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НА СОФИЙСКИЯ УНИВЕРСИТЕТ
„СВ. КЛИМЕНТ ОХРИДСКИ“

Биологически факултет

Книга 2 – Ботаника

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

БИОЛОГИЧЕСКИ ФАКУЛТЕТ

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FIRST APPLICATION OF A DRONE FOR STUDIES OF THE BIODIVERSITY OF BULGARIAN EXTREMOPHILIC ALGAE IN THE MARIKOSTINOV THERMAL COMPLEX

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*The paper is dedicated to Assoc. Prof. Tanyo Michev (1939-2018),
the first biologist who invented aerophotos in the studies
of ecosystem changes in Bulgaria*

Abstract. The paper presents results from the first application of a drone in the studies of the biodiversity of extremophilic thermal algae in Bulgaria. The drone was used to choose the sampling sites in the Marikostinovo thermal complex (South-Western Bulgaria). From the eight samples chosen in this way, totally 54 algal taxa (except diatoms) were identified. As it was expected, a comparison between species composition of the collected samples (52) and that of the cultured at room temperature material (14) showed significant difference in the registered biodiversity with only seven algae found in both types of samples. Among all algae found, 3 genera and 17 species are new for Bulgaria, 48 taxa from 22 genera are new for the Marikostinovo thermal complex, and 35 species from 9 genera are new for the thermal flora of Bulgaria. In this way, the total number of algae recorded in Bulgarian thermal waters increased from 206 to 241, from which the current biodiversity of Marikostinovo (54 taxa) comprises 29%, and the total number of algae determined in the complex during the last hundred years became 70. Four of the species found during this study were declared as threatened in the Red List of Bulgarian microalgae (two *Endangered* and two *Near Threatened*). In the same time, 40 species

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are potential cyanotoxin-producers, which requires further studies of Bulgarian thermal waters in this aspect. All obtained results showed the great potential of application of drones in the studies related with biodiversity of extreme and vulnerable habitats, and threatened species as well.

Key words: cyanobacteria, cyanoprokaryotes, cyanotoxins, thermal algae, threatened species, vulnerable habitats

INTRODUCTION

In the last years, the application of Unmanned Aerial Vehicles (UAV) in biological studies have increased rapidly. Currently, a special issue of the journal *Drones* was targeted on the usage of drones for Biodiversity Conservation and Ecological Monitoring. The Special Issue information starts with the following words of the Guest Editors DR. R. DÍAZ-DELGADO & DR. C. A. MÜCHER (2018): “Unmanned Aerial Vehicles (UAV) have already become an affordable and cost-efficient tool to quickly map a targeted area for many emerging applications in the arena of Ecological Monitoring and Biodiversity Conservation. Managers, owners, companies and scientists are using professional drones equipped with high-resolution visible, multispectral or thermal cameras to assess the state of ecosystems, the effect of disturbances, or the dynamics and changes of biological communities inter alia. We are now at a tipping point on the use of drones for these type of applications over natural areas...”. Doubtless, UAV and drones in particular can help in better studies of ecosystems and their biodiversity both through mapping of the areas and through enhancing searching for proper habitats in the biodiversity studies, especially when microscopic organisms like microalgae are investigated. In Bulgaria, our first application of a drone in the studies of aquatic harmful algal blooms (HABs) proved to be successful and promising (STOYNEVA-GÄRTNER ET AL. 2019). Therefore, drone was further tested in a study of the biodiversity of more peculiar ecological group of algae, this of extremophilic thermal algae. The paper presents results from the study of the algal biodiversity of the thermal complex Marikostinovo, which is used as a balneotherapy sanatorium (mainly for mud treatment) with a limited access, when a drone was applied for selection of the sampling sites. Current obtained data are compared with earlier studies of this thermal complex (PETKOFF 1925; GEORGIEV 1948) and of other Bulgarian thermal springs, summarized and taxonomically updated by STOYNEVA-GÄRTNER ET AL. (2018). Taking into account the increasing role of thermal waters and related SPA centers in the life of the Bulgarian society and the hazardous role, which their main inhabitants – cyanoprokaryotes - can play in human life and health (e.g. MERILUOTTO ET AL. 2017), we outlined the potential cyanotoxin producing algae.

MATERIAL AND METHODS

Marikostinovo thermal complex is situated in South-Western Bulgaria, 2 km

North-West from the village Marikostinovo, in the valley of the river Struma (**Fig. 1**). It is among the 35 thermal complexes of Bulgaria, for which data on algal biodiversity, obtained by conventional light microscopy, are available (STOYNEVA-GÄRTNER ET AL. 2018). The Marikostinovo thermes are well-known due to the thermal springs (sparkling at 59-63°C) and geyser mineral mud, used since centuries for balneotherapy. Before 1907 there was a building of the Turkish bath with some additional free water basins outside (ANONYMOUS 2019). Currently, the Turkish bath is transformed in a modern SPA center, in which algae are permanently cleaned. Another building contains a mineral pool and many separate bath tubes used for mud treatment (op. cit.). The open water area, which remained relatively less anthropogenically touched, is situated outside of the mud center and is surrounded by a fence (**Fig. 2a**). It is alternatively used as a male and a female bath several times during the day. There, thermal springs are constantly sparkling at several small spots causing permanent moving of the mud layers, and algal mats are raising from the bottom (**Fig. 2b**). The samples (8) for this study were taken from the algal mats floating on



Fig. 1. Map of Bulgaria with the Capital and village Marikostinovo pointed on it (after d-maps.com).

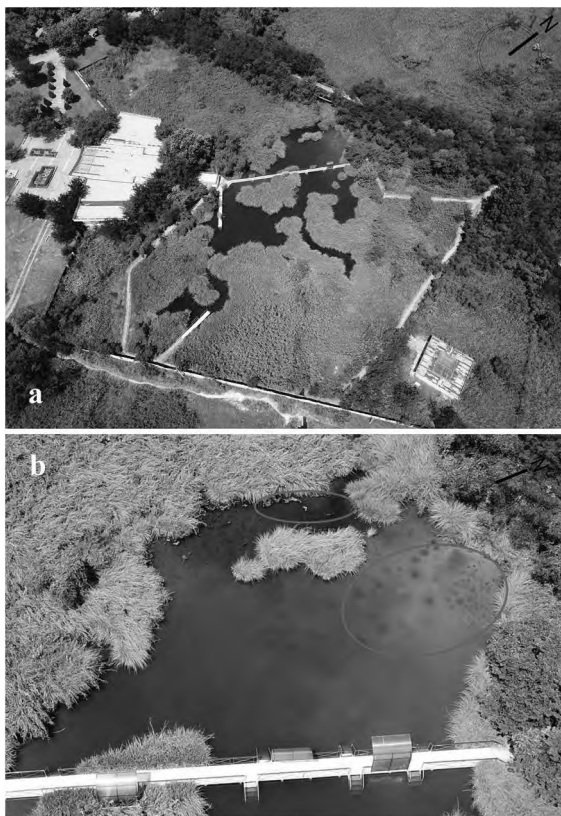


Fig. 2. Photos of Marikostinovo thermal complex, obtained by drone: **a** - thermal complex with the building of mud bath center; **b** - algal sampling areas.

the surface or mats laying on the bottom, and from the muddy bottom on 26.07.2018 (**Fig. 2b**). The natural thermal effluents, which were situated out of the region of the baths and formed small water pools, described in the study by PETKOFF (1925), were not found by us during the pilot visit of the first author in 2003 and during the visit of both authors in 2018. Most probably, the decrease of surrounding thermal effluents was due to the use of thermomineral waters for heating large areas of greenhouses in the region during the last decades (ANONYMOUS 2019).

Before the sampling, a drone supplied with a photo camera, was sent to observe and document the whole water body and sites with visible differences in the color or floating mats of algae. The drone used was DJI Mavic Pro, Model: M1P GL200A, Manufactured by SZ DJI Technology Co., LTD. The records are stored as photos and videos. The site coordinates (N 41°26.3186 E 23°19.0194), altitude (99 m a.s.l.), water temperature (47-59°C), pH (7.74), water hardness (TDS – 1470 mg l⁻¹), oxygen content (DO -85.2% and 5.61 mg l⁻¹) and conductivity (2263 µs cm⁻¹) were measured in situ by Aquameter AM-200 and Aquaprobe AP-2000 from Aquaread water monitoring instruments, 2012 Aquaread Ltd.

The material was collected in plastic tubes and on the next day, 1/3 part of it was inoculated in Petri dishes with BBM agar for culturing in the Algal collection of Sofia University (ACUS – UZUNOV ET AL. 2012), 1/3 part was fixed in 2% formalin and 1/3 part was frozen for further investigations.

Microscopic identification of algae (except diatoms) followed the standard taxonomic literature (e.g. GEITLER 1931, 1942; GOLLERBAKH ET AL. 1953; KOMÁREK & FOTT 1983; KOMÁREK & ANAGNOSTIDIS 1999, 2005; KOMÁREK 2013) with updates from ALGAEBASE (GUIRY & GUIRY 2019). It was done on 32 non-permanent slides from row cultures and fixed material using conventional light microscopy (LM) on Motic BA 4000 microscope with magnification 100x and immersion. Microphotographs were taken by Moticom 2000 camera supplied by Motic Images 2 Plus software program. In spite of the fact that some specimens could not be easily focused due to development in mucilaginous tufts or mats over inorganic particles, the photos were not additionally processed by any specialized program. In the prepared taxonomic list for each taxon the habitat type and general distribution is shown with enlisting especially the European countries in alphabetical order, but in some cases, it follows exactly the order provided in the cited literature. Since distribution in Bulgaria is discussed separately, the country is not mentioned among the other European countries in the texts on general distribution. The number of samples in which each species was found is indicated in brackets, with outlining the findings in cultured and fixed samples.

Threatened status of the recorded taxa is provided after the Red List of Bulgarian microalgae (STOYNEVA-GÄRTNER ET AL. 2016).

The potentially toxic cyanoprokaryotic taxa are indicated after CATHERINE ET AL. (2013) and BERNARD ET AL. (2017) with some additions from the papers by MOHAMED & AL-SHEHRI (2015) and STOYNEVA ET AL. (2015).

RESULTS

The total list of the species from the Marikostinovo thermal complex, determined during this study, contains 54 algal taxa from three divisions – Cyanoprokaryota (52), Chlorophyta (1) and Streptophyta (1). However, a part of the algal material remained absolutely unidentified, even at generic level, and it has to be noted that in some cases these unidentified specimens were even quite abundant. Therefore, the identification work is in a progress.

DIVISION CYANOPROKARYOTA

CLASS CYANOPHYCEAE

Order Chroococcales

Family Aphanothecaceae

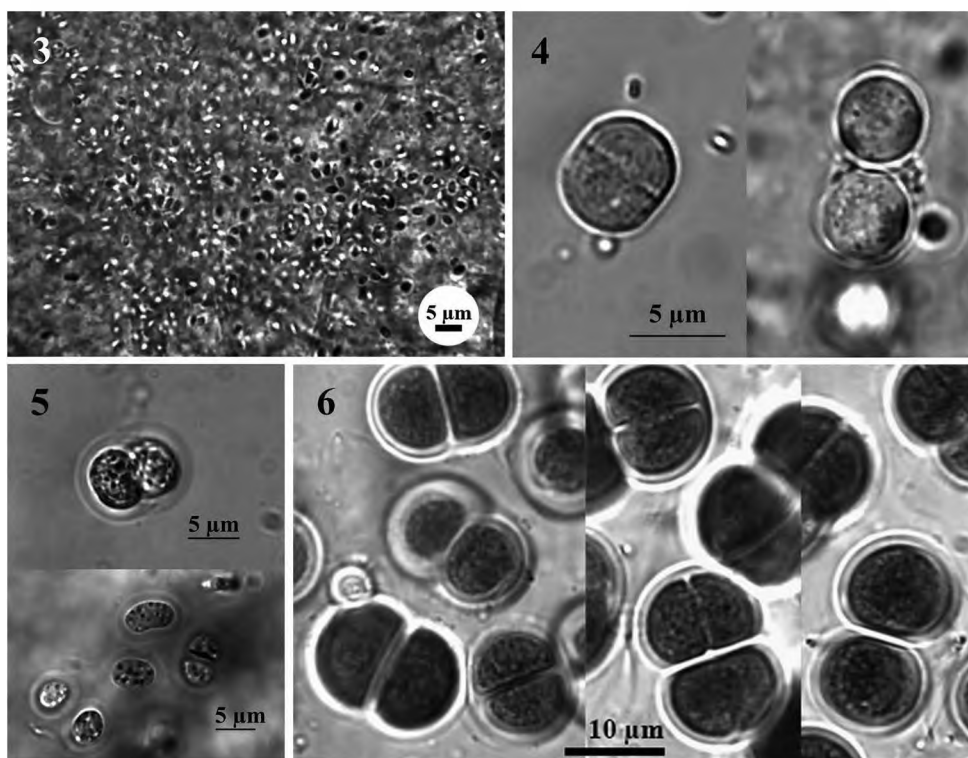
Aphanothece sp. – The cells very small, up to 2 µm long and up to 1 µm wide, loosely arranged in groups of irregular shape, possible kept together by common colorless very fine mucilage (**Fig. 3**). Representatives of this genus have been found in thermal waters (KOMÁREK & ANAGNOSTIDIS 1999) and are known from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018), but this is the first record for Marikostinovo thermal complex. Up-to-now found by LM in cultured samples (1). Representatives of *Aphanothece* are known as producing microcystins (CATHERINE ET AL. 2013).

Family Chroococcaceae

Chroococcus globosus (Elenkin) Hindák 1978 – Cells solitary, rounded oval, 5–(6) µm, blue-green, with homogenous protoplast and colorless, thin, structureless envelopes; the cells reach spherical shape before the next division (**Fig. 4**). The species is thermophilic, described from the mud of Eurasian hot springs (Kamchatka) and found also in Europe (Slovakia) - KOMÁREK & ANAGNOSTIDIS (2005), GUIRY & GUIRY (2019). It was not recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and according to our knowledge, from Bulgaria so far. Up-to-now found by LM in fixed samples (1).

Chroococcus membraninus (Meneghini) Nägeli 1849 – Cells with a diameter 4-5-8 µm, in process of division in twos in the colonies with colorless lamellate mucilage (**Fig. 5**). The species is distributed “near thermal waters, subaerophytic on wet mud, usually mixed with other cyanoprokaryotes” (KOMÁREK & ANAGNOSTIDIS 2005, p. 296) and was found in Europe (Britain, Slovakia), North America, Asia, Australia and New Zealand (GUIRY & GUIRY 2019). It is known from thermal springs of Bulgaria, including Marikostinovo (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (2). It was included as *Near Threatened* in the Red List of Bulgarian microalgae [NT – A4 B3 C4 D1 E1 F2 G1 T16].

Chroococcus thermalis (Meneghini) Nägeli 1849 – Cells in colonies, the colorless mucilage envelopes follow the cell outlines, cells with finely granulated



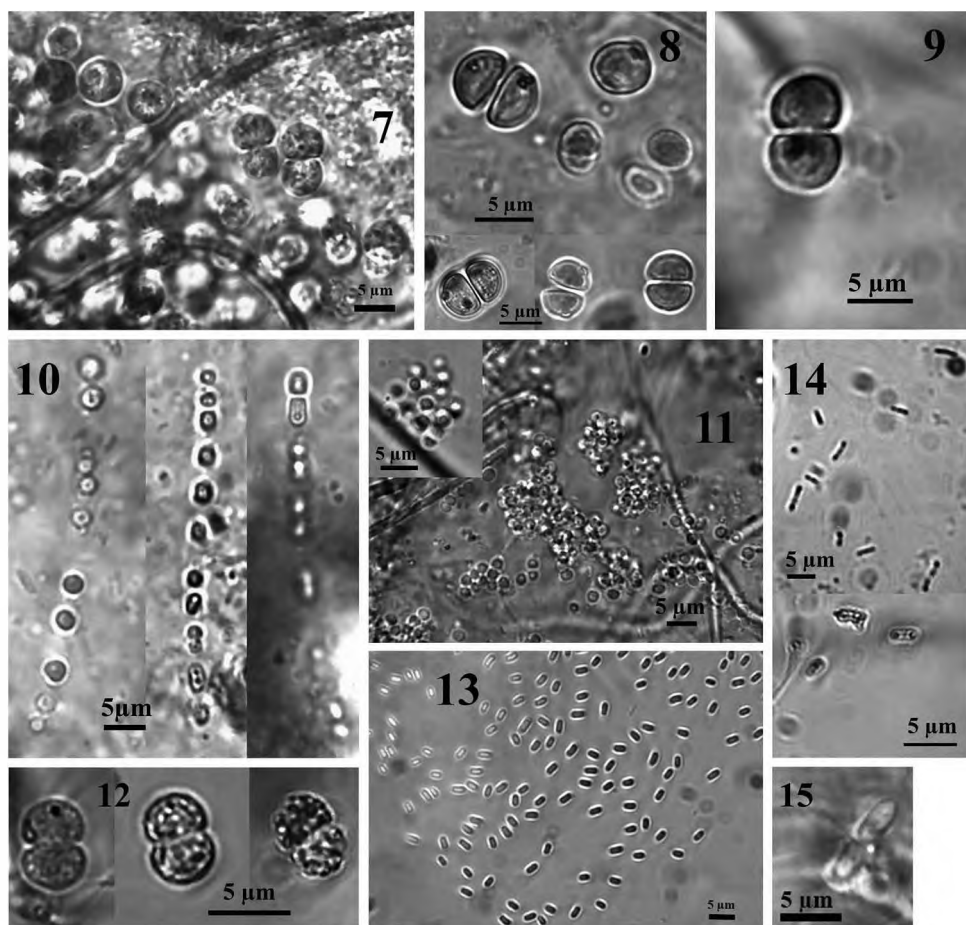
Figs. 3-6. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **3** - *Aphanothece* sp.; **4** - *Chroococcus globosus* (Elenkin) Hindák 1978; **5** - *Chroococcus membraninus* (Meneghini) Nägeli 1849; **6** - *Chroococcus thermalis* (Meneghini) Nägeli 1849.

content. The cell dimensions were (7) 8 (14) x 12 (13-22). In one of the cultures dimensions varied from 10 (12-13) µm width to 20-27 µm length (**Fig. 6**). The species is “widely distributed in thermal waters” (KOMÁREK & ANAGNOSTIDIS 2005, p. 304) as shown in GUIRY & GUIRY (2019) also. It is known from thermal springs of Bulgaria, including Marikostinovo (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in both fixed (4) and cultured samples (1).

***Chroococcus* sp. 1** – Cells with a diameter 5-6 µm, in process of division in twos (up to 12 µm long) in the colonies with thin, colorless not lamellate mucilage; cell content not keritomised or net-like, with visibly granules (?cyanophycin) (**Fig. 7**). Up-to-now found by LM in fixed samples (1).

***Chroococcus* sp. 2** – Cells with a diameter 5-8 µm, in process of division in twos in the colonies with thin, colorless not lamellate mucilage (**Fig. 8**). Up-to-now found by LM in fixed samples (4).

***Chroococcus* sp. 3** - Only solitary cells, with a diameter 5 µm, in process of division



Figs. 7-15. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **7** - *Chroococcus* sp. 1; **8** - *Chroococcus* sp. 2; **9** - *Chroococcus* sp. 3; **10** - *Johannesbaptistia* sp.; **11** - *Aphanocapsa thermalis* Brügger 1863; **12** - *Synechocystis aquatilis* Sauvageau 1892; **13** - *Cyanobium eximium* (Copeland) Komárek, J. Copecký & Cepák - dark granules at cell poles; **14** - *Synechococcus bigranulatus* Skuja 1933; **15** - *Synechococcus* cf. *lividus* J. J. Copeland 1936.

in twos, with very thin, colorless mucilage; cell content not keritomised or net-like but with visible centropalsma and chromatoplasma (Fig. 9). Up-to-now found by LM in fixed samples (1).

Family Cyanotrichaceae

Johannesbaptistia sp. – Solitary short, straight unbranched pseudofilaments, consisting of one row of clearly separated sphaerical blue-green cells with diameter 2.5 (3 µm) and visible granules in the protoplast, connected by tiny colorless mucilage; before division cells enlarged and two conspicuous granules are visible (Fig. 10). Representatives of this genus were reported from

thermal waters (KOMÁREK & ANAGANOSTIDIS 1999) but not from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018) and from Bulgaria so far (STOYNEVA 2014). Up-to-now found by LM in cultured samples (1).

Order Synechococcales

Family Merismopediaceae

***Aphanocapsa thermalis* Brügger 1863** – Cells spherical, densely packed in groups (colonies) with spherical form or more irregularly spread, without individual gelatinous envelopes, (1.5)-2-2.5-(3) μm in diameter, without aerotopes but with cell content separated in centroplasma and chromatoplasma (**Fig. 11**). According to KOMÁREK & ANAGNOSTIDIS (1999) the species is spread as subaerophytic, but also as aquatic in running hot water, on wet rocks in thermal springs (up to 68.7°C), common in the thermal springs of the northern hemisphere and recorded also in Argentine and New Zealand. It was found in Europe (Greece, Slovakia, Turkey), North America, Asia, Australia and New Zealand (GUIRY & GUIRY 2019). The species was not reported from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018) and from Bulgaria so far. This is also the first record of a representative of the genus *Aphanocapsa* from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in cultured samples (1).

***Synechocystis aquatilis* Sauvageau 1892** – Cells widely oval, solitary, in a process of cell division, 3-4 μm in diameter (**Fig. 12**). The species has a broad distribution, but has not been pointed for thermal springs by KOMÁREK & ANAGNOSTIDIS (2005) or GUIRY & GUIRY (2019). It was reported from thermal springs of Bulgaria, including Marikostinovo (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1).

Family Synechococcaceae

***Cyanobium eximium* (COPELAND) KOMÁREK, J. COPECKÝ & ČEPÁK** – Cells loosely spread in aggregates, rod-shaped with rounded poles, 1 μm wide and 1.5-2-3 μm long, bright to olive blue-green, in some cells with two darker granules at each pole (**Fig. 13**). The species is considered thermophilic, spread “outside Europe” (KOMÁREK & ANAGANOSTIDIS 1999), as shown in GUIRY & GUIRY (2019) also. It has not been recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and from Bulgaria so far (STOYNEVA 2014). Up-to-now found by LM in cultured samples (1).

***Synechococcus bigranulatus* Skuja 1933** – Cells loosely spread in aggregates, rod-shaped, straight or slightly curved, 0.8-1 μm wide and (2.5) 3-4.5 (5) μm long, blue-green, with two darker granules at each pole and distinct chromatoplasma (**Fig. 14**). The species is known from temperature springs all over the world from (20)30 to 75.8°C (KOMÁREK & ANAGANOSTIDIS 1999). According to GUIRY & GUIRY (2019) it was found in Europe (Greece) and Asia (India, Israel). *S. bigranulatus* was reported from thermal springs of Bulgaria (STOYNEVA-GÄRTNER ET AL. 2018) but the present record is first for the Marikostinovo

thermal complex. Up-to-now found by LM in fixed samples (1). Microcystin and anatoxin A producing species (CATHERINE ET AL. 2013).

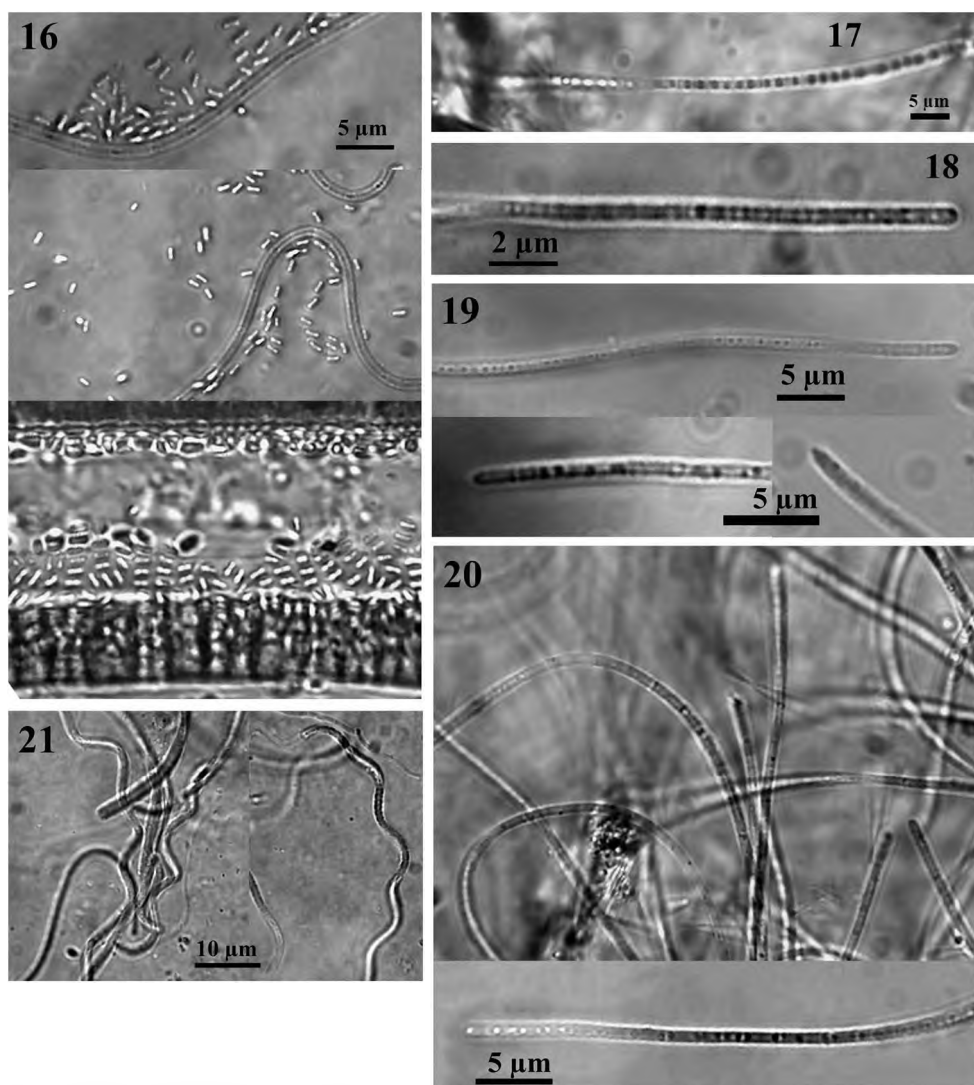
***Synechococcus cf. lividus* J. J. Copeland 1936** – Cells rod-shaped, bright blue-green, 2-2.5 μm wide and 5(6) long μm long, reproduced by division in twos (Fig. 15). Considering the note of KOMÁREK & ANAGNOSTIDIS (1999, p. 126) that the species has not been recorded from Europe, the very low number of specimens found in the fixed samples (1) and similarities with other thermobiotic species (e.g. *Synechococcus vulcanos* J. J. Copeland 1936, *S. viridissimus* J. J. Copeland 1936 and *S. caldarius* Okada 1939), we decided to include the material found with some uncertainty. According to GUIRY & GUIRY (2019) *S. lividus* was found in Europe (Spain), South America, Asia, Australia and New Zealand. The species was included in the Checklist of Bulgarian thermal algae (STOYNEVA-GÄRTNER ET AL. 2018) due to photo published without any comments by STRUNECKÝ ET AL. (2018). Up-to-now found by LM in fixed samples (1). Microcystin-producing species (CATHERINE ET AL. 2013; BERNARD ET AL. 2017).

***Synechococcus* sp.** – The cells were numerous, mostly attached in groups or rows to the mucilage of other filamentous algae, rod-shaped, 1.5-2-(2.5-3) μm long and 0.5-(1-2) μm wide with very thin colorless individual mucilage envelopes (Fig. 16). Our material resembles in part the thermophilic species *Cyanobium gracile* Rippka et Cohen-Bazire 1983, especially by its small dimensions, but differs by the presence of thin mucilage layer around the cells (a feature which was not observed in *Cyanobium*) and by the shape of the cells – rod-shaped in our material and oval in *C. gracile* (Komárek & Anagnostidis 1999). In part, by the type of aggregation it coincides with representatives of the genus *Bacularia* and therefore further identification of our material is needed. Up-to-now found by LM in cultured samples (1).

Разред Synechococcales

Family Leptolyngbyaceae

***Leptolyngbya gelatinosa* (Woronichin) Anagnostidis & Komárek 1988** – Filaments thin, with colorless diffluent sheaths; trichomes are straight, not constricted, slightly thinner (1.2 μm) in comparison with species dimensions provided by GOLLERBAKH ET AL. (1963, p. 488) and KOMÁREK & ANAGNOSTIDIS (2005, p. 189): 1.6-2 μm wide; cells isodiametric to longer than wide, with distinct granules (?sulphur granules) and rounded apical cells (Fig. 17). The species is known from thermal and mineral springs of Europe (Georgia, Austria, Russia) and New Zealand (KOMÁREK & ANAGNOSTIDIS 2005), and more recently from North America, Asia and Australia (GUIRY & GUIRY 2019). It was not recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and according to our knowledge, in Bulgaria so far. Up-to-now found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).



Figs. 16-21. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **16** - *Synechococcus* sp.; **17** - *Leptolyngbya gelatinosa* (Woronichin) Anagnostidis & Komárek 1988; **18** - *Leptolyngbya geysericola* (J. J. Copeland) Anagnostidis 2001; **19** - *Leptolyngbya granulifera* (J. J. Copeland) Anagnostidis 1936; **20** - *Leptolyngbya thermalis* Anagnostidis in Anagnostidis et Komárek 1988; **21** - *Leptolyngbya thermobia* Anagnostidis 2001.

***Leptolyngbya geysericola* (J. J. Copeland) Anagnostidis 2001** – Filaments long, straight; sheaths hyaline; trichomes pale blue-green, not constricted, 0.4-0.6 µm wide with isodiametric or, rarely, twice longer cells with homogenous content; apical cells rounded, without calyptra or thickened outer cell wall (**Fig.**

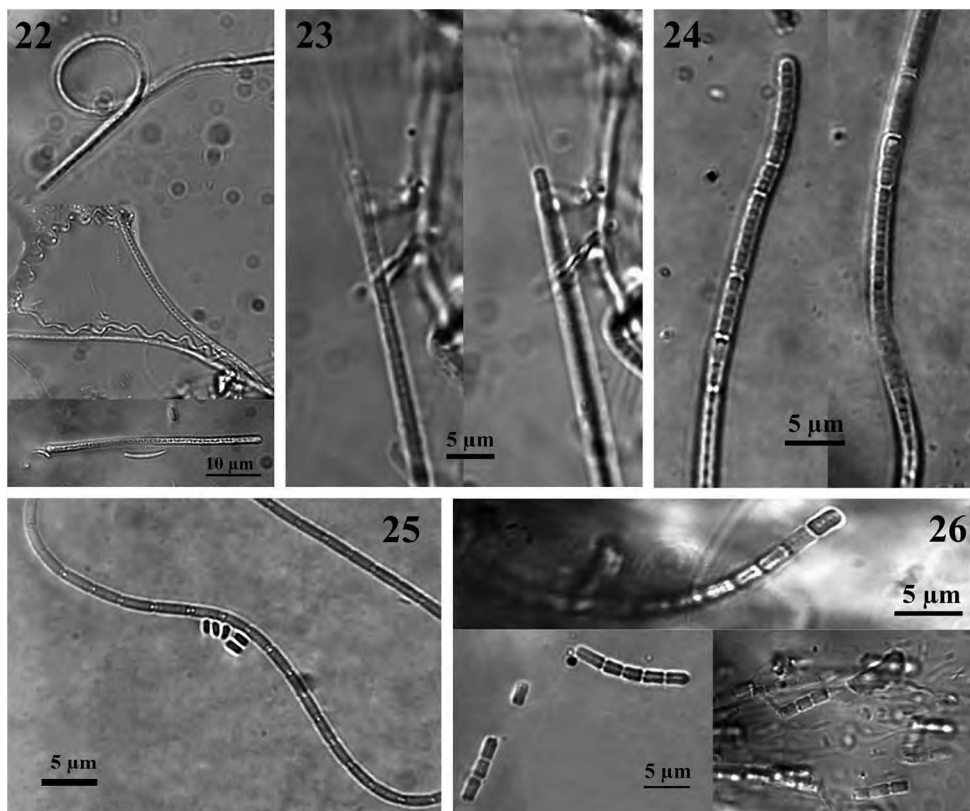
18). It was described from Yellowstone geysers (59-84°C) and was included by KOMÁREK & ANAGNOSTIDIS (2005) as unrevised species, with possible relations with *Jaaginema geysericola* L. It was recorded also in Asia (India) - GUIRY & GUIRY (2019). The species is known from thermal springs of Bulgaria (STOYNEVA-GÄRTNER ET AL. 2018), proved in Rupite samples by single-cell PCR (STRUCEKÝ ET AL. 2018), but this is the first record from Marikostinovo thermal complex. However, there are morphological differences between our material and photo provided in STRUNECKÝ ET AL. (2018) with cells much longer than wide. Up-to-now found by LM in fixed samples (3). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).

***Leptolyngbya granulifera* (J. J. Copeland) Anagnostidis 1936** – Filaments with very thin sheath, trichomes bright blue-green, 1-1.3 µm with cells 2-3 times longer than wide, with rounded conical apical cells. Granules at each side of the transversal walls are well visible. The slight tapering at the ends, noted by KOMÁREK & ANAGNOSTIDIS (2005, p. 213) could not be seen from their drawings (Fig. 262) and was not seen in our materials, except for the apical cells (**Fig. 19**). The species was described from the Yellowstone thermal springs, and afterwards was recorded also from alkaline thermal springs in Greece, “in both cases frequently together with *Mastigocladus laminosus*” (op. cit.). In ALGAEBase (GUIRY & GUIRY 2019) distribution in North America and Asia is added. It was not recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and according to our knowledge, in Bulgaria so far. Up-to-now found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).

***Leptolyngbya thermalis* Anagnostidis in Anagnostidis et Komárek 1988** (as *Leptolyngbya thermalis* Anagnostidis 1988: 393, nom. inval. in ALGAEBase (GUIRY & GUIRY 2019) - Filaments solitary, with very thin, colorless difficultly visible sheath; trichomes pale blue-green, not constricted, not attenuated at the ends, 1-1.3 µm with cylindrical cells up to 3 times longer than wide and refractile granules at the cross walls; rounded apical cells (**Fig. 20**). According to KOMÁREK & ANAGNOSTIDIS (2005) the species was described from thermal springs in Greece, and was found also in thermal springs of France, Hungary, former Yugoslavia (?Croatia), and in Algeria and Argentina as well. In ALGAEBase (2019) distribution in Asia is added. The species was not recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and according to our knowledge, in Bulgaria so far. Up-to-now found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).

***Leptolyngbya thermobia* Anagnostidis 2001** – Filaments solitary or entangled, 1.8-2 µm wide, irregularly or regularly screw-like coiled with colorless sheaths; trichomes constricted or not constricted, and apical cells rounded, without calyptra; granules in cells were seen (**Fig. 21**). The species was described

from thermal waters in Greece (salty springs) and was recorded also from Hungary, Russia, Japan and USA (KOMÁREK & ANAGNOSTIDIS 2005). It was not known from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and according to our knowledge, in Bulgaria so far. Up-to-now found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).



Figs. 22-26. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **22** - *Leptolyngbya valderiana* (Gomont) Anagnostidis & Komárek 1988; **23** - *Leptolyngbya* sp.; **24** - *Jaaginema geminatum* (Schwabe ex Gomont) Anagnostidis & Komárek 1988; **25** - *Jaaginema thermale* Anagnostidis 2001; **26** - *Pseudanabaena catenata* Lauterborn 1915.

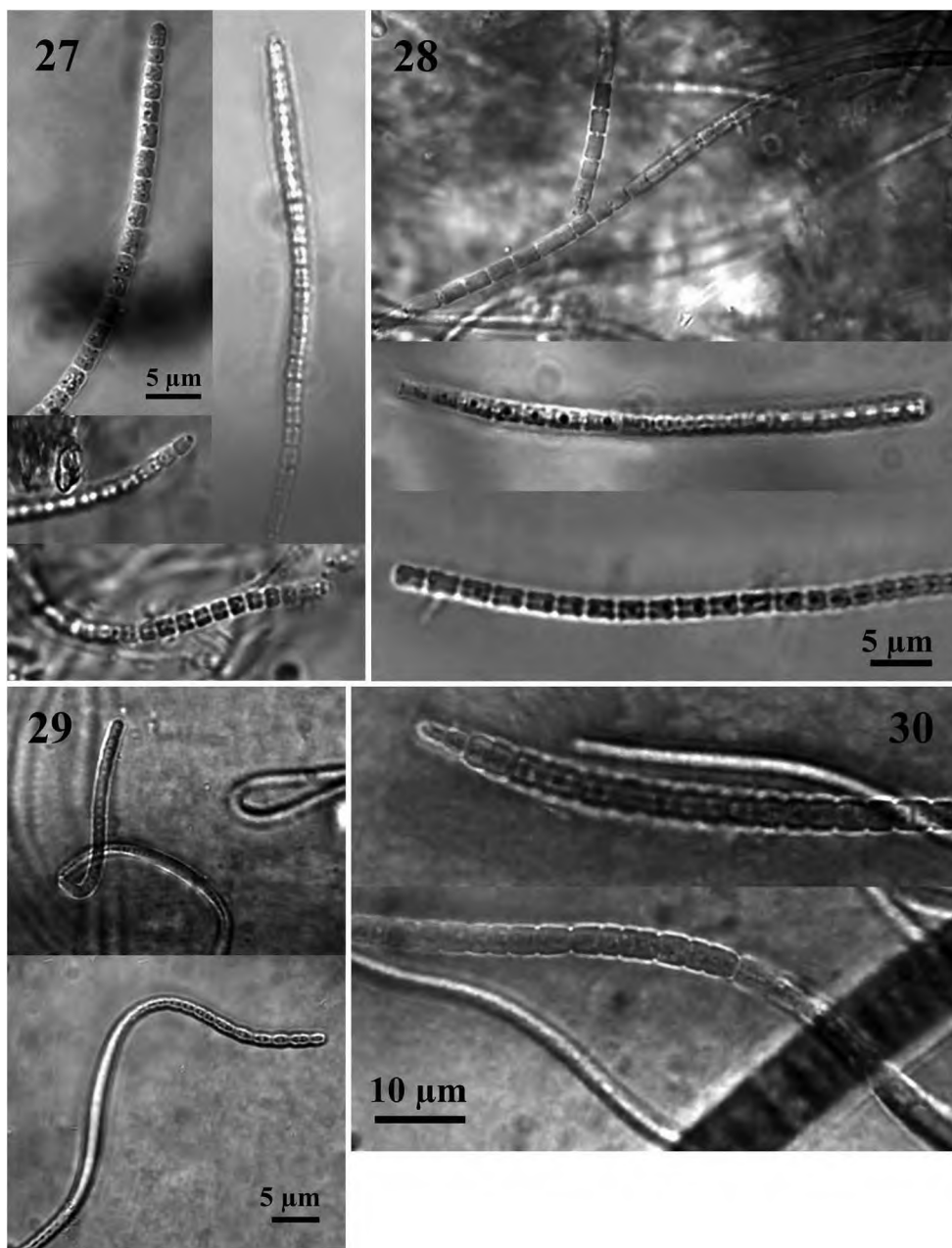
***Leptolyngbya valderiana* (Gomont) Anagnostidis & Komárek 1988** – Filaments singular or entangled, frequently snake-like or ring-like coiled; sheaths thin, colorless, sometimes difficultly distinguishable; trichomes (1.8)-2-(2.5) µm, not constricted and not attenuated to the ends, cells isodiametric or longer than wide, with granules near to the cross walls; apical cells rounded, without calyptra (**Fig. 22**). According to KOMÁREK & ANAGNOSTIDIS (2005, p. 211)

this species is mainly freshwater benthic and “unrevised populations are recorded from cataracts and thermal springs (at the margins and on stones and walls) ...distributed worldwide, probably cosmopolitan. probably in different morphotypes... However, records from...thermal waters in Iceland and USA (Yellowstone Nat. Park), *etc.*, need revision.” According to GUIRY & GUIRY (2019) *L. valderiana* was found in Europe (Britain, Czech Republic, Georgia, Portugal, Romania, Russia, Slovakia, Spain, Turkey and Scandinavian countries), North America, South America, Pacific Islands, Asia, Australia and New Zealand. The species is known from thermal springs of Bulgaria (STOYNEVA-GÄRTNER ET AL. 2018) but it is the first record for Marikostinovo thermal complex. Up-to-now found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).

***Leptolyngbya* sp.** – Filaments solitary, 1.5 µm wide, with thin, colorless but not diffuent sheaths; trichomes 0.8–1 µm wide; cells cylindrical, longer than wide with homogenous content; apical cell rounded (**Fig. 23**). Our material completely fits to the diagnosis of *Leptolyngbya erebi* (W. et G. S. West) **Anagnostidis et Komárek 1988**. We did not indicate it with this name due to the notes in KOMÁREK & ANAGNOSTIDIS (2005), according to which the species is typical for Antarctica and the records from tropics and thermal springs in Hungary are debatable. Up-to-now rarely found by LM in fixed samples (1). Representatives of *Leptolyngbya* are known as producing nodularin (CATHERINE ET AL. 2013).

Family Incertae sedis

***Jaaginema geminatum* (Schwabe ex Gomont) Anagnostidis & Komárek 1988** – Trichomes blue-green, mostly straight, unbranched, 2-2.5 µm wide, constricted, with rounded apical cells; cells isodiametric or slightly longer than wide; necridic cells visible; cell content separated in centroplasma and chromatoplasma, showing the parietal orientation of thylakoids (**Figs. 24**). The type of the reproduction and presence/absence of necridic cells in the whole genus are not clarified yet (KOMÁREK & ANAGNOSTIDIS 2005). The species, described from thermal springs in France is considered as a “thermophilic species, often accompanying *Mastiglocladus laminosus*” broadly distributed in the thermal springs of Europe (including neighboring to Bulgaria Greece and former Yugoslavia) and “known from almost all investigated thermal springs in the world” - KOMÁREK & ANAGNOSTIDIS (2005, p. 117). According to GUIRY & GUIRY (2019) *J. geminatum* was found in Europe (Czech Republic, France, Germany, Greece, Lithuania, Netherlands, Romania, Russia, Spain, Turkey, Ukraine and Scandinavian countries), South America, Asia, Australia and New Zealand. The species was recorded from Bulgarian thermal springs, including Marikostinovo thermal complex (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found in fixed samples (1). It was included as *Near Threatened* in the Red List of Bulgarian microalgae [NT - A3 B4 C3 D2 E1 F1 G1 T15]. Representatives



Figs. 27-30. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **27** - *Pseudanabaena papillateterminata* (Kisselev) Kukk 1959; **28** - *Pseudanabaena thermalis* Anagnostidis 2001; **29** - *Pseudanabaena* sp.; **30** - *Geitlerinema numidicum* (Gomont) Anagnostidis 1989.

of *Oscillatoria*, from which *Jaaginema* has been taxonomically separated, are known as microcystins and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017).

***Jaaginema thermale* Anagnostidis 2001** - Trichomes solitary or aggregated together, straight or variously curved, not attenuated at the ends, blue-green to pale blue-green, 0.8-1.2 µm wide with cells 2-2.5-(3) times longer than wide, distinctly separated at the translucent cross walls, with a granule at each side of the cross walls, with a rounded apical cell (**Fig. 25**). The species occurs in thermal springs, probably widely distributed – known from hot springs in Europe (Austria, Croatia, France, Greece, Hungary) and Asia (KOMÁREK & ANAGNOSTIDIS 2005). It was not recorded from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018), and, according to our knowledge, also from Bulgaria so far. Up-to-now found by LM in fixed samples (1). Representatives of *Oscillatoria*, from which *Jaaginema* has been taxonomically separated, are known as microcystins and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017).

Family Pseudanabaenaceae

***Pseudanabaena catenata* Lauterborn 1915** – Material from Marikostinovo fits completely with the species description: trichomes solitary or aggregated, long, straight, strongly constricted at the hyaline, translucent cell walls, not attenuated at the ends, with rounded apical cells and intercalary cells 1-1.2-2 µm wide and about 3 times longer than wide (**Fig. 26**). This species shares many morphological features with the thermal *Leptolyngbya granulifera*, but differs by the lack of thin diffluent sheaths and by conical apical cell. *P. catenata* is worldwide distributed in different types of habitats (for details see GUIRY & GUIRY 2019), including thermal waterbodies (KOMÁREK & ANAGNOSTIDIS 2005). It was recorded many times in different Bulgarian water bodies (STOYNEVA 2014), but not in thermal habitats so far (STOYNEVA-GÄRTNER ET AL. 2018). Microcystin producing species (CATHERINE ET AL. 2013) and “harmful species” (GUIRY & GUIRY 2019 after MOHAMED & AL-SHEHRI 2015). Up-to-now found by LM in fixed samples (1).

***Pseudanabaena papillaterminata* (Kisselev) Kuk 1959** – Trichomes solitary, mainly pale blue-green (but dark blue-green in one of the samples), 2.5-3 µm wide, distinctly constricted at the cross-walls, not attenuated at the ends; cells slightly longer than wide or isodiametric; the apical cell with a clearly visible protrusion (**Fig. 27**). Despite the differences in the colors of trichomes and shapes of apical cells, this species resembles *Pseudanabaena thermalis*, with which it was found together in one of the samples and in our opinion, both species need taxonomic reconsideration and clarification of relation to *Komvophoron*, with which many features are shared (including shape of the cells, presence of hyaline bridges and differentiation into chromatoplasma and centroplasma). It is possible to suppose, that the protrusion found by us and

other authors, is an artefact from trichome fragmentation. The species has not been pointed as thermophilic and is considered benthic, spread in the mud of mainly haline biotopes (mainly in central Asia, and rarely in Europe and Central America) but also in stagnant freshwaters (KOMÁREK & ANAGNOSTIDIS 2005). According to GUIRY & GUIRY (2019) *P. papillaterminata* was found in Europe (Georgia, Lithuania, Romania, Russia, Spain) and Asia (Tadjikistan). It has been recorded in Bulgaria (STOYNEVA 2014), but not in thermal waters (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now, rarely found in fixed samples (2). Representatives of *Pseudanabaena* are known as producing microcystins (CATHERINE ET AL. 2013).

***Pseudanabaena thermalis* Anagnostidis 2001** – Trichomes constricted, with hyaline bridges; cells clearly differentiated in centro- and chromatoplasma, 2-3 µm wide and 4-9 (10) µm long, or 2 µm wide and up to 3.5 µm long; apical cell flat-rounded, mostly with aerotopes (**Fig. 28**). The species is particularly common for alkaline springs of *Mastigocladus*-type, frequently also in other sulphide-containing thermal waters (28-54°C, pH 7-9.5), known from thermal springs of Europe (Greece, Germany, Hungary, Switzerland), probably widely distributed in corresponding biotopes (known from USA, probably also New Zealand, India and Iceland) - KOMÁREK & ANAGNOSTIDIS (2005), but was not recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and in Bulgaria so far. Up-to-now found by LM in fixed samples (4), in one of them quite abundant. Representatives of *Pseudanabaena* are known as producing microcystins (CATHERINE ET AL. 2013).

***Pseudanabaena* sp.** – The material coincides with the drawing provided by KOMÁREK & ANAGNOSTIDIS (1999 – fig. 73) for unidentified species, commonly found in thermal springs of Greece together with *Mastigocladus laminosus*, or separately from it. Trichomes are blue-green, clearly constricted, cells are 1.5-2 µm wide with content separated in centroplasma and chromatoplasma (**Fig. 29**). Up-to-now found in fixed samples (1). Representatives of *Pseudanabaena* are known as producing microcystins (CATHERINE ET AL. 2013).

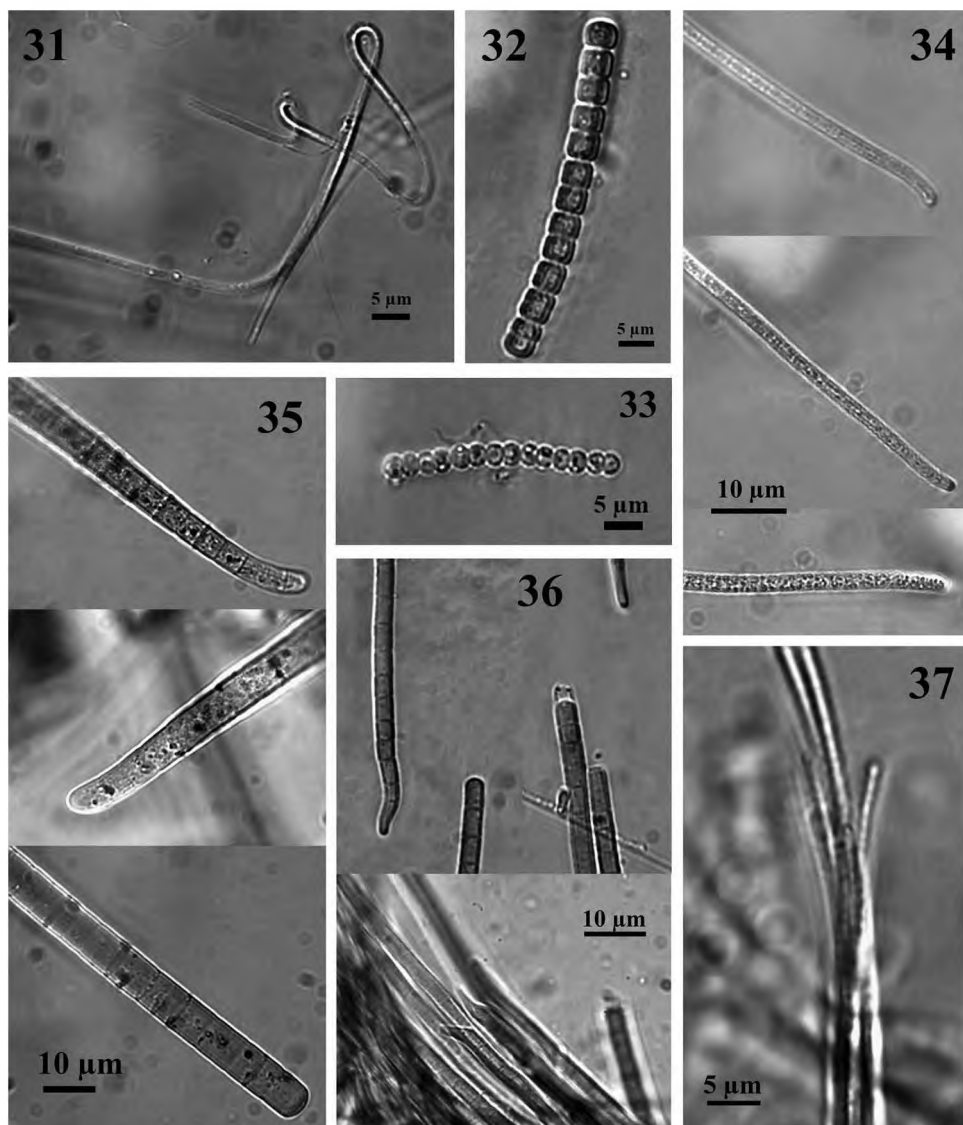
Order Oscillatoriales

Order Oscillatoriales

Family Coleofasciculaceae

***Geitlerinema numidicum* (Gomont) Anagnostidis 1989**, non *Phormidium numidicum* (Gomont sensu Welsh) Anagnostidis 2001 – Trichomes bright blue-green, 3-4 µm wide, clearly constricted and gradually attenuated to the ends; cells mostly isodiametric and more rare twice longer than wide (?before division); apical cells straight, conical and rounded at the top, without calyptra (**Fig. 30**). According to KOMÁREK & ANAGNOSTIDIS (2005) this species is known in various concepts, incl. referring to *Phormidium*. Our material is in coincidence with their text description and fig. 139, and not with the description of *Phormidium numidum* and fig. 632. The same authors (p. 131) provided

species occurrence in “thermal springs (atmophytic sites near thermal waters), geysers, ... Greece, Hungary, Iceland, ... Germany, N Africa (Morocco) and N



Figs. 31-37. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **31** - *Geitlerinema thermale* Anagnostidis 2001; **32** - *Konvophoron* sp.; **33** - “*Isocystis pallida* Voronichin 1927”; **34** - *Kamptonema jasorvense* (Vouk) Strunecký, Komárek & J. Smarda 2014; **35** - *Kamptonema cortianum* (Meneghini ex Gomont) Strunecký, Komárek & J. Smarda 2014; **36** - *Oxynema acuminatum* (Gomont) Chatchawan, Komárek, Strunecky, Smarda & Peerapornpisal 2012; **37** - *Symploca* cf. *elegans* Kützing ex Gomont 1892.

America (?).” In ALGAEBASE (GUIRY & GUIRY 2019) distribution in Romania and South America is added. The species was not recorded from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018), and, according to our knowledge, also from Bulgaria so far. Up-to-now found by LM in cultured samples (1). Representatives of *Geitlerinema* are known as producing microcystins (CATHERINE ET AL. 2013).

***Geitlerinema thermale* Anagnostidis 2001** – Trichomes solitary, near to the ends with coils, pale blue-green, 1-5-1.8 (2) μm wide, not constricted, cross-walls ungranulated or near to them some tiny refractive granules exist; cells up to 2 times longer than wide or isodiametric; cell content mostly homogenous; apical cell rounded, without calyptra or thickened outer cell wall (**Fig. 31**). The species was described from thermal saline springs in Greece, and later from thermal springs in Georgia, Indonesia and Japan (KOMÁREK & ANAGNOSTIDIS 2005). It is not known from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and, according to our knowledge, has not been reported from Bulgaria so far (STOYNEVA 2014). Up-to-now found by LM in fixed samples (1). Representatives of *Geitlerinema* are known as producing microcystins (CATHERINE ET AL. 2013).

Family Gomontiellaceae

***Komvophoron* sp.** – Trichomes solitary, found as relatively short (12-15 cells) chain of blue-green cells 5 μm wide and 2.5-3-4 μm long, constricted at the ungranulated cell walls; apical cells rounded; the cell content clearly divided in chromatoplasma and centropasma, with solitary granules (**Fig. 32**). Representatives of this genus are known from thermal waters (KOMÁREK & ANAGNOSTIDIS 2005) but have not been recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1).

“*Isocystis pallida* Voronichin 1927” – Trichomes as relatively short moniliform chain of 10-14 pale blue-green cells, strongly constricted; cells sphaerical, 2.5-3 μm in diameter with large granules; apical cells do not differ from the other cells in the chain (**Fig. 33**). This species was originally described from thermal waters but is yet taxonomically debatable and possibly belongs to the genus *Komvophoron* (for details see KOMÁREK & ANAGNOSTIDIS 2005 and KOMÁREK 2013). Here, we keep it with its original name, but have to note some similarities with *Komvophoron jovis* (J. J. Copeland) Anagnostidis & Komárek 1988, from which it differs by smaller dimensions in our material (cells 3.4-4.5 μm wide and 3-6 μm long in typical *K. jovis*), lack of conical apical cells and finding of trichomes with spherical cells only in different samples. By these features, the material found in Marikostinovo is close also to *Komvophoron breve* (Carter) Anagnostidis (found in coastal swamps or on tidal mud in Essex, England and California, USA - KOMÁREK & ANAGNOSTIDIS 2005) and to *Komvophoron groenlandicum* Anagnostidis et Komárek, described and up to now known

only from a subarctic shallow lake in Greenland (KOMÁREK & ANAGNOSTIDIS 2005). Due to these differences in the ecological characteristics, we would like to note that *K. jovis* was recorded from different types of thermal springs, frequently with *Mastigocladus laminosus* in a broad range of temperatures and pH in America and Asia, and in Europe known from Nigrita in Greece (KOMÁREK & ANAGNOSTIDIS 2005). *Isocystis pallida* was recorded in Europe (Greece, Turkey) - GUIRY & GUIRY (2019) but is not known from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and from Bulgaria so far. Up-to-now rarely found by LM in fixed samples (2).

Family Microcoleaceae

***Kamptonema jasorvense* (Vouk) Strunecký, Komárek & J. Smarda 2014**

(Syn. *Oscillatoria jasorvensis* Vouk 1919, *Phormidium jasorvense* (Vouk) Anagnostidis & Komárek 1988, *Geitlerinema jasorvense* (Vouk) Anagnostidis 1989) – Trichomes blue-green to slightly yellowish-green, 3 µm wide, cells isodiametric or slightly longer than wide, 3-4 µm, straight but hooked at the apex; apical cell rounded (**Fig. 34**). The species is known from thermal springs of Europe (Croatia, Greece, Romania, Russia, Slovenia, Spain and rarely – Czech Republic), Asia, Africa and USA (KOMÁREK & ANAGNOSTIDIS 2005, p. 130, 418; ALGAEBASE 2019), but was not recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and according to our knowledge, in Bulgaria so far. Up-to-now found by LM in fixed samples (2). Representatives of the genus *Oscillatoria* were pointed as microcystins and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017), and representatives of *Phormidium* – as anatoxin A, homoanatoxin A, microcystins and nodularins (CATHERINE ET AL. 2013).

***Kamptonema cortianum* (Meneghini ex Gomont) Strunecký, Komárek & J.**

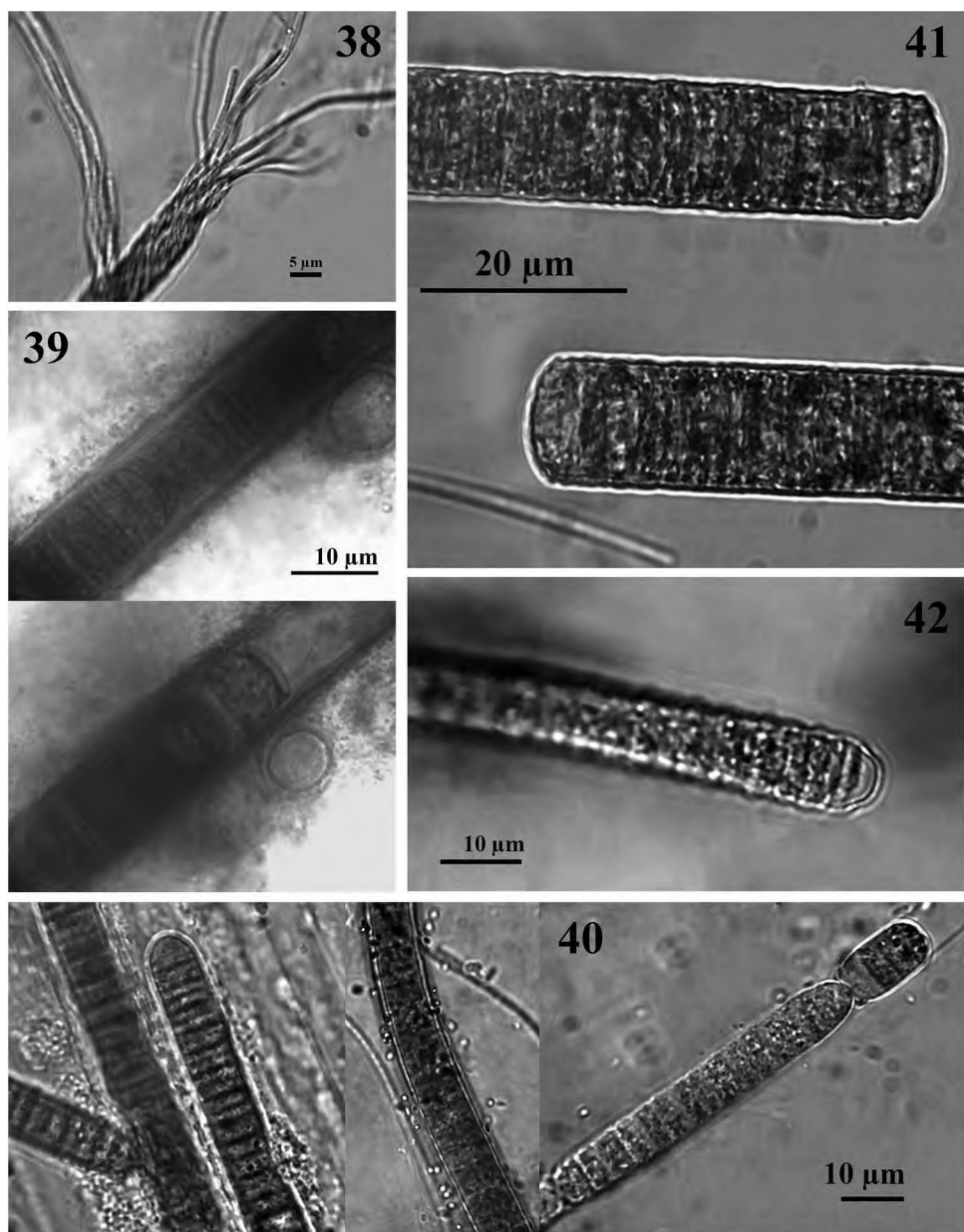
Smarda 2014 – identified by LM in fixed samples (7) after (KOMÁREK & ANAGNOSTIDIS 2005) as *Phormidium cortianum* (Meneghini ex Gomont) Anagnostidis & Komárek 1988: Trichomes straight, blue-green, (5) 6-9 µm wide with cells up to 7 µm, (6)7-8(9)x5 µm and 6-7-9x4-5 µm, slightly but distinctly constricted, or 9x4-5 µm and unconstructed, with prominent cyanophycin granules; apical cell obtuse conical, not capitated, or more rarely, flat conical, without calyptra (**Fig. 35**). The species is known from thermal and mineral springs of Europe (Italy, Croatia, Czech Republic, Georgia, Greece, Hungary), Africa, Asia and USA (KOMÁREK & ANAGNOSTIDIS 2005). According to GUIRY & GUIRY (2019) *K. cortianum* was found in Europe (Britain, Czech Republic, Greece, Romania, Russia, Slovakia, Spain, Turkey), South America, Africa, Asia, Australia and New Zealand. The species was published for Bulgaria as *Oscillatoria cortiana* Meneghini ex Gomont 1892 (for thermal springs see comments in STOYNEVA-GÄRTNER ET AL. 2018). According to KOMÁREK & ANAGNOSTIDIS (2005) this species embraces also *Kamptonema okenii* (C. Agardh ex Gomont) Strunecký, Komárek & J. Smarda

2014 recorded from thermal springs. As “*Oscillatoria okeni* Ag.” it was also pointed for the Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018). The present record of *K. cortianum* is the first for Marikostinovo thermal complex. Up-to-now frequently found by LM in both fixed (4) and cultured samples (2). Representatives of the genus *Oscillatoria* were pointed as microcystins and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017), and representatives of *Phormidium* – as anatoxin A, homoanatoxin A, microcystins and nodularins (CATHERINE ET AL. 2013).

***Oxynema acuminatum* (Gomont) Chatchawan, Komárek, Strunecky, Smarda & Peerapornpisal 2012** – identified by LM in cultured samples (2) after (KOMÁREK & ANAGNOSTIDIS 2005) as *Phormidium acuminatum* (Gomont) Anagnostidis & Komárek 1988, which is currently regarded as its homotypic synonym. Filaments with fine, firm colorless sheaths (which were pointed as rare for the species - KOMÁREK & ANAGNOSTIDIS 2005, p. 400). Trichomes bright blue-green, almost not constricted; at the ends abruptly attenuated with a bended sharply pointed characteristic apical cell; trichome cells 4 µm wide, 1.5(2) times longer than wide (**Fig. 36**). The species is widely distributed in thermal springs of Europe (Austria, Greece, Hungary, Italy), Africa, Asia and USA (KOMÁREK & ANAGNOSTIDIS 2005). According to GUIRY & GUIRY (2019) *O. acuminatum* was found in Europe (Czech Republic, Greece, Italy, Lithuania, Romania, Russia, Spain, Turkey), South America, Carribean islands, Africa, Asia, Australia and New Zealand. As *Oscillatoria acuminata* Gomont 1892 it is known from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), but the present record is first for Marikostinovo thermal complex. Up-to-now found in both fixed (1) and cultured (1) samples. Saxitoxin-producing species (MOHAMED & AL-SHEHRI 2015).

***Symploca cf. elegans* Kützing ex Gomont 1892** – Trichomes 1 µm wide, which is slightly less than the minimum width (1.3 µm) pointed by KOMÁREK & ANAGNOSTIDIS (2005), cells 2-3 times longer than wide or isodiametric in a common colorless sheath; apical cell slightly conically rounded, not calyptated or capitated (**Fig. 37**). This is mainly a freshwater/terrestrial species, which is known also from the outflows of thermal springs; distribution is in Europe (Czech Republic, Greece, Italy), Africa and Asia (KOMÁREK & ANAGNOSTIDIS 2005; GUIRY & GUIRY 2019). The species was not recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and according to our knowledge, in Bulgaria so far. KOMÁREK & ANAGNOSTIDIS (2005) pointed that this species is not well-known and possibly belongs to the genus *Leptolynghya*. Up-to-now found by LM in fixed samples (1).

***Symploca thermalis* Gomont 1892** – Trichomes blue-green, 1-1.2 µm wide, with almost isodiametric or slightly longer cells, constricted, straight or slightly curved and entangled as a network in the colorless sheaths; apical cells rounded, without calyptra. Granules at the cross walls were not seen in



Figs. 38-42. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **38** - *Symploca thermalis* Gomont 1892; **39** - *Lyngbya aestuarii* Liebman ex Gomont 1892; **40** - *Lyngbya thermalis* Kützing ex Gomont 1892; **41** - *Oscillatoria princeps* Vaucher ex Gomont 1892; **42** - *Oscillatoria sancta* Kützing ex Gomont 1892.

the cultured material (**Fig. 38**). The species is known from thermal springs (with steam, up to 52°C) and is cosmopolitan, distributed worldwide; Europe (Austria, Croatia, Czech Republic, France, Georgia, Greece, Hungary, Iceland, Italy, Portugal - KOMÁREK & ANAGNOSTIDIS 2005) and Bulgaria (STOYNEVA-GÄRTNER ET AL. 2018) but this record is the first for Marikostinovo thermal complex. *Endangered* in the Red List of Bulgarian microalgae [EN - A4 B3 C4 D3 E1 F4 G4 T23]. Up-to-now found by LM in both cultured (1) and fixed (2) samples.

Family Oscillatoriaceae

***Lyngbya aestuarii* Liebman ex Gomont 1892:** 152, nom. inval. - Filaments 12-13 µm, sheaths structured in layers, violet-brownish, trichomes 9-10 µm wide. The apical cell more or less truncate with a thickened cell wall (**Fig. 39**). According to KOMÁREK & ANAGNOSTIDIS (2005, p. 621) the species is “marine and halophilic...data from fresh and thermal springs must be revised... Possibly collective species...” Earlier GOLLERBAKH ET AL. (1953, p. 543) wrote that it is distributed “...also in thermal springs”. The morphology of this species is quite similar to *L. thermalis*, but the general difference is in the apical cells. Therefore, due to the finding of truncate apical cells with thickened cell walls, we refer our material to *L. aestuarii*. This species is enlisted in Bulgarian Algal Flora (VODENICHAROV ET AL. 1971) with a text showing the occurrence in thermal springs, but without pointing the exact localities. Because of this, we included this species in the Checklist of Bulgarian thermal algae (STOYNEVA-GÄRTNER ET AL. 2018) but it is possible that the present finding in Marikostinovo is the first real confirmation for the occurrence of the species in Bulgarian thermal springs. According to GUIRY & GUIRY (2019) *L. aestuarii* was found in Europe (Britain, Czech Republic, Georgia, Portugal, Romania, Russia, Slovakia, Spain, Turkey), South America, Asia, Australia and New Zealand. Up-to-now found in fixed samples (1). Representatives of the genus *Lyngbya* are known as microcystin producers (CATHERINE ET AL. 2013).

***Lyngbya thermalis* Kützing ex Gomont 1892:** 152, nom. inval. – Filaments 12-13 µm, trichomes 9-10 µm wide. In one of the cultures, the color of sheaths varied from colorless to yellow (**Fig. 40**). The species is known from thermal waters, at the margins of the springs or in their vicinity on warm moistened soil and is spread in Europe (Czech Republic, Greece, Hungary, Italy and possibly in Slovakia) - KOMÁREK & ANAGNOSTIDIS 2005; GUIRY & GUIRY 2019). The species is known from thermal springs of Bulgaria (STOYNEVA-GÄRTNER ET AL. 2018) but the present record is the first for Marikostinovo thermal complex. Up-to-now found by LM in both cultured (1) and fixed samples (2). Representatives of the genus *Lyngbya* are known as microcystin producers (CATHERINE ET AL. 2013).

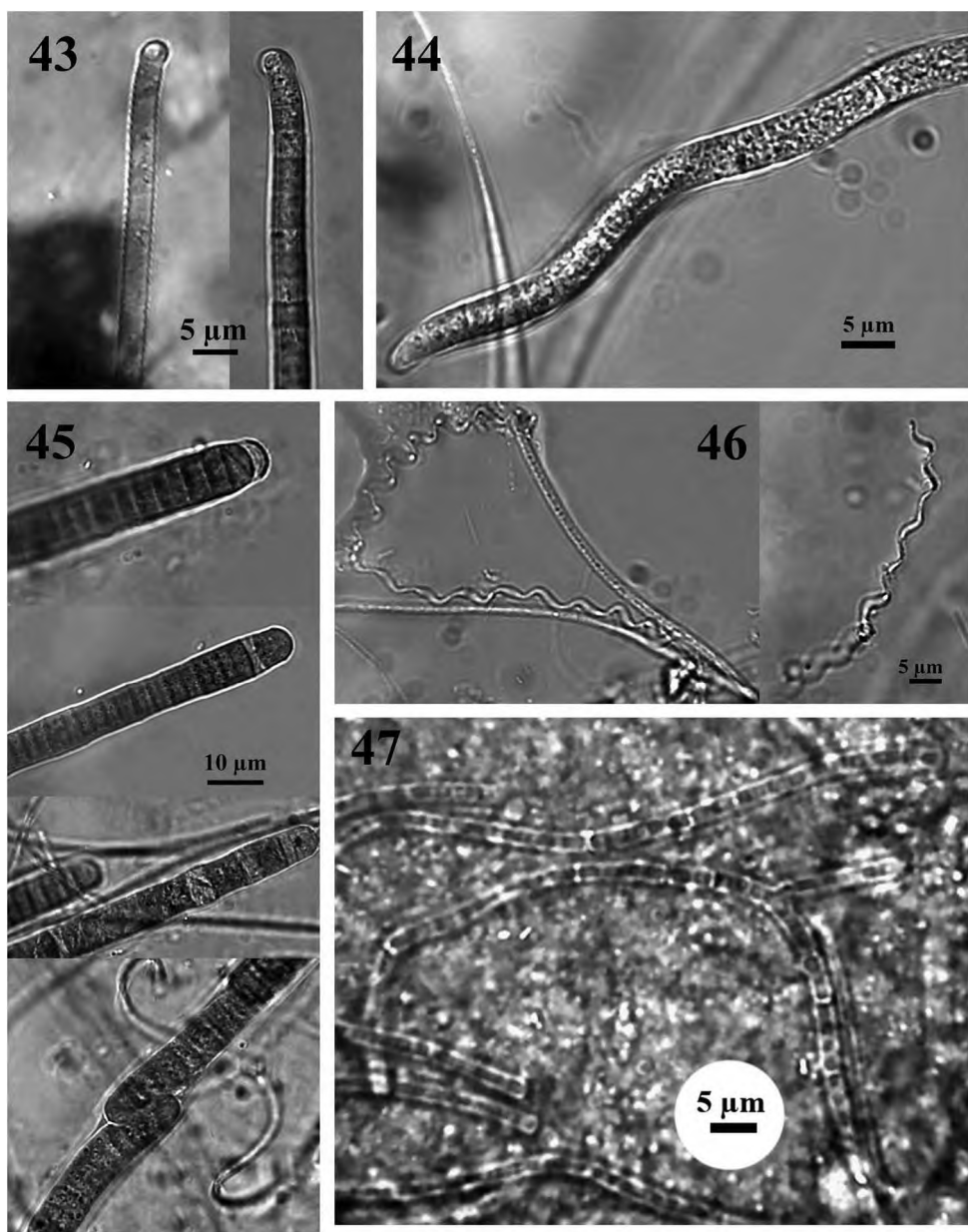
***Oscillatoria princeps* Vaucher ex Gomont 1892** – Trichomes dark blue-green, (15)16-17(20) µm wide, not constricted, very long, with typical rounded,

hemispherical, depressed-hemispherical or truncate apical cells and short discoid cells with granular content in the main trichome (**Fig. 41**). According to KOMÁREK & ANAGNOSTIDIS (2005, p. 590-592) this species is mainly freshwater benthic “and possibly (?) from thermal springs (mostly at lower temperatures....) ...distributed worldwide, perhaps cosmopolitan. ...An extremely variable and collective species.” It is ubiquitous in freshwaters of the world (GUIRY & GUIRY 2019). *O. princeps* is known from thermal springs of Bulgaria, including Marikostinovo and is wide spread in the whole country (STOYNEVA-GÄRTNER ET AL. 2018). The representatives found resemble *Lyngbya anomala* (Rao) Anagnostidis 2001, which has very short cells and flattened, bluntly rounded apical cells, and has been recorded from different biotopes, including thermal springs in Himalaya (KOMÁREK & ANAGNOSTIDIS 2005). The difference between our material and *L. anomala* is in the much narrower trichomes of the last species (-8-10(10.5) µm wide). Up-to-now found by LM in fixed samples (1). Representatives of the genus *Oscillatoria* were pointed as microcystin and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017).

***Oscillatoria sancta* Kützinger ex Gomont 1892** – Trichomes straight, blue-green, slightly constricted, 8 µm wide with cells distinctly shorter than wide, not granulated at the transverse walls but with some scattered solitary granules, apical cells rounded with very distinctly thickened outer cell wall (**Fig. 42**). This is a “possibly collective species”, which possibly occurs “also in thermal springs, especially at lower temperatures” (KOMÁREK & ANAGNOSTIDIS 2005, pp. 593-594), broadly distributed (GUIRY & GUIRY 2019). The species was not recorded in Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) and in Bulgaria so far. Up-to-now found by LM in fixed samples (2). Representatives of the genus *Oscillatoria* were pointed as microcystin and anatoxin A producers (CATHERINE ET AL. 2013; BERNARD ET AL. 2017).

***Phormidium chalybeum* (Mertens ex Gomont) Anagnostidis & Komárek 1988** - Trichomes mostly straight, blue-green, 5-(7) µm wide, cells 1.5-2-2.5 shorter than wide, apical cells widely rounded and typically hooked, without calyptra (**Fig. 43**). The species is freshwater with broad distribution (GUIRY & GUIRY 2019) but according to KOMÁREK & ANAGNOSTIDIS (2005) the identity of populations from thermal springs must be revised. As *Oscillatoria chalybea* Mertens ex Gomont 1892 it was recorded from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018), but the present record is the first for Marikostinovo thermal complex and, most probably, the first real record from Bulgarian thermal waters. Up-to-now found by LM in both fixed (1) and cultured samples (1). Representatives of *Phormidium* are known as producing anatoxin A, homoanatoxin A, microcystins and nodularins (CATHERINE ET AL. 2013).

***Phormidium lucidum* Kützinger ex Gomont 1892** – Trichomes curved, blue-green, 5 µm wide, cells 2-2.5 shorter than wide, apical cells with a conical calyptra,



Figs. 43-47. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **43** - *Phormidium chalybeum* (Mertens ex Gomont) Anagnostidis & Komárek 1988; **44** - *Phormidium lucidum* Kützinger ex Gomont 1892; **45** - *Pseudophormidium* sp.; **46** - *Spirulina magnifica* (J. J. Copeland) Anagnostidis 2001; **47** - *Mastigocladus laminosus* Cohn ex Kirchner 1898.

mucilage sheaths thin (**Fig. 44**). The species is known mainly from thermal springs, growing on wetted mud along the margin or on walls exposed to steam (KOMÁREK & ANAGNOSTIDIS 2005). The same authors pointed the need to revise the identity of populations from different localities and habitats (especially from cold waters) and therefore the broad distribution shown by GUIRY & GUIRY (2019) is not represented here in details. The species is not known from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018), and, according to our knowledge, has not been reported from Bulgaria so far (STOYNEVA 2014). Up-to-now found by LM in fixed samples (1). Representatives of *Phormidium* are known as producing anatoxin A, homoanatoxin A, microcystins and nodularins (CATHERINE ET AL. 2013).

***Pseudophormidium* sp.** – Filaments with firm, but very thin and difficultly visible sheaths, 7.5-11 µm wide; trichomes in diverse colors – from blue-green, yellowish-green to purple-reddish, 7-10 µm wide, cells 2-2.5 times shorter than wide, apical cell rounded, without calyptra although after release of the uppermost hormogonia, initially the necridic band remnant looks similar to calyptra; reproduction through hormogonia with formation of necridic cells and bands (**Fig. 45**). The species strongly resembles *Lyngbya thermalis* Kützing ex Gomont 1892, but initial false branching was observed (**Fig. 45**). The representatives of this genus, according to our knowledge, have not been recorded in thermal springs so far and have not been reported for Bulgaria. The material found in Marikostinovo, most probably, belongs to a new species, but this can be proved by further more detailed investigations. Up-to-now found by LM in fixed samples (1).

Order Spirulinales

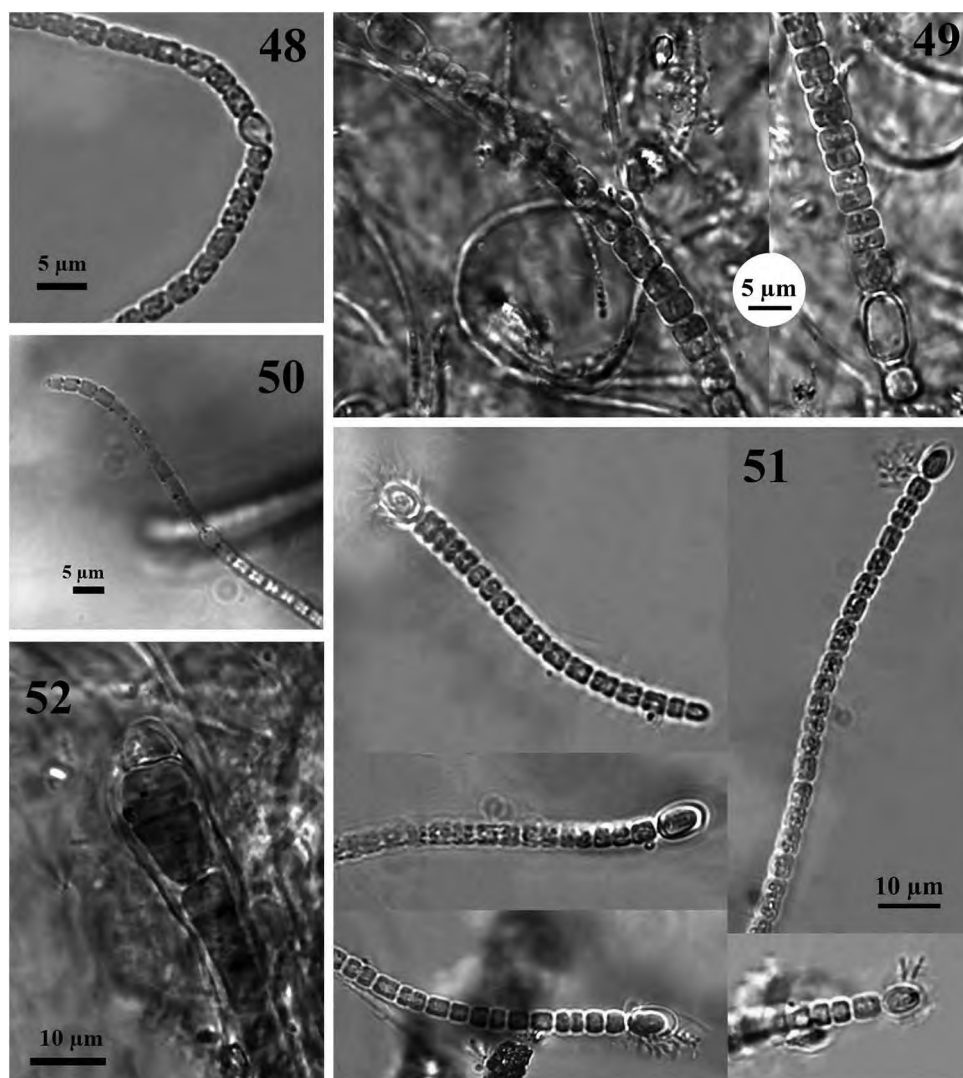
Family Spirulinaceae

***Spirulina magnifica* (J. J. Copeland) Anagnostidis 2001** – Trichomes loosely spirally coiled, pale blue-green, 0.8-1-1.2 µm wide (**Fig. 46**). The species was described as widely spread in Yellowstone National Park, at temperatures from 16.9 to 57°C, and afterward found in thermal springs of Nigrita (Greece), at temperature 53.6°C (KOMÁREK & ANAGNOSTIDIS 2005) and in Arkanzas (GUIRY & GUIRY 2019). It was not recorded from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018), and, according to our knowledge, also from Bulgaria so far. Up-to-now found by LM in fixed samples (2).

Order Nostocales

Family Hapalosiphonaceae

***Mastigocladus laminosus* Cohn ex Kirchner 1898** – Trichomes bright blue-green to dark green, with single true branches 2.5-3 µm wide and cylindrical to barrel-shaped cells. The material weekly developed in cultures (1) after cultivation at room temperature, and some empty filaments occurred (**Fig. 47**). In a formalin sample, cells of the trichome were darker, 5 µm in diameter, resembling cells of *Anabaena* s.l. The species is considered by KOMÁREK



Figs. 48-52. Microphotographs of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **48** - *Anabaena* sp. ster. 1; **49** - *Anabaena* sp. ster. 2; **50** - *Anabaena* sp. ster. 3; **51** - *Cylindrospermum* sp.; **52** - *Calothrix thermalis* Hansgirg ex Bornet & Flahault 1886.

(2013) as cosmopolitan but known only from thermal waters (usually in 37-55°C), and the same characteristic is given in ALGAEbase (Guiry & Guiry 2019). Data from Bulgaria are available (see comments in Stoyneva-Gärtner et al. 2018), but the present record is first for Marikostinovo thermal complex. Found by LM in both fixed (1) and cultured samples (1) from the same site,

with rarely visible heterocytes. The development in slimy tufts over the mud, makes microscopical observations very difficult and leads to disintegration of filaments, when the material is smashed under the cover glass.

Family Nostocaceae

***Anabaena* sp. ster. 1** – Trichomes blue-green, constricted, cells almost cylindrical, (2.5)-3 μm wide), without gas vesicles; heterocytes (4 μm) oval, intercalary (**Fig. 48**). The lack of akinetes does not allow species identification and the name *Anabaena* is used in its broadest sense. Species from this genus have not been recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1). Representatives of *Anabaena* are known as producing apoptogen toxin (CATHERINE ET AL. 2013).

***Anabaena* sp. ster. 2** – Trichomes blue-green, constricted, cells almost cylindrical, 3-3.5(4) μm wide, 2-(4) μm long, without gas vesicles; heterocyte (5x9 μm) oval, intercalary (**Fig. 49**). The lack of akinetes does not allow species identification and the name *Anabaena* is used in its broadest sense. Species from this genus have not been recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1). Representatives of *Anabaena* are known as producing apoptogen toxin (CATHERINE ET AL. 2013).

***Anabaena* sp. st. 3** – Trichomes (3 μm wide) strongly resemble *Komvophoron* (originally *Pseudanabaena*) with hyaline bridges between the cells but clearly differ by presence of heterocytes (**Fig. 50**). According to KOMÁREK & ANAGNOSTIDIS (2005, p. 334) the species with heterocytes in concept of previous authors clearly “belong to benthic *Anabaena* species without akinetes”. Therefore, here the name *Anabaena* is used in its broadest sense. As it was mentioned above, species from this genus have not been recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1). Representatives of *Anabaena* are known as producing apoptogen toxin (CATHERINE ET AL. 2013).

***Cylindrospermum* sp.** – Trichomes blue-green, clearly constricted, 3-4 μm wide; cells isodiametric or slightly longer than wide; with terminal ellipsoidal heterocytes 5 μm wide (often with bacteria on the surface) and 7 μm long; without akinetes (**Fig. 51**). The lack of akinetes does not allow species identification. Species from this genus have not been recorded from Bulgarian thermal springs (STOYNEVA-GÄRTNER ET AL. 2018). The genus is not typical for thermal waters – only one species from such habitats was described (*Cylindrospermum thermophilum* Schwabe 1936). It is similar in some of its morphological characteristics to our material, but according to KOMÁREK (2013, p. 897) this species “very probably is related to *Trichormus* complex”. Although rarely, *Cylindrospermum stagnale* (Kützing) ex Bornet et Flahault 1888 was also reported from thermal waters (COPELAND 1936; FRÉMY & RAYSS 1938) but in the opinion of KOMÁREK (2013) these records need revision.

In part, our material is close to the description of the soil *Cylindropermum gregarium* (Zakrzewski) Elenkin 1938. Up-to-now found by LM in fixed samples (2).

Family Rivulariaceae

Calothrix thermalis Hansgirg ex Bornet & Flahault 1886 – Trichomes found in a fixed sample were 10 µm wide at the basis and 6-7 µm at the middle, with a heterocyte 8 µm in diameter and ending at the opposite side with a hair-like protrusion (Fig. 52). Taxon with the same name was published for Bulgarian thermal waters, but needs revision (see comments in STOYNEVA-GÄRTNER ET AL. 2018). This species is known as attached to substrates in thermal waters and is considered cosmopolitan in thermal springs (KOMÁREK 2013). In ALGAEBASE (2019) it is indicated for European countries Greece, Czech Republic and Slovakia. For records in Bulgarian thermal waters see the comments in STOYNEVA-GÄRTNER ET AL. (2018). *Endangered* in the Red List of Bulgarian microalgae [EN - A4 B3 C4 D3 E1 F4 G4 T23]. Up-to-now found by LM in fixed samples (1). Microcystin producing species (CATHERINE ET AL. 2013).

Calothrix sp. – Filaments with heterocytes but without akinetes were observed. Trichomes at the basis were 5 µm wide and 3-2 µm at the upper part, where no hair-like protrusion was seen (Fig. 53). Although mucilage sheath was not seen, this material is close to the description provided by LUKAVSKY ET AL. (2011, p. 8) for Pancharevo thermal spring under the name *Calothrix thermalis* Hansgirg ex Bornet & Flahault 1886. Future more detailed investigations with a polyphasic approach can reveal the similarity or differences between these two algae. Up-to-now found by LM in fixed samples (1). Representatives of *Calothrix* are known as producing nodularins (CATHERINE ET AL. 2013), and one species (*C. convolvicola* Agardh ex Bornet et Flahault 1886) was shown as possible causative agent for seaweed dermatitis (STOYNEVA ET AL. 2015).

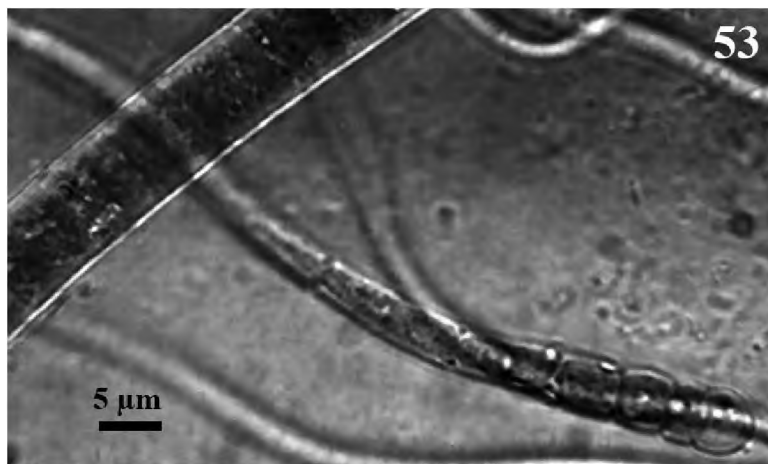


Fig. 53. Microphotograph of the alga from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: *Calothrix* sp.

Family Scytonemataceae

Scytonema sp. – Identification at species level was impossible because of finding of small fragments with broad yellow sheaths and akinetes only; the generic name is also used in a broad sense. The representatives of *Scytonema*/*Heteroscytonema* were reported from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018) but this is the first record from Marikostinovo thermal complex. Up-to-now found by LM in fixed samples (1). Representatives of *Scytonema* are known as producing saxitoxins and gonyotoxin (CATHERINE ET AL. 2013).

DIVISION CHLOROPHYTA

CLASS CHLOROPHYCEAE

Order Sphaeropleales

Family Microsporaceae

Microspora sp. – Some unbranched filaments were found in the fixed samples with partially shrunk plastid, which makes generic identification disputable and species identification impossible. Representatives of this genus were not recorded from Bulgarian thermal waters (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1).

DIVISION STREPTOPHYTA

CLASS CHAROPHYCEAE

Order Zygnemales

Family Zygnemataceae

Spirogyra sp. st. – cells 41 µm wide, with one chloroplast. The representatives of the genus were often found in sterile forms in different thermal springs of Bulgaria, with *Spirogyra jugalis* (Dillwyn) Kützing 1845 recorded in Marikostinovo (STOYNEVA-GÄRTNER ET AL. 2018). Up-to-now found by LM in fixed samples (1).

DISCUSSION

During the study, totally 54 algal taxa from 27 genera of three divisions (one of blue-green prokaryotic algae/cyanobacteria and two from the eukaryotic green algal evolutionary line) were identified. There was a difference in species numbers between the cultures (14 taxa) and fixed field samples (52 taxa) with only seven species found in both types of samples. The significantly lower number of taxa in the cultures is easily explainable with our recent facilities of culturing at room temperatures in ACUS (UZUNOV ET AL. 2012) and agrees with the results of STRUNECKÝ ET AL. (2018). Although the identification of the collected material by LM is still in progress, we would like to note that the total number of 54 species (except diatoms) in Marikostinovo during this study exceeds the 11 species from the same groups reported earlier (for details see STOYNEVA-GÄRTNER ET AL. 2018). The detected differences in the current species composition with the biodiversity

reported by previous authors (PETKOFF 1925; GEORGIEV 1948) with only five common taxa could be explained by the developed taxonomy of cyanoprokaryotes with many new taxa described and improved microscopy techniques. Most probably, they also reflect the changes in the studied habitats during the last 70 years caused by the increased anthropogenic impact.

Among the currently identified species, four were threatened and thus conservationally important: two *Endangered* and two *Near Threatened* in the Red List of Bulgarian microalgae (STOYNEVA-GÄRTNER ET AL. 2016). According to the results from this study, 15 taxa are possibly new for science, 3 genera and 17 species are new for Bulgaria, 48 taxa from 22 genera are new for the Marikostinovo thermal complex, and 35 species from 9 genera are new for the thermal flora of Bulgaria. In this way, the species composition of algae in Bulgarian thermal waters has been accomplished and their total number increased from 206 to 241: Cyanoprokaryota (118), Rhodophyta (4), Ochrophyta (44: 3 - Tribophyceae, 40 - Bacillariophyceae), Chlorophyta (33) and Streptophyta (44). Thus, the current algal biodiversity of Marikostinovo comprises 29% of the total algal biodiversity in Bulgarian thermal waters recorded during more than one century, and 35% of this diversity when diatoms (which are out of the scope of this study) are not considered. More, the order of Bulgarian thermal complexes according to their algal biodiversity, evaluated by STOYNEVA-GÄRTNER ET AL. (2018) has been changed and Marikostinovo thermal complex occupies the first place with a total of 70 taxa.

All these results, obtained from the processing of eight samples only, clearly show the rich biodiversity of Marikostinovo thermal complex with presence of conservationally important species despite the strong anthropogenic pressure in the region. Moreover, these data indicate inevitably the great potential of using of a drone for choosing of sampling sites during field studies of thermal habitats, as was already shown for other freshwater habitats by STOYNEVA-GÄRTNER ET AL. (2019). The role of a drone application for speeding-up the process of field work, with saving time for running through the whole investigated area and for seeing sites (or points, or mats) invisible from the shores, has to be outlined. Since the importance of biodiversity data for monitoring and relevant management decisions is well-known, the application of a drone could be also recommended in future for targeted monitoring and management studies, which are particularly important in the cases of threatened species and vulnerable habitat types, among which are the Bulgarian thermal springs (STOYNEVA & GÄRTNER 2004; STOYNEVA 2014; BISERKOV ET AL. 2015).

The results on the algal biodiversity in Marikostinovo showed that similarly to all thermal waters in the world, the most rich and abundant group in the whole complex was Cyanoprokaryota/Cyanobacteria (e.g. ROUND 1981). This peculiar and important prokaryotic algal lineage contains many hazardous for human life species due to production of peculiar toxins, named cyanotoxins (e.g. MERILUOTTO ET AL. 2017). Therefore, considering the increasing role of thermal waters and SPA

centers in the daily life of modern Bulgarian society, we compared our species list with current data on cyanotoxin producers. The results from this checking revealed that 40 species (or 74% of all algae found) could be outlined as potential producers (separately or in combination) of anatoxin A, apoptogen toxin, gonyotoxin, homoanatoxin, microcystins, nodularins and saxitoxins. This inevitably requires further purposive investigations of the extremophilic thermal algae of Bulgarian thermo-mineral waters in this health-care aspect.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the paper preparation.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

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MACROFUNGI AND LICHEN-FORMING FUNGI ON THE TERRITORY OF IBUR RESERVE, RILA NATIONAL PARK (BULGARIA)

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Abstract. Data on the species diversity of macrofungi and lichen-forming fungi recorded in Ibur Reserve (Rila Mts) are presented in the paper. All taxa belong to Ascomycota and Basidiomycota. One species (*Russula sphagnophila*) is new for Bulgaria. Four macrofungi (*Galerina paludosa*, *Lactarius sphagneti*, *Limacellopsis guttata* and *Mitrula paludosa*) are of high conservation value, included in the Red List of fungi of Bulgaria. The lichen *Nephromopsis chlorophylla* is a rare species, known as an old-growth forest indicator.

Key words: *Agaricomycotina*, ascomycetes, basidiomycetes, fungal conservation, *Pezizomycotina*, Rila Mts

INTRODUCTION

This work reports the preliminary data on the species diversity of macrofungi and lichen-forming fungi in the Reserve Ibur (known also as Ibar) on Rila Mts. A list of 384 macrofungi from the eastern part of Rila Mts was published by HINKOVA

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(1958). However, there is no information about the fungal diversity of the reserve. Later, data about conservationally important fungi of the region were presented by GYOSHEVA ET AL. (2017). The Catalogue of the lichenized and lichenicolous fungi in Bulgaria (MAYRHOEFER ET AL. 2005) includes a total number of 388 taxa, known from Rila Mts. To our knowledge, there is no information available in the previous lichenological studies about the lichen-forming fungi from Ibur Reserve. The only exception is the report of tree lungwort found on maple along the Kraina River (СТОЙКОВ 2018). Therefore, the information concerning macrofungi and lichens presented in this paper is the first report for the Reserve Ibur.

MATERIAL AND METHODS

The reserve Ibur is situated on the northern slopes of Rila Mts (Western Bulgaria) on the territory of Rila National Park. It was declared as such in 1985, with a total area of 2263.2 ha dominated by forests. Among them the coniferous forests prevail (75% of the whole area). Typical for the reserve are *Picea abies* (L.) Karst. forests, code 34G3 and *Pinus sylvestris* L. forests, code 35G3 (Natura 2000 habitats). The communities of *Fagus sylvatica* L. dominated in the broadleaf forests. Scrublands of *Pinus mugo* Turra cover large areas in the subalpine belt.

Most of the materials have been collected in the framework of the project for updating the Management Plan of the Rila National Park, conducted in 2015. During the field studies the transect methods were applied with main attention to the forest communities in the valley of the Kraina River and the coniferous bog woodlands in the locality *Shavaritoto Dere*. Most of the color photographs were taken in the field using Canon PS digital cameras. The threat status was determined following the Red List of fungi in Bulgaria (GYOSHEVA ET AL. 2006). Microscopic examinations of the lichen-forming fungi were done on the light microscope Boeco BM-180/T/SP LM. Cross-sections of thalli were prepared in tap-water, and lactophenol was added for better visualization of the asci and spores. Spot tests with C, K and Lugol's solution (after DOBSON 2011) were applied *ex situ*. The studied specimens of fungi (new for Bulgaria and those of conservation significance) are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (SOMF).

RESULTS AND DISCUSSION

Species diversity of macrofungi

Seventy-nine macrofungal species were recorded in the reserve with the greatest fungal diversity in the spruce and beech forests, and in peat habitats. The species found are enlisted alphabetically in phyla, subphyla, classes, orders and families.

In the list provided below, nine species belong to the division Ascomycota,

subdivision Pezizomycotyna (3 classes, 4 orders, 7 families, 8 genera), and 71 species belong to the division Basidiomycota, subdivision Agaricomycotina (2 classes, 11 orders, 32 families, 49 genera). Order Agaricales dominated by the number of taxa (37 species and 1 variety). The most abundant were the families Agaricaceae (9 species), Russulaceae (7 species), Amanitaceae (6 species), Cortinariaceae, Mycenaceae and Tricholomataceae (each with 5 species). The richest genera were *Amanita* Pers. and *Cortinarius* (Pers.) Gray (each with 5 species), followed by *Mycena* (Pers.) Roussel and *Russula* Pers. (each with 4 species). One species – *Russula sphagnophila* Kauffmann (**Plate I, Figs 1-2**) is a new macrofungus for Bulgarian mycota, and its brief description is provided below. Three rarely found species in Bulgaria: *Cortinarius coniferarum* (M. M. Moser) Moëgne-Locc. & Reumaux, *Gymnopus aquosus* (Bull.) Antonín & Noordel. and *Xylaria longipes* Nitschke were collected in the valley of the Kraina River in 2015.

PHYLUM ASCOMYCOTA

SUBPHYLLUM PEZIZOMYCOTINA

CLASS LEOTIOMYCETES

Order Helotiales

Family Chlorociboriaceae: *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C. S. Ramamurthy, Korf & L. R. Batra

Family Cudoniaceae: *Cudonia circinans* (Pers. : Fr.) Fr.

Family Helotiaceae: *Bisporella citrina* (Batch : Fr.) Korf. & S. E. Carp., *Mitrula paludosa* Fr. : Fr.

CLASS PEZIZOMYCETES

Order Pezizales

Family Discinaceae: *Gyromitra esculenta* (Pers. : Fr.) Fr.

Family Helvellaceae: *Helvella lacunosa* Afzel. : Fr.

CLASS SORDARIOMYCETES

Order Xylariales

Family Hypoxylaceae: *Hypoxylon fuscum* (Pers. : Fr.) Fr.

Family Xylariaceae: *Xylaria hypoxylon* (L. : Fr.) Grev., *X. longipes* Nitschke (**Plate I, Fig. 3**)

PHYLUM BASIDIOMYCOTA

SUBPHYLLUM AGARICOMYCOTINA

CLASS AGARICOMYCETES

Order Agaricales

Family Agaricaceae: *Agaricus augustus* Fr., *A. silvaticus* Schaeff., *A. xanthodermus* Genev., *Chlorophyllum rhacodes* (Vittad.) Vellinga, *Cystoderma carcharias* (Pers.) Fayod, *Lycoperdon perlatum* Pers. : Pers., *L. excipuliforme* (Scop. : Pers.) Perdeck, *Apioperdon pyriforme* (Schaeff. : Pers.) Vizzini, *Macrolepiota procera* (Scop. : Fr.) Singer

Family Amanitaceae: *Amanita excelsa* (Fr.) Bertill., *A. muscaria* (L. : Fr.) Lam.,



Plate I, Figs. 1-4: **1** - *Russula sphagnophila* – basidioma (pileus); **2** - *Russula sphagnophila* – basidioma (stipe and gills); **3** - *Xylaria longipes*– ascomata; **4** - *Galerina paludosa* – basidiomata.

A. rubescens Pers. : Fr., *A. submembranacea* (Bon) Gröger, *A. vaginata* (Bull. : Fr.) Lam., *Limacellopsis guttata* (Pers. : Fr.) Zhu L. Yang, Q. Cai & Y. Y. Cui

Family Cortinariaceae: *Cortinarius bivelus* (Fr. : Fr.) Fr., *C. brunneus* (Pers. : Fr.) Fr., *C. coniferarum* (M. M. Moser) Moënne-Loec. & Reumaux, *C. multiformis* (Fr.) Fr., *C. sanguineus* (Wulfen. : Fr.) Gray

Family Entolomataceae: *Entoloma vernalis* S. Lundell

Family Hydnangiaceae: *Laccaria amethystina* Cooke

Family Hygrophoraceae: *Hygrophorus piceae* Kühner

Family Marasmiaceae: *Mycetinis alliaceus* (Jacq. : Fr.) Earle ex A. W. Wilson & Desjardin

Family Mycenaceae: *Hemimycena lactea* (Pers. : Fr.) Singer, *Mycena epipterygia* (Scop. : Fr.) Gray, *M. pura* (Pers. : Fr.) P. Kumm., *Xeromphalina campanella* (Batsch : Fr.) Künner & Maire

Family Omphalotaceae: *Gymnopus androsaceus* (L.) Della Magg. & Trassin, *G. aquosus* (Bull. : Fr.) Antonín & Noordel., *Rhodocollybia butyracea* (Bull.

: Fr.) Lennox

Family Physalacriaceae: *Armillaria mellea* (Vahl. : Fr.) P. Kumm.

Family Hymenogastraceae: *Galerina paludosa* (Fr.) Kühner (**Plate I, Fig. 4**),
Hypholoma capnoides (Fr. : Fr.) P. Kumm.

Family Tricholomataceae: *Clitocybe ditopa* (Fr.) Gillet, *C. gibba* (Pers. : Fr.) P. Kumm., *C. odora* (Bull. : Fr.) P. Kumm., *Tricholoma saponaceum* (Fr. : Fr.) P. Kumm., *T. vaccinum* (Schaeff. : Fr.) P. Kumm.

Order Auriculariales

Family Auriculariaceae: *Exidia glandulosa* (Bull. : Fr.) Fr.

Order Boletales

Family Boletaceae: *Boletus edulis* Bull. : Fr., *Chalciporus piperatus* (Bull. : Fr.) Bataille, *Xerocomus subtomentosus* (L. : Fr.) Quél.

Family Gomphidiaceae: *Chroogomphus helveticus* (Singer) M. M. Moser, *C. rutilus* (Schaeff. : Fr.) O. K. Mill., *Gomphidius glutinosus* (Schaeff. : Fr.) Fr.

Family Suillaceae: *Suillus luteus* (L. : Fr.) Roussel

Order Cantharellales

Family Cantharellaceae: *Cantharellus cibarius* Fr. : Fr.

Family Hydniaceae: *Hydnum repandum* L. : Fr.

Order Gloeophyllales

Family Gloeophyllaceae: *Gloeophyllum abietinum* (Bull. : Fr.) P. Karst.

Order Gomphales

Family Gomphaceae: *Ramaria aurea* (Schaeff. : Fr.) Quél., *R. formosa* (Pers. : Fr.) Quél.

Order Hymenochaetales

Family Hymenochaetaceae: *Coltricia perennis* (L. : Fr.) Murrill

Family Repetobasidiaceae: *Rickenella fibula* (Bull. : Fr.) Raithelh.

Order Polyporales

Family Fomitopsisaceae: *Fomitopsis pinicola* (Sw. : Fr.) P. Karst.

Family Ganodermataceae: *Ganoderma applanatum* (Pers.) Pat.

Family Polyporaceae: *Polyporus leptcephalus* (Jacq. : Fr.) Fr., *Trametes hirsuta* (Wulfen : Fr.) Lloyd, *Trichaptum abietinum* (Pers. ex J. F. Gmel.) Ryvarden

Order Russulales

Family Albatrellaceae: *Albatrellus ovinus* (Schaeff. : Fr.) Kotl. & Pouzar

Family Bondarzewiaceae: *Heterobasidion annosum* (Fr. : Fr.) Bref.

Family Peniophoraceae: *Peniophora pini* (Schleich. ex DC.) Boidin

Family Russulaceae: *Lactarius badiosanguineus* Kühner & Romagn., *L. scrobiculatus* (Scop.) Fr., *L. sphagnetii* (Fr.) Neuhoff, *Russula delica* Fr., *R. densifolia* Secr. ex Gillet, *R. queletii* Fr., **R. sphagnophila* Kauffmann (**Plate I, Figs. 1-2**)

Order Thelephorales

Family Bankeraceae: *Sarcodon imbricatus* (L. : Fr.) P. Karst.

Family Thelephoraceae: *Thelephora palmata* (Scop.) Fr.

Class Dacrymycetes

Order Dacrymycetales

Family Dacrymycetaceae: *Calocera viscosa* (Pers. : Fr.) Fr

Description of the species, new for the Bulgarian mycota

Russula sphagnophila Kauffmann, Report Mich. Acad. Sci 11: 86 (1909) (**Plate I, Figs 1-2**).

Pileus was up to 4 cm in diameter, initially convex to plane, depressed in the center, pale-pinkish, pink-violaceous to ochraceous-pink, darker in the center, viscid, margin furrowed striate. Gills were distant, loosely attached to almost free, white to cream in colour. Stipe was to 4 cm long and 1 cm thick, cylindrical to slightly clavate, smooth, white, fragile. Context was whitish, very fragile. Smell was slightly fruity. Basidia were clavate, 4-spored. Basidiospores were 7.5-10×6-8.5 µm, broadly-ellipsoidal, ornamented, consisting of warts, which formed in places a partial reticulum. Cheilocystidia were fusiform. Pileipellis was with cylindrical to clavate, septate (one or two septa) pileocystidia.

Habitat: In peaty habitats and in moist spruce forests in mountains, among *Sphagnum* spp., solitary or in groups, summer to autumn. Mycorrhizal fungus, associated with birch and spruce (COURTECUISE & DUHEM 1995; KRIEGLSTEINER 2000; KRÄNZLIN 2005).

The examined specimen was found in the locality *Shavaritoto Dere*, in a bog coniferous forest (*P. abies* and *P. sylvestris*), solitary among peat mosses, at ca. 1652 m a.s.l., on 08.06.2015, leg. & det. M. GYOSHEVA (SOMF 30028).

Species of conservation importance

Four macrofungi with high conservation value, included in the Red List of fungi in Bulgaria have been recorded in Ibur Reserve so far: *Mitrula paludosa* Fr. – Critically Endangered (CR), *Galerina paludosa* (Fr.) Kühner – Endangered (EN), *Limacellopsis guttata* (Pers.) Zhu L. Yang, Q. Cai & Y. Y. Cui – Vulnerable (VU), and *Lactarius sphagneti* (Fr.) Neuhoff – Data Deficient (DD). *M. paludosa* and *G. paludosa* are included also in the Red Data Book of the Republic of Bulgaria (PEEV ET AL. 2015). All species were recorded in peat habitats on the territory of the reserve. Macrofungi with conservation value were found in other localities on the territory of Rila National Park as well, by the authors (GYOSHEVA ET AL. 2017).

Species diversity of lichen-forming fungi

Forty-three species of lichen-forming fungi were collected from the reserve area during field trips in the vicinity of the stream *Shavaritoto Dere* and close to the Kraina River, performed in June 2015. They belong to the division Ascomycota, subdivision Pezizomycotina, 3 classes (Arthoniomycetes, Coniocybomycetes and Lecanoromycetes), 4 subclasses, 10 orders, 20 families and 29 genera and are enlisted below in alphabetical order.

PHYLUM ASCOMYCOTA

SUBPHYLUM PEZIZOMYCOTINA

CLASS ARTHONIOMYCETES

SUBCLASS ARTHONIOMYCETIDAE

Order Arthoniales

Family Arthoniaceae: *Arthonia radiata* (Pers.) Ach. (**Plate II, Fig. 1**)

Family Chrysotrichaceae: *Chrysothrix chlorina* (L.) J. R. Laundon

Family Roccellaceae: *Alyxoria varia* (Pers.) Ertz & Tehler, s.l. (after ERTZ & TEHLER 2011). Note: Thallus growing on bark of old spruce, not surrounded by black prothallus. Species with positive reaction of the hymenium in Melcer's reagent. Asci are about $37\text{--}40 \times 16\text{--}18.5\ \mu\text{m}$, with 4-celled hyaline spores: $(17.5\text{--})\ 20\text{--}22.5 \times 5\text{--}7\text{--}(7.5)\ \mu\text{m}$, with second cell widened. (**Plate II, Fig. 2**)

CLASS CONIACYBOMYCETES

Order Coniocybales

Family Coniocybaceae: *Chaenotheca chrysocephala* (Turner ex Ach.) Th. Fr.

CLASS LECANOROMYCETES

Subclass Lecanoromycetidae

Order Candelariales

Family: Candelariaceae: *Candelariella aurella* (Hoffm.) Zahlbr., *C. vitellina* (Hoffm.) Müll. Arg.

Order Lecanorales

Family Cladoniaceae: *Cladonia coccifera* (L.) Willd., *C. coniocraea* (Flörke) Spreng., *C. fimbriata* (L.) Fr., *C. macilenta* Hoffm., s.l.

Family Lecanoraceae: *Lecanora albella* (Pers.) Ach., *Lecidella elaeochroma* (Ach.) M. Choisy

Family Parmeliaceae: *Bryoria capillaris* (Ach.) Brodo & D. Hawksw., s.l., *B. fuscescens* (Gyeln.) Brodo & D. Hawksw., *Evernia divaricata* (L.) Ach., *E. prunastri* (L.) Ach., *Hypogymnia physodes* (L.) Nyl., *H. tubulosa* (L.) Hue, *Parmelia saxatilis* (L.) Ach., *P. sulcata* Taylor, *Pseudevernia furfuracea* (L.) Zopf, *Melanohalea elegantula* (Zahlbr.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch, *Nephromopsis chlorophylla* (Willd.) Divakar, A. Crespo & Lumbsch (after DIVAKAR ET AL. 2017; **Plate II, Fig. 3**), *Usnea florida* (L.) F.H. Wigg. emend. Clerc, s.l., *Vulpicida pinastri* (Scop.) J.-Mattson & M.J. Lai, *Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch

Family Ramalinaceae: *Ramalina farinacea* (L.) Ach.

Family Stereocaulaceae: *Lepraria incana* (L.) Ach., s.l.

Order Lecideales

Family Lecideaceae: *Porpidia macrocarpa* (DC.) Hertel & A. J. Schwab

Order Peltigerales

Family Lobariaceae: *Lobaria pulmonaria* (L.) Hoffm.

Family Peltigeraceae: *Peltigera aphthosa* (L.) Willd., *P. canina* (L.) Willd.

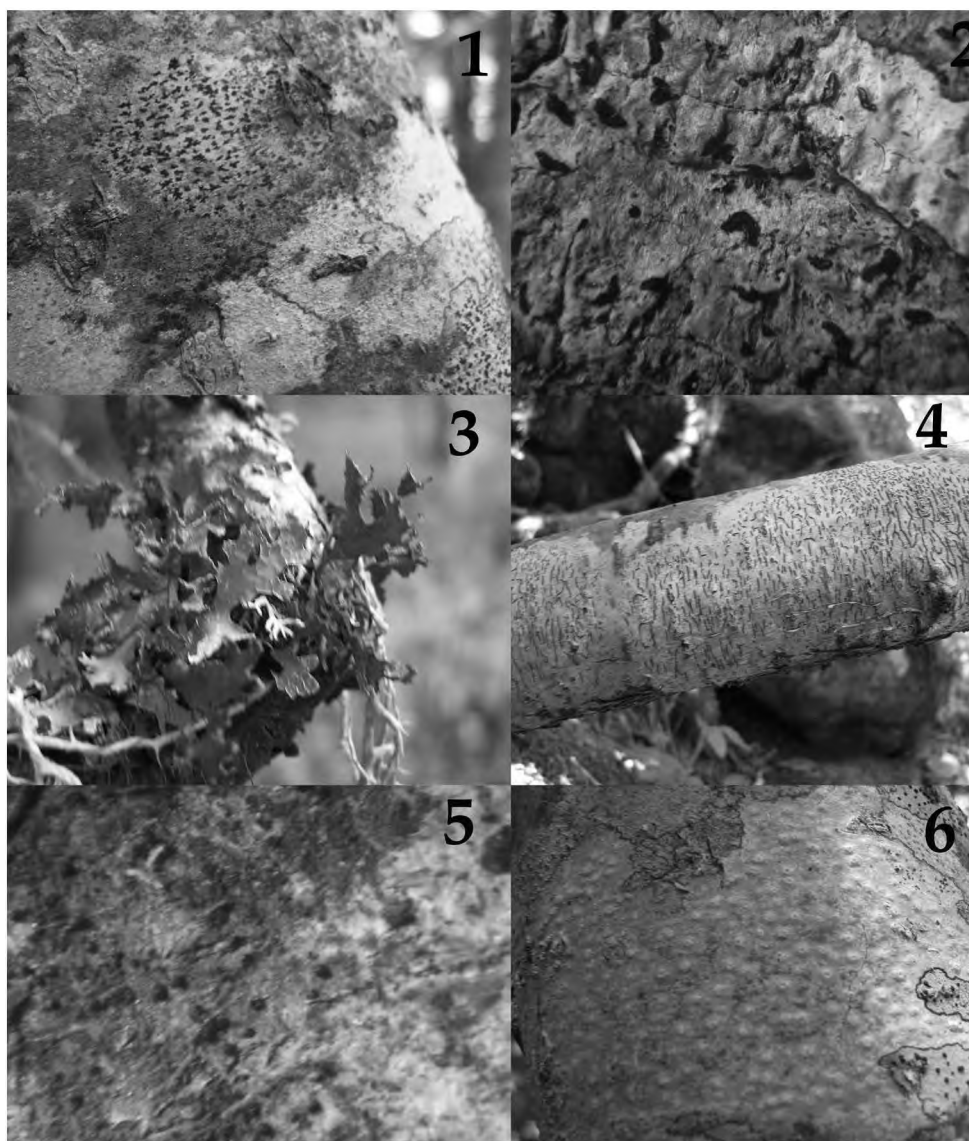


Plate II, Figs. 1-6: **1** - *Arthonia radiata* – thalli; **2** - *Alyxoria varia*, s.l. – thallus, *ex situ*; **3** - *Nephromopsis chlorophylla* – thallus, along with *Parmelia sulcata* and *Evernia divaricata*; **4** - *Graphis scripta* – thallus; **5** - *Pseudosagedia aenea* – thalli, *ex situ*; **6** - *Pertusaria leioplaca* – thallus.

Order Rhizocarpales

Family Rhizocarpaceae: *Rhizocarpon geographicum* (L.) DC., *R. hochstetteri* (Körb.) Vain.

SUBCLASS OSTROPOMYCETIDAE

Order Ostropales

Family Graphidaceae: *Graphis scripta* (L.) Ach. (Plate II, Fig. 4)

Family Phlyctidaceae: *Phlyctis argena* (Spreng.) Flot. s.l.

Family Porinaceae: *Pseudosagedia aenea* (Wallr.) Hafellner & Kalb. Note: Thallus thin, smooth, green-brown, without prothallus, on bark of beech. Perithecia black, up to 200 µm diam. Ascospores hyaline, 3-septate, mostly 11-13.5(-15) × 3.5-4 µm. (Plate II, Fig. 5)

Order Pertusariales

Family Ochrolechiaceae: *Ochrolechia alboflavescens* (Wulfen) Zahlbr., *O. parella* (L.) A. Massal.

Family Pertusariaceae: *Pertusaria lactea* (L.) Arnold, *P. leioplaca* DC. (Plate II, Fig. 6)

SUBCLASS UMBILICARIOMYCETIDAE

Order Umbilicariales

Family Umbilicariaceae: *Umbilicaria cylindrica* (L.) Delise ex Duby, *U. deusta* (L.) Baumg.

The richest order was Lecanorales (5 families, 15 genera, 22 species), with most species belonging to the families Parmeliaceae (10 genera, 14 species) and Cladoniaceae (1 genus, 4 species). *Pseudosagedia aenea* (Wallr.) Hafellner & Kalb. (Porinaceae), *Alyxoria varia* (Pers.) Ertz & Tehler, s.l. (Rocellaceae) and *Phlyctis argena* (Spreng.) Flot., s.l. (Phlyctidaceae) are new records for Rila Mts.

The lung lichen, *Lobaria pulmonaria* (L.) Hoffm. was found by STOYKOV (2018) on a bark of maple along the Kraina River. *Arthonia radiata* (Pers.) Ach., *Graphis scripta* (L.) Ach., *Pertusaria leioplaca* DC., *Phlyctis argena* (Spreng.) Flot., s.l. and *Pseudosagedia aenea* were recorded on twigs or trunks of smooth-bark trees in the region of the Kraina River. *P. aenea*, along with *Lecidella elaeochroma* (Ach.) M. Choisy, grows on bark of old *Fagus sylvatica*.

Graphis scripta, recorded during this study on the twigs of *Corylus avellana* L. near the Kraina River, is known as an important component of epiphytic climax lichen communities and as a typical inhabitant of old beech forests (RAVERA ET AL. 2010). From Bulgaria it was known in Belasitsa Mts (STOYKOV 2014), while in the Eastern Forebalkan (Northern Bulgaria), *G. scripta* occurred on the bark of beeches, with *Acrocordia gemmata* (Ach.) A. Massal (STOYKOV, unpubl.). *Phlyctis argena* found during this study, was recorded earlier (2013) on the bark of an old maple tree in Central Stara Planina Mts (Nature Park *Balgarka*, above the hut *Bulgarka*, STOYKOV, unpubl.).

The powdered ruffle lichen *Nephromopsis chlorophylla* (Willd.) Divakar, A. Crespo & Lumbsch is an epiphyte, known as an old-growth forest indicator (ELLIS ET AL. 2015). It was reported as a lichen of conservation value in the North European countries - Scotland (GRAHAM 2012), Poland (CZARNOTA 2012). In Central Europe (Czech Republic, Hungary and Slovakia) it is considered as infrequent and

rare species from old oak forests, with occurrence in the studied areas below 20% (SVOBODA ET AL. 2011). During this study, *N. chlorophylla* was recorded only once on a twig of old spruce only in the locality *Shavaritoto Dere*.

CONCLUSION

The data presented in the paper, show that the Reserve Ibur contains rich species diversity of ascomycetous and basidiomycetous macrofungi, and lichen-forming fungi, some of which are of conservational importance or are good ecological indicators. Therefore, it is necessary to ensure regular systematic studies, including monitoring and mapping, especially of the conservationally significant fungi.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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ГОДИШНИК НА СОФИЙСКИЯ УНИВЕРСИТЕТ „СВ. КЛИМЕНТ ОХРИДСКИ“

БИОЛОГИЧЕСКИ ФАКУЛТЕТ

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PILOT INVESTIGATIONS OF LICHENS IN 20 BULGARIAN PROTECTED TERRITORIES ALONG THE BLACK SEA COAST, ALONG THE DANUBE RIVER, AND IN THE MOUNTAINS STRANDZHA, STARA PLANINA, SREDNA GORA AND VITOSHA. IMPLICATIONS FOR SPECIES CONSERVATION.

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Abstract. The studies of lichenized fungi (called hereafter with shorter common term *lichens*) in Bulgaria date back more than a century. However, according to the available literature, so far there are no specialized lichen studies in most Bulgarian protected areas. At the same time, lichens are included as a mandatory element of biodiversity in the templates of modern management plans of the Ministry of Environment and Waters of the country. Therefore, the purpose of this article is to give information on lichen species and their conservation significance in 20 Bulgarian protected natural areas, collected for the preparation of their first or updated management plans, with proposals for their conservation assessment and for considering the ecosystem services they provide in the harboring ecosystems. During the study totally 84 lichen species from 46 genera, 21 families, 8 orders and 2 classes were found. Their distribution by numbers and relevant ecological groups in each protected area with a complete species list is provided below. As it could be expected, the number of species varied strongly in different protected territories, depending on their areas and main subject of conservation. For almost

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all studied protected areas, the data provided in the present paper are practically the first ones. Exceptions are the Reserves *Bogdan*, *Bistrishko Branishte* and *Torfeno Branishte*. In addition, the threats on the species *Lobaria pulmonaria* and *Cetraria islandica* are discussed with underlining the need for their protection.

Key words: conservation, managed reserve, protected locality, reserve, threatened species

INTRODUCTION

The studies of lichenized fungi (called hereafter with shorter common term *lichens*) in Bulgaria date back more than a century (KAZANDJIEV 1900) and a specialized flora on these symbiotic organisms has been published (POPNIKOLOV & ZHELEZOVA 1964). At the beginning of the 21st century appeared a catalog of all lichenized and lichenicolous fungi of Bulgaria with showing their spread in 14 regions, outlined in accordance with the floristic zoning of the country (MAYRHOFER ET AL. 2005). In the paper of the fungal diversity of the Rodopi Mts, 484 lichens and lichenicolous fungi were included (DENCHEV ET AL. 2006). Later, the first checklist of lichens and lichenicolous fungi of Pirin Mts, which contained 514 taxa, was published (IVANOV 2010). Data on wetlands lichen species were collected in the first Bulgarian database on wetlands and their biodiversity (STOYNEVA & MICHEV 2007), and a summary of knowledge on lichens in wetlands and related protected areas was provided by STOYNEVA (2007). However, according to the available literature, so far there are no specialized lichen studies in most Bulgarian protected areas (the few exceptions related with studied areas are given in the discussion of the results). At the same time, lichens are included as a mandatory element of biodiversity in the templates of modern management plans of the Ministry of Environments and Waters of the country. Therefore, the purpose of this article is to give information on lichen species and their conservation significance in 20 Bulgarian protected natural areas, collected for the preparation of their first or updated management plans, with proposals for their conservation assessment and for considering the ecosystem services they provide in the harboring ecosystems.

MATERIAL AND METHODS

According to the geographic location and conservation status, the studied protected areas are distributed as follows (Fig. 1):

1. along the Black Sea coast – Reserve *Ropotamo*, Managed Reserves *Velyov Vir (Vodnite Lili)*, *Pyasuchna Liliya* and *Atanasovsko Ezero*;
2. along the River Danube – Reserve *Beli Lom*, Managed Reserve *Ibisha* and Protected Locality *Lomiya*;
3. in Strandzha Mt - Reserves *Silkosiya*, *Uzunbudzhak*, *Vitanovo*, *Sredoka* and *Tisovitsa*;
4. in Stara planina Mts – a) in Eastern Stara planina - Reserve *Orlitsata* and

- Managed Reserve *Ardachluka*; 6) in Western Stara planina - Reserves *Chuprene* and *Gornata Koriya*, Managed Reserve *Uchilishtna Gora*;
 5. in Sredna Gora Mts – Managed Reserve *Bogdan*;
 6. in Vitosha Mt. – Reserves *Bistrishko Branishte* and *Torfeno Branishte*.

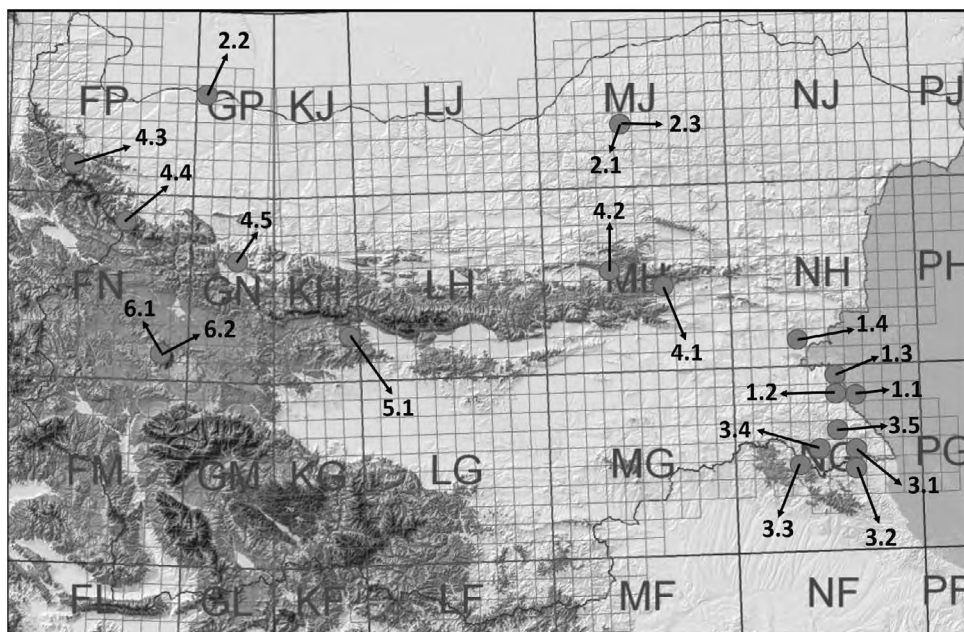


Fig. 1. Distribution of the studied protected areas in Bulgaria: **1.1** - Reserve *Ropotamo*; **1.2** - Managed Reserves *Velyov Vir (Vodnite Lili)*; **1.3** - *Pyasuchna Liliya*; **1.4** - *Atanasovsko Ezero*; **2.1** - Reserve *Beli Lom*; **2.2** - Managed Reserve *Ibisha*; **2. 3** - Protected locality *Lomiya*; **3. 1** - Reserve *Silkosiya*; **3.2** - Reserve *Uzunbudzhak*; **3.3** - Reserve *Vitanovo*; **3.4** Reserve *Sredoka*; **3.5** - Reserve *Tisovitsa*; **4.1** - Reserve *Orlitsata*; **4.2** - Managed Reserve *Ardachluka*; **4.3** - Reserve *Chuprene*; **4.4** - Reserve *Gornata Koriya*; **4.5** - Managed Reserve *Uchilishtna Gora*; **5. 1** - Managed Reserve *Bogdan*; **6.1** - Reserve *Bistrishko Branishte*; **6.2** - Reserve *Torfeno Branishte*.

All materials were collected in the periods June-September 2014 and April-June 2015 following the transect method, applied for collecting of all floristic and mycological materials at the same sites. Most materials were collected by the field teams which participated in the preparation of the relevant management plans. Materials from *Torfeno Branishte*, *Uchilishtna Gora* and *Bogdan* were collected by three of the authors of this paper (GG, MSt-G and BU on 2-4.06.2015 on Vitosha Mt, 1.04.2015r. and 2-3.04. 2015, respectively for the last two areas), materials from the Reserve *Beli Lom* and Protected Locality *Lomiya* were collected by DR K. VASSILEV (10-17.06.2014), materials from the Reserve *Chuprene* were collected mainly by one of the authors (MSt-G on 3-4.07.2014) and some other were collected by PROF. D. PEEV and MAG. N. VULYOVSKA on 7.07.2014, materials from

Gornata Koriya were collected by PROF. D. PEEV and MAG. N. VULYOVSKA in the period 8-12.07.2014, materials from the Reserve *Ibisha* - PROF. D. PEEV, MAG. N. VULYOVSKA and PROF. P. MITOV in the period 20-22.07.2014, and materials from the managed reserve *Atanasovsko ezero* – by the late ASSOC. PROF. T. MICHEV on 8.08.2014. The materials were collected from different types of habitats (deciduous, mixed and coniferous forests, open rocky terrains, sands, etc.), relevant for each site and contained the following ecological groups of lichens: epilithic (on rocks and stones), epiphleodic (on tree barks) and epigeous (on the soil surface and among mosses) – **Table 1**. The paper contains data on species from all ecological groups, found personally by one of the authors (M St-G) in the months June-July during the annual summer student practices in the period 1988-2002 on the territory of the Reserve *Bistrishko Branishte* along the tourist path to the village Bistritsa.

The identification of the collected material was done in the lab due to the need for microscopic observations on lichen slits, for wetting and coloration of the thalli according to standard methods (POPNIKOLOV & ZHELEZOVA 1964; NASH III 1996, 2008; WIRTH ET AL. 2013). The classification system used is based generally on WIRTH ET AL. (2013) with considering the most recent update to the classification of lichen fungi in the Ascomycota and Basidiomycota to genus level, with species numbers and references to changes compared to the *2010 Outline of Ascomycota* and other recent classifications (LÜCKING ET AL. 2017) and consulting INDEX FUNGORUM.

For comparisons of taxa found in this study with literature data we took into account only species, which have been indicated for the relevant protected area or species supplied with some location details, which can indicate their presence in each of the studied areas but not species with distribution in the whole floristic region (e.g. Black Sea coastal region), or for the whole mountains (e.g. Stara Planina, Sredna Gora, Strandzha) or the whole river flow (e.g. Ropotamo).

In Bulgaria lichens have never been included in any lists of protected species and in the Bulgarian Red Data Books (VELCHEV 1984; PEEV 2015). The single published proposal for protection of some lichen species was made in 1992 (DRAGANOV & STOYNEVA 1994), and a list of rare species was proposed by D. IVANOV in VODENICHAROV ET AL. (1993). In the present paper, both publications were considered in the discussion of the conservational significance of the species.

RESULTS AND DISCUSSION

During the study totally 84 lichen species from 46 genera, 21 families, 8 orders and 2 classes were found (**Table 1**). Their distribution by numbers and relevant ecological groups in each protected area is shown in **Table 1**, and the complete species list is provided below. As it could be expected, the number of species varied strongly in different protected territories, depending on their areas and main subject of conservation. For example, it is easy to explain the finding of a single

species in the small sandy Reserve *Pyasuchna Liliya*, created to protect the plant *Pancratium maritimum* L., or in the Managed Reserves *Atanasovsko Ezero* or *Velyov Vir*, declared to protect peculiar coastal wetlands. By contrast, in larger and higher mountainous Reserves like *Chuprene* and *Bistrishko Branishte*, the diversity of lichens is significantly higher – 38 and 32 species, respectively (**Table 1**).

Table 1. Taxonomic structure of the lichen flora in 20 Bulgarian protected areas according to the sampled ecological groups. Abbreviations: **EL** – epilithic lichens, **EP** – epiphytic lichens, **EG** – epigeous lichens; **Ord** – orders, **Fam** – families, **Gen** – genera, **Sp** – species. Note: *Incertae sedis* orders are counted as one.

Protected area	Ecological groups			Taxonomic structure				
	EL	EP	EG	Total				
				Class-es	Ord	Fam	Gen	Sp
<i>Ardachluka</i>		x	x	1	3	5	7	7
<i>Atanasovsko Ezero</i>		x		1	1	1	1	1
<i>Beli Lom</i>	x	x	x	1	3	5	8	9
<i>Lomiya</i>	x	x	x	1	2	5	5	8
<i>Chuprene</i>	x	x	x	1	6	10	22	38
<i>Gornata Koriya</i>	x	x	x	1	4	9	17	27
<i>Ibisha</i>		x	x	1	2	4	4	7
<i>Orlitsata</i>	x	x	x	1	3	6	9	13
<i>Pyasuchna Liliya</i>			x	1	1	1	1	1
<i>Ropotamo</i>	x	x	x	1	3	6	8	10
<i>Silkosiya</i>		x	x	1	3	3	5	5
<i>Uzunbudzhak</i>				1	4	7	9	10
<i>Vitanovo</i>		x	x	1	3	5	6	6
<i>Sredoka</i>	x	x	x	1	3	6	7	10
<i>Tisovitsa</i>		x	x	1	3	5	9	9
<i>Velyov Vir</i>		x	x	1	2	3	5	5
<i>Uchilishtna Gora</i>	x	x	x	1	4	10	17	20
<i>Bogdan</i>	x	x	x	2	5	9	11	12
<i>Torfeno Branishte</i>				1	4	9	15	20
<i>Bistrishko Branishte</i>	x	x	x	1	4	12	22	32
Total				2	8	32	56	84

Species list of lichens in different protected areas, organised in alphabetical order at the level of classes, orders, families, genera, species and subspecies:

Class Arthoniomycetes

Order Arthoniales

Family Arthoniaceae

Genus *Arthonia* Ach.

Arthonia radiata (Pers.) Ach. - Bogdan

Class Lecanoromycetes

Order Candellariales

Family Candelariaceae

Genus *Candelariella* Müll. Arg.

Candelariella coralliza (Nyl.) H. Magn. - Bistrishko Branishte, Torfeno Branishte

Candelariella xanthostigma (Pers. ex Ach.) Lettau - Chuprene, Bogdan

Order Lecanorales

Family Alectoriaceae

Genus *Alectoria* Ach.

Alectoria spp. (juv.) – Chuprene, Gornata Koriya, Bistrishko Branishte

Family Cladoniaceae

Genus *Cladonia* Hill. ex G. H. Web

Cladonia caespiticia (Pers.) Flörke - Chuprene, Gornata Koriya

Cladonia coniocraea (Flörke) Spreng. – Beli Lom, Lomiya, Uzunbudzhak, Chuprene, Gornata Koriya, Ardachluka, Uchilishtna Gora, Bistrishko Branishte

Cladonia fimbriata (L.) Fr. – Orlitsata, Chuprene, Gornata Koriya, Bistrishko Branishte

Cladonia foliacea (Huds.) Willd. – Pyasuchna Liliya, Ropotamo

Cladonia furcata (Huds.) Schrad. - Ropotamo, Uzunbudzhak, Vitanovo, Sredoka, Orlitsata, Chuprene, Gornata Koriya, Bistrishko Branishte

Cladonia pyxidata (L.) Hoffm. subsp. *pyxidata* – Sredoka, Orlitsata, Chuprene, Gornata Koriya, Bistrishko Branishte

Cladonia pyxidata subsp. *chlorophaea* (Flörke ex Sommerf.) V. Wirth – Chuprene

Cladonia pyxidata (L.) Hoffm. s.l. - Uchilishtna Gora, Torfeno Branishte

Cladonia rangiformis Hoffm. - Sredoka

Cladonia stellaris (Opiz) Pouzar & Vězda - Chuprene

Cladonia spp. (juv.) – Tisovitsa, Chuprene, Gornata Koriya, Bistrishko Branishte

Family Lecanoraceae

Genus *Lecanora* (Ach.) Th. Fr.

Lecanora argentata (Ach.) Röhl. (Syn. *Lecanora argentata* (Ach.) Malme)
– Ardachluka, Uchilishtna Gora, Chuprene, Gornata Koriya
Lecanora carpinea (L.) Vain. – Chuprene, Bogdan, Bistrishko Branishte,
Torfeno Branishte

Lecanora cf. *conizaeoides* Nyl. ex Croub. - Uzunbudzhak

Genus *Myriolecis* Clem.

cf. *Myriolecis dispersa* (Pers.) Šliwa, Zhao Xin & Lumbsch (Syn. *Lecanora*
cf. *dispersa* (Pers.) Sommerf.) - Chuprene, Gornata Koriya

Genus *Rhizoplaca* Zopf

Rhizoplaca melanophthalma (Ram.) Leuckert - Torfeno Branishte

Family **Parmeliaceae**

Genus *Brodoa* Goward

Brodoa intestiniformis (Vill.) Goward - Torfeno Branishte

Genus *Bryoria* Brodo et D. Hawksw.

Bryoria capillaris (Ach.) Brodo & D. Hawksw. - Chuprene, Gornata
Koriya

Bryoria fuscescens (Gyeln.) Brodo et D. Hawksw. – Chuprene, Gornata
Koriya, Bogdan, Bistrishko Branishte

Bryoria implexa (Hoffm.) Brodo et D. Hawksw. - Bistrishko Branishte

Genus *Cetraria* Ach.

Cetraria islandica (L.) Ach. – Chuprene, Gornata Koriya, Bistrishko
Branishte, Torfeno Branishte

Genus *Cornicularia* (Schreb.) Ach.

Cornicularia normoerica (Gunnerus) Du Rietz - Torfeno Branishte

Genus *Evernia* (L.) Ach.

Evernia prunastri (L.) Ach. – Atanasovsko ezero, Ropotamo, Velyov
Vir, Beli Lom, Ibisha, Silkosiya, Uzunbudzhak, Vitanovo,
Sredoka, Tisovitsa, Orlitsata, Ardachluka, Uchilishtna Gora,
Chuprene, Bistrishko Branishte

Genus *Flavoparmelia* Hale

Flavoparmelia caperata (L.) Hale – Beli Lom, Silkosiya, Sredoka,
Tisovitsa, Ardachluka, Uchilishtna Gora

Genus *Hypogymnia* Nyl.

Hypogymnia physodes (L.) Nyl. – Ardachluka, Uchilishtna Gora,
Chuprene, Gornata Koriya, Bistrishko Branishte

Hypogymnia tubulosa (Schaer.) Hav. - Uchilishtna Gora, Chuprene,
Gornata Koriya, Bogdan, Bistrishko Branishte

Genus *Imshaugia* S. L. F. Mey

Imshaugia aleurites (Ach.) S. L. F. Mey. – Silkosiya, Chuprene, Gornata
Koriya

Genus *Melanelia* Essl.

Melanelia hepaticum (Ach.) A. Thell. - Bistrishko Branishte, Torfeno

Branishte

Genus **Melanelixia** O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. et Lumbsch
Melanelixia subaurifera (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D.
Hawksw. & Lumbsch - Velyov Vir, Tisovitsa

Genus **Parmelia** Ach.

Parmelia saxatilis (L.) Ach. - Chuprene, Bistrishko Branishte,
Parmelia sulcata Tayl. – Ropotamo, Velyov Vir, Beli Lom, Lomiya,
Uzunbudzhak, Vitanovo, Tisovitsa, Orlitsata, Uchilishtna
Gora, Bistrishko Branishte, Torfeno Branishte

Genus **Parmelina** Hale

Parmelina carporrhizans (Taylor) Hale – Uchilishtna Gora, Bogdan
Parmelina quercina (Willd.) Hale – Orlitsata
Parmelina cf. *quercina* (Willd.) Hale - Tisovitsa
Parmelina tiliacea (Hoffm.) Hale – Uchilishtna Gora

Genus **Parmeliopsis** Nyl.

Parmeliopsis ambigua (Wulf.) Nyl. - Chuprene, Gornata Koriya,
Bistrishko Branishte

Genus **Platismatia** W. Culb. et C. Culb.

Platismatia glauca (L.) W. L. Culb. & C. F. Culb. – Chuprene, Gornata
Koriya

Genus **Protoparmeliopsis** M. Choisy

Protoparmeliopsis muralis (Schreb.) M. Choisy - Bistrishko Branishte

Genus **Pseudevernia** Zopf

Pseudevernia furfuracea (L.) Zopf - Orlitsata, Uchilishtna Gora, Chuprene,
Gornata Koriya, Bistrishko Branishte

Genus **Pseudephebe** M. Choisy

Pseudephebe pubescens (L.) M. Choisy - Torfeno Branishte

Genus **Usnea** Dill ex Adans

Usnea dasypoga (Ach.) Nyl. - Chuprene
Usnea hirta (L.) Weber ex F. H. Wigg. (juv.) – Uchilishtna Gora
Usnea cf. *subfloridana* Stirt - Bogdan
Usnea spp. (juv.) – Chuprene, Gornata Koriya, Bistrishko Branishte

Genus **Vulpicida** Mattson & Lai

Vulpicida pinastri (Scop.) J.-E. Mattsson & M. J. Lai – Chuprene, Gornata
Koriya, Bistrishko Branishte

Family **Ramalinaceae**

Genus **Ramalina** Ach.

Ramalina capitata (Ach.) Nyl. - Torfeno Branishte
Ramalina farinacea (L.) Ach. – Velyov Vir, Sredoka, Tisovitsa (juv.),
Uchilishtna Gora, Bistrishko Branishte
Ramalina fastigiata (Pers.) Ach. – Ropotamo, Sredoka, Orlitsata

Family **Stereocaulaceae**

Genus *Lepraria* Ach.

Lepraria incana (L.) Ach. – Uchilishtna Gora, Gornata Koriya, Bogdan,
Bistrishko Branishte

Lepraria spp. - Beli Lom, Lomiya, Ibisha, Uzunbudzhak, Chuprene,
Gornata Koriya

Genus *Squamarina* Poelt

Squamarina cartilaginea (With.) P. James (Syn. *Lecanora crassa* (Huds.)
Ach.) - Orlitsata

Family Umbilicariaceae

Genus *Umbilicaria* (Hoffm.) Fw.

Umbilicaria crustulosa (Ach.) Lamy - Torfeno Branishte

Umbilicaria cylindrica (L.) Delise - Bistrishko Branishte,

Umbilicaria decussata (Vill.) Zahlbr. - Torfeno Branishte

Umbilicaria deusta (L.) Baumg. - Torfeno Branishte

Umbilicaria torrefacta (Lightf.) Schrad. - Torfeno Branishte

Order Ostropales

Family Graphidaceae

Genus *Graphis* Adans.

Graphis scripta (L.) Ach. - Sredoka

Family Phlyctidaceae

Genus *Phlyctis* (Wallrot.) Flot.

Phlyctis argena (Ach.) Flot. – Uchilishtna Gora, Chuprene

Order Peltigerales

Family Lobariaceae

Genus *Lobaria* (Schreb.) A. Z.

Lobaria pulmonaria (L.) Hoffm. – Uzunbudzhak, Gornata Koriya

Family Peltigeraceae

Genus *Peltigera* Willd.

Peltigera canina (L.) Willd. - Ropotamo, Sredoka, Bistrishko Branishte

Peltigera horizontalis (Huds.) Baumg. – Orlitsata, Ardachluka

Peltigera malacea (Ach.) Funck - Chuprene

Peltigera membranacea (Ach.) Nyl. – Chuprene, Gornata Koriya

Peltigera neopolydactyla (Gyeln.) Gyeln. – Chuprene, Bistrishko
Branishte

Peltigera polydactylon (Neck.) Hoffm., ster. - Chuprene

Peltigera praetextata (Flörke ex Sommerf.) Zopf – Silkosiya, Vitanovo,
Orlitsata, Chuprene, Gornata Koriya

Peltigera rufescens (Weiss) Humb. - Bistrishko Branishte

Order Pertusariales

Family Ochrolechiaceae

Genus *Ochrolechia*

Ochrolechia arborea (Kreyer) Almborn (cf.!) – Bogdan

Family **Pertusariaceae**

Genus ***Lepra*** Scop.

Lepra amara (Ach.) Hafellner (Syn. *Pertusaria amara* (Ach.) Nyl.) –
Uzunbudzhak, Vitanovo, Uchilishtna Gora

Genus ***Pertusaria*** DC

Pertusaria cf. *hymenea* (Ach.) Schaer. - Orlitsata

Pertusaria pertusa (Weigel) Tuck. - Uzunbudzhak, Vitanovo, Sredoka,
Orlitsata, Ardachluka

Pertusaria pustulata (Ach.) Duby – Uchilishtna Gora

Order **Teloschistales**

Family **Caliciaceae**

Genus ***Amandinea*** M. Choisy ex Scheid. & M. Mayrhofer

Amandinea punctata (Hoffm.) Coppins & Scheid - Bogdan

Genus ***Buellia*** De-Not

Buellia schaeferi De Not. – Uchilishtna Gora

Family **Physciaceae**

Genus ***Physcia*** Ach. emend. Vain.

Physcia adscendens H. Olivier - Ropotamo, Beli Lom, Lomiya, Ibisha,
Chuprene, Gornata Koriya, Bistrishko Branishte

Physcia aipolia (Ehrb. ex Humb.) Fűrnr. - Ropotamo, Ibisha

Physcia stellaris (L.) Nyl. - Beli Lom, Lomiya, Ibisha, Chuprene,
Bistrishko Branishte

Physcia tenella (Scop.) DC. – Lomiya, Tisovitsa

Genus ***Physconia*** Poelt

Physconia distorta (With.) R. Laundon - Ropotamo

Physconia enteroxantha (Nyl.) Poelt. - Lomiya

Physconia grisea (Lam.) Poelt – Beli Lom, Uchilishtna Gora, Bogdan

Family **Teloschistaceae**

Genus ***Xanthoria*** (Fr.) Th. Fr.

Xanthoria elegans (Link) Th. Fr.) - Ibisha

Xanthoria parietina (L.) Th. Fr. – Ropotamo, Beli Lom, Lomiya, Ibisha,
Uchilishtna Gora, Bogdan, Bistrishko Branishte, Torfeno
Branishte

Orders **incertae sedis**

Family **Lecideaceae**

Genus ***Lecidea*** Ach.

Lecidea lapicida (Ach.) Ach. - Chuprene, Torfeno Branishte

Genus ***Lecidella*** Körber em. Hertel et Leuck.

Lecidella elaeochroma (Ach.) M. Choisy – Velyov Vir, Uzunbudzhak,
Tisovitsa

Lecidella stigmata (Ach.) Hertel & Leucke - Chuprene, Gornata Koriya

Family **Ophioparmaceae**

Genus *Hypocenomyce* M. Choisy

Hypocenomyce scalaris (Ach. ex Lilj.) M. Choisy - Silkosiya

Family **Rhizocarpaceae**

Genus *Rhizocarpon* Ramond ex DC

Rhizocarpon alpicola (Wahlenb.) Rabenh. - Torfeno Branishte

Rhizocarpon badioatrum (Flörke ex Spreng.) Th. Fr. - Torfeno Branishte

Rhizocarpon geographicum (L.) DC - Bistrishko Branishte, Torfeno Branishte

For almost all studied protected areas, the data provided in the present paper are practically the first ones. Exceptions are: 1) the managed reserve *Bogdan*, for which three species were indicated by ŽELEZOVA (1960, 1963) – *Heterodermia speciosa* (Wulfen) Trevis. (Syn. *Anaptychia speciosa* (Wull.) Vain., *Leptogium saturninum* (Dicks.) Nyl. and *Nephroma parile* (Ach.) Ach.; 2) the reserve Ropotamo for which *Collema flaccidum* (Ach.) Ach., *Graphis scripta* (L.) Ach. (Syn. *Graphis scripta* var. *pulverulenta* (Pers.) Ach.), *Pertusaria flavida* (DC.) J. R. Laundon (Syn. *P. lutescens* (Hoffm.) Lamy) was pointed by ŽELEZOVA (1963) and 3) the both Vitosha reserves *Bistrishko Branishte* and *Torfeno Branishte*. Although the lichens of Vitosha Mt were the first investigated in Bulgaria (KAZANDJIEV 1900, 1906), and the mountain, due to its close situation to the capital Sofia, is one of the best studied in the country (e.g. P. NIKOLOFF 1931, 1932, 1935, PODPERA 1911, SUZA 1929, CRETZOUI 1936, SUZA 1929, ŽELEZOVA 1956, 1960, 1962, 1963, LAMBREV ET AL. 1962, KLOSS 1962, MOTYKA & ŽELEZOVA 1962, SHIVAROV & STOYKOV 2012, KOVACHEV, unpubl.), only few data on the lichens of *Bistrishko Branishte* exist and there is no purposive detailed study, which covers its whole territory. In the Contribution to the lichen flora of Bulgaria, among the species found on Vitosha Mt, ŽELEZOVA (1960) pointed only *Diploschistes muscorum* (Scop.) R. Sant. (Syn. *D. bryophilus* (Ehrenb.) A. Z.) as found at altitude of 700 m a.s.l. in the territory of this reserve. In the monographic study of the genus *Usnea* in Bulgaria, MOTYKA & ŽELEZOVA (1962) provide data on Vitosha Mt, but especially for the reserve they indicate only one variety - *Usnea faginea* var. *cirrhosa* Mot. However, from the text of both authors, considering the substrates and altitudes pointed, it is possible to suppose that there were two more species found in the reserve *Bistrishko Branishte*. These are *Usnea barbata* (L.) Wigg. emend. Mot. and *Usnea florida* (L.) Wigg., the first of which was not proved by the authors. About 350 taxa are included in the unpublished Report: *The lichens of Vitosha* of Mag. A. KOVACHEV to the Directorate of the Nature Park Vitosha, but according to his descriptions of the studied sites (altogether 30), only two of them (numbers 29 and 30) with 32 lichen taxa found are relevant for the reserve. We prepared a list of all lichen taxa mentioned by ŽELEZOVA (1960), MOTICA & ZHELEZOVA (1962) and KOVACHEV (unpubl.), for which the finding on the reserve territory was explicitly pointed, accomplished by taxa found in this study (*Appendix 1*). As a result, the

list contains 49 lichens, 15 of which are new for the reserve territory and 17 are in accordance with former studies. There are no purposive data on lichens of the reserve *Torfeno Branishte*. However, using the study by P. NIKOLOFF (1931), we prepared a list of lichen species for which he provided ecological details (including broad distribution, substrates and altitude) which allowed to suppose finding on the reserve territory, accomplished by taxa found in this study (*Appendix 2*). As a result, the list contains 69 lichens, 16 of which are new for the reserve territory and 4 are in accordance with former studies.

In most of the studied areas there were no species of conservation significance according to the papers by DRAGANOV & STOYNEVA (1994) and VODENICHAROV ET AL. (1993). Exceptionally, *Lobaria pulmonaria* (L.) Hoffm. (tree lungwort, lung lichen, lung moss, lungwort lichen, oak lungs or oak lungwort) was found in the reserves *Uzunbudzhak* and *Gornata Koriya*. This extremely sensitive epiphytic/epiphleodic species was proposed for protection in 1992 (for details see DRAGANOV & STOYNEVA 1994) because of two reasons: a) the species inhabiting of mesophyllous beech forests of high air humidity and clean air; b) its medicinal properties as a curative means against cough as long-ago known remedy for the treatment of lung diseases. Nowadays, the medicinal properties of the lichen are “rediscovered” by modern Bulgarians due to the broad advertisement and usage of the homeopathic anticough syrups (UZUNOV & STOYNEVA-GÄRTNER 2015). *L. pulmonaria* is accepted as threatened and included in the Red Lists in a number of countries and regions in Europe (e.g. TÜRK & HAFELLNER 1999; JÜRIADO & LIIRA 2010; JÜRIADO ET AL. 2011; BENESPERI ET AL. 2018). Therefore, we believe that this species, which is relatively rare in the territory of both reserves, should be subject to special conservation and permanent monitoring.

Cetraria islandica (L.) Ach. (Iceland moss) was found in small amounts (single specimens) in the reserves *Chuprene*, *Gornata Koriya*, *Bistrishko Branishte* and *Torfeno Branishte*. We believe that this species has long been among the endangered species in the country due to its including in herbal manuals among the folk medicines for treatment of different diseases (EUROPEAN MEDICINE AGENCY 2014; UZUNOV & STOYNEVA-GÄRTNER 2015). Modern Bulgarian people use the lichen mainly as herbal cough remedy (UZUNOV & STOYNEVA-GÄRTNER 2015). In the reserves *Bistrishko Branishte* and *Torfeno Bransihte* the population strongly decreased also due to collection of the species for students herbaria during students practices conducted there, according to our knowledge, for more than 25 years (1975-2000). Therefore, it can be argued that among the lichens in both reserves there is a species of conservation significance, the collection of which must be especially strictly forbidden.

Although during the present study we did not find new taxa for the territory of Bulgaria, and there are not officially accepted threatened species, our pilot results indicate the presence of significant number of lichenized fungi in the studied protected areas. Doubtless, further more detailed targeted studies could

reveal higher biodiversity of these organisms and therefore we can strongly recommend conducting of systematic inventories and continuous mapping of the species. Considering the extremely slow growth of these organisms (NASH III 2008) and their sensitivity to the air pollution which makes them valuable air quality indicators, combined with their low amounts in the studied sites, we find it necessary to outline the need of their permanent monitoring and taking measures for preventing any air pollution (and especially the appearance of acid rains) in the studied regions.

Lichenised fungi (lichens) are often neglected in analyses of ecosystem services and in nature conservation management, mostly due to the underestimation of their importance, to the lack of monitoring data for many regions, and to the difficulties in species identification (ZEDDA ET AL. 2014; ZEDDA & RAMBOLD 2015). However, lichens and their symbionts underpin a great number of ecosystem functions, among which are the rock decomposition, soil formation, carbon, and nitrogen fixation. In addition, they support the diversity of numerous organisms through the provision of food, habitat, shelter, camouflage, or nesting material. Furthermore, lichens provide numerous direct and indirect ecosystem services (*e.g.* the provision of lichen secondary metabolites and other compounds for medicinal and other purposes, the use of lichens as bioindicators of environmental changes, and as inspiration source in the context of culture, arts and design) - ZEDDA & RAMBOLD (2015). Although included in the templates of management plans of the Bulgarian Ministry of Environment and Waters, lichens have not been evaluated in any of them in respect of their ecosystem functioning and of their role as providers of ecosystem services. Therefore, we underline this omission and we believe that in future this aspect will find place in nature conservation documents and assessments.

CONCLUSION

Data on lichen biodiversity in 20 protected areas in Bulgaria, obtained during this study, revealed the need of conducting more detailed systematic and purposive inventory of these organisms, continuous mapping of species and their permanent monitoring in all Bulgarian protected areas. In addition, we propose assessment of their role as providers of ecosystem services in future natural legislative documents and urgent including relevant lichens in lists of threatened and protected species in Bulgaria. Since the general protection of habitats in the protected areas of the country is ensured by the law, we would like to stress the need for taking measurements for preventing any air pollution since it can harm in an irreversible way these slowly growing and very sensitive organisms, affecting in this way the function of their harboring ecosystems.

CONFLICT OF INTERESTS

The authors declare lack of conflict of interests. Identification of the lichen material was done by G. GÄRTNER and M. P. STOYNEVA-GÄRTNER, who also collected part of the materials. B. A. UZUNOV participated in part in the field work with collection of the materials and in the preparation of the illustrations. All authors worked equally in literature search and writing of the manuscript.

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List of lichens of the reserve *Bistrishko Branishte*. The list is organized in alphabetical order of the species Latin names. Data on lichen species provided by ŽELEZOVA (1960), MOTYKA & ŽELEZOVA (1962) and KOVACHEV (unpubl.), for which the finding on the reserve territory was explicitly pointed, were accomplished with taxa found in this study (indicated by * when they are reported for first time, and by (*) when they coincide with previously reported taxon). In quotes are indicated taxa taken from ŽELEZOVA (1960), MOTYKA & ŽELEZOVA (1962) and KOVACHEV (unpubl.), which were not found with the same writing, combinations or author names in INDEX FUNGORUM.

1. **Alectoria* spp. (juv.)
2. (*)*Bryoria fuscescens* (Gyeln.) Brodo et D. Hawkw.
3. (*)*Bryoria implexa* (Hoffm.) Brodo et D. Hawkw.
4. *Bryoria subcana* (Nyl. ex Stiz.) Brodo et D. Hawkw.
5. **Candelariella coralliza* (Nyl.) H. Magn.
6. *Candelariella vitellina* (Hoffm.) Müll. Arg. (as “*C. vitellina* (Ehrh.) Müll. Arg.”)
7. (*)*Cetraria islandica* (L.) Ach.
8. *Cladonia coccifera* (L.) Willd.
9. **Cladonia coniocraea* (Flörke) Spreng.
10. (*)*Cladonia fimbriata* (L.) Fr.
11. **Cladonia furcata* (Huds.) Schrad.
12. (*)*Cladonia pyxidata* (L.) Hoffm. subsp. *pyxidata*
13. **Cladonia* spp. (juv.)
14. *Diplochistes muscorum* (Scop.) R. Sant. (Syn. *D. bryophilus* (Ehrenb.) A. Z.)
15. (*)*Evernia prunastri* (L.) Ach.
16. (*)*Hypogymnia physodes* (L.) Nyl.
17. (*)*Hypogymnia tubulosa* (Schaer.) Havass.
18. *Lecanora albella* (Pers.) Ach.
19. *Lecanora allophana* (Ach.) Nyl. (as “*Lecanora allophana* (Nyl.) Röhl.”)
20. (*)*Lecanora carpinea* (L.) Vain.
21. *Lecidella euphorea* (Förke) Hertel
22. (*)*Lepraria incana* (L.) Ach.
23. *Melanelia fuliginosa* (Fr. ex Duby) Essl. in Egan
24. **Melanelia hepatizon* (Ach.) A. Thell.
25. **Parmelia saxatilis* (L.) Ach.
26. (*)*Parmelia sulcata* Tayl.
27. (*)*Parmeliopsis ambigua* (Wulf.) Nyl.
28. *Parmeliopsis hyperopta* (Ach.) Vain. (as “*Parmeliopsis hyperopta* (Ach.) Arnold”)
29. (*)*Peltigera canina* (L.) Willd.
30. **Peltigera neopolydactyla* (Gyeln.) Gyeln.

31. **Peltigera rufescens* (Weiss) Humb.
32. *Peltigera subcanina* Gyeln.
33. **Physcia adscendens* H. Olivier
34. **Physcia stellaris* (L.) Nyl.
35. *Platismatia glauca* (L.) W. Culb. et C. Culb.
36. **Protoparmeliopsis muralis* (Schreb.) M. Choisy
37. (*)*Pseudevernia furfuracea* (L.) Zopf.
38. (*)*Ramalina farinacea* (L.) Ach.
39. (*)*Rhizocarpon geographicum* (L.) DC
40. *Scoliosporum umbrinum* (Ach.) Arn.
41. *Tuckermanopsis chlorophylla* (Willd.) Hale
42. **Umbilicaria cylindrica* (L.) Delise
43. *Usnea faginea* var. *cirrhusa* Mot.
44. *Usnea filipendula* Stirt.
45. *Usnea florida* (L.) Weber ex F. G. Wigg. (as "*U. florida* (L.) Wigg. emend Clere")
46. **Usnea* spp. (juv.)
47. (*)*Vulpicida pinastri* (Scop.) J. –E. Mattson et Lai
48. *Xanthoparmelia mougeotii* (Schaer. ex D. Dietr.) Hale
49. **Xanthoria parietina* (L.) Th. Fr.

Appendix 2

List of lichens of the reserve *Torfeno Branishte*. The list is organized in alphabetical order of the species Latin names. List of lichens for which the ecological data (incl. broad distribution, substrates and altitude) provided by P. NIKOLOFF (1931) allowed to suppose finding on the reserve territory, were accomplished with taxa found in this study (indicated by * when they are reported for first time, and by (*) when they coincide with previously reported taxon). In quotes are indicated taxa taken from P. NIKOLOFF (1931), which were not found with the same writing, combinations or author names in INDEX FUNGORUM.

1. *Alectoria implexa* (Hoffm.) Röhl. (as "*A. implexa* (Hoffm.) Nyl.")
2. *Alectoria jubata* (L.) Ach.
3. *Aspicilia cinerea* (L.) Korb.
4. *Baeomyces byssoides* (L.) P. Gaertn., G. Mey. & Scherb. (as "*B. byssoides* (L.) Pers.")
5. *Biatorella testudinea* (Ach.) Mass.
6. **Brodoa intestiniformis* (Vill.) Goward
7. "*Buellia etrata* Mudd."
8. *Buellia parasema* f. *saprophila* (Ach.) Stein (as "*B. parasema* f. *saprophila* Ach.")
9. *Caloplaca aurantiaca* (Lightf.) Th. Fr. (as "*C. aurantiacum* (Lightf.) Th. Fr.")
10. *Caloplaca ferruginea* (Huds.) Th. Fr. (as "*C. ferrugineum* var. *genuinum* (Kbr.) Th. Fr.")

11. *Caloplaca vitellinula* (Nyl.) Oliv.
12. **Candelariella coralliza* (Nyl.) H. Magn.
13. *Calvitimela aglaea* (Sommerf.) Hafellner (Syn. *Lecidea aglaea* Somf.)
14. **Cetraria islandica* (L.) Ach.
15. *Cetraria pinastri* (Scop.) Grey (as “*C. pinastri* (Scop.) Ach.”)
16. *Cladonia coccifera* (L.) Willd. (as “*C. coccifera* (L.) Schaer.”)
17. *Cladonia gracilis* f. *aspera* Boistel (as “*C. gracilis* f. *aspera* Flk.”)
18. *Cladonia fimbriata* (L.) Fr. (Syn. “*C. fimbriata* f. *radiata* Schaer.”)
19. *Cladonia fimbriata* var. *tubaeformis* (Hoffm.) Fr. (as “*C. fimbriata* f. *tubaeformis* Hoffm.”)
20. *Cladonia fimbriata* f. *prolifera* Retz. (as “*C. fimbriata* f. *prolifera* Flk.”)
21. *Cladonia furcata* f. *polyphylla* (Flörke) Jatta (as “*C. furcata* f. *polyphylla* Flk.”)
22. (*)*Cladonia pyxidata* (L.) Hoffm. (as “*C. pyxidata* (L.) Fr.”)
23. *Cladonia pyxidata* var. *neglecta* (Flörke) Mass. (as “*C. pyxidata* var. *neglecta* Schaer.”)
24. **Cornicularia normoerica* (Gunnerus) Du Rietz
25. *Dermatocarpon miniatum* (L.) W. Mann. (as “*D. miniatum* (Lin.) Ach.”)
26. “*Dermatocarpon miniatum* var. *complicatum* (Sw.) Fr.”
27. **Lecanora carpinea* (L.) Vain.
28. *Lecanora cenisia* Ach.
29. *Lecanora rupicola* (L.) Zahlbr. (Syn. *Lecanora sordida* (Pers.) Th. FR.
30. *Lecanora sulphurea* (Hoffm.) Ach.
31. **Lecidea lapicida* (Ach.) Ach.
32. **Melanelia hepatizon* (Ach.) A. Thell.
33. *Melanelia stygia* (L.) Essl. (Syn. *Parmelia stygia* (L.) Ach.)
34. *Parmelia conspersa* var. *latior* Schaer.
35. “*Parmelia encaustra* (Smrft.) Nyl.”
36. *Parmelia isidiata* (Anzi) Gyel.
37. *Parmelia pubescens* Pers. (as “*P. pubescens* L. (Syn. *Parmelia stygia* var. *lanata* Sydow)”))
38. *Parmelia saxatilis* (L.) Fr.
39. **Parmelia sulcata* Tayl.
40. *Parmelina tiliacea* (Hoffm.) Hale (Syn. *Parmelia tiliacea* (Hoffm.) Ach. as “*P. tiliacea* (Hoffm.) Fr.”)
41. *Peltigera leucophlebia* (Nyl.) Gyeln. (as “*Peltigera variolosa* (Mass.) Gyel.) (Syn. *Peltigera aphotosa* (L.) Hoffm.)”)
42. *Peltigera canina* (L.) Willd. (as “*P. canina* (L.) Hoffm.”)
43. *Peltigera horizontalis* (Huds.) Baumg. (as “*P. horizontalis* (L.) Hoffm.”)
44. *Peltigera polydactyla* (Neck.) Hoffm.
45. *Peltigera polydactyla* f. *microcarpa* (Ach.) Mérat
46. *Protopannaria pezizoides* (Weber) P. M. Jorg. & S. Ekman (Syn. *Pannaria brunnea* (Sw.) Mass.)

47. *Protoparmeliopsis muralis* (Schreb.) M. Choisy (Syn. “*Placodium saxicolum* (Poll.) Kbr.”)
48. **Pseudephebe pubescens* (L.) M. Choisy
49. (*)*Ramalina capitata* (Ach.) Nyl. (incl. Syn. *Ramalina strepsilis* (Ach.) A. Z.)
50. “*Ramalina carpathica* Kbr.”
51. *Ramalina pollinaria* (Westr.) Ach. (as “*R. pollinaria* (Mester.) Ach.”)
52. *Ramalina polymorpha* (Lilj.) Ach. (as “*R. polymorpha* Ach.”)
53. **Rhizocarpon alpicola* (Wahlenb.) Rabenh.
54. **Rhizocarpon badioatrum* (Flörke ex Spreng.) Th. Fr.
55. **Rhizocarpon geographicum* (L.) DC
56. *Rhizocarpon geographicum* f. *contiguum* (Schaer.) Mass. (as “*R. geographicum* f. *contiguum* Fr.”)
57. **Rhizoplaca melanophthalma* (Ram.) Leuckert
58. *Sporastatia cinerea* (Schaer.) Körb.
59. *Tephromela atra* (Huds.) Hafellner (Syn. *Lecanora atra* (Huds.) Ach.)
60. (*)*Umbilicaria crustulosa* (Ach.) Lamy (incl. Syn. *Gyrophora cirrhosa* (Hoffm.) Wain.)
61. *Umbilicaria cylindrica* (L.) Delise (Syn. *Gyrophora cylindrica* (L.) Ach.)
62. **Umbilicaria decussata* (Vill.) Zahlbr.
63. (*)*Umbilicaria deusta* (L.) Baumg. (incl. Syn. *Gyrophora deusta* (L.) Fw.)
64. **Umbilicaria torrefacta* (Lightf.) Schrad.
65. *Umbilicaria vellea* (L.) Ach. (Syn. *Gyrophora vellea* (L.) Ach.)
66. *Umbilicaria pustulata* (L.) Hoffm.
67. *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale (Syn. *Parmelia conspersa* var. *stenophylla* (Ach.) Heugel.
68. *Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch (Syn. *Parmelia prolixa* Ach.)
69. **Xanthoria parietina* (L.) Th. Fr.

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FLORA, VEGETATION AND HABITATS OF *KAYLUKA* PROTECTED AREA

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Abstract. The paper presents results from a study of the flora, vegetation and habitats of *Kayluka* Protected Area (Pleven District, Danube Plain, Bulgaria). It is one of the first protected areas in Bulgaria, with high regional and national significance. The vascular flora is presented by 844 species and subspecies from 99 families. The vegetation cover consists of various communities, but is mostly related to the deciduous forests and calcareous rocky terrains. The ruderalisation is also high because of the transformation of the biggest part of this area into recreational urban territory. Eleven habitat types (from totally 22 types), according to the Annex I of Habitats Directive, were recorded in the protected area, which is a part of the NATURA 2000 site *Studentets*.

Key words: conservation significance, plant communities, Pleven district, species diversity

INTRODUCTION

Kayluka (**Fig. 1**) is one of the first protected territories in Bulgaria (TODOROV 1982). It was designated with an area of 300 ha and a status of National Park by

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Fig 1. Map of the Protected Area *Kayluka* (based on Google Earth) with the localities of vascular plants with conservation significance: **1** - *Galanthus elwesii*; **2** - *Spiranthes spiralis*; **3** - *Himantoglossum caprinum*; **4** - *Celtis glabrata*; **5** - *Anemone sylvestris*; **6** - *Pulmonaria mollis*; **7** - *Jurinea ledebourii*.

Decree №21496/17.10.1939 of the Ministry of Agriculture and State properties. Later, it was transformed into Protected Area and its borders were expanded (999.8 ha) under an Order №3700/29.12.1972 of the Ministry of Forestry and Conservation of Environment. According to the Order №800/04.11.2008, it is a part of NATURA 2000 sites: SPA and SCI BG0000240 *Studenets*. During the period 1946–1971, following the efforts of a special committee led by General IVAN VINAROV, a big part of the valley was transformed into an urban park area. Numerous forest plantations, tree and shrub compositions have replaced or affected significantly its natural vegetation. This was also accompanied by the construction of many chalets, summer houses, hotels, touristic infrastructure such as alleys, artificial lakes and dams, *etc.* Actually, the main part of the protected areas has been used as urban park for different types of recreation. On spring days, thousands of people visit this area (MANAGEMENT PLAN OF *KAYLUKA* PROTECTED AREA AND *RAZBITITSA* NATURAL MONUMENT 2011-2021, hereafter abbreviated as MPKPARNM 2011-2021).

In spite of the importance of the protected area (for the conservation of the nature, as well as for a recreational site), its flora, vegetation and habitats have never been studied in any specialized work. This is probably due to the fact that the Protected Area *Kayluka* is located in a region of Bulgaria, which has remained peripheral for the active biodiversity studies. The earliest data on the flora is contained in the work of URUMOV (1925), which is generally dedicated to the Pleven District, but *Kayluka* locality was mentioned in relation to many vascular plants. Some of them which were not confirmed by our recent study, could also indicate a possible negative successional changes of the ecosystems there. The main source of information on the flora and vegetation is the PhD thesis by TZONEV (2002). Although it refers to the Middle Danube Plain, there are described many communities found in *Kayluka* Protected Area. It was followed by several works, also by TZONEV (2004, 2009, 2013, 2017), where plant associations or protected species from this region (including *Kayluka*) were published. There are data about the flora of *Kayluka* in some shorter floristic and taxonomic notes as well (PANOV 1996; TZONEV ET AL. 2017). The information about the floristic diversity is also provided in the biggest unpublished inventory of the biota (including flora, vegetation and habitats) - the Management Plan (2011-2021) of the Protected Area *Kayluka* and of the Natural Monument *Razbititsa* (MPKPARNM 2011-2021).

MATERIAL AND METHODS

The largest part (12 km long) of the studied Protected Area *Kayluka* is occupied by the canyon-like Tuchenitsa River valley, a right tributary of the Vit River. It has typical karst structure and its slopes are represented by 40-50 m high steep rocks and walls of organogennic limestone at Upper Cretaceous age (mostly Maastricht), rich in macrofossils (TZANKOV ET AL. 1981). According to VELEV (1990) the climate is temperate-continental, with western and north-western winds, and the average of

rainfalls 500-600 mm/year. Due to the high runoff of the Maastricht limestone in the Pleven syncline and the alluvial deposits of the Vit River, the Pleven Heights are characterized by greater soil humidity of 40-50 mm per year. There are also karst springs - the largest one is Srebrostruy (MIHAYLOV ET AL. 1989). Tuchenitsa River is with a short spring freshet and a long summer-autumn low water flow. Specifically, for the rivers crossing the Pleven syncline, the karst feeding significantly influences their flow. Because of the numerous small valleys and steep slopes, there are also episodic torrential floods (MIHAYLOV ET AL. 1989). According to NINOV (2002), the dominating soil type is Chromic Luvisol. However, some skeletal soils, such as Rendzic Leptosols, are also common.

The study was carried out over a long period of personal observations performed by the authors (1997-2017), as well as during a special inventory in the period 2008-2009 for the purposes of the special Management Plan. The identification of taxa (*Appendix 1*) and the nomenclature of the vascular plants were according to KOZHUHAROV (1992) and DELUPAVLOV & CHESHMEDZHIEV (2003). The full checklists of established taxa were completed with data for endemic and subendemic (noted with E) (PETROVA & VLADIMIROV 2010), protected species (Annex II, III and IV of BULGARIAN BIOLOGICAL DIVERSITY ACT), species with conservation significance according to the Bulgarian Red Lists and Red Data Books (PETROVA & VLADIMIROV 2009; PEEV ET AL. 2015). The categories were provided according to PETROVA & VLADIMIROV (2009): CR – *Critically Endangered*; EN – *Endangered*; VU – *Vulnerable*; NT – *Near Threatened*, DD – *Data deficient*, LC – *Least Concern*. For each vascular plant species the frequency and distribution on the territory of the protected area was given. The following groups were presented in the checklist with their abbreviations: Common (C), Comparatively Rare (CoR), Rare (R), Very Rare (VR), Possibly Extinct (PE), and Probably Distributed (PD). The syntaxonomy followed the methodology of BRAUN-BLANQUET (1964). The syntaxonomical nomenclature was based on the latest revision of the European vegetation (MUCINA ET AL. 2016). The habitats were determined according to EUNIS habitat classification (DAVIES ET AL. 2004) and the corresponding units with the Habitats Directive (COUNCIL DIRECTIVE 92/43/EEC) were also given.

RESULTS AND DISCUSSION

Flora

On the territory of the studied protected area, 844 species and subspecies of vascular plants belonging to 406 genera and 99 families were identified by the existing literary data and recent study. Most of the vascular plant species in *Kayluka* are widespread in the country as weeds, ruderals or dominant species in the natural and secondary vegetation. However, some of them are rare for this area, especially in the cases when it is located on the periphery of their range of distribution in the country. Such are some species common to the mountain and foothill regions of Bulgaria like

Hepatica nobilis Mill., *Phyllitis scolopendrium* (L.) Newn., *Dryopteris filix-mas* (L.) Shott., and *Ceterach officinarum* DC. They have reached the Danube Plain only in single localities and form small populations limited to the dark and moist valleys. Some of the species noted for the area or its surroundings in the past by URUMOV (1925) currently are possibly extinct from the area.

The checklist of flora does not include dozens of exotic and introduced species, although some of them are spread spontaneously, such as: *Celtis australis* L., *Cercis siliquastrum* L., *Koeleruteria paniculata* Laxm., etc. Most of these species, however, do not really spread within the studied protected area and exist only as cultivated single trees or small groups. Such species are: *Celtis occidentalis* L., *Chamecyparis lawsoniana* (A. Murray) Parl., *Ginkgo biloba* L., *Juniperus virginiana* L., *Picea pungens* Engelm., *Pinus strobus* L., *Taxus baccata* L., *Pseudotsuga douglasii* (Sabine ex D. Don) Carrière and many others.

Most of the species (467 taxa) were defined as Common in the whole protected territory. Comparatively Rare were 159 species, Rare - 144, Very Rare - 61 species (with one or two localities) and 7 were Possibly Extinct. One species (*Dictamnus albus* L.) noted by URUMOV (1925) could possibly be found, but has not been confirmed for a long period now and was accepted by us as Probably Distributed. Seven species are included in the Red Data Book of Bulgaria and the Red List as *Vulnerable*, *Endangered* and *Near Threatened*. The population in Kayluka of one of them, *Galanthus elwesii* Hook f., is of national significance. Kayluka is also one of the few protected areas in Bulgaria with a population of *Jurinea ledebourii* Bunge. The localities of species enlisted in the Red Data Book and the Red List are shown on Fig. 1 with numbers. One species, *Orchis papilionacea* L. (Vu), was found in the vicinities of Pleven, Brestovets and Radishevo (URUMOV 1925), i.e. area, which possibly includes Kayluka. However, this species has not been confirmed anywhere in the last decades, and probably is *Extinct*.

1. ***Galanthus elwesii* Hook f.** - EN. It was found in forests of *Tilia tomentosa* Moench or *Carpinus orientalis* Mill. and has numerous populations in the protected area.
2. ***Spiranthes spiralis* (L.) Chevall.** - VU. A single very small population was found in the protected area, in the shrubs mostly of *Fraxinus ornus* L. (TZONEV ET AL. 2017).
3. ***Himantoglossum caprinum* (M. Bieb.) Spreng** - VU. The species is also a target for BG0000240 *Studenets*. The established population was numerous and located mostly in secondary habitats - forest plantations and lilac shrubs (TZONEV ET AL. 2017).
4. ***Celtis glabrata* Stev. ex Planch.** - EN. A single specimen was found on Popskata Skala locality (the Big Dam) in the protected area.
5. ***Anemone sylvestris* L.** - NT. One very small population was found in the protected area. However, it could be of anthropogenic origin.
6. ***Pulmonaria mollis* Velen.** - It was in the old Red Data Book of Bulgaria (VELCHEV

1984), but in the current Red List was assessed as Least Concern. Because of the construction activities around Orbita Hotel (single locality in the area), this population has not been confirmed in the last 15 years.

7. *Jurinea ledebourii* Bunge. – EN. Several small populations were found in the protected area, mostly in shrubs and rocky grasslands.

Amongst these species, only *Himantoglossum caprinum* (L.) Spreng. is enlisted both in Annex II and III of the BIODIVERSITY ACT. A total of 9 species are protected (enlisted in *Appendix 3*), and 19 species are in its Annex IV (controlled use).

Vegetation and habitats of the Protected Area Kayluka

The vegetation of the Kayluka Protected Area is characterized by a great diversity - 10 classes, 11 orders, 14 alliances, 15 associations and 3 communities (*Appendix 2*), but predominantly the artificial and highly anthropogenically influenced communities are dominant. The vegetation and habitats could generally be divided into the following main groups: aquatic, grassland, chasmophytic, forest and shrub. There are a lot of artificial communities such as forest plantations, ruderal vegetation, etc. The checklist of habitats is presented in *Appendix 3*. It includes 22 natural, semi-natural and artificial habitats. The corresponding habitat types (11 types) according to the Annex I of HABITAT DIRECTIVE and BIODIVERSITY ACT were also shown in this checklist.

1. Aquatic vegetation - There are no large ponds in Kayluka. The Tuchenitsa River flows through it and three small dams were built. The diversity of aquatic vegetation is poor. Typical are the communities dominated by *Myriophyllum spicatum* L., *Potamogeton nodosus* Poir., *P. pusillus* L., *Zannichellia palustris* subsp. *pedicellata* (Wahleb et Rosén) Hook. The vegetation along the periphery of wetlands is dominated by *Typha latifolia* L., *Phragmites australis* (Cav.) Trin. ex Stend., *Schoenoplectus lacustris* (L.) Pall., and *Sparganium erectum* L. Along the Tuchenitsa River there are communities of *Angelica sylvestris* L., *Epilobium hirsutum* L. and *Scrophularia umbrosa* (Stoj.) Peev.

2. Grasslands - This vegetation is presented by petrophytic (rocky) steppes, dry pastures and mesophytic meadows.

2. 1. The petrophytic (rocky) steppes are spread on the slopes above the rocks. They are dominated by mostly aromatic herbs and semi-shrubs such as *Achillea clypeolata* S. et S., *Satureja montana* ssp. *kitaibelii* (Wierzb. ex Heuff.) Ball., *Teucrium polium* ssp. *capitatum* (L.) Arcangeli.

2. 2. Dry pastures are relatively rare in the protected area and most of them are degraded. They do not occupy large areas and, in most cases, their floristic composition is highly ruderalized. The dominated grass species are *Poa pratensis* L., *Festuca valesiaca* Schleich. ex Gaud., *Chrysopogon gryllus* (L.) Trin. and *Dichanthium ischaemum* (L.) Roberty.

2. 3. The mesophilous meadows were mostly distributed in the valley of the Tuchenitsa River in the past. After the transformation of the protected area

for recreational purposes, they were almost completely destroyed. Today, despite the presence of some species, such as *Arhenatherum elatius* (L.) Beauv ex J. et C. Presl., this vegetation has been replaced by ruderal and semi-ruderal communities.

3. Chasmophytic vegetation - It occurs on the vertical limestone walls on both sides of the valley, but mostly on the eastern part. The most widespread species in their composition are *Asplenium trichomanes* L., *A. ruta-muraria* L., *Galium lucidum* All., *Seseli rigidum* Waldst. et Kit., etc. Some rare or endemic species also participate in its composition, such as *Centaurea affinis* ssp. *balcanica* (Urum. et Wagn.) Dost., *Dianthus petraeus* ssp. *noeanus* (Boiss.) Tutin, *Celtis glabrata* Stev. ex Planch. *Kayluka* is *locus classicus* of *Parietaria erronea* Panov - local endemic for this region of the country (PANOV 1996), assessed as *Data Deficient* in the Red List of Bulgarian vascular plants.

4. Forests - Although there are a lot of forests in the protected area, most of them are plantations, and the natural forest vegetation is highly fragmented, isolated and subject to rapid degradation. Only the massif of the forest *Bohotska Gora* represents a larger complex of natural predominantly oak forest vegetation.

4.1 Natural forests - The largest areas in *Kayluka* are occupied by the forests dominated by *Quercus cerris* L., in some places co-dominated by *Q. frainetto* ten. *Tilia tomentosa* forests have limited distribution on the northeast slopes of the canyon near the Vinarov House. The forests of *Carpinus orientalis* are the second widespread natural forests after the oak forests in *Kayluka*. They occur on the slopes of the canyon, on shallow and eroded soils and on the rocks. Their flora is a rich in ephemeroïdes like *Galanthus elwesii*, *Scilla bifolia* L., *Anemone ranunculoides* L., *Isopyrum thalictroides* L., *Corydalis* spp. In *Kayluka*, there is also one small intra-zonal forest of *Carpinus betulus* L. - on the eastern slope of the canyon behind the chalet *Srebrostruy*. The alluvial willow-poplar forests have been widespread in the valley of the Tuchenitsa River. Today they practically do not occur in *Kayluka*, mostly only groups of willows and black poplars have been found. In many places, the natural riparian vegetation has been replaced by some not native species like *Acer negundo* L. or planted hybrid poplars.

4.2 Forest plantations - The most common plantations in the area are these ones of *Robinia pseudoacacia* L., *Pinus nigra* L., *Picea abies* (L.) H. Karst., *Populus x canadensis*, *Fraxinus pennsylvanica* Marshall, etc. The plantations of *Robinia pseudoacacia* are the most widespread amongst the planted alien species. In some places of *Kayluka* there are also *Juglans regia* L. plantations. These plantations are very dry and overgrown with natural tree, shrub and grass species. The coniferous plantations (mainly *Pinus nigra*) have been planted not only because of their rapid growth and their wood, but also in many places, because of aesthetic reasons.

Everywhere, outside of the valley bottom, reaching a certain age, due mostly to the summer drought, these forests begin to dry up. Today, in the most places coniferous plantations are in poor condition, often burned by summer fires. Some other natural and planted species have, also have negligible participation in the forest vegetation of the area. They are *Quercus robur* L. (single or group of threes), *Q. pubescens* Willd., *Ulmus minor* L., *Fraxinus ornus* (common as single three or small groups), *Picea abies*, *Gleditsia triacanthos* L. Very high degree of presence of exotic, non-European species represented as single trees, small groups or even small-scale plantations is characteristic to the park area. Such species are *Pseudotsuga douglasii*, *Picea pungens*, *Juniperus virginiana*, and many others.

- 5. Shrubs** - In the Protected Area *Kayluka*, there are many shrubs, which are of natural origin and, could rarely be decorative species. The natural shrub vegetation is secondary due to the forest degradation on the steep slopes of the valley. These degraded forests are represented by mixed or monodominant communities of *Paliurus spina-christi* Mill. and *Syringa vulgaris* L. There are also communities of *Mahonia aquifolium* (Pursh.) Nutt, *Lycium barbarum* L. (Archeological site *Storgozia*), *Cercis siliquastrum* (the dams) and others, some of which spontaneously spread in the park.

CONCLUSION

Although *Kayluka* is one of the first protected areas, it is highly urbanized and used as a recreational park of town Pleven. However, it still has a great floristic, taxonomic and syntaxonomic diversity, and is important for the conservation of many protected and endangered plant species, as well as for plant communities and habitats with European nature conservation significance.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

This paper is prepared by ROSSEN TZONEV. RUMYANKA BALEVA and IVAN PURVANOV completed the manuscript after check in the herbarium of Regional Historical Museum of Pleven, and they also determined the categories of presence and rarity of all plant taxa.

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Appendix 1.

Checklist of vascular plants in the Protected Area *Kayluka*. For abbreviations, please follow the text of the paper.

POLYPODIOPHYTA

Aspidiaceae: *Dryopteris filix-mas* (L.) Shott. – VR; BA IV; **Aspleniaceae:** *Asplenium trichomanes* L. – C; *A. adianthum-nigrum* L. – R; *A. ruta-muraria* L. – C; *Phyllitis scolopendrium* (L.) Newn. – VR; *Ceterach officinarum* DC – VR; **Athyriaceae:** *Cystopteris fragilis* (L.) Bernh. – R; **Polypodiaceae:** *Polypodium vulgare* L. – VR.

EQUISETOPHYTA

Equisetaceae: *Equisetum arvense* L. – C; *E. telmateja* Ehrh. – CoR; *E. ramosissimum* Desf. – R; **MAGNOLIOPHYTA:** Magnoliopsida: **Juglandaceae:** *Juglans*

regia L. – R; **Salicaceae:** *Populus alba* L. – CoR; *P. nigra* L. – CoR; *Salix fragilis* L. – CoR; *S. alba* L. – C; *S. purpurea* L. – R; **Betulaceae:** *Alnus glutinosa* (L.) Gaertn. – VR; *Carpinus betulus* L. – VR; *C. orientalis* Mill. – C; *Corylus avellana* L. – R; **Fagaceae:** *Quercus cerris* L. – C; *Q. dalechampii* Ten. – VR; *Q. robur* L. – R; *Q. frainetto* Ten. – CoR; *Q. virgilliana* (Ten.) Ten. – R; *Q. pubescens* Willd. – R; **Ulmaceae:** *Ulmus minor* L. – C; *Celtis glabrata* Stev. ex Planch. – VR; EN; E; **Moraceae:** *Morus alba* L. – C; *Ficus carica* L. – C; **Canabaceae:** *Humulus lupulus* L. – C; *Canabis sativa* L. – C; **Urticaceae:** *Urtica urens* L. – C; *U. dioica* L. – C; *Parietaria erronea* Panov – C; DD; E; *P. officinalis* L. – C; **Santalaceae:** *Comandra elegans* (Roch. et Rchb.) Reichenb. – C; *Thesium simplex* Velen. ssp. *moesiaceum* (Velen.) Koz. et Kuzm. – VR; E; **Polygonaceae:** *Rumex acetosella* L. – R; *R. tuberosus* L. – C; *R. crispus* L. – C; *R. pulcher* L. – C; *R. obtusifolius* L. – C; *Polygonum aviculare* L. – C; *P. rurivagum* Boreau. – C; *Persicaria maculata* (Raf.) S. F. Gray – C; *P. hydropiper* (L.) Opiz. – C; *P. mitis* (Schränk) Assen. – C; *Bilderdykia convolvulus* (L.) Dum. – C; *B. aubertii* (Louis Henry) Moldenke – C; **Phytolaccaceae:** *Phytolacca americana* L. – C; **Portulacaceae:** *Portulacca oleraceae* L. – C; **Caryophyllaceae:** *Paronychia cephalotes* (Bieb.) Bess. – C; *Herniaria hirsuta* L. – C; *Scleranthus perennis* L. – C; *S. annuus* L. – C; *Minuartia setacea* (Thurill) Hay. ssp. *setacea* – C; *Arenaria serpyllifolia* L. – C; *Holosteum umbellatum* L. – C; *Stellaria holostea* L. – C; *S. graminea* L. – C; *S. media* (L.) Vill. – C; *Myosoton aquaticum* (L.) Moench. – C; *Moenchia mantica* (L.) Bartl. – CoR; *Cerastium glomeratum* Thuill. – C; *C. brachypetalum* Pers. – C; *Gypsophilla muralis* L. – CoR; *Saponaria officinalis* L. – C; *Petrorrhagia saxifraga* (L.) Link. – C; *P. prolifera* Ball. et Heyw. – CoR; *P. illyrica* Ball. et Heyw. – CoR; *Dianthus armeria* L. – PE; *D. pallens* S. et S. – C; *D. petraeus* Waldst. et Kit. ssp. *noeanus* (Boiss.) Tutin – R; E; *D. giganteus* D'Urv. ssp. *giganteus* – R; *D. moesiacus* Vis. et Panc. ssp. *moesiacus* – R; E; *Cucubalus baccifer* L. – CoR; *Silene alba* (Mill.) E. Krause – C; *S. conica* L. ssp. *conica* – CoR; *S. dichotoma* Ehrh. ssp. *dichotoma* – C; *S. dichotoma* ssp. *racemosa* (Otth.) Grbn. – C; *S. noctiflora* L. – C; *S. vulgaris* (Moench.) Garcke. ssp. *vulgaris* – C; *S. italica* (L.) Pers. – C; *S. viridiflora* L. – C; *Lychnis coronaria* (L.) Desr. – C; *Viscaria vulgaris* Rohl. ssp. *atropurpurea* (Griseb.) Stoj. – CoR; **Chenopodiaceae:** *Chenopodium botrys* L. – C; *C. vulvaria* L. – CoR; *C. polyspermum* L. – C; *C. hybridum* L. – C; *C. ficifolium* Sm. – C; *C. glaucum* L. – C; *C. album* L. – C; *Atriplex hortensis* L. – C; *A. hastata* L. – CoR; *A. patula* Waldst. et Kit. – C; *Kochia scoparia* (L.) Schra – C; *Salsola rutenica* Iljim. – CoR; *Polycnemum arvense* L. – CoR; **Amaranthaceae:** *Amaranthus hybridus* L. – C; *A. retroflexus* L. – C; *A. albus* L. – C; *A. lividus* L. – CoR; **Ranunculaceae:** *Helleborus odoratus* Waldst. et Kit. – C; *Isopyrum thalictroides* L. – C; *Nigella arvensis* L. – C; *Consolida regalis* S. F. Gray. – C; *C. hispanica* (Costa) Greut. et Burd. – C; *Thalictrum aquilegifolium* L. ssp.

storgosiacum P. Pan. – CoR; E; *T. minus* L. – CoR; *Anemone sylvestris* L. – VR; NT; BA III; *A. ranunculoides* L. – C; *Clematis vitalba* L. – C; *C. recta* L. – VR; *Ranunculus trichophyllus* Chaix. – CoR; *R. ficaria* L. – C; *R. pedatus* Waldst. et Kit. – VR; *R. illyricus* L. – C; *R. millefoliatus* Vahl. – C; *R. repens* L. – C; *R. sardous* Crantz – VR; *R. acris* L. – C; *R. sceleratus* L. – C; *R. arvensis* L. – C; *R. auricomus* L. – VR; *R. villosus* DC. ssp. *constantinopolitanus* A. Elen. – VR; E; *R. falax* (Wimm. et Grab.) Sloboda – VR; *R. polyanthemus* L. – C; *Hepatica nobilis* Mill. – VR; *Adonis flammea* Jacq. – C; *A. aestivalis* L. – C; **Berberidaceae:** *Berberis vulgaris* L. – VR; *Mahonia aquifolium* (Pursh.) Nutt. – C; **Ceratophyllaceae:** *Ceratophyllum demersum* L. – C; **Aristolochiaceae:** *Aristolochia clematitis* L. – C; **Paeoniaceae:** *Paeonia peregrina* Mill. – VR; BA IV; **Hypericaceae:** *Hypericum hirsutum* L. – CoR; *H. perforatum* L. – C; *H. elegans* Stephan. ex Willd. – VR; **Papaveraceae:** *Chelidonium majus* L. – C; *Glaucium corniculatum* (L.) J. H. Rudolph. – VR; *Papaver dubium* L. – C; *P. rhoeas* L. – C; *Corydalis solida* (L.) Swartz. – C; *C. bulbosa* (L.) DC – C; *Fumaria vaillantii* Loisel. – CoR; *F. officinalis* L. – C; **Brassicaceae:** *Sisimbrium officinale* (L.) Scop. – C; *S. orientale* L. – C; *Descurainia sofia* (L.) Webb. ex Prantl. – C; *Alliaria petiolata* (Bieb.) Cavara et Grande – C; *Myagrum perfoliatum* L. – C; *Erysimum repandum* L. – C; *E. diffusum* Ehrh. – C; *E. cuspidatum* (Bieb.) DC – C; *Hesperis sylvestris* Crantz. ssp. *sylvestris* – CoR; VU; BA III *Rorripa sylvestris* (L.) Bess. – C; *R. pyrenaica* (L.) Reichenb. – CoR; *Turritis glabra* L. – CoR; *Cardamine bulbifera* (L.) Crantz. – CoR; *Arabis turrita* L. – CoR; *A. recta* Vill. – CoR; *A. sagittata* (Bertol.) DC – CoR; *Lunaria annua* L. – CoR; *Alyssum saxatile* L. – C; *A. minutum* Schlecht. ex DC. – C; *A. alyssoides* L. – C; *A. minus* (L.) Rothm. ssp. *hirsutum* (Bieb.) Stoj. et Stev. – C; *Berteroa incana* (L.) DC – C; *Draba muralis* L. – VR; *Erophila verna* (L.) Bess. ssp. *verna* – C; *Camelina microcarpa* Andz. ex DC. – VR; *C. sativa* (L.) Crantz. ssp. *sativa* – VR; *Capsella bursa-pastoris* (L.) Medic – C; *Thlaspi perfoliatum* L. – C; *T. arvense* L. – C; *T. alliaceum* L. – C; *Lepidium perfoliatum* L. – CoR; *L. campestre* (L.) R. Br. – C; *Cardaria draba* (L.) Desv. – C; *Conringia orientalis* (L.) Dum. – C; *Brassica nigra* (L.) Koch – C; *Sinapis arvensis* L. – C; *Calepina irregularis* (Asso) Thell. – C; *Raphanus raphanistrum* L. – C; **Resedaceae:** *Reseda lutea* L. – C; **Crassulaceae:** *Sedum maximum* (L.) Suter. – C; *S. hispanicum* L. – C; *S. album* L. – VR; *S. ochroleucum* Chaix. – VR; *S. sartorianum* Boiss. – CoR; **Saxifragaceae:** *Saxifraga tridactylites* L. – CoR; **Rosaceae:** *Filipendula vulgaris* Moench. – C; *Rubus caesius* L. – C; *R. lloydianus* Genev. – R; *Rosa myriacantha* DC ex Lam. et DC. – R; *R. arvensis* Huds. – R; *R. gallica* L. – C; *R. canina* L. – C; *R. vosagiaca* Desp. – VR; *Agrimonia eupatoria* L. ssp. *eupatoria* – C; *Aremonia agrimonoides* (L.) DC. – R; *Sanguisorba minor* Scop. ssp. *minor* – C; *Geum urbanum* L. – C; *Potentilla micrantha* Ramond ex DC. – CoR; *P. supina* L. – CoR; *P. argentea* L. – C; *P. neglecta* Baumg. – C; *P. bornmuelleri* Borb. – VR;

P. reptans L. – C; *P. pedata* Willd. – VR; *P. pilosa* Willd. – CoR; *P. obscura* Willd. – C; *Fragaria vesca* L. – CoR; *F. moschata* Duch. – C; *Pyrus pyraeaster* Burgsd. – C; *P. nivalis* Jacq. – R; *Malus sylvestris* Mill. – R; *M. praecox* (Pall.) Borkh. – VR; *Sorbus domestica* L. – R; *S. torminalis* (L.) Crantz – VR; *Cotoneaster niger* (Thunb.) Fries – PE; *C. integerrimus* Medic. – VR; *Crataegus monogyna* Jacq. – C; *Prunus machaleb* L. – VR; *P. avium* L. – VR; *P. spinosa* L. – C; *P. cerasifera* Ehrh. – C; **Fabaceae:** *Chamaecytisus hirsutus* (L.) Link. – C; *Bituminaria bituminosa* (L.) Stirt. – C; *Amorpha fruticosa* L. – C; *Galega officinalis* L. – C; *Robinia pseudoacacia* L. – C; *Astragalus onobrychis* ssp. *chlorocarpus* (Griseb.) Stoj. et Stef. – C; *A. cicer* L. – CoR; *A. glycyphyllos* L. – CoR; *Dorycnium herbaceum* Vill. – C; *Lotus corniculatus* L. – C; *Coronilla scorpioides* (L.) Koch – C; *C. varia* L. – C; *Onobrychis vicifolia* Scop. – R; *O. arenaria* (Kit.) DC – R; *Ononis arvensis* L. – R; *Trigonella coerulea* (L.) Ser. – R; *Medicago lupulina* L. – C; *M. falcata* L. – C; *M. arabica* (L.) Huds. – R; *M. orbicularis* (L.) Bartal. – R; *M. minima* (L.) Bartal. – CoR; *M. rigidula* (L.) All. – R; *Melilotus alba* Medic. – C; *M. officinalis* (L.) Pall. – C; *Trifolium campestre* Schreb. – C; *T. repens* L. ssp. *repens* – C; *T. alpestre* L. ssp. *lanigerum* (Ser.) Hayek – C; E; *T. ochroleucon* Huds. ssp. *caucasicum* (Tausch.) Koz. – VR; *T. pratense* L. – C; *T. echinatum* Bieb. – C; *T. incarnatum* L. ssp. *molinerii* (Babb. ex Hornem.) Syme – R; *T. arvense* L. – C; *Vicia serratifolia* Jacq. – CoR; *V. narborensis* L. – C; *V. tetrasperma* (L.) Schrb. – C; *V. lathyroides* L. – C; *V. peregrina* L. – C; *V. pannonica* Crantz ssp. *panonica* Crantz – C; *V. pannonica* ssp. *striata* (Bieb.) Nyman – C; *V. grandiflora* Scop. – C; *V. sativa* L. – C; *V. hirsuta* (L.) S. F. Gery. – CoR; *V. varia* Host. – C; *V. cracca* L. – C; *Lathyrus nissolia* L. – C; *L. aphaca* L. – C; *L. pannonicus* (Jacq.) Garcke ssp. *varius* (C. Koch) Ball. – VR; *L. sylvestris* L. – R; *L. sativus* L. – R; *L. hirsutus* L. – C; *L. pratensis* L. – R; *L. tuberosus* L. – C; *L. sphaericus* Retz. – C; *L. laxiflorus* (Desf.) O. Kuntze – R; *L. niger* (L.) Bernh. – R; *L. vernus* (L.) Berhn. – VR; **Oxalidaceae:** *Oxalis corniculata* L. – C; **Geraniaceae:** *Geranium sanguineum* L. CoR; *G. lucidum* L. – CoR; *G. robertianum* L. – C; *G. phaeum* L. – VR; *G. columbinum* L. – CoR; *G. dissectum* L. – R; *G. pusillum* L. – C; *G. pyrenaicum* Burm. – C; *G. rotundifolium* L. – C; *Erodium cicutarium* (L.) L' Her – C; **Zygophyllaceae:** *Tribulus terrestris* L. C; **Linaceae:** *Linum hirsutum* L. ssp. *hirsutum* – R; *L. tenuifolium* L. – CoR; **Euphorbiaceae:** *Mercurialis ovata* Sternb. et Hoppe – R; *M. perennis* L. – R; *Euphorbia chamaesyce* L. – C; *E. polychroma* Kern. – CoR; *E. helioscopia* L. – C; *E. amygdaloides* L. – R; *E. nicaensis* All. ssp. *nicaensis* – C; *E. cyparissias* L. – C; *E. esula* L. ssp. *tommasiniana* (Bertol.) Nyman – C; *E. agraria* Bieb. – C; **Rutaceae:** *Haplophyllum suaveolens* (DC) G. Don. – CoR; *Dictamnus albus* L. – PD; **Simaroubaceae:** *Ailanthus altissima* (Mill.) Swingle – C; **Polygalaceae:** *Polygala vulgaris* L. – R; *P. major* Jacq. – R; **Anacardiaceae:** *Cotinus coggygria* Scop. – C; **Aceraceae:** *Acer tataricum*

L. – C; *A. campestre* L. – C; **Celastraceae:** *Euonymus verrucosus* Scop. – CoR; *E. europaeus* L. – CoR; **Staphyleaceae:** *Staphylea pinnata* L. – R; **Rhamnaceae:** *Rhamnus catharticus* L. – R; *Rh. saxatilis* Jacq. ssp. *saxatilis* – R; *Paliurus spina-christi* Mill. – C; **Tiliaceae:** *Tilia tomentosa* Moench. – C; *T. cordata* Mill. – R; **Malvaceae:** *Abutilon theophrastii* Medic. – C; *Lavatera thuringiaca* L. – C; *Althaea hirsuta* L. – CoR; *A. officinalis* L. – CoR; *A. cannabina* L. – CoR; *Alcea rosea* L. – R; *A. pallida* (Waldst. et Kit. ex Wild.) Waldst. et Kit. – R; *Malva sylvestris* L. – C; *Hibiscus trionum* L. – C; **Thymeleaceae:** *Thymelaea passerina* (L.) Coss. et. Germ. – R; **Eleagnaceae:** *Elaeagnus angustifolia* L. – R; **Violaceae:** *Viola mirabilis* L. – VR; *V. reichenbachiana* Jord. ex Boreau. – CoR; *V. sieheana* Becker – R; *V. jordanii* Henry. – C; *V. suavis* Bieb. – C; *V. odorata* L. – C; *V. alba* L. – C; *V. hirta* L. – C; *V. kitaibeliana* Schult. – CoR; *V. arvensis* Murr. – C; **Cistaceae:** *Helianthemum nummularium* (L.) Mill. – R; *Rhodax canus* (L.) Fuss. – PE; **Tamaricaceae:** *Tamarix ramosissima* Ledeb. – R; **Cucurbitaceae:** *Bryonia alba* L. – VR; **Lythraceae:** *Lythrum salicaria* L. – R; **Onagraceae:** *Epilobium hirsutum* L. – C; *E. parviflorum* Schreb. – CoR; **Haloragaceae:** *Myriophyllum spicatum* L. – CoR; **Cornaceae:** *Cornus mas* L. – C; *C. sanguinea* L. – C; **Araliaceae:** *Hedera helix* L. – C; **Apiaceae:** *Eryngium campestre* L. – C; *Myrrhoides nodosa* (L.) Cann. – C; *Anthriscus sylvestris* (L.) Hoffm. – R; *A. nemorosa* (Bieb.) Spreng. – R; *A. cerefolium* (L.) Hoffm. – C; *A. caucalis* Bieb. – R; *Torilis ucranica* Spreng. – R; *T. japonica* (Houtt.) DC – R; *T. arvensis* (Huds.) Link. ssp. *arvensis* – C; *T. arvensis* ssp. *neglecta* (Schult.) Thell. – C; *Caucalis platycarpos* L. – C; *Turgenia latifolia* (L.) Hoffm. – VR; *Orlaya grandiflora* (L.) Hoffm. – C; *Bifora radians* Bieb. – C; *Conium maculatum* L. – C; *Bupleurum praealtum* L. – C; BA IV; *B. affine* Sald. – VR; BA IV; *Falcaria vulgaris* Bernh. – C; *Pimpinella saxifraga* L. – CoR; *Aegopodium podagraria* L. – CoR; *Sium latifolium* L. – VR; *Berula erecta* (Huds.) Coville – C; *Seseli rigidum* Waldst. et Kit. ssp. *rigidum* – CoR; *Physospermum cornubiensis* (L.) DC – VR; *Angelica pancicii* Vand. – VR; VU; E; *A. sylvestris* L. – R; *Ferulago sylvatica* (Bess.) Reichenb. – R; *Peucedanum longifolium* Waldst. et Kit. – VR; *P. alsaticum* L. – CoR; *Pastinaca hirsuta* Panc. – C; *P. sativa* L. ssp. *sativa* – C; *Heracleum sibiricum* L. – C; *Tordilium maximum* L. – C; *Laser trilobum* (L.) Borkh. – C; *Daucus carota* L. – C; **Primulaceae:** *Lysimachia nummularia* L. – C; *L. vulgaris* L. ssp. *glandulo-villosa* (Beck.) Peev – R; *L. punctata* L. – VR; *Anagalis arvensis* L. ssp. *arvensis* – C; *A. arvensis* L. ssp. *foemina* (Mill.) Schinz. et Thell. – C; *Androsace maxima* L. – R; *Primula veris* L. ssp. *canescens* (Len.) Ludi – R; BA IV; **Plumbaginaceae:** *Plumbago europaea* L. – R; **Oleaceae:** *Fraxinus ornus* L. – C; *Syringa vulgaris* L. – C; *Ligustrum vulgare* L. – C; **Gentianaceae:** *Centaurium erythraea* Rafn. ssp. *erythraea* – C; **Apocynaceae:** *Vinca herbacea* Waldst. et Kit. – CoR; *V. minor* L. – CoR; **Asclepiadaceae:** *Vincetoxicum*

hirundinaria L. – CoR; **Convolvulaceae:** *Convolvulus arvensis* L. – C; *C. cantabrica* L. – C; *Calystegia sepium* (L.) R. Br. – C; *C. sylvatica* (Kit.) Griseb. – C; **Cuscutaceae:** *Cuscuta monogyna* Vahl. – C; *C. europaea* L. – C; *C. epithymum* L. ssp. *epithymum* – CoR; **Rubiaceae:** *Sherardia arvensis* L. – C; *Crucianella angustifolia* L. – R; *Asperula purpurea* (L.) Ehrend. – R; *A. taurina* L. ssp. *leucanthera* (G. Beck.) Hayek – CoR; E; *A. tenella* Heuff. ex Deg. – R; *A. cynanchia* L. – C; *Galium humifusum* Bieb. – C; *G. octonarium* (Klokov) Soo – C; *G. pseudoaristatum* Schur. – C; E; *G. verum* L. – C; *G. lucidum* All. – C; *G. album* Mill. ssp. *album* – C; *G. aparine* L. – C; *Cruciata pedemontana* (Bell.) Ehrend. – C; *C. laevipes* Opiz. – C; *C. glabra* (L.) Ehrend. – R; **Boraginaceae:** *Heliotropium europaeum* L. – C; *Buglossoides purpureocaerulea* (L.) Johnst. – C; *B. arvensis* (L.) Johnst. – C; *Cerinthe minor* L. – C; *Echium italicum* L. – C; *E. vulgare* L. – C; *Pulmonaria officinalis* L. – C; *P. mollis* Wulf. ex Horn. – VR; LC; *Nonea pulla* (L.) DC – R; *Symphitum officinale* L. – R; *Anchusa procera* Bess. – CoR; *A. officinalis* L. – C; *Asperugo procumbens* L. – C; *Myosotis laxa* Lehm. ssp. *caespitosa* (C. F. Schultz.) Hyl. ex Nordhl. – R; *M. scorpiodes* L. – R; *M. stricta* Link. et Koem. et Schult. – R; *M. ramosissima* Roch. – CoR; *Lappula marginata* (Bieb.) Gurke. – CoR; *L. squarosa* (Retz.) Dum. – R; *Cynoglossum officinale* L. – R; *C. creticum* Mill. – R; *C. hungaricum* Simk. – R; **Verbenaceae:** *Verbena officinalis* L. – C; **Lamiaceae:** *Teucrium polium* L. ssp. *capitatum* (L.) Arcangeli – C; *T. montanum* L. ssp. *montanum* R; *T. chamaedrys* L. – C; *Ajuga chamaeepytis* (L.) Schreb. ssp. *chia* (Schreb.) Arcangeli – C; *A. laxmanii* (L.) Benth. – CoR; *A. genevensis* L. – C; *A. reptans* L. – C; *Mentha pulegium* L. – C; *M. arvensis* L. – C; *M. aquatica* L. – CoR; *M. spicata* L. ssp. *spicata* – C; *M. longifolia* (L.) Huds. – C; *Lycopus europaeus* L. – C; *L. exaltatus* L. – CoR; *Origanum vulgare* L. ssp. *vulgare* – CoR; *Thymus callierii* Borb. ex Velen. ssp. *urumovii* Velen. – C; E; *T. moesiacus* Velen. – R; E; *T. sibthorpii* Benth. – R; E; *Melissa officinalis* L. ssp. *officinalis* – R; *Satureja montana* L. ssp. *kitaibelii* (Wierzb. ex Heuff.) Ball. – C; E; *Calamintha sylvatica* Bromf. ssp. *sylvatica* – CoR; *C. nepeta* (L.) Savi. ssp. *glandulosa* (Req.) Ball. – CoR; *Acinos arvensis* (Lam.) Dandy – CoR; *A. rotundifolius* Pers. – CoR; *A. alpinus* (L.) Moench. ssp. *hungaricus* (Simonk.) Sojak – C; *Clinopodium vulgare* L. – C; *Marrubium peregrinum* L. – PE; *Sideritis montana* L. – C; *Glechoma hederacea* L. – C; *G. hirsuta* Waldst. et Kit. – C; *Melittis melissophyllum* L. ssp. *albida* (Guss.) Ball. – R; *Leonurus cardiaca* L. – CoR; *L. marrubiastrum* L. – CoR; *Ballota nigra* L. ssp. *nigra* – C; *Stachys officinalis* (L.) Trev. – CoR; *S. germanica* L. – C; *S. annua* L. – C; *S. recta* L. ssp. *subcrenata* (Vis.) Briq. – C; E; *S. palustris* L. – CoR; *S. sylvatica* L. – R; *Phlomis tuberosa* L. – CoR; *Lamium maculatum* L. – CoR; *L. amplexicaule* L. – C; *L. purpureum* L. – C; *Lamiastrum galeobdolon* (L.) Ehrend. et Polaschek. ssp. *galeobdolon* – CoR; *Galeopsis ladamum* L. – R; *Prunella laciniata* L. – CoR; *P. vulgaris* L. – C; *Salvia verticillata* L. – C; *S.*

aethiopsis L. – C; *S. amplexicaulis* Lam. – R; *S. virgata* Jacq. – R; *S. nemorosa* L. – C; *Scutellaria columnae* All. – R; *S. altissima* L. – R; **Solanaceae:** *Hyoscyamus niger* L. – R; *Physalis alkekengi* L. – R; *Solanum dulcamara* L. – C; *S. nigrum* L. – C; *S. schultesii* Opiz. – C; *Datura stramonium* L. – C; *Lycium barbatum* L. – C; **Scrophulariaceae:** *Verbascum blattaria* L. – CoR; *V. phoeniceum* L. – C; *V. phlomoides* L. – C; *V. banaticum* Schrad. – C; *V. nigrum* L. – CoR; *V. lychnitis* L. – CoR; *Kickxia spuria* (L.) Dum. – R; *K. elatine* (L.) Dum. – C; *Cymbalaria muralis* Gaertner. – R; *Linaria vulgaris* Mill. – C; *L. genistifolia* (L.) Mill. ssp. *linifolia* (Boiss.) Davis. – C; *L. genistifolia* ssp. *genistifolia*. – C; *Scrophularia umbrosa* (Stoj.) Peev – CoR; *Gratiola officinalis* L. – CoR; *Pseudolysimachion orhideum* (Crantz) Wraber – R; *Veronica austriaca* L. ssp. *jaquinii* (Baugm.) Maly – C; *V. prostrata* L. – R; *V. chamaedrys* L. – R; *V. teucrium* L. ssp. *teucrium* – R; *V. anagalis-aquatica* L. ssp. *anagalidiformis* (Boreau.) Jav. et Soo – C; *V. triphyllus* L. – C; *V. arvensis* L. – C; *V. verna* L. ssp. *verna* – C; *V. triloba* (Opiz.) Kern. – C; *V. hederifolia* L. – C; *V. persica* Poir. – C; *V. polita* Fries – C; *Digitalis lanata* Ehrh. – C; *Melampyrum arvense* L. ssp. *arvense* – CoR; *M. arvense* ssp. *pseudobarbatum* (Schur.) Wettst. – CoR; *M. cristatum* L. ssp. *solstitiale* (Ronn.) Ronn. – CoR; *Odontites verna* (Bell.) Dum ssp. *serotina* (Dum) Corb. – C; *Rhinanthus rumelicus* Vel. – C; E; **Orobanchaceae:** *Lathraea squamaria* L. – VR; *Orobanche ramosa* L. – R; *O. alba* Steph. ex Willd. – R; *O. caryophyllaceae* Sm. – R; *O. gracilis* Sm. – R; **Acanthaceae:** *Acanthus balcanicus* Heyw. – R; E; **Plantaginaceae:** *Plantago lanceolata* L. – C; *P. major* L. – C; *P. media* L. – C; **Caprifoliaceae:** *Sambucus ebulus* L. – C; *S. nigra* L. – CoR; *Viburnum lantana* L. – C; **Valerianaceae:** *Valerianella coronata* (L.) DC – C; *V. carinata* Loisel. – C; *Valeriana officinalis* L. ssp. *officinalis* – CoR; **Dipsacaceae:** *Cephalaria transilvanica* (L.) Roem. et Schult. – C; *Dipsacus laciniatus* L. – C; *Knautia arvensis* (L.) Coult. – C; *Scabiosa ochroleuca* L. ssp. *ochroleuca* – C; *S. micrantha* Desf. – R; *S. argentea* L. – CoR; **Campanulaceae:** *Campanula persicifolia* L. – CoR; *C. lingulata* Waldst. et Kit. – CoR; *C. sibirica* L. – C; *C. bononiensis* L. – C; *C. trachelium* L. ssp. *trachelium* – CoR; *C. rapunculoides* L. – CoR; **Asteraceae:** *Eupatorium cannabinum* L. – C; *Solidago canadensis* L. – R; *Bellis perennis* L. – C; *Aster amellus* L. – VR; *Erigeron acer* L. – C; *E. amius* (L.) Presl. – C; *Filago vulgaris* Lam. C; *Inula helenium* L. – VR; *I. conyza* DC. – CoR; *I. germanica* L. – CoR; *I. bifrons* L. – CoR; *I. ensifolia* L. – CoR; *I. hirta* L. – CoR; *I. salicina* L. ssp. *aspera* (Poir.) Hay. – CoR; *I. britanica* L. – CoR; *Pulicaria dysenterica* (L.) Bernh. – C; *Galinsoga parviflora* Cav. – CoR; *Xanthium spinosum* L. – C; *X. strumarium* L. – C; *X. italicum* Moret. – C; *Bidens tripartita* L. – CoR; *B. frondosa* L. – C; *Anthemis tinctoria* L. – C; *A. austriaca* Jacq. – CoR; *A. arvensis* L. – CoR; *Achillea nobilis* L. ssp. *neilreichii* (A. Kern.) Velen. – R; *A. millefolium* L. – C; *A. clypeolata* S. et S. – C; E;

Matricaria perforata Merat. – C; *M. trichophylla* (Boiss.) Boiss. – C; *Leucanthemum vulgare* Lam. – R; *Chamomilla recutita* (L.) Rausch. – CoR; *Tanacetum vulgare* L. – CoR; *T. corymbosum* (L.) Schultz-Bib. – CoR; *Artemisia vulgaris* L. – C; *A. absinthium* L. – C; *A. annua* L. – C; *A. scoparia* Waldst. et Kit. – C; *Tussilago farfara* L. – CoR; *Doronicum hungaricum* L. – CoR; *D. orientale* Hoffm. – VR; *Senecio vulgaris* L. – C; *S. vernalis* Waldst. et Kit. – C; *S. jacobaea* L. ssp. *jacobaea* – CoR; *Echinops banaticus* Roch. ex. Schrad. – C; BA IV; *E. ritro* L. – R; BA IV; *E. sphaerocephalus* L. ssp. *sphaerocephalus* – R; BA IV; *Xeranthemum annuum* L. – C; *Carlina vulgaris* L. – CoR; *Arctium lappa* L. – C; *A. minus* Bernh. – C; *Jurinea ledebourii* Bunge. – R; EN; BA III; *Carduus nutans* L. – C; *C. thoermeri* Weinm. – C; *C. acanthoides* L. – C; *Cirsium vulgare* (Savi.) Ten. – C; *C. ligulare* Bois. – C; *C. arvense* (L.) Scop. – C; *C. creticum* (L.) D"Urv. – C; *Onopordum acanthium* L. – C; *Crupina vulgaris* Cass. – CoR; *Serratula tinctoria* L. – CoR; *Centaurea jacea* L. – R; *C. calcitrapa* L. – C; *C. solstitialis* L. – C; *C. cyanus* L. – C; *C. orientalis* L. – C; *C. salonitana* Vis. – CoR; *C. scabiosa* L. ssp. *spinulosa* (Roch. ex Spreng.) Dost. – C; *C. diffusa* Lam. – C; *C. rutifolia* S. et S. ssp. *jurineifolia* (Boiss.) N – C; *C. affinis* Friv. ssp. *affinis* – R; *C. affinis* Friv. ssp. *balcanica* (Urum. et Wagn.) Dost. – R; E; *C. rhenana* Boreau – CoR; *C. iberica* Trev. ex Spreng. – C; *Carthamus lanatus* L. – C; *Conyza canadensis* (L.) Cronq. – C; *Cichorium intybus* L. – C; *Lapsana communis* L. – C; *Leontodon hispidus* L. ssp. *hispidus* – C; *L. crispus* Vill. ssp. *crispus* – C; *Picris hieracioides* L. ssp. *hieracioides* – C; *Tragopogon dubius* Scop. – C; *Scorzonera lhispanica* L. – R; *Chondrilla juncea* L. – C; *Taraxacum serotinum* (Waldst. et Kit.) Poir. – C; *T. officinale* Web. – C; *Sonchus oleraceus* L. – C; *S. arvensis* L. ssp. *arvensis* – C; *S. arvensis* L. ssp. *uliginosus* (Bieb.) Nym. – CoR; *Lactuca serriola* L. – C; *L. saligna* L. – C; *L. quercina* L. ssp. *quercina* – R; *L. viminea* (L.) J. et C. Presl. – R; *Mycelis muralis* (L.) Dum. – CoR; *Crepis sancta* (L.) Babcock. – R; *C. foetida* L. ssp. *foetida* – C; *C. foetida* ssp. *rhoedifolia* (Bieb.) Celak – C; *C. setosa* Hall. – C; *C. pulchra* L. – CoR; *Hieracium hoppeanum* Schult. – R; *H. praealtum* Vill. ex Goch. ssp. *bauchinii* (Bess.) Petum. – R; *H. cymosum* L. ssp. *cymosum* – R; *H. echioides* Lunm. – R; *H. umbellatum* L. – R; *H. racemosum* Waldst. et Kit. – R.

Liliopsida: Butomaceae: *Butomus umbellatus* L. – C; **Alismataceae:** *Alisma plantago-aquatica* L. – C; *A. lanceolatum* With. – C; **Hydrocharitaceae:** *Elodea canadensis* Michx. – CoR; **Potamogetonaceae:** *Potamogeton pectinatus* L. – C; *P. pussilus* L. – R; *P. natans* L. – C; *P. crispus* L. – C; **Zannichelliaceae:** *Zannichellia palustris* L. ssp. *pedicelata* Wahl. et Rosen – C; **Liliaceae:** *Colchicum autumnale* L. – CoR; *Gagea pratensis* (Pers.) Dum. – C; *G. lutea* (L.) Ker- Gaw. – R; *G. minima* (L.) Ker- Gaw. – C; *G. arvensis* (Pers.) Dum. – C; *Allium scordoprasum* L. – C; *A. sphaerocephalon* L. – R; *A. moschatum* L. – C; *A. flavum* L. – CoR; *A. rotundrum* L. – C; *A. paniculatum*

ssp. *fuscum* (Waldst. et Kit.) Arcang. – CoR; E; *Lilium martagon* L. – R; BA IV; *Scilla bifolia* L. – C; BA IV; *Ornithogalum nutans* L. ssp. *nutans* – R; *O. pyrenaicum* L. – CoR; *O. narbonense* L. – C; *O. refractum* Kit. ex Schecht. – R; *O. sibthorpii* Greut. – R; *O. kochii* Parl. – R; *O. umbellatus* L. – C; *Hyacinthella leucophaea* (Stev.) Shur. – C; *Muscari botryoides* (L.) Mill. – VR; *M. racemosum* (L.) Lam. et DC. – C; *M. tenuiflorum* Tausch. – C; *Asparagus tenuifolius* Lam. – C; BA IV; *A. officinalis* L. – C; BA IV; *Ruscus aculeatus* L. – R; BA IV; *Polygonatum latifolium* (Jacq.) Desf. – C; *Convallaria majalis* L. – VR; **Amarillidaceae:** *Galanthus elwesii* Hook fil. ssp. *minor* Welb. – R; EN; BA III; *Sternbergia colchiciflora* Waldst. et Kit. – C; **Dioscoreaceae:** *Tamus communis* L. – R; **Iridaceae:** *Crocus flavus* West. – C; BA IV; *Iris sintenisii* Janka – CoR; *I. pseudacorus* L. – R; *I. pumila* L. – VR; *I. variegata* L. – CoR; **Orchidaceae:** *Orchis papilionacea* L. – PE; BA III; *O. simia* L. – R; BA IV; *O. purpurea* Huds. – R; BA IV; *Ophrys scolopax* Cav ssp. *cornuta* (Stev.) Camus. – PE; BA III; *Himantoglossum caprinum* (L.) Spreng. – BA II III; *Cephalanthera damasonium* (Mill.) Druce. – R; *Spiranthes spiralis* (L.) Chevall. – VR; VU; BA III; **Juncaceae:** *Juncus effusus* L. – R; *J. inflexus* L. – C; *J. conglomeratus* L. – C; *J. compressus* Jacq. – C; *Luzula campestris* (L.) DC – VR; **Cyperaceae:** *Schoenoplectus lacustris* (L.) Pall. – C; *Eleocharis palustris* (L.) R. Br. – C; *Bolboschoenus maritimus* (L.) Palla – C; *Carex praecox* Schreb. – C; *C. hirta* L. – R; *C. remota* L. – R; *C. muricata* L. – C; *C. divisa* Huds. – CoR; *C. michelii* Host. – CoR; *C. flacca* Schreb. – CoR; *C. caryophyllaea* Latourr. – C; *C. digitata* L. – VR; *C. tomentosa* L. – R; *C. riparia* Curt. – CoR; *C. pseudocyperus* L. – C; *C. pilosa* Scop. – VR; *C. melanostachya* Bieb. ex Willd. – R; *C. distans* L. – R; **Poaceae:** *Dichanthium ischaemum* (L.) Roberty. – C; *Chrysopogon gryllus* (L.) Trin. – C; *Tragus racemosus* (L.) Desf. – C; *Echinochloa crus-galli* (L.) Beauv. – C; *Setaria verticillata* (L.) Beauv. – C; *S. pumila* (Poir.) Schult. – C; *S. italica* (L.) Beauv. – R; *S. viridis* (L.) Beauv. – C; *Anthoxanthum odoratum* L. – CoR; *Stipa capillata* L. – C; BA IV; *S. pulcherrima* L. – C; BA IV; *Piptatherum virescens* (Trin.) Boiss. – C; *Phleum phleoides* (L.) Karst. – CoR; *P. montanum* C. Koch. – CoR; *P. pratense* L. ssp. *pratense* – C; *P. graecum* Boiss. et Heldr. – CoR; *P. paniculatum* Huds. – CoR; *Alopecurus myosuroides* Huds. – C; *A. pratensis* L. – C; *Agrostis stolonifera* L. – C; *A. castellana* L. – VR; *Calamagrostis epigeios* (L.) Roth. – C; *Vulpia myurus* (L.) C. C. Gmel. – CoR; *Avena fatua* L. – C; *Arrhenatherum elatius* (L.) Beauv ex J. et C. Presl. – C; *Cynodon dactylon* (L.) Pers. – C; *Phragmites australis* (Cav.) Trin. ex Stend. – C; *Cleistogenes serotina* (L.) Keng. – CoR; *Eragrostis minor* Host. – C; *E. pilosa* (L.) Beauv. – C; *Koeleria macrantha* (Ladab.) Schult. et Schult. – C; *Melica ciliata* L. – C; *M. transsylvanica* Schur. – CoR; *M. uniflora* Reutz. – C; *Briza media* L. – CoR; *Dactylis glomerata* L. ssp. *glomerata* – C; *Cynosurus echinatus* L. – CoR; *Sclerochloa dura* (L.) Beauv. – C; *Poa compressa* L. – CoR; *P. bulbosa* L. – C; *P. angustifolia* L. – C;

P. annua L. – C; *P. nemoralis* L. – C; *P. pratensis* L. – C; *Puccinellia limosa* (Schur.) Holmb. – CoR; *Festuca pratensis* L. – CoR; *F. heterophylla* Lam. – C; *F. valesiaca* Schleich. ex Gaud. – C; *Bromus sterilis* L. – C; *B. tectorum* L. – CoR; *B. inermis* Leyss. – C; *B. ramosus* Huds. – VR; *B. arvensis* L. – C; *B. secalinus* L. – CoR; *B. mollis* L. – C; *B. racemosus* L. – C; *B. squarrosus* L. – CoR; *Brachypodium sylvaticum* (Huds.) Beauv. – C; *Lolium perenne* L. – C; *Dasyphyrum villosum* (L.) Caud. – C; *Aegilops cylindrica* Host. – C; *Hordeum bulbosum* L. – R; *H. leporinum* Link. – R; *H. murinum* L. – C; *Elymus hispidus* (Opiz.) Meld. ssp. *barbulatus* (Schur.) Meld. – CoR; *E. repens* (L.) Gould. – C; *E. elongatus* (Host.) Greut. ssp. *elongatus* – CoR; *Digitaria sanguinalis* (L.) Scop. – C; *Glyceria plicata* (Fries.) Fries. – C; *Sorghum halepense* (L.) Pers. – C; *Taeniatherum caput-medusae* (L.) Nevski. – CoR; **Araceae:** *Arum maculatum* L. – C; **Lemnaceae:** *Lemna minor* L. – C; **Typhaceae:** *Typha angustifolia* L. – C; *T. latifolia* L. – C; **Sparganiaceae:** *Sparganium erectum* L. ssp. *neglectum* (Beeby) Schinz. et Thell. – C; *S. erectum* ssp. *erectum* L. – C.

Appendix 2.

Synoptic scheme of the Protected Area *Kayluka*

Class *Lemnetea minoris* (R. Tx. 1955) de Bolos et Masclans 1955
Order *Lemnietalia minoris* (R. Tx. 1955) de Bolos et Masclans 1955
Alliance *Lemnion gibbae* R. Tx. et Schwabe-Braun in R. Tx. 1974
Association *Lemnetum minoris* Th. Müller et Görs 1960
Class *Potamogetonetea pectinati* R. Tx. et Prsg. 1942
Order *Potamogetonetalia pectinati* W. Koch 1926
Alliance *Potamogetonion pectinati* (Koch 1926) Görs 1977
Association *Myriophylletum spicati* Soó 1927
Association *Ceratophylletum demersi* Corillion 1957
Class *Phragmitetea communis* Tx. et Prsg. 1942
Order *Phragmitetalia communis* Koch 1926
Alliance *Phragmition communis* Koch 1926
Association *Typhetum latifoliae* G. Lang 1973
Association *Phragmitetum communis* von Soó 1927
Order *Nasturtio-Glyceiretalia Pignatti* 1953
Alliance *Glycerio-Sparganion* Br.-Bl. et Sissingh in Boer 1942
Association *Leersietum oryzoidis* Eggler 1933
Class *Epilobietea angustifolii* Tx. et Preising ex von Rochow 1951
Order *Convolvuletalia sepium* Tx. ex Moor 1958
Alliance *Archangelicion litoralis* Scamoni et Passarge 1963
Association *Brachypodio sylvatici-Angelicetum sylvestris* Tzonev 2017
Class *Montio-Cardaminetea* Br.-Bl. et Tx. ex Klika et Hadac 1944

Order *Montio-Cardaminetalia* Pawlowski in Pawlowski et al. 1928
 Alliance *Cratoneurion commutati* W. Koch 1928
 Communities of spring calciphite mosses (*Cratoneuron* spp.)
 Class *Thlaspietea rotundifolii* Br.-Bl. 1947
 Chasmophytic communities *Parietaria erronea*, *Galium lucidum*, *Aspenium rutamuraria*, *A. trichomanes*
 Class *Festuco-Brometea* Br. Bl. et Tx. 1943
 Order *Festucetalia valesiacae* Klika 1931
 Alliance *Festucion rupicolae* Soó 1940
 Association *Bothriochloetum (Andropogonetum) ischaemi* (Krist. 1937) Pop 1977
 Communities of *Chrysopogon gryllus*
 Alliance *Saturejon montanae* Horvat 1962
 Association *Potentillo pilosae-Achilleetum clypeolatae* Tzonev 2013
 Class *Quercio-Fagetea* Br.-Bl. et Vlieg. 1937
 Order *Quercetalia pubescentis* Br.-Bl. (1931) 1932
 Alliance *Quercion frainetto* Ht. 1954
 Association *Cotino-Quercetum cerris* Rousakova et Tzonev 2003
 Order *Fagetalia sylvaticae* Pawlowski et al. 1928
 Alliance *Carpinion betuli* Issler 1931
 Association *Carpinetum betuli* Dinic 1977 s.l.
 Alliance *Aceri tatarico-Quercion* Zól. - Jakucs 1957
 Association *Staphyleo-Tilietum tomentosae* Tzonev 2013
 Alliance *Syringo-Carpinion orientalis* Jakucs 1959
 Association *Arabio turritae-Carpinetum orientalis* Tzonev 2013
 Association *Syringo-Paliuretum* nom. prov.
 Class *Salicetea albae* Moor 1958
 Order *Salicetalia purpureae* Moor 1958
 Alliance *Salicion albae* Soo 1930
 Association *Salici-Populetum* Isl. 1958
 Class *Robinietea* Jurko ex Hadac et Sofron 1980
 Order *Chelidonio-Robinietalia* Jurko ex Hadac et Sofron 1980
 Alliance *Balloto nigrae-Robinion* Hadac et Sofron 1980
 Communities of *Robinia pseudoacacia*

Appendix 3

Checklist of habitats from EUNIS classification and related units from the Habitat Directive in the Protected Area Kayluka

C2.12 Hard water springs - 7220 *Petrifying springs with tufa formation (*Cratoneurion*).

C2.2 Permanent non-tidal, fast, turbulent watercourses + E5.42 Tall-herb communities of humid meadows - 6430 Hydrophilous tall herb fringe

communities of plains and of the montane to alpine levels.

E1.21 Helleno-Balkan savory steppes - **6240*** Sub-pannonic steppic grasslands

E1.22 Arid subcontinental steppic grassland - **6210** Semi-natural dry grasslands
and scrubland facies on calcareous substrates (Festuco-Brometalia)

E2.7 Unmanaged mesic grasslands - **6510** Lowland hay meadows

F3.24 Subcontinental and continental deciduous thickets

FB.4 Vineyards

G1.111 Middle European *Salix alba* forests - **91E0*** Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)

G1.7A Steppe oak woods - **91I0*** Euro-Siberian steppic woods with *Quercus* spp.

G1.A3 *Carpinus betulus* woodland - *91G0** Pannonic woods with *Quercus petraea* and *Carpinus betulus*

G1.7C2 *Carpinus orientalis* woods

G1.7C41 Silver lime woods - **91Z0** Moesian Silver lime woods

G1.C3 [*Robinia*] plantations

G1.D2 [*Juglans*] groves

G1.C4 Other broadleaved deciduous plantations

G3.F1 Native conifer plantations

G3.F2 Exotic conifer plantations

H1 Terrestrial underground caves, cave systems, passages and waterbodies - **8310**
Caves not open to the public

H3.2A Illyrio-Helleno-Balkan cinquefoil cliffs - **6110*** Rupicolous calcareous or basophilic grasslands of the *Alyso-Sedion albi* + **8210** Calcareous rocky slopes with chasmophytic vegetation

I2 Cultivated areas of gardens and parks

J5 Highly artificial man-made waters and associated structures

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IMPROVING THE CONSERVATION EFFECTIVENESS OF WETLANDS – WETMAINAREAS: PURPOSE AND CONTRIBUTION OF PROJECT FUNDED UNDER TRANSNATIONAL COOPERATION PROGRAMME BALKAN – MEDITERRANEAN 2014-2020

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Abstract. This paper aims to announce the purpose and contribution of the transnational cooperation project *Improving the conservation effectiveness of wetlands – WetMainAreas*. The project with a duration of two years (01.09.2017-31.08.2019) is funded under TNCP Balkan-Mediterranean 2014-2020 in accordance with Subsidy Contract BMP1/2.1/2342/2017. The *WetMainAreas* brings together research partners, users and observer partners from Bulgaria, Greece, Albania and Republic of North Macedonia. The main purpose of the project is protection, conservation and development of the wetlands as a shared asset of the Balkan-Mediterranean territory, ensuring expansion of ecological connectivity and transnational ecosystems integration of designated areas with a target value of 3 568 225 ha. The project methodology is highly innovative – based on remote Earth observation techniques and testing of a novel method for proving the wetlands role as connecting units within the frame of ecosystem services. The main focus is on the „small wetlands“ and their maintaining role for improving the conservation effectiveness. The project contribution is: 1) Update of the picture of wetlands through mapping and field studies; 2) Mapping and assessment of wetland ecosystems connectivity and analysis of their integration in the designated areas and networks (NATURA 2000); 3) Demonstration of joined wetlands conservation techniques at 4 pilot sites; 4) Development of good practices for promotion of environmental and cultural heritage; 5) Improvement of wetlands conservation policies.

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INTRODUCTION

The paper presents main aims and methodology of a recent transnational project oriented towards effective management and protection of Balkan wetlands with a duration of two years (01.09.2017-31.08.2019). It is named *Project Improving the conservation effectiveness of wetlands – WetMainAreas*, and is funded under Transnational Cooperation Programme Balkan – Mediterranean 2014-2020, in accordance with Subsidy Contract № BMP1/2.1/2342/2017. Details on the project are available at:

- <http://www.interreg-balkanmed.eu/approved-project/10/>; <https://www.KEEP.eu/project/19704/improving-the-conservation-effectiveness-of-wetlands>;
- <https://wetmainareas.com/wp-content/uploads/2018/10/leaflet.pdf>;
- <http://fe.swu.bg/en/scientific-activity/eu-and-international-funded-projects/eu-and-international-funded-projects/project-improving-the-conservation-effectiveness-of-wetlands-wetmainareas/>.

Letters of support have been received from the Ministry of Environment and Water, Bulgaria, from the coordinator of Horizon 2020 European research project *Satellite based wetland observation service – SWOS*, and from the initiative coordinator of *Ramsar regional initiative for the Mediterranean Wetlands* (MedWet).

WetMainAreas brings together 10 partners from the Balkan-Mediterranean territory, as follows:

- 1) University of Forestry, Faculty of Ecology and Landscape Architecture – Lead Partner (Bulgaria);
- 2) Goulandris Natural History Museum/ Greek Biotope - Wetland Centre – Project partner 2 (Greece);
- 3) Faculty of Economics, South-West University "Neofit Rilski" – Project partner 3 (Bulgaria);
- 4) Management Unit of Special Research Account, National Observatory of Athens – Project partner 4 (Greece);
- 5) National Environmental Agency – Project partner 5 (Albania);
- 6) “St. Kliment Ohridski” University – Bitola, Faculty of Tourism and Hospitality – Ohrid – Project partner 6 (Republic of North Macedonia, formerly FYROM);
- 7) Region of Thessaly – Project partner 7 (Greece);
- 8) Municipality of Gotse Delchev – Project partner 8 (Bulgaria);
- 9) Ministry of Environment and Energy – Observer partner (Greece);
- 10) State Environmental Inspectorate – Observer partner (Republic of North Macedonia).

All of the above researchers, users and observers have been brought together

for dealing with the transnational challenge of promoting ecological connectivity and transnational ecosystems integration by focusing on wetlands conservation and scientific policy improvement.

Within the EU directives and under the umbrella of EU Biodiversity Strategy 2020, wetland ecosystems are of major importance for wild fauna and flora, and they host a range of habitats and species of Community interest. By their function as stepping stones or corridors, they are essential for migration, dispersal and genetic exchange of wild species, and they have to be conserved as key landscape features for enhancing the coherence, connectivity and resilience of the broader protected areas network. Despite this importance, there is no general overview of the BalkanMed wetlands spatial distribution and the biodiversity they host. Data exist at country or region level, and for individual sites in various formats, but often they are not easily accessible and occur in non-compatible formats. Definitely, the lack of comprehensive knowledge could lead to inadequate protection and poor planning. WetMainAreas tackles the lack of data and the assessment on wetlands connectivity.

The main purpose of the project is protection, conservation and development of wetlands as shared asset of the Balkan-Mediterranean territory.

The specific objectives are:

- 1) To assess the BalkanMed wetland connectivity beyond the boundaries of the protected areas and national borders in order to improve the knowledge on ecological connectivity and transnational ecosystem integration in the BalkanMed territory.

- 2) To support the policy and action plans by integrating scientific knowledge into guidance documents.

WetMainAreas contributes to 3 EU Macroregional Strategies: a) through the participation of Bulgaria in the EU Strategy for the Danube Region (EUSDR)/ Priority Area 06 “To preserve biodiversity, landscapes and the quality of air and soil”; b) through Greece and Albania - in the EU Strategy for the Adriatic-Ionian Region (EUSAIR) /T.O. 6 “Tackling the environment & resources efficiency” (in the aspect of terrestrial part of coastal zone); c) through Bulgaria and Greece - in the EU Strategy for the Black Sea Region (EUSBS)/ Priority 2 “Energy, transport and environment” (in the aspect of protection of natural resources).

The WetMainAreas capitalizes on existing knowledge and tools developed in previous EU funded projects and initiatives – the inventory tools that were developed and promoted by the Ramsar regional initiative for the Mediterranean Wetlands (MedWet), the research work of the Horizon 2020 European research project *Satellite based wetland observation service – SWOS* (ABDUL MALAK ET AL. 2019) .

MATERIALS AND METHODS

The project territory covers Bulgaria, Greece, Cyprus, Albania and Republic of North Macedonia ensuring expansion of ecological connectivity and transnational ecosystems integration of the designated areas with a target value of 3 568 225 ha (**Figs. 1-4**). WetMainAreas adopts the widely accepted Ramsar wetlands definition and typology with taking into consideration all wetlands with area of at least 1 ha.

The project consists of 5 work packages (WP), as follows: WP 1: Project management and coordination; WP 2: Project communication and dissemination; WP 3: BalkanMed wetland identification and connectivity; WP 4: Joint pilot BalkanMed wetland assessments, and WP 5: Support in policy and action plan.

The core of the project are work packages 3 to 5. Project methodology is articulated around: knowledge improvement (Work package 3 and Work package 4) and scientific knowledge uptake into policy (Work package 5). It is highly innovative, based on pilot technologies and methods.

Work Package 3 includes the following tasks:

- Production of Remote Sensing intermediate wetland layer per country (Sentinel-2 analysis results for water and wetness detection in 2017).

- Evaluation of the Remote Sensing intermediate wetland layer (photointerpretation via GoogleEarth).

Production of Final GIS wetland layer per country based mainly on the existing wetlands inventories and other GIS layers – Copernicus Hydro Data for Europe (COPERNICUS 2015), Global surface water Explorer, Open street map data for European countries. Compilation of a site code and name per each wetland. The key source for producing the final GIS wetland layer per Bulgaria is the DataBase from the Inventory of Bulgarian wetlands and their biodiversity (MICHEV & STOYNEVA 2007).

- Production of harmonized wetland inventory datasets per wetland site and population of a common database. For this a minimum harmonized dataset per wetland site is compiled, including: site code, wetland name, general category (marine, coastal, inland), protection status, Ramsar wetland type.

- Assessment of ecosystem potential at national level (biodiversity state, impact of anthropogenic pressure, ecosystem potential at country level to activate the flow of the ecosystem services).

- Assessment of wetlands as connecting units at country and at Balkan-Mediterranean level. This is an approach set by SWOS Horizon 2020 project (ABDUL MALAK ET AL. 2019) to develop a widely applicable methodology for the assessment of wetlands role in the supply of the Ecosystem Service maintaining nursery populations and habitats (including gene pool protection) according to CICES V5.1 (CZÚCZ ET AL. 2016; HAINES-YOUNG & POTSCHIN 2018).

Work package 4 zooms the experience and results from Work package 3 into

4 pilot sites through development of joint wetland conservation techniques and eco-tourism opportunities. The pilot sites are:

1. Mesta /Nestos River basin (Bulgaria and Greece, **Fig. 1**);
2. Vardar/Axios River basin (Republic of North Macedonia and Greece, **Fig. 2**);
3. Vjosa/Aoos River basin (Albania and Greece, **Fig. 3**);
4. Thessaly region (Greece, **Fig. 4**).

Work package 4 includes the following tasks:

- Desk study on all existing data on biodiversity, environment, land management, etc. in the pilot sites.
- Field studies within the pilot sites.
- Population of additional datasets in the common database.
- Production of Reports on the pilot sites.
- Production of a Guideline, including conservation techniques for wetlands ecosystems that are ecologically and functionally connected - WetMainAreas
 - Inventory of cultural heritage in the four pilot sites.
 - Production of a Tourist guide promoting sustainable cultural and ecotourism in the four pilot sites.

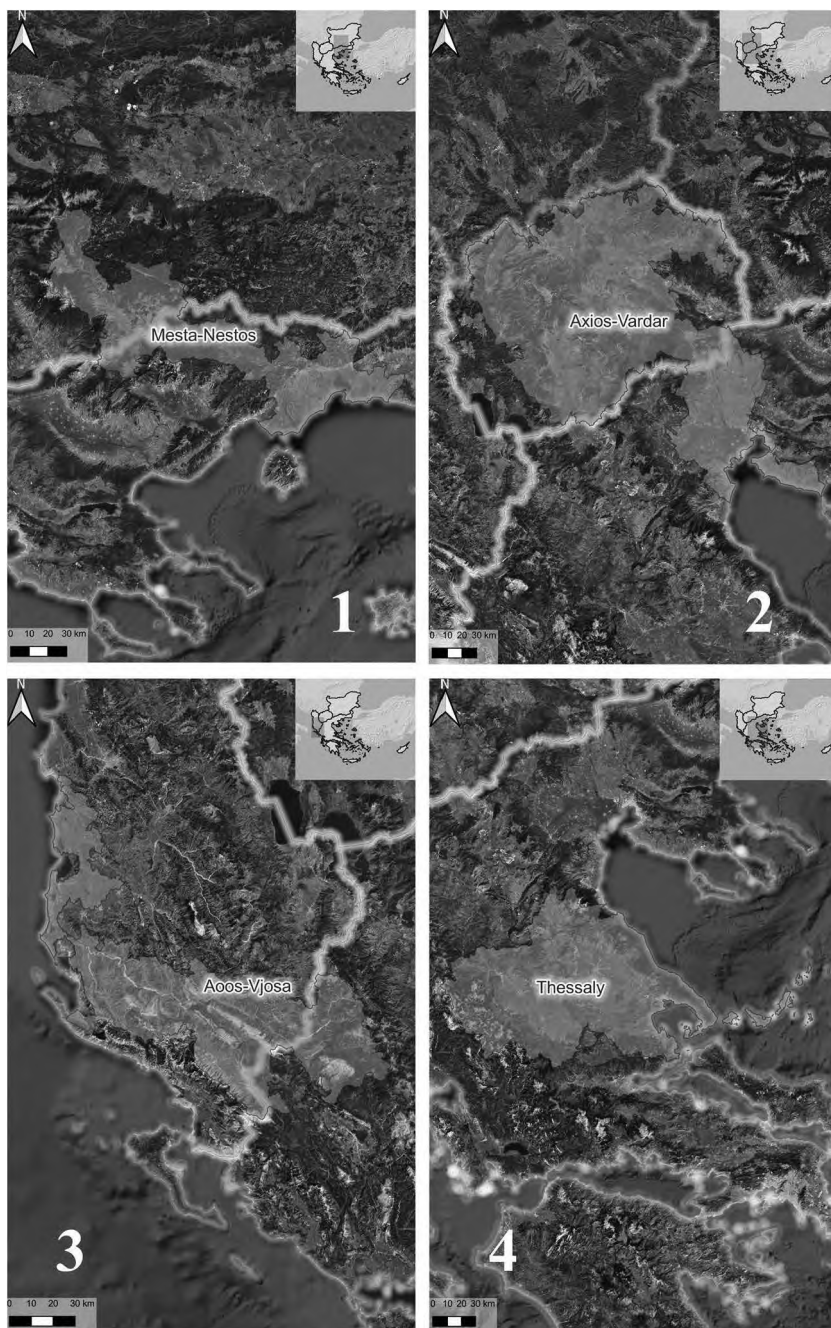
Work package 5 includes the following tasks:

- Study on the state-of-art on wetland relevant policies and links with other sectoral and territorial plans such as agriculture, fishery, water management.
- Living lab workshop on transnational wetland ecosystems.
- Integration of project results into policy and action plan.

RESULTS AND DISCUSSIONS

In 2019, the first project results will be available – lists of wetlands within the project area, populated common database with a geoportal, pilot assessments on wetland ecosystems connectivity and analysis on their integration to the designated areas and networks. The project will end with combination of data from all partners and with a common publication with a free on-line access.

The implementation of the WetMainAreas project leads to the following contribution: 1) Update of the picture of wetlands through mapping and field studies; 2) Mapping and assessment of wetland ecosystems connectivity and analysis on their integration to the designated areas and networks (NATURA 2000); 3) Demonstration of joined wetlands conservation techniques at four pilot sites; 4) Development of good practices for promotion of environmental and cultural heritage; 5) Improvement of wetlands conservation policies.



Figs. 1-4. Pilot sites of the Project: **1** - Mesta /Nestos River basin (Bulgaria and Greece); **2** - Vardar/Axios River basin (Republic of North Macedonia and Greece); **3** - Vjosa/Aoos River basin (Albania and Greece); **4** - Thessaly region (Greece).

CONCLUSION

The main idea of the project is to outline not only the conservation value of the wetlands, but also their maintaining role for the designated areas as well. Our approach aims at development of practices and techniques for conservation of these maintaining wetlands and thus at improving the conservation effectiveness. This idea has to be disseminated as wide as possible. As many key actors as possible need to be involved in order to ensure sustainability of this understanding and to ensure proper management that will lead to improving the conservation effectiveness of wetlands.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the paper preparation.

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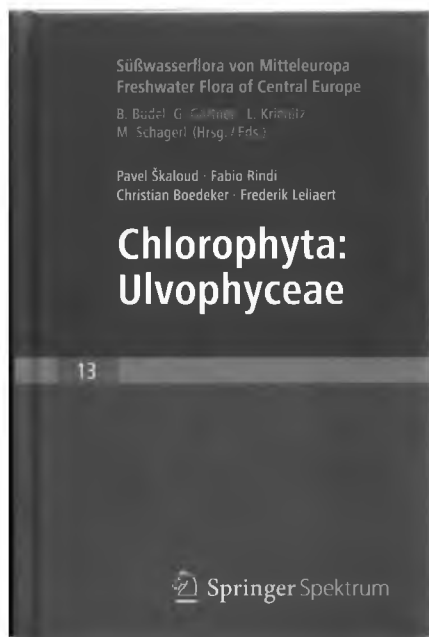
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Book review: ŠKALOUD P., RINDI F., BOEDEKER C. & LELIAERT 2018. Volume 13. Chlorophyta: Ulvophyceae. - In: BÜDEL B., GÄRTNER G., KRIENITZ L. & SCHAGERL M. (Hrsg./Eds.), Süßwasserflora von Mitteleuropa (Freshwater Flora of Central Europa), Springer Spektrum, Berlin, Germany, 289 pp.



In 2018, a new, 13th volume of the Süßwasserflora von Mitteleuropa (Freshwater Flora of Central Europa), edited by B. BÜDEL, G. GÄRTNER, L. KRIENITZ & M. SCHAGERL, was issued. It covers one of the main classes of the green algal evolutionary lineage (Chlorophyta) - the natural class Ulvophyceae, which according to GUIRY & GUIRY (2019: GUIRY M. D. & GUIRY G. M. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>) consists of about 1700 species, some of which are exclusively marine. In accordance with the main idea and subject covered by the Central European Freshwater Flora, the present volume treats only the freshwater, aerophytic and terrestrial algae of the class. The authors, stepping on the recent molecular phylogeny,

significantly revise the taxonomy of ulvophycean algae and provide an updated classification with one order and one family resurrected (Chlorocystidales, Chlorocystidaceae), and one order and five families newly described (Ignatiiales, Ignatiaceae, Binucleariaceae, Planophilaceae, Hazeniaceae, Sarcinofilaceae, and Tupiellaceae). This modern and comprehensive approach of work is not surprising, considering the extant experience of the authors: PAVEL ŠKALOUD – Associate Professor at the Charles University, Prague, Czech Republic, FABIO RINDI – Senior Lecturer at the University Politecnica delle Marche, Ancona, Italy, CHRISTIAN BOEDEKER – Postdoctoral Fellow at Victoria University of Wellington, New Zealand, and FREDERIK LELIAERT – Research Director at the Botanic Garden Meise and visiting professor at Ghent University, Belgium.

The volume under review is based on the latest knowledge in phylogeny, ultrastructure and morphology, briefly and clearly outlined in the *Introduction to the freshwater Ulvophyceae* in the beginning of the book, supplied by very good and expressive color illustrations. It is followed by a useful *Key to the Ulvophycean genera included in the volume* and ends with a *Systematic part*, which comprises

nine orders and 22 families, each supplied by very helpful keys for generic and species delimitation and identification. In this modern reference work for identifying ulvophycean algae, descriptions, drawings and photos of more than 100 European species and descriptions of more than hundred non-European taxa are provided. Where possible, texts on species are supplied with biogeographical and ecological data increasing the value of this useful tool for scholars in phycology worldwide.

Mag. rer. nat., Dr. rer. nat. Wolfgang Karl Hofbauer,
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Fraunhofer Institute for Building Physics, Valley, Germany

Book review: ASEN ASENOV 2019. Biogeography. St Kliment Ohridski University Press, Sofia, 430 pp. (In Bulgarian)



The book of ASEN ASENOV, titled *Biogeography*, was issued by the Publishing House of Sofia University *St Kliment Ohridski* in 2019. This comprehensive overview comprises 424 pages, based on 322 references and is illustrated with 98 figures and 10 tables. The text is presented in two main parts. The first part focuses on the theoretical foundations of biogeography science and is based on system approach with major weight on the system of areas. The second part steps on the ecosystem approach and analyzes the nature and structure of planetary biomes. The value of this monographic work, oriented towards scholars dealing with biogeography and biodiversity, besides the provided facts and descriptions, lies in the author's

personal analyses, reflections and conclusions. In the same time, written in Bulgarian language, with the highlights on the new accents and trends in the development of biogeographic science, it can be strongly recommended as a textbook for students in Geography, Biology or other similar subjects in Bulgarian universities and other higher educational institutions. In this aspect it is to underline the provided description of the biogeography since ancient times till nowadays, which ends with an assessment of the main textbooks and monographs on the topic. The importance of biogeographical research is reflected in the book by representation and discussion on new build-up concepts such as *natural capital* and *ecosystem goods and services*. This logically leads to outlining of the significance of biogeography for UNESCO MAB Program and for operation of NATURA 2000 ecological network, and to the hot topic of *invasive species* as well. In this aspect, the complementation by Bulgarian experience in invasive species research has to be underlined. Another essential value of the book is the author's precision in terminology, writing of Latin names and authors, nomenclatural names of syntaxa, *etc.*, which is guaranteed also by the work of both prominent reviewers – Assoc. Prof. PETAR BERON, PhD and Prof. DIMITAR BECHEV, DrSc. Last but not least, it is noteworthy to mention the significant role of the years long scientific and teaching experience of the author ASEN ASENOV, an Associated Professor, PhD in the Department for Landscape ecology and environmental protection of the Faculty of

Geology and Geography, Sofia University *St Kliment Ohridski* for creation of such useful book for Bulgarian scientific community.

Prof. Dobri L. Ivanov, PhD
Head of the Department of Biology,
Medical University “Prof. P. Stoyanov” - Varna

In Memoriam Prof. Velcho Velchev (1928-2019)



Prof. Velcho Velchev passed away in January 2019. After his graduation the Faculty of Biology, Geology and Geography at the Sofia State University "Kliment Ohridski", in 1951 he dedicated all his life time to the development of Bulgarian botany. Being one of the worthy and authoritative Bulgarian botanists, with more than 160 publications, he contributed to the education and supported the professional development of generations of scholars in the field. Prof. Velchev was the responsible editor of the volume dedicated to plants in the first Red Data Book of P. R. Bulgaria, issued in 1984 and the fifth Chairman of the Bulgarian Botanical Society (1979-1991). His competence and

qualification were the basis of his different administrative positions in respectful botanical centres:

- Director of the Institute of Botany with a Botanical Garden of the Bulgarian Academy of Sciences (1974-1993);
- Head of the Department of Botany in the Faculty of Biology of Sofia University (1975-1994);
- Director of the University Botanical Garden of Balchik (1974-1993).

Editorial note

In accordance with the new Rules for approving and printing of the Annual of Sofia University, accepted on the meeting of the Academician Council of Sofia University “St. Kliment Ohridski” (Protokol 5/19.12.2018), this volume of the Annual’s Book 2 – Botany was approved by the Faculty Council of the Faculty of Biology in May 2019 instead of November 2018. The same procedure will be followed for all next volumes of the Annual of Sofia University, Faculty of Biology, Book 2- Botany.

INSTRUCTIONS FOR AUTHORS

Book 2 – Botany of the Annual of Sofia University is a peer-reviewed periodical, issued yearly in one volume, which is published on-line with an open access and in a printed version with two relevant IUSSNs.

Original papers covering the entire field of scientific botany and mycology with a worldwide geographical scope are published with special encouragement to the papers of students and young scientists. Five categories of contributions are published: 1) Research articles; 2) Review articles (invited or published with the editors' consent); 3) Short communications; 4) Book reviews; 5) Information about scientific events, past or forthcoming or, preferably, overview of the topics and contributions of the scientific meetings, as well as obituaries.

Manuscripts have to be written **in English** and first three categories of contribution 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 the **copyright and all rights** of reproduction of any kind, translations and distribution to the publisher.

Manuscripts should be **submitted as electronic file/files** (e-mail attachment). Text, references, tables and figure captions should be submitted as **.doc/docx (Times New Roman 12, double-spaced, A4 with margins 3cm all around)**. In case of tables, when necessary, the font size could be smaller. Figures should be provided in **.tif** or **.jpg** format (min 300 dpi required). Details on their formatting and presentation are described in the end of the instructions.

Incoming manuscripts are **initially judged by the editor**. If the manuscript does not meet the criteria and standards for publication it may be rejected without being sent out for review. If the manuscript is acceptable as corresponding to the scope of the journal and representing a major contribution deserving publication in an international journal, it will be forwarded to reviewers for evaluation. **The editors decide on acceptance after the recommendation of international expert referees** and on corrections and alterations of the manuscript thought to be advisable. Final responsibility for acceptance of all submissions rests with the Editor-in-Chief. After the approval of the final version by the Editorial Board, the manuscript will be accepted for publication. The editor reserves the right to make **editorial changes**. Authors agree, with the acceptance of the manuscript, that the copyright is transferred to the publisher. **The editorial policy does not support any form of plagiarism and requires correct citations from the authors.**

In preparing the manuscripts, the authors are kindly requested to adhere to the following instructions:

As a rule, the **size** of the contributions should not exceed 16 printed pages. If a paper exceeds the pointed limits, the authors are requested to obtain the editors' consent in advance.

The text must be accurate and the language clear and correct.

The **title** of the paper must be concise, but informative, describing the matter of the contribution as well as possible. If a Latin name of a species is used in the title, it is recommended to indicate the division, class, order, or family to which it belongs.

The **authors' given names** must be spelled in full, while a middle name should be abbreviated: full first name(s), middle initials and surname(s). The authors' **address(es)** should be stated on the first page of the paper below the title. The addresses should be as complete as possible (affiliation, street, postal code, town, country). In case of authors from different affiliations, a number (superscript) should be put in the end of the authors name and the same number with a normal font size should be placed before the address. The postal address and the email of the corresponding author should be indicated as a footnote on the first page.

Example:

* *corresponding author*: M. P. Stoyneva – Sofia University “St. Kliment Ohridski”, Faculty of Biology, Department of Botany, 8 Blvd. Dr. Tsankov, BG-1164, Sofia, Bulgaria; mstoyneva@uni-sofia.bg

The proper paper text must be preceded by an English summary („**Abstract**“), which should express the important new results precisely and should be limited to 300 words. Please, remember that the abstract will be seen and used by many more people than the full paper will! Subsequently up to 6 **key words** (or key word combinations) suitable for information-retrieval system are to be listed (in alphabetical order). The key words should not repeat those, which already are mentioned in the title. The disposition of the paper sections should be in agreement with common use. The „**Introduction**“ should outline the essential background for the work and the reasons why it was undertaken. It should clearly explain the purpose of the work and its relations to other studies in this field. Before the material and method description, optionally, due to author's decision, a description of the **studied site/s** could be included. Descriptions of **materials and methods** should provide sufficient information to permit repetition of the experimental work. This includes proper documentation of the sources of cultures, plants and fungi used in the work. Authors should consider depositing voucher material in an internationally reputable museum, collection or herbarium and the relevant numbers or codes should be provided in the text. All new gene or protein sequences should be submitted to major databases (DDBJ, EMB, GenBank) before the submission of manuscripts and the accession codes should be indicated in the manuscript. The geographic names should be transliterated from the common geographic names used in the certain country (e.g. Rodopi Mts instead of Rhodopes). The proper Bulgarian legislative documents for translation and transliteration are cited at the end of this Instruction. The origin of the material investigated, methods of preparation and the herbaria and collections in which the vouchers are deposited, should be indicated completely. In case of work with threatened species and protected areas

it is recommended to provide the permission data. For the metric measurements **SI-units** are requested. They should not be followed by full stops and slashes have to be replaced by minus index (*e.g.* mg l⁻¹ should be used instead of mg/l). Please, use % instead of *per cent*.

Not commonly used abbreviations should be explained at the end of the chapter „**Material and methods**“. The „**Results**“ preferably have to be separated from the „**Discussion**“. The discussion should deal with the interpretation of the results, not only recapitulate them. It should evaluate the results in relation to the reasons why the study was undertaken, place the results in the context of the other work, and point out their significance.

The beginning of a paragraph should be indicated by indenting the first line.

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, cultivar etc.) and the author, when mentioned for 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 in brackets 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: 149–150, or IVANOV 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). In case of editor/editors, they have to be indicated only in the reference list, but not in the text of the paper (*e.g.* PETROV (2013) in the text and PETROV I. A. (Ed.) 2013....in the references). In the same way, in the references, but not in the text, the Editor in chief is abbreviated as Ed-in-Chief and Compiler/Compilers are abbreviated as Comp. and Comps respectively (*e.g.* PETROV I. P. (Ed-in-Chief) 2013; PETROV A. V. (Comp.) 2015; PETROV A. V. & DRAGANOV I. P. (Comps) 2017).

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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 CO₂-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

IVANOV I. P. & PETROV P. I. 2013. Photosynthetic CO₂-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

IVANOV I. P., PETROV P. I. & DIMITROV V. N. 2013. Photosynthetic CO₂-fixation pathways. – Ann. Rev. Plant Physiol. 21 (2): 141–263.

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Books:

DIMITROV D. G. & IVANOV A. N. 2017. Biodiversity of the seashores of Bulgaria. Springer, Heidelberg, 405 pp.

IVANOV W. H., STOYANOV H. M. & PETROV F. B. (Eds) 2000. Water ecosystems. Elsevier, New York, 265 pp.

Book chapters:

PETROV F. K. 2000. Grazing in water ecosystems. – In: IVANOV W. J., STOYANOV H. P. & PETROV F. B. (Eds), Water ecosystems, Elsevier, New York, 59–105.

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Or, alternatively, depending on the order of date and place in the original title of the Proceedings/Abstract books:

BOGDAN D. M. 2017. Biosphere reserves and special legislation for environmental protection. - In: VENEV N. (Ed-in-Chief), Book of Abstracts First European

Symposium *Conservation and management of biodiversity in the European seashores*, 8-12 May 2017, Primorsko, Bulgaria, p. 36.

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INDEX FUNGORUM. Retrieved from <http://www.indexfungorum.org/Names/Names.asp> on 19.11.2017.

Or, alternatively

INDEX FUNGORUM. <http://www.indexfungorum.org/Names/Names.asp> (Last accessed on 19.11.2017).

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References to manuscripts in preparation should not be included in the text and in the reference list, except for extremely significant data. Other data should be cited as unpublished (unpubl. or unpubl. data) or as manuscripts (diploma works, *etc.*), personal communications (pers. comm.) or written documents (in litt.) in the text, but not in the references.

Titles of the **papers in cyrillic** should be translated (or their relevant German, French or English titles provided by authors in abstracts should be used with indicating of the original language and the language/s of the summary/summaries (see the examples below and, please, note the places of dots). The title of the journal and/or publishing house should be transliterated in case that there is no accepted international journal abbreviation:

Journal:

PETKOV N. H. 1915. La flore algologique du mont Pirin-planina.- Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian).

PETKOV N. H. 1915. La flore algologique du mont Pirin-planina.- Sbornik na Bulgarskata Akademiya na Naukite 20: 1–128 (In Bulgarian, French and Russian summ.).

Book:

VALKANOV D. E., DRAGANOVA P. M. & TSVETKOVA B. B. 1978. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian)

VALKANOV D. E., DRAGANOVA P. M. & TSVETKOVA B. B. 1978. Flora of Bulgaria. Algae. Izd. Narodna Prosveta, Sofia, 642 pp. (In Bulgarian, English summ.)

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