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FIRST APPLICATION OF A DRONE FOR STUDIES OF THE BIODIVERSITY OF BULGARIAN EXTREMOPHILIC ALGAE IN THE MARIKOSTINOVO 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).

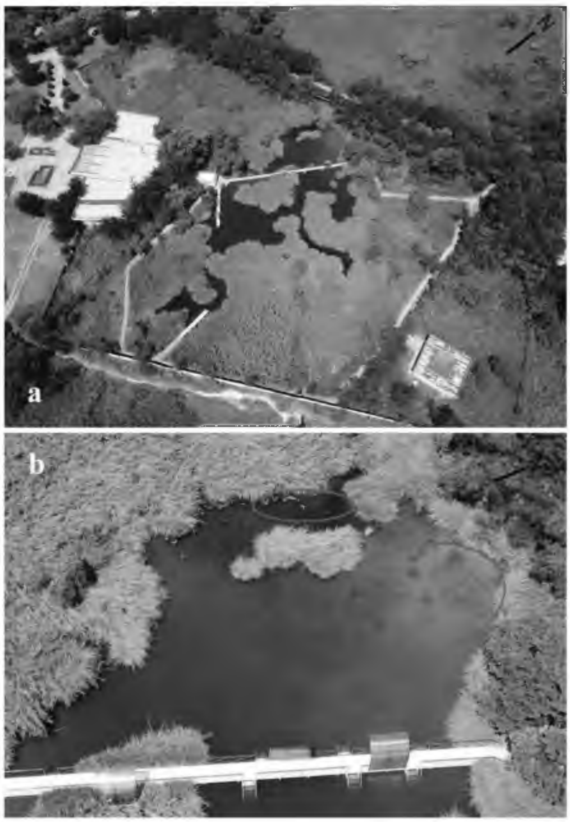


Fig. 2. Photos of Marikostinovo thermal complex, obtained by drone: **a** - thermal complex with the building of mud bath center; **b** - algal sampling areas.

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 send 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 Moticam 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

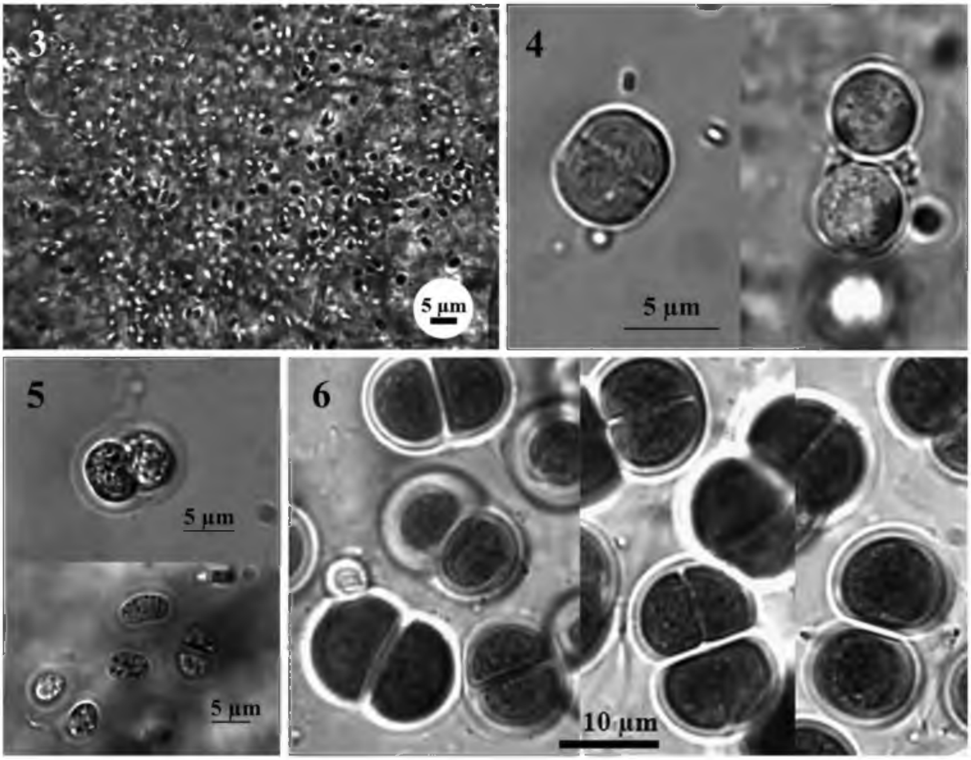
***Aphanothece* sp.** – The cells very small, up to 2 µm long and up to 1 µm wide, loosely arranged in groups of irregular shape, possible kept together by common colorless very fine mucilage **(Fig. 3)**. Representatives of this genus have been found in thermal waters (Komárek & Anagnostidis 1999) and 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



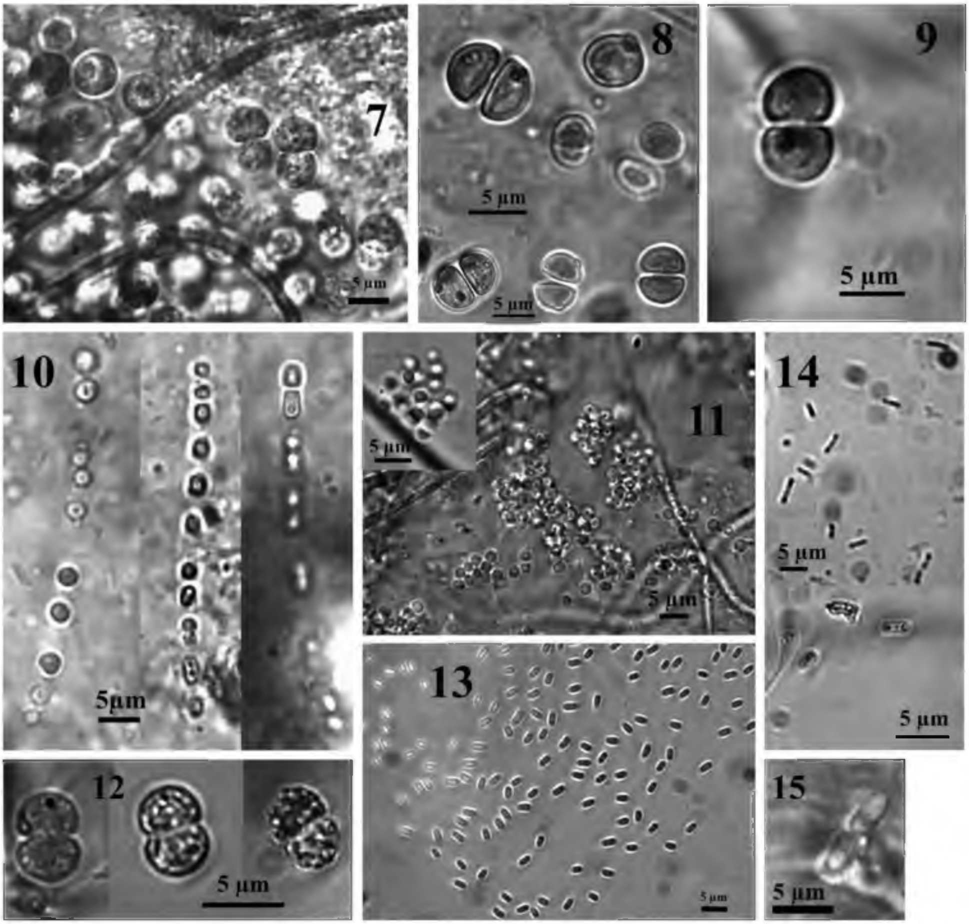
Figs. 3-6. Microphotograps of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **3** - *Aphanothece* sp.; **4** - *Chroococcus globosus* (Elenkin) Hindák 1978; **5** - *Chroococcus membraninus* (Meneghini) Nägeli 1849; **6** - *Chroococcus thermalis*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

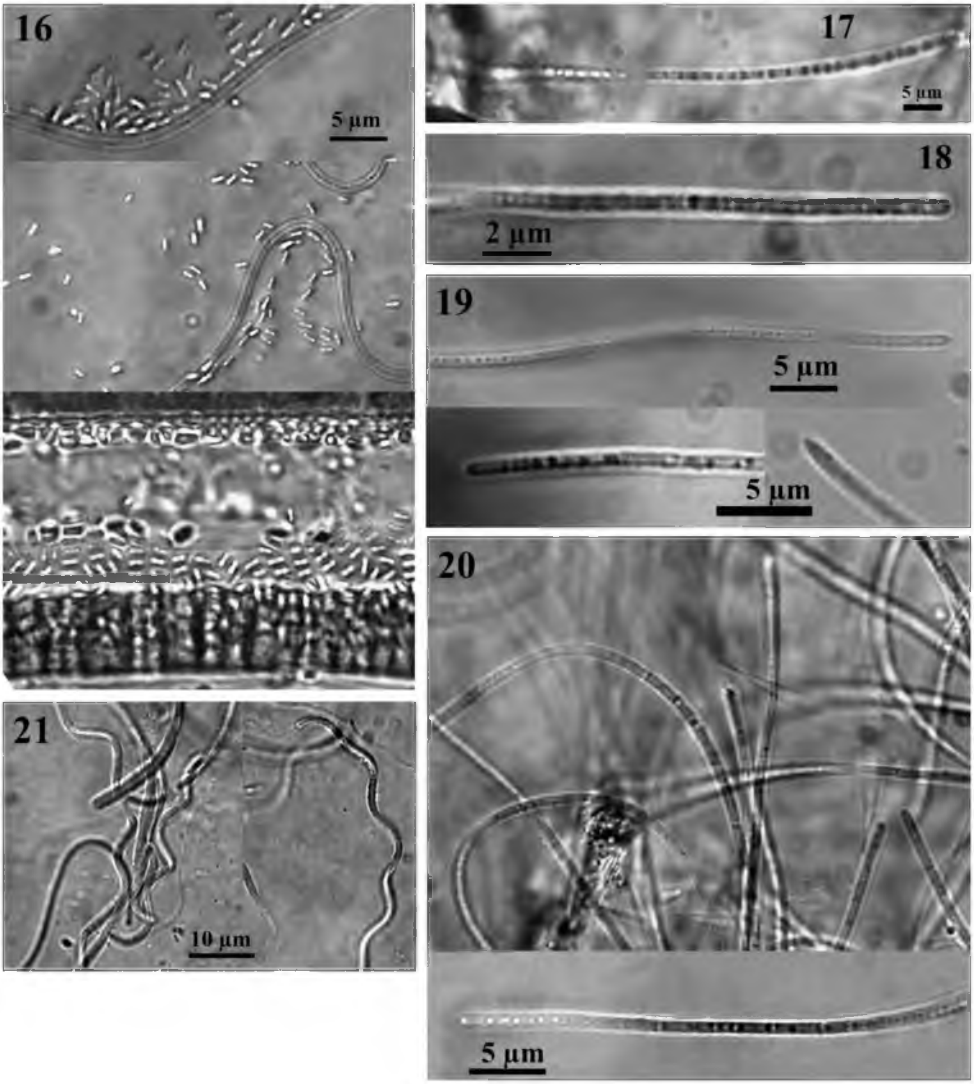
***Synechococcus* cf. *lividus* J. J. Copeland 1936** – Cells rod-shaped, bright blue-green, 2-2.5 µm wide and 5(6) long µm long, reproduced by division in twos (**Fig. 15**). Considering the note of Komárek & Anagnostidis (1999, p. 126) that the species has not been recorded from Europe, the very low 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. Microphotograps 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.

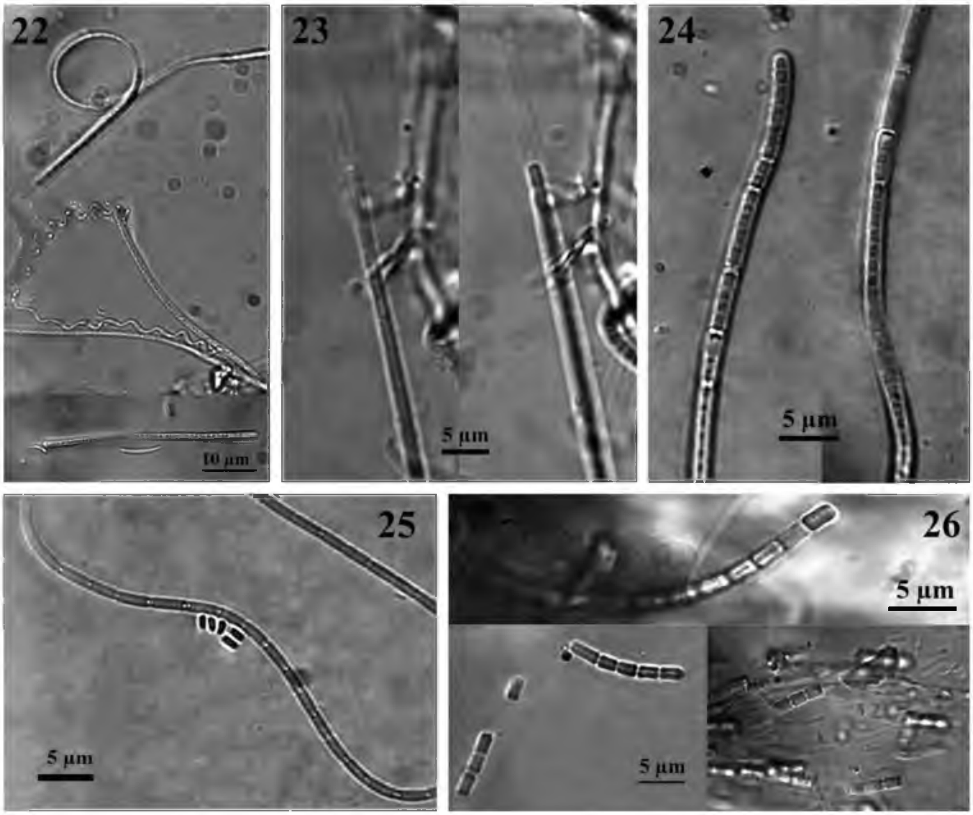
Leptolyngbya geysericola **(J. J. Copeland) Anagnostidis 2001** – Filaments long, straight; sheaths hyaline; trichomes pale blue-green, not constricted, 0.4-0.6 µm wide with isodiametric or, rarely, twice longer cells with homogenous content; apical cells rounded, without calyptra or thickened outer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



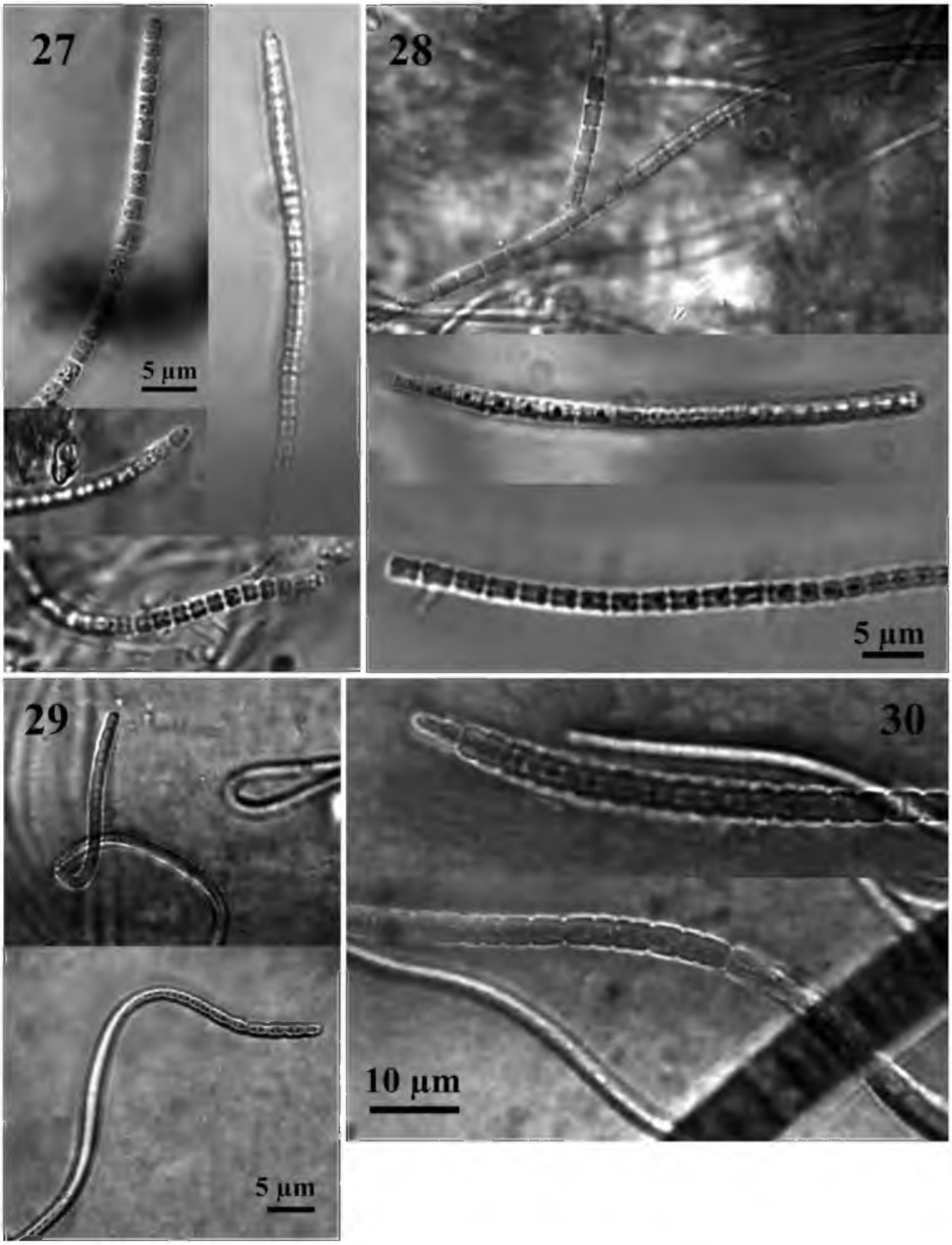
Figs. 22-26. Microphotograps of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **22** - *Leptolyngbya valderiana* (Gomont) Anagnostidis & Komárek 1988; **23** - *Leptolyngbya* sp.; **24** - *Jaaginema geminatum* (Schwabe ex Gomont) Anagnostidis & 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 diffluent 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. Microphotograps of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **27** - *Pseudanabaena papillaterminata* (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) Kukk 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).

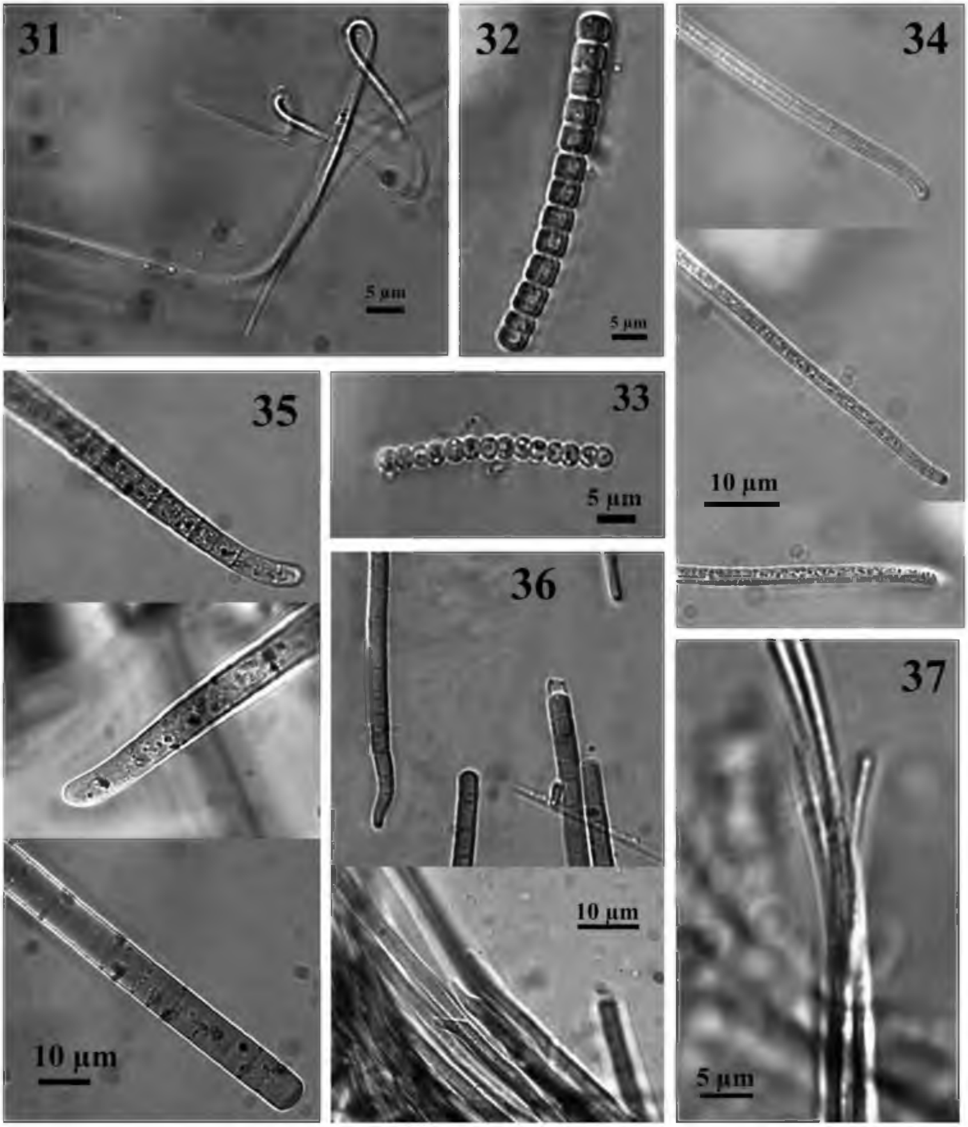
***Pseudanabaena* sp.** – The material coincides with the drawing provided by Komárek & Anaganostidis (1999 – fig. 73) for unidentified species, commonly found in thermal springs of Greece together with *Mastigocladus laminosus*, or separately from it. Trichomes are blue-green, clearly constricted, cells 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. Microphotograps of the algae from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: **31** - *Geitlerinema thermale* Anagnostidis 2001; **32** - *Komvophoron* 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).

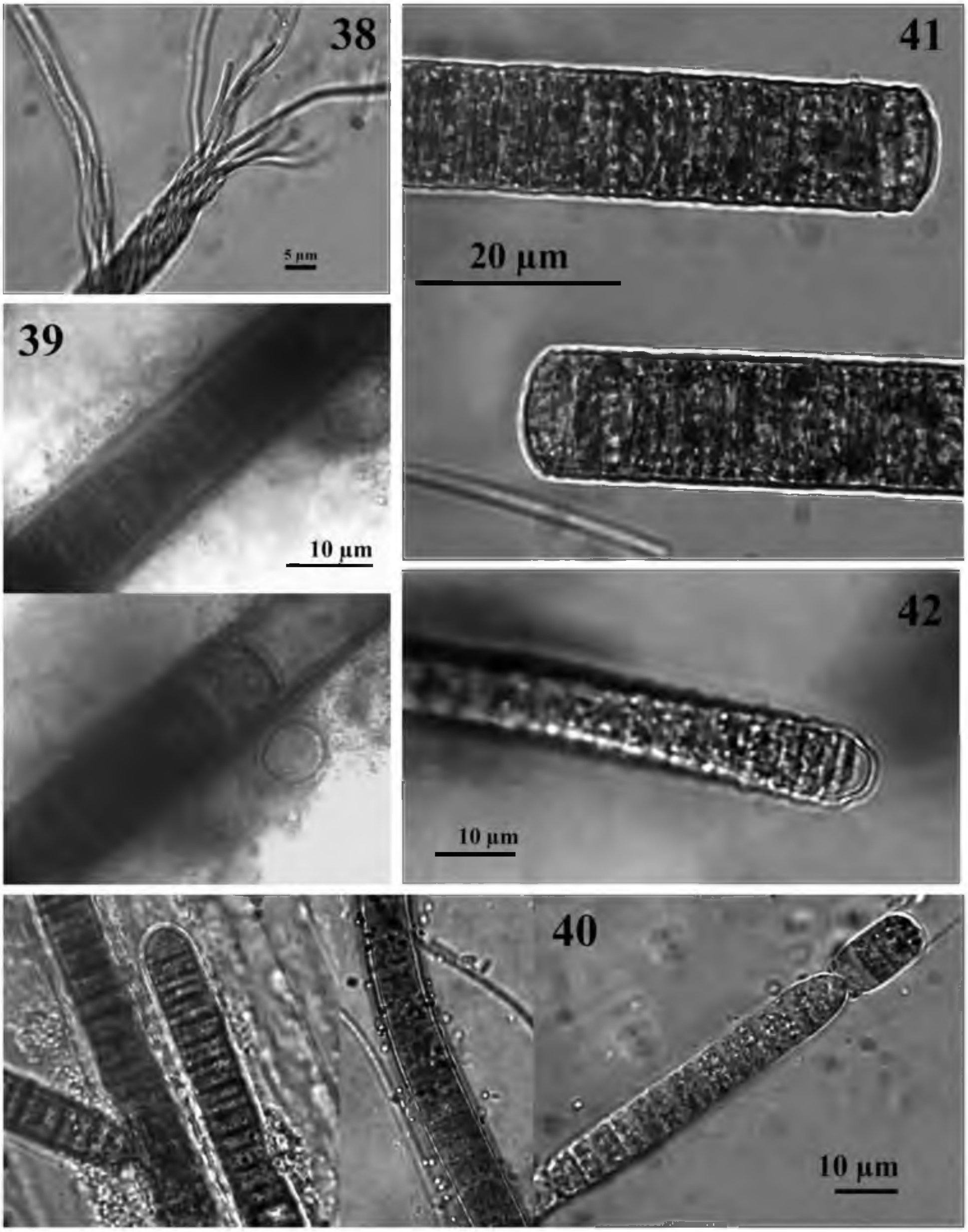
Kamptonema cortianum **(Meneghini ex Gomont) Strunecký, Komárek & J. Smarda 2014** – identified by LM in fixed samples (7) after (Komárek & Anagnostidis 2005) as *Phormidium cortianum* (Meneghini ex Gomont) Anagnostidis & Komárek 1988: Trichomes straight, blue-green, (5) 6-9 µm wide with cells up to 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 calyptrated 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 ***Leptolyngbya. 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. Microphotograps 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).

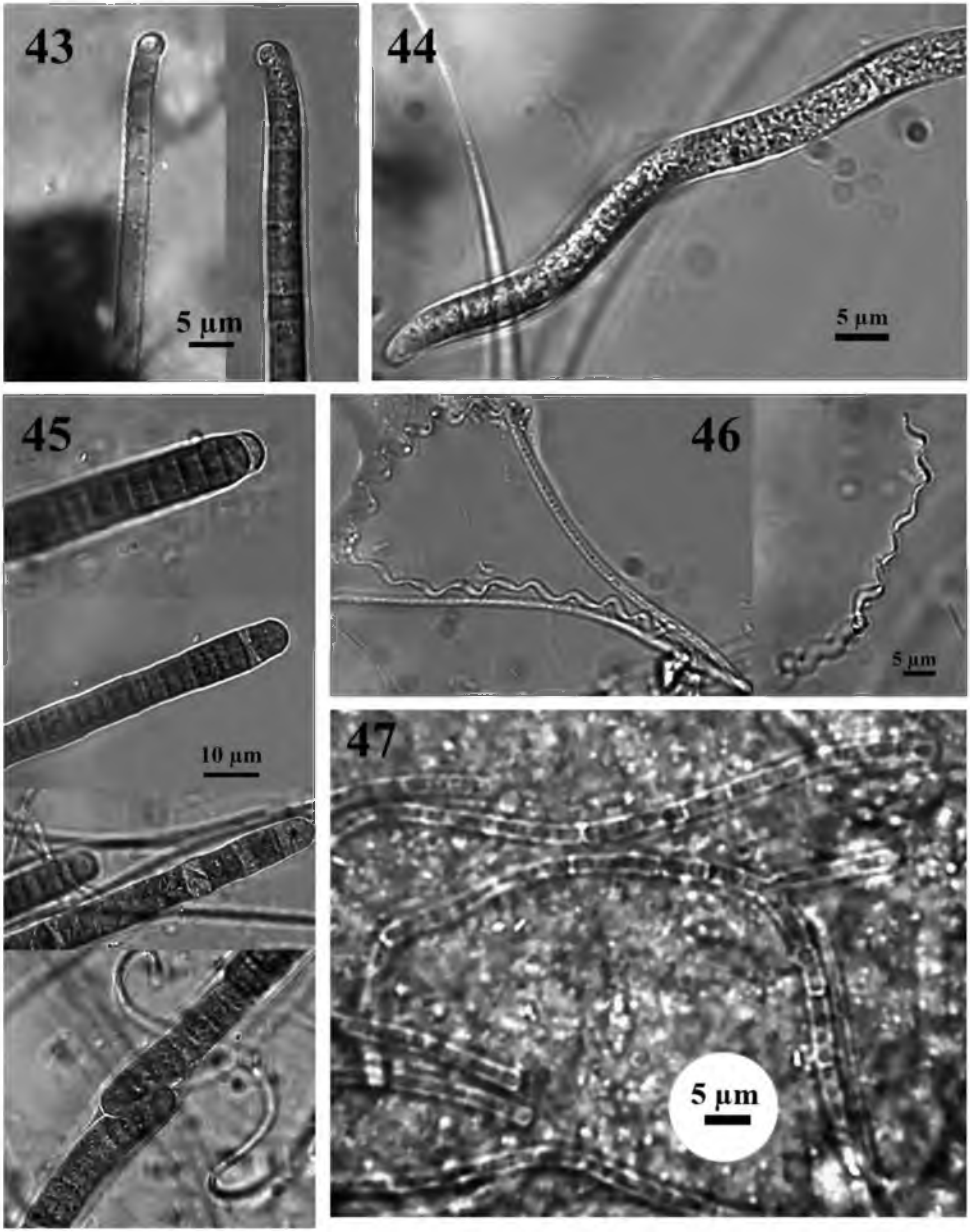
***Lyngbya thermalis* Kützing ex Gomont 1892**: 152, nom. inval. – Filaments 12-13 µm, trichomes 9-10 µm wide. In one of the cultures, the color of sheaths varied from colorless to yellow (**Fig. 40**). The species is known from thermal waters, at the margins of the springs or in their vicinity on warm 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 **Kützing 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 **Kützing 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. Microphotograps 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* Kützing 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

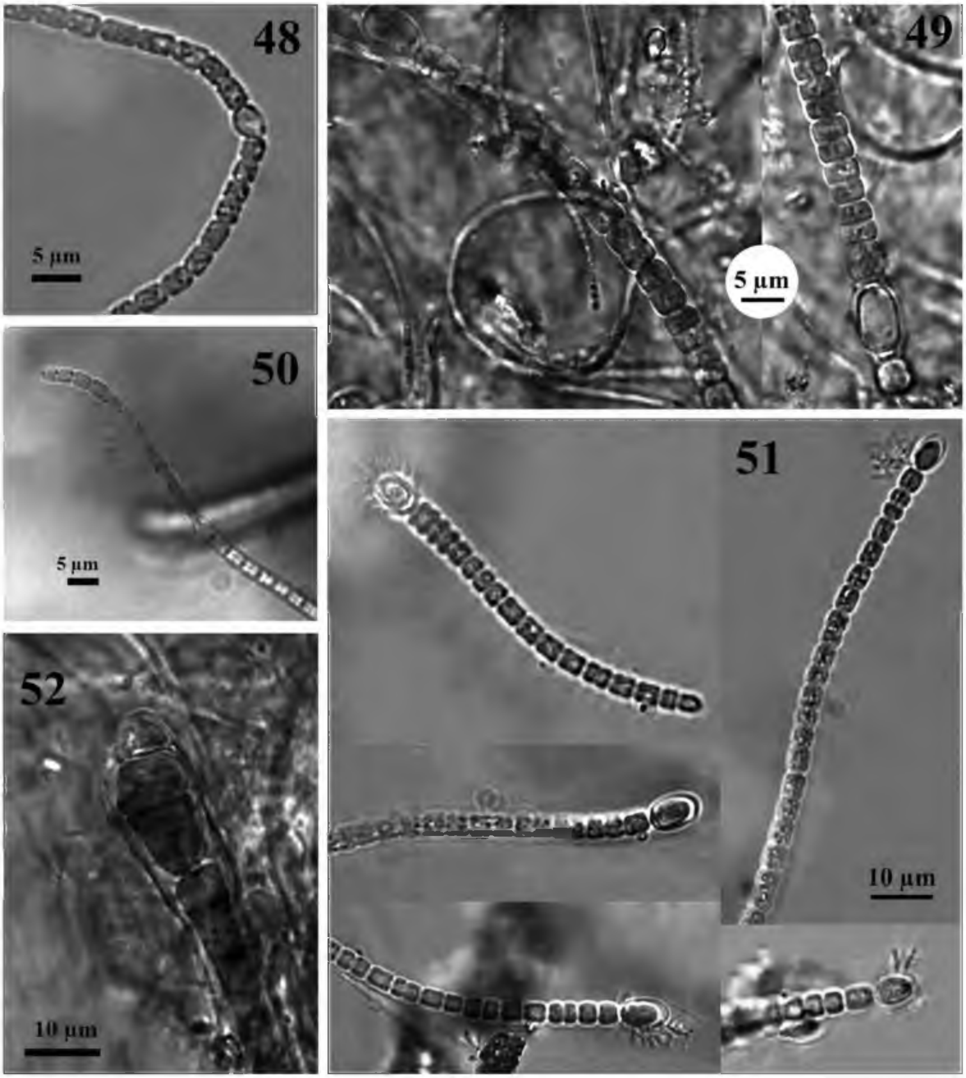
Family Spirulinaceae

***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. Microphotograps 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 *Pseudanabaeana*) 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. convervicola* Agardh ex Bornet et Flahault 1886) was shown as possible causative agent for seaweed dermatitis (Stoyneva et al. 2015).

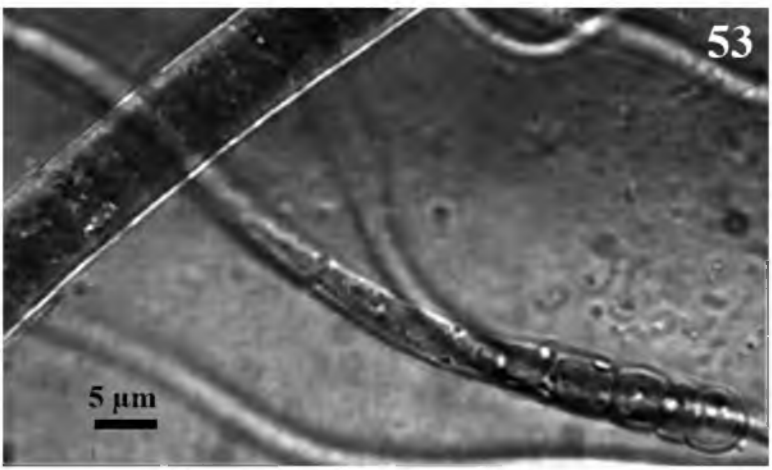


Fig. 53. Microphotograp of the alga from the thermal complex Marikostinovo. For taxonomic details and dimensions see the text: *Calothrix* sp.

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 shrinked 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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