meaningful and full utilisation of the potential of the databases in the learning activities of primary and secondary schools and universities. Three Bulgarian universities joined the project as associate members: Sofia University "St. Kliment Ohridski", Medical University – Sofia, and University of Shumen "Bishop Konstantin Preslavski".

An interactive portal for schools and universities was developed during the project. The universities' part of the portal offered opportunities for free use of a number of electronic identification keys (e-keys) in the fields of Botany, Zoology, Mycology, published in different languages, mainly in English. The advantage of

e-keys is that they can be used on a portable computer (laptop) with Internet access, or with a CD-version of the respective e-key. Versions have been developed for mobile phones as well.

## MATERIALS AND METHODS

An e-Learning course was conducted by the authors of the article during the period September 2010 – February 2011, the first semester of the academic year in Bulgaria.

The aim was to use the opportunities provided by the project “KeyToNature”, funded by the EC eContentPlus programme), and the e-learning platform Moodle (<http://k2n.dmaster.org/moodle-new/>, Cole & Foster 2008) to create specialised courses and to conduct training through them. To realise this goal, the authors have developed an exemplary course in Botany (Dendrology), (currently available at <http://k2n.dmaster.org/moodle-new/course/view.php?id=45>, access as a “Guest”). Before to run the course, described in the current article, an appropriate national teacher training was conducted for selected Bulgarian schools and universities (MIHNEV & RAYCHEVA 2010).

Thirty six first-grade university students participated in the course from two different specialties: “Biology and Chemistry”, and “Physics and Biology”. Each student had to gather information for a tree or shrub species found in the park of the University of Shumen. The tasks were formulated in a special page on the platform. A special electronic key (e-key) for identification of trees and shrubs in Bulgaria (in Bulgarian) was used, developed under the project (NIMIS ET AL. 2010). The key was developed in three versions – as online Internet key ([http://dbiodbs.units.it/carso/chiavi\\_pub21?sc=324](http://dbiodbs.units.it/carso/chiavi_pub21?sc=324)), in a CD-version, and in a version for mobile phones.

A final result that has been aimed at, was the development of a profile of plant species, which had to be published in the e-learning platform. At the end of the course each student completed an electronic “opinion questionnaire”, aimed to assess the quality of the course by end-users point of view. The COLLES instrument (Taylor & Maor 2010) was used for processing the results of the survey. The COLLES survey consists of 24 statements grouped into six scales: relevance, reflective thinking (self-reflection), interactivity, support of teacher support from other participants in the course and interpretation. The specific questions, the research findings, and the grouping by categories can be viewed in the online edition of TAYLOR & MAOR (2010).

The course ended with a final assessment, which was included in the formation of the final exam mark of the Botany course.

## RESULTS AND DISCUSSION

Students who were enrolled in the course received a personal password by the administrator of the course MR. PENCHO MIHNEV. Of course, then they had the right to change it. The course was publicly accessible by non-participants who were able to enter as “Guests”. Unlike the students, they were unable to publish information, and to see the submitted student assignments.

The work assignments that were given to the students were two types: field-work and cameral work. The cameral work was divided in two phases: preliminary (training) and individual (execution of tasks). The fieldwork was carried out between the two phases. DR. DIMCHO ZAHARIEV headed, commissioned and oversaw the proper fulfilment of the work.

The course included several themes. Different study tasks were assigned to each theme, namely:

### **Theme 1. Working with an electronic key**

*Activity 1.* Identify one tree or shrub species.

Use the electronic key on trees and shrubs in Bulgaria. Link to the electronic key is included.

*Activity 2.* Fill in the worksheet.

Download the file that is attached to the theme. Link to the worksheet file is included.

### **Theme 2. Collecting information about the species of place**

*Activity 1.* Take photos of the tree or shrub species, assigned to you: habitus, twig with buds and leaves, leaf, flower, inflorescence, fruit.

*Activity 2.* Determine the location of the tree/shrub.

Use GPS or interactive Internet map showing the coordinates providing the location (eg. GoogleMap). Link to GoogleMap is included.

### **Theme 3. Collection of additional information on the species**

*Activity 1.* Gather text information about species:

- 1.1. Spreading.
- 1.2. Morphological description.
- 1.3. Period of flowering and fruiting.
- 1.4. Importance.

Use books from the library and materials from the Internet.

*Activity 2.* Gather information on the type of pictures. Use images from the web.

*Activity 3.* Gather mapping information on the species:

- 3.1. Spreading in Europe.
- 3.2. Spreading in Bulgaria.

### **Theme 4. Storage of collected information**

*Activity 1.* Submit the completed worksheet to the Moodle platform. A submission link is included.

*Activity 2.* Fill in a Data Base record with your collected data. Use the link provided in the theme.

*Activity 3.* Application of the identified species' location coordinates on the map. A link to the map is included.

**Theme 5. Create a profile of the species.**

*Activity 1.* Create a profile of your plant. Use the template file "Profile of the species".

*Activity 2.* Develop the profile of the species as a multimedia product.

*Activity 3.* Send the file by using the Upload button, related to the theme.

**Theme 6. Creation of an electronic key**

*Activity 1.* Create an electronic key of trees and shrubs in the Park of Shumen University. Use The Editor of electronic keys. A link to the Online Editor of e-keys is included.

*Activity 2.* Fill in the new key information collected by you.

*Activity 3.* Save created key to your computer.

After the last topic a link to the opinion survey is included that each student must complete after the completion of his work in the e-Learning course.

The preliminary cameral work consisted of the following:

1. Training to work with electronic keys. The students were divided into 3 groups by 12 persons each. The training was conducted in a computer lab. During the training several different plant species were identified by using the e-key. As a result, the students discovered that work with electronic key is much easier than the use of conventional printed keys.

2. Training for mastering the steps to perform the tasks. It was necessary because e-learning is still not very much used in Bulgaria. During the training the opportunities which it provides were shown. The basic steps that should be followed in order to meet the objectives of the course were demonstrated to the students. To answer the questions that may arise during their independent work a contact e-mail of the teacher was provided to all participants.

Fieldwork consisted of determining the species identification of tree or shrub species. The place of work was the park in front of the main building of the University of Shumen. Each student had to work with a separate species, different from that of all other students. Who exactly will be the plant species was determined randomly by the students themselves. The identification of plant species was performed by using the electronic key installed either on laptop or on mobile phone. The students filled out "in place" in the park a worksheet with the most important morphological features of the studied individual plants. They took photos of vegetative and/or generative organs of "their" species. They were asked to describe also as additional information the specific habitat around their plant and to determine the presence or absence of relationships with other organisms (plants and animals) in its surrounding environment.

The individual cameral work included collection of textual and illustrative information in order to develop a profile of the plant species. The profile included the following data: Latin name of the tree or shrub species; its Bulgarian name; name of the family to which the species belongs; classification with level of detail at the student's discretion; photos of the tree/shrub (minimum 2 made by the student, and 2 from other sources); description (morphology, habitat, altitude, spreading in Europe and in Bulgaria, preference for soil conditions); importance for the humans; interesting facts; more information (personal comments); description of the resources used during the work (electronic keys, online resources, print resources).

The information required had to be from several print and electronic sources.

As an additional benefit to students was offered the proposal to develop PowerPoint presentations of their identified plant species that could bring to them additional award points.

The collected data were entered in an interactive geographical map embedded into Moodle and into prepared multimedia database for the course.

The final students' opinion survey included 48 questions, divided into six groups: relevance, reflective thinking (self-reflection), interactivity, support of teacher support from other participants in the course and interpretation. The possible answers to each question were as follows: "almost never", "rarely", "sometimes", "often" and "almost always". At the end of the survey the students had to enter the time required for its completion. There was a possibility to also add a free-text comment at the end of the survey.

## CONCLUSION

The delivery of an e-learning course on biodiversity was performed for the first time in Shumen University among universities in Bulgaria. The results showed that the course can be used successfully for remote collection, publication and verification of information of different nature. The electronic course offered a number of advantages as a form of individual work for the students in comparison to the conventional course forms used in Bulgarian Universities. The course was characterized with attractiveness: the used tools for learning and studying were high-tech, similarly to those that students use in their everyday life. Another feature is the achieved learning dynamics: the variety of tasks that were allocated throughout the course. The third course advantage was the accessibility: students performed the tasks at a satisfactorily level. One of the most important features was that the electronic course provided opportunities for creativity that took the learning and training to a higher level. In the future, we expect that the role of e-learning in Bulgarian universities will increase and even replace some (parts) of the conventional study forms.

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**Book review: Ettl H. & Gärtner G. 2014. Syllabus der Boden-,  
Luft- und Flechtenalgen. 2 Auflage. Springer Spektrum,  
New York, 773 pp.**



Recently we often speak about the “craft of scientific writing” and many books, lectures and internet sites provide significant and interesting guides on the topic. One of them suggests the following to those, who want to write a book review, or book report: “Try to appreciate the book: It will teach you something if you are open to learning.” (<http://www.wikihow.com/Write-a-Book-Report>, accessed 24.10.2014). The book, which is the focus of this brief review, is easy to be appreciated. In fact, there is no other way to accept it, since it is the most profoundly presented collection of aero-terrestrial eukaryotic algae of the Earth, including lichen photobionts. Nowadays, when so-called “classical” phycology, based mostly on algal morphology, cytology, reproduction and ecology, is generally considered obsolete, but still could not be fully and sufficiently replaced by “modern” studies alone, books like this one are strongly needed. The explanation is easy – it provides a reasonable bridge between these two lines in algal knowledge, linking, as far as it is possible, the classical system with recent molecular phylogenetic results, still keeping strong point of support on use of living cultures and proper terminology. Appreciation to this book comes also from the fact, that Univ. Prof. DrSc Georg Gärtner – the co-author, who updated it and prepared this second edition, fully kept the structure, keys, texts and figures of the first edition, and, following the model of classical algal flora of Pierre Bourrelly (1968–1972), made an appendix with recent data on transformed or newly described taxa, as well as on newly recorded for aero-terrestrial habitats species with relevant references. In this way, it introduces the recent knowledge, but in the same time gives a possibility to young users to become acquaint with this well-known and widely used Syllabus, commonly smilingly named “*Ettl & Gärtner 1995*”. The positive criticism, which has a purpose to increase the use of this book, could be expressed in two wishes: to see it as soon as possible translated in English language and to see it enlarged with data on cyanoprokaryotes, which are quite common and abundant aero-terrestrial inhabitants. But even in German language, the recently published second edition of Syllabus is of inestimable value for all those, who are open to learning.

Dobri Ivanov



## Annual of Sofia University, Faculty of Biology, Book 2 – Botany

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**Book 2 – Botany of the Annual of Sofia University** is issued yearly in one volume. Original papers covering the entire field of scientific botany and mycology are published in the journal with special encouragement to the papers of students and young scientists. Reviews may be published with the editors' consent. Papers have to be written in English and must present new and important research findings that have not been published or submitted for publication elsewhere. By submitting a manuscript the author expresses his agreement to transfer all rights of reproduction of any kind, translations and distribution.

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As a rule, the contributions should not exceed 16 printed pages. If a paper exceeds this limit, the authors are requested to obtain the editors' consent in advance. The text must be accurate and the language correct.

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The scientific names of the taxa (genera, species and lower ones) must be quoted completely, denominating the name of the genus, species epitheton (if necessary subspecies, cultivarq *etc.*) and the author, when mentioned the first time in the text. Full scientific names, as a rule, should be mentioned in the summary also. The author names in the scientific names should not be formatted. The classification system used is up to the authors, but in case of different from commonly approached, should be properly indicated.

The **Acknowledgments** may be inserted at the end of the text, before the literature references. Their correctness and ethics are total responsibility of the authors.

**References** to the works cited are given **in the text** by the name of the author and the year of publication, e.g., IVANOV 1971, or (IVANOV 1971) and IVANOV & PETROV 1942 or (IVANOV & PETROV 1942), respectively. When more authors have to be cited semicolons between them should be used and works should be listed in chronological (not in alphabetical!) order, e.g. (IVANOV & PETROV 1942; IVANOV 1971; BABOV 1987). In case of unchanged citation certain pages of a paper referenced should be indicated as follows: IVANOV 2013: 149–151, or IVANOV & PETROV 2013: 169. The abbreviated citation "et al." should be used in the text only in cases where three and more authors are involved, e.g. IVANOV ET AL. 1971, or IVANOV ET AL. (1971). **References** to the cited works (and only those) are to be arranged alphabetically **at the end of the paper**, the papers of the same author(s) should be listed in chronological order and according to the number of co-authors. The well-known journals should be enlisted with their common abbreviations; the other journals should be written in full titles. The form of citations should conform to general use, as the following examples, organized for one, two and three or more authors (please note that after a punctuation mark an interval should be used):

#### Journals:

- IVANOV I. P. 2013. Photosynthetic CO<sup>2</sup>-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.
- IVANOV I. P. & PETROV P. I. 2013. Photosynthetic CO<sup>2</sup>-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.
- IVANOV I. P., PETROV P. I. & DIMITROV V. N. 2013. Photosynthetic CO<sup>2</sup>-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

#### Books:

- PETROV F. 2000. Grazing in water ecosystems. – In: IVANOV W., STOYANOV H. & PETROV F. (eds.), Water ecosystems, Elsevier, New York, 59–105.

Alternatively, we accept full text citations of journal titles. However, the reference list must be consistent in this regard.

References to manuscripts in preparation and personal communications should not be included in the reference list, except for extremely significant data.

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#### Journal:

- PETKOFF S. 1925. La flore algologique du mont Pirin-planina.- Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian, French summ.).

#### Book:

- VODENICHAROV D., DRAGANOV S. J. & TEMNISKOVA D. 1971. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian).

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- HINDÁK F. 1996. Klúč na určovanie nerozkonárených vláknitých zelených rias (Ulotrichineae, Ulotrichales, Chlorophyceae) [Key to unbranched filamentous green algae (Ulotrichineae, Ulotrichales, Chlorophyceae)]. – Bull. Slov. Bot. Spol., Bratislava, Suppl. 1: 1–77 (in Slovakian).

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The number of both tables and illustrations must be restricted to the indispensable amount. Repeated presentation of the same fact by tables and figures will not be accepted. In case of doubt, figures (line drawings) should be preferred.

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