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Taxonomic and Ecological Notes to the List of Green Algal Species from Bulgarian Thermomineral Waters

by

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Synopsis: Among the algal flora of Bulgarian thermal springs, green algae were documented by 75 taxa which represent a significant part of the total algal diversity of more than 200 species, varieties and forms described in literature. In addition to a list of green algal taxa already published (STOYNEVA 2003), some comments on 51 species recently rediscovered or seemingly disappeared within the last decades are given. The decrease of algal biodiversity in many Bulgarian thermal springs may be caused also by the unauthorized use of these habitats.

Keywords: Green algae, thermal springs, Bulgaria.

1. Introduction:

Bulgaria is rich in heterogeneous thermomineral waters, which belong to 4 hydromineral formations: a) formation of nitrogen thermal waters or akratothermes; b) formation of carbohydrate (incl. carbon-nitrogenous) waters and thermes; c) haline formation and d) soda-glauber formation (SHTEREV 1964). In the hydrogeological tract each of these formations forms hydromineral province with relevant sub-provinces, which cover about 3/4 of the territory of Bulgaria (Fig. 1). Most of the Bulgarian hot springs in the traditional balneotherapy resorts and mineral baths belong to the subprovince of akratothermes of silica type (SHTEREV 1982; Fig. 1). Most of them have been investigated hydrogeologically and hydrochemically (e.g. VATEV 1904, DOBREV 1904-1905, ISCHIRKOFF 1908-1909, ANONYMOUS 1996) but studies on their algal flora are quite scarce. According to an analysis of the Bulgarian phycological literature for the period 1898-2000 it was shown that of 56 thermal spring systems only 26 have been studied from an algological point of view and more than 200 taxa were recorded (STOYNEVA 2003). Green algae have been documented only for 18 thermal springs and were already published in a complete list of 75 taxa (STOYNEVA 2003).

Dedicated to Prof. D. Temniskova in honour of her 70th anniversary.

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In the present paper some additional comments are provided on 51 species which were documented in the literature and were again or no longer rediscovered.

2. Material and Methods:

The Bulgarian phycological literature for the period 1898-2001 was reviewed by the first author who provided earlier also the geographical data combined with the main abiotic parameters of Bulgarian thermes (STOYNEVA 2003). Investigations of taxa were made on living material sampled between March 2002 – July 2002 or on permanent slides with light microscope (Amplival Jena). Taxonomic determination was done following the taxonomic system in STOYNEVA (2003) with additions according to the literature cited.

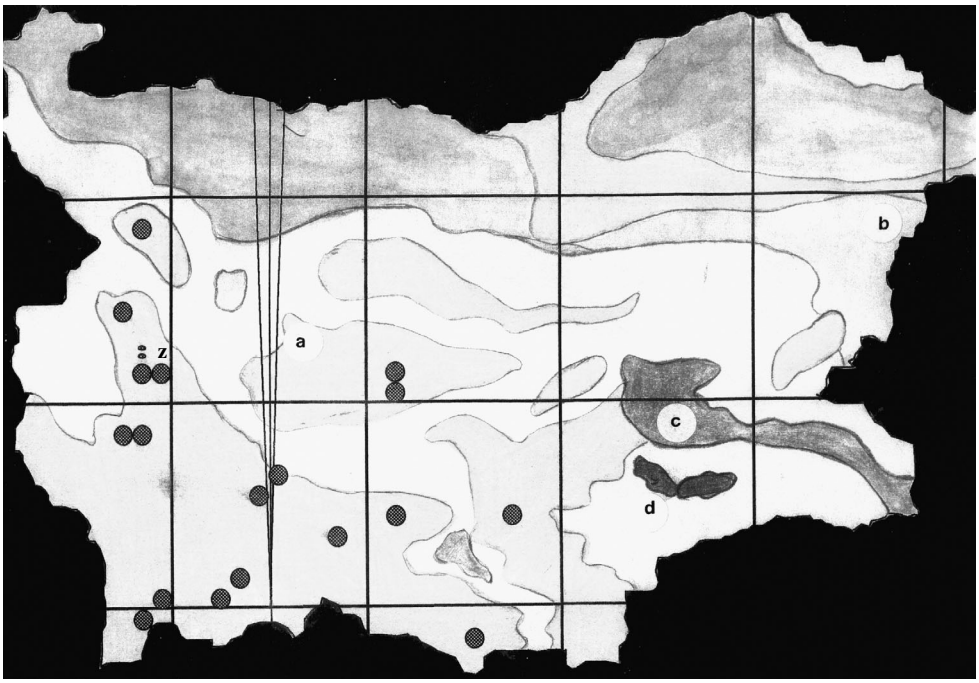


Fig. 1: Map of Bulgaria (UTM-grid map by Bulgarian UTM Directory computer programme (MICHEV 1999) with the studied sites and limits of main formations, following SHTEREV (1982); **a**) formation of nitrogen thermal waters of akratothermes; **b)** formation of carbohydrate (incl. carbon-nitrogenous) waters and thermes; **c)** haline formation; **d)** soda-glauber formation; **z** = Thermal spring Zheleznitsa (figs. 5-8).

3. Results and Discussion:

***Chaetomorpha herbipolensis* LAGERHEIM**

According to PETKOFF (1908-1909) in the material from thermal spring complex between the villages Slivnitsa, Bezden and Opitsvet (named by him "toplitsi Opitsvet") "the thallus consisted of young cylindrical and old barrel-shaped cells, with diameter which varied between 38 and 90 μm, but commonly was 40-72 μm. It occurred in free-floating mats together with *Vaucheria geminata* and *V. sessilis*, and even mixed with them in the thermal springs below the village of Opitsvet... The material corresponds completely to that of LAGERHEIM in the warm aquarium water.... The only difference from the typical species is in the width, since the diameter did not exceed 90 μm. But it could be that we came on and collected only young filaments."

In 2002, this thermal spring system was visited and it was found that two of the spring complexes (between Slivnitsa and Bezden) were captured and the habitats were destroyed. Only Bistritsa springs near to Opitsvet village remained in almost natural state, but *C. herbipolensis* was not found there.

***Chara braunii* GMELIN (Syn.: *Chara coronata* ZIZ ex BISCHOFF)** – Fig. 2: 1

According to LUKOV (1964: 24, Fig. 9) the oospores in the materials from the Hisarya thermal springs (Samodivski Izvor and nameless spring in front of Tinkova Cheshma) were black, 409-448 μm long and 339-354 μm wide and each one posed 8-9 slightly protruded ribs; the branchlets were 8-11, each with a ring of 3 mucronate short cells. According to DAMBSKA (1964) the oospores have dimensions 420-550x275-370 μm and posses 7-10 ribs, while according to KRAUSE (1997) the oospores are 450-750 μm long, 275-350 μm wide and posses 8-11 ribs.

The species was abundantly developed on the bottom of insolated floods of two Hisariya springs (Samodivski Izvor and spring in front of Tinkova Cheshma) with depth up to 30 cm at temperature ranges 24-41 °C. In the spring Samodivski Izvor it is combined with *Pithophora oedogonia* (WITTROCK) MONT.

***Chara coronata* ZIZ ex BISCHOFF f. *intermedia* PETKOFF 1913 (forma inter f. *humilior* A. BRAUN et f. tenuior A. BRAUN)**

These forms were not included in the floras by DAMBSKA (1964) and KRAUSE (1997) and, most probably, they comprise the variability of the species *Chara braunii* GMELIN. PETKOFF (1913: 12-13) wrote: "According to the dimensions of the thalli, which, in most cases do not exceed 10 cm and also according to the width of the branchlets of the last nodule, our form is similar with f. *humilior* A. BR. but according to all other features it resembles f. *tenuior* A. BR. (MIGULA 1897: 331)... Distribution: Together with one species of *Bulbochaete* (*sterilis*) and *Gloeotrichia rufescens* developed abundantly in the mires (swampy floods) below Karlovski Bani. July 1906."

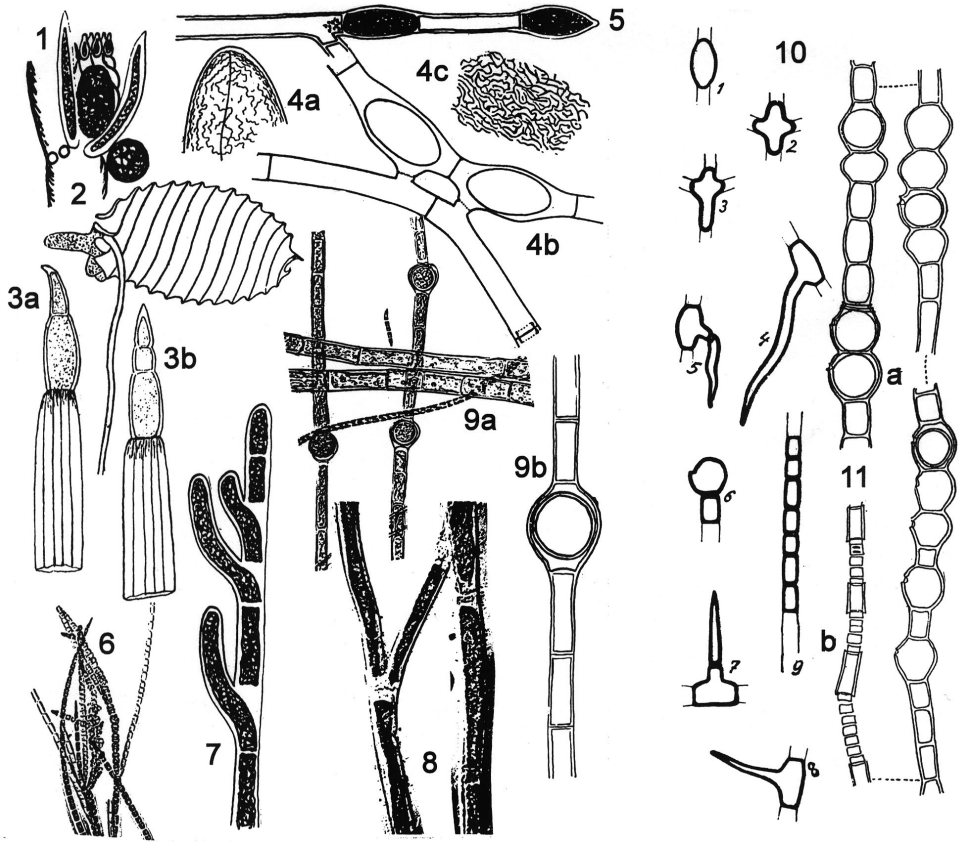


Fig. 2: Original figures and notes of S. PETKOFF, A. VALKANOV and S. LUKOV: **1** – *Chara braunii* GMELIN by LUKOV (1964); **2** - *Chara foetida* f. *thermalis* PETKOFF by PETKOFF 1913 ("magnif. 90 x"); **3** - *Chara foetida* A. BR. α) *subinermis* f. *longibracteata* A. BR. – by PETKOFF 1913 ("magnif. 50 x"); **a** - "terminal part with 2 cells and curved tapered terminal cell"; **b** – "terminal part with 3 cells and straight tapered terminal cell"; **4** - *Spirogyra reticulata* NORDSTEDT forma PETKOFF (**4c** – original fig. 2 of the cell wall sculpture by PETKOFF 1934/35 with "magnif. 540/1") and *S. willei* CZURDA (**4 a-b** – orig. figs. 772-773 of KOLKWITZ & KRIEGER, 1944); **5 – 9** - orig. figs by LUKOV (1964) without provided magnification: **5** - *Pithophora oedogonia* (MONT) WITTROCK; **6** - *Stigeoclonium thermale* A. BRAUN (photo); **7** - *Cladophora* sp. I; **8** - *Cladophora glomerata* (L.) KÜTZING; **9** - *Oedogonium intermedium* WITTROCK in WITTROCK et NORDSTEDT (**a** – photo; **b** – drawing); **10 (1-9)** - *Pithophora oedogonia* (MONT) WITTROCK – orig. drawings by VALKANOV (1955): "**1**- completely developed ellipsoidic akinete; **2 – 3** – completely developed akinetes with irregular shape; **4** – akinetes with well formed initial branch; **5** – akinete with already formed branch – cell which is also transformed into akinete; **6** – akinete formed in the end of interrupted filament; spherical shape; **7** – as fig. 5; **8** – as fig. 4, but the initial branch is significantly shorter; **9** – seven akinetes with origin from the same vegetative cell"; **11** – *Oedogonium cardiacum* (Hass.) WITTR. f. *thermalis* PETKOFF – orig. figs of PETKOFF (1922) : "magnif. 420x: **a** - ♀ filament with 1-4 oogonia, some of which with spores; **b** - ♂ filament with 2-6 celled antheridia".

***Chara foetida* A. BRAUN f. *macrostephana* WAHLDSTEDT**

According to PETKOFF (1908-1909: 77) this form "somewhere with smaller, somewhere with greater dimensions formed a large submerged field on the muddy bottom of the floods below Malo Belovo thermal springs. At the almost opened insolated bottom parts, it formed yellow-green tufts, which were up to 5 cm high."

In the opinion of KRAUSE (1997: 81, 83), *Ch. foetida* A. BRAUN is synonymous to *Ch. vulgaris* L. and the same is valid for the "modifications" from thermal waters. DAMBSKA (1964) transformed different varieties of *Ch. foetida* into different species. The precise taxonomical decision could be taken only after checking of the original material. The attempts to find this taxon in 2002 failed due to complete destruction of the habitat.

Chara foetida* A. BR. f. *macroptila* MIG. 2. MINOR, *humilior*, *pauci-ramosa*, *brevipapillosa

According to PETKOFF (1934: 25) "this form occurred in specimens, which were very fragile, only slightly branched, up to 15 cm long and extremely fertile and which differ from f. *macroptila* MIG. (1897: 576) by their relatively smaller length dimensions, by the absence of well developed thorns, by the sterile part of the leaves, which commonly consisted of 3 cells unequal in length and width, and mainly by the fact that each leave which is normally fertile posses at least one node with two antheridia and two oogonia (normally developed) – as it is in the f. *batrachosperma* (MIGULA 1897: 590). Difference existed also in the dimensions of their relatively big egg. And these are the dimensions of the reproductive organs and of the egg: antheridia (in matured conditions) were normally spherical, 320-340 μm in diameter; the oogonia were elongated-ellipsoidic, with short and almost equal in width flat coronula, 740-800 μm long and 420-492 μm wide; coronula 92-100 μm high and 360-400 μm wide; egg was black, elongated- ellipsoidic, 520-580 μm long and 320-360 μm wide". At the same page, describing the distribution of this taxon, PETKOFF wrote that it was "abundant in stagnant water below the village of Belovo, 20.06.1905". The reason to refer this form among the thermal algae is its pointing out in another work of PETKOFF (1929:105) particularly for the thermal springs of Malo Belovo and their floods.

***Chara foetida* A. BRAUN f. *microptila* MIGULA**

According to PETKOFF (1913: 12-13) "there is almost complete conformity of the material found in the floods of the Malo Belovo warm spring (on 23.05.1909) with the form described by MIGULA (1897: 587). Difference could be find only in the following insignificant features: the length of stem never exceeded 15 cm, while in the mentioned form it reached up to 20 cm; the spines of the young internodes were developed in a less degree in comparison to the described in the material from Sweden; the bract-ring was developed in a lower degree; at last the shape and dimensions of branchlets were the same as these in from Sweden, but the first leaves were relatively longer in comparison with the lateral ones; besides this the non-fruitful part consisted of 3-4 cells and not of 1-3 cells. However, it is notable to mention that according to the stem diameter (460-500 μm in our specimens) and according to the "egg" (which in our material was auburn-yellow, about 400 μm long

and about 300 μm wide) there is not any difference. It is true that our specimens live in a thermal water, but at a distance from the main spring, where the water is cooled..."

"*Chara foetida* A. Br. α) *subinermis* β) *longibracteata* A. Br." – Fig. 2: 3a, b

These taxa were mentioned only in this way by Petkoff (1929: 105) for Malo Belovo thermal springs and their floods, without any other note. In Petkoff (1913) "*Chara foetida* A. Br. α) *subinermis* ad f. *longibracteata* A. Br. (Migula 1897:567) was described from the floods of rivulet Mutnitsa near to Krioder village". This locality is not related to thermal waters, however we include here the original drawings provided by Petkoff (1913).

***Chara foetida* A. Braun f. *thermalis* Petkoff 1913** – Fig. 2: 2

Besides the short description in Latin (Petkoff 1913: 31) of the material collected from the Ovcha Kupel thermal springs, Petkoff (1913: 31-33) added more detailed notes in Bulgarian, which are provided below: "The stem is densely branched, weekly incrusted with $CaCO_3$, 20 cm and more in length, up to 1 mm wide. The warts on the cortex were quite small. The internodes were up to 4.5 cm long. The leaves were 8-9 in a nodule, up to 2.5 cm in length (especially the non-fertile leaves), at young nodules they were erected, while in the more developed ones they were more inclined. The non-fertile leaves were without cortex, while the fertile have 1-3 basal segments covered by cortex and 1-3 fertile nodules and one apical segment without cortex, which consisted of 3-4 cells. This last naked segment occupied 2/3 from the length of the leaves and generally is longer that the fertile parts. The leaves of the fertile branches resembled these of f. *macroptila* Mig. – i.e. the lateral leaves were longer than the first leaves and are longer than the oogonium. Antheridia and oogonia were situated by one at each nodule; the antheridia are spherical, cinnabar-reddish, 269-320 and more rarely up to 350-468 μm in diameter; the oogonia are oval elongated with 10-12 spiral curves, 740-760 and more rarely up to 800 μm long and 460-480 μm wide. The oospore is elongated, barrel-shaped, black, 460-680 μm long and 320-400 μm wide (Fig. 5)". In the opinion of Petkoff (1913), this form was close to the f. *gracilis* Mig. (Migula 1897: 581), but clearly differed from it according to the dimensions of the thalli, of the leaves and oogonia.

Later on, Petkoff (1922: 240) made additional notes and pointed out that "on the muddy bottom of the lowest and most dirty floods of the springs developed some specimens, which leaves reach up to 4 cm in length. Besides this, they could be absolutely naked and without branchlets".

The precise taxonomical decision could be taken only after checking of the original material. The attempts to find this taxon in 2002 failed due to change of the habitat. Petkoff (1913) described his observations on the development of this form since 1896 and changes in its occurrence after capturing of the springs and transforming of the sources into a bath-complex. In the beginning, this form was abundantly developed in the main floods below the springs of Ovcha Kupel in Sofia with water temperature 32-34 °C (and even hig-

her) but after turning of the springs into official baths, it started to flag and was near to complete disappearance.

***Chara fragilis* Desvaux f. *normalis* Migula**

According to Petkoff (1913: 41) the dimensions of thalli found in the thermal baths of Eli-Dere (in September 1905) fitted completely to the description of Migula (1897: 729) – "15 cm long and 5 mm in diameter. However, the oogonia and oospores (eggs) were smaller: the coronula was of two types – elongated and narrow with dimensions 120x200 μm or with equal length and width of 200 μm; oospore (egg) was spherical, about 280 μm in diameter or elongated–oval with dimensions 240x300 μm. Antheridia were spherical with diameter up to 400 μm. All parts of the thalli incrusted with $CaCO_3$".

This form was accepted by Dambska (1964), but was not discussed by Krause (1997). He included *Ch. fragilis* Desvaux as synonym to *Ch. globularis* Thuillier. The dimensions pointed out for *Ch. globularis* and for *Ch. fragilis* f. *normalis* were: up to 300 μm in diameter for antheridia; 600-700x700-1000 μm for the oogonia and 350-450x500-800 μm for the oospores (eggs).

***Chara gymnophylla* A. Braun f. *thermalis* Petkoff 1934 (proxima f. *pulchella* Migula)**

Besides the short description in French (Petkoff 1934: 62) of the material collected from the Malo Belovo thermal springs, Petkoff (1934: 15-16) added more detailed notes in Bulgarian, which are provided below: "the internodes were 3-7 cm long (only very rarely 8 cm in the lower parts of the thalli and down to 0.5 cm only in the most upperly situated 5-6 internodes) and leaves were 0.5-1 cm long. Thallus is monoecious, strongly incrusted with $CaCO_3$. The "stem" is densely branched, about 25 cm long and 750 μm in diameter. The internodes were covered by cortex of tube-like cells with equal diameter. The number of these cells is twice bigger than the number of the leaves of the upper nodule (8-10). On the cortex there were conical elongated wart-shaped isodiametrical cells. The bract-ring was well developed (particularly below the young nodules at the top of the stem and leaves) with cells up to 3 times longer than wide. The leaves generally 10 in number, but sometimes 6 with total length up to 1 cm, but commonly shorter (down to 0.5 cm). The fertile basal parts of these leaves consisted of two short internodes and 2 fertile nodules with leaves. Their non-fertile part normally consisted of 3 cells and is longer in comparison with the fertile one; the cell on the top is short and taped. The leaves were with different length in comparison with the oogon. The antheridia and oogonia were situated singularly at each fertile nodule. The antheridia were spherical, colorless, 420-400 μm. The oogonia are widely–ellipsoidic with dimensions 480x660 μm (780 μm long with the coronula). The coronula was 120 μm in height and at the base and at the top was with equal width of 280 μm. The oospore (egg) was widely ellipsoidic, dark brown to black with dimensions 320x480-520 μm. This form on first glimpse was close to f. *pulchella* Mig. mainly according to the length of the stem and dimensions of the egg. But in all other features it differed and this mainly concerned the following features: 1) the length of leaves, which did not

exceeded 1 cm and was quite variable; 2) the fertile nodules which were 2 (rarely 1) at each leave and were always fertile; 3) the internodes, which reached up to 8 cm in length". This form developed abundantly, completely submerged in the main floods of the Malo Belovo springs in water with temperature 24 °C.

In fact, this form was firstly mentioned by PETKOFF (1913: 22) as *Ch. gymnophylla* A. BR. f. *pulchella* MIG. with brief notes on some deviations from the description of MIGULA (1897: 553). Later on, in the description of changes in the ecological situation of the Malo Belovo habitat, PETKOFF (1929: 101, 105) listed again *Ch. gymnophylla* f. *pulchella* MIG. among the abundantly developed stoneworts. After the publication from 1934, there is no reason to list f. *pulchella* MIG. among the green algae in Bulgarian thermomineral waters. DAMBSKA (1964) and KRAUSE (1997) in their floras did not comment the both forms discussed by MIGULA (1897) and PETKOFF (1934).

***Cladophora glomerata* (L.) KÜTZING** – Fig. 2: 8

According to LUKOV (1964: 22) it "forms dense tufts, formed by branched filaments. In the basal part of the filaments cells were 59 μm wide and at the apical part – 23. 5 μm wide. The length of cells was 413-800 μm." The species grew abundantly on the well insolated stonewalls of the Hisariya spring Tinkova Cheshma at temperature 24 °C (together with cyanoprokaryotes *Calothrix* and *Gloeocapsa*). At water temperature 27 °C in the floods of the Hisarya spring 13 it dominated in the shore ecotone zone, where together with *Stigeoclonium thermale* A. BRAUN and *Oedogonium intermedium* WITTROCK it formed 1-3 cm high tufts. It is interesting to note that in the spring Tinkova Cheshma this species occurred also together with *Stigeoclonium thermale* and *Oedogonium intermedium*. At the Hisariya spring Havuz-Dere at 28 °C it developed on the insolated spring stone walls (together with cyanoprokaryotes *Oscillatoria* and *Phormidium*).

VODENICHAROV et al. (1971) and VINOGRADOVA et al. (1980) pointed out the following dimensions for *C. glomerata*: cells of main axis 65-275 μm wide and cells of apical parts – 19-100 μm wide and the following dimensions for *C. fracta* (MÜLLER ex VAHL) KÜTZING cells of main axis 45-85 μm wide and cells of apical parts – 17.5-38 μm wide. According to the dimensions and due to the fact that in the Bulgarian algal flora VODENICHAROV et al. (1971) noted the occurrence of *C. fracta* in thermal springs of Bulgaria, in our opinion, the species found by LUKOV (1964) has to be referred to *C. fracta*. The only microphotograph provided by him is not enough for taking a final decision, more over that JOHN et al. (2002: 469) wrote: "main branches of profusely branching forms (of *C. glomerata*) up to 150 μm wide; lesser branched forms somewhat narrower...".

***Cladophora* sp**. I – Fig. 2: 7

According to LUKOV (1964: 22, Fig. 4) this alga formed dense tufts, which consisted of abundantly branched filaments. Filaments in the base were 55-60 μm wide and at the apex – 45-48 μm wide. The length of the cells ranged between 170-236 μm. The material

was found in the effluent of the spring Chair Banya (water temperature 41 °C) in Hisarya spring complex.

***Closterium acerosum* (SCHRANK) EHRENBERG**

According to PETKOFF (1925) in the floods below Dobrinishka Banya the species occurred only rarely, the cells were typical for the species, 40 μm wide and 430 μm long.

Closterium closterioides* (RALFS) LOUIS et PEETERS var. *closterioides

In the material from shadowed effluents of Zheleznitsa thermal springs below the laundry with water temperature 29 °C single broadly spindle-shaped cells with the following dimensions were found: 55x370 μm. RUZICKA (1977) and LENZENWEGER (1996) referred *C. libellula* FOCKE as synonym to *C. closterioides*. But LENZENWEGER(1996) provided smaller dimensions: 35-45x200-300 μm. VODENICHAROV et al. (1971) gave the following dimensions for *C. libellula*: 30-52x170-450 μm, RUZICKA(1977) and JOHN et al. (2002) – (30)-35-45-(54)x(170)-200-300-(400) μm and 38-46x270-315 μm, respectively for *C. closterioides*.

***Closterium delponte*i (KLEBS) WOLLE**

PETKOFF (1925: 62) described *C. decorum* BRÉBISSON f. *minor* PETKOFF 1925 in the material from the floods of Bansko spring: "cells were narrowly lanceolate, slightly twisted, delicately furrowed, strongly narrowed at the ends and widely swelled. Pyrenoids were in one line, 6-8 in each semi-cell. Each vacuole was with 10-15 moving grains. The cells were 304 μm long (in the diagnosis – 370-450 μm), 27 μm wide (in the diagnosis 30-41 μm) with 6 μm wide ends. Our form differs from the typical only by the much smaller dimensions in length and width". *C. decorum* BRÉBISSON f. *minor* PETKOFF 1925 was pointed out by RUZICKA (1977: 187) as homonym for f. *minus* W.& G.S.WEST 1907 and in this way it belongs to *C. delpontei* (KLEBS) WOLLE with a slightly difference in the measurements at the apex – 7-11 μm in *C. delpontei* var. *delpontei* and 6 μm in the form described by PETKOFF.

"Closterium digitus"

According to GEORGIEV (1948) it was found in the floods below the springs of Marikostinovo together with 11 species with temperature range 40-58 °C and pH=7,7. Most probably, it is a typographical error and the species has to be referred to *Netrium digitus* (EHRENBERG ex RALFS) ITZIGSOHN et ROTHE.

Closterium pritchardianum* ARCHER var. *pritchardianum – Fig. 3: 13

In the material from Ovcha Kupel floods, collected in 2002, cells were very slightly curved, 38-44-55.5 μm wide, 414-440 μm long, with cone-shaped apex (3.8-4-5 μm wide) and 20-24 axile pyrenoids per cell. Some of the cells were with destroyed cell content.

***Cosmarium botrytis* MENEGHINI ex RALFS**

According to PETKOFF (1925: 79) in the floods below the spring of Bansko the cells were "with typical shape and with maximal dimensions pointed for the species: cells 72-88 μm long, 58-64 μm wide; isthmus 20-24 μm wide, more rarely 18 μm wide."

PALAMAR-MORDVINTZEVA (1982) mentioned particularly the occurrence of this widely distributed species in hot springs.

***Cosmarium botrytis* MENEGHINI ad var. *paxilosporum* WEST & G. S. WEST**

According to PETKOFF (1925: 79) in the floods below the spring of Bansko "the cell wall was fine punctate with irregularly scattered warts. The cells were 88 μm long, 60 μm wide; isthmus 18 μm wide; hemicells 40 μm thick. Zygote was not observed... According to the dimensions our material was close to the typical species, but according to the sculpture of the cell wall it was close to the pointed out variety (fig. 3-4, pl. XCVI: 4)."

PALAMAR-MORDVINTZEVA (1982) did not give data to the cell wall sculpture, but pointed out smaller length (72-80 μm) and thickness (29-36 μm) for this variety. LENZENWEGER(1999) did not discuss this variety. In their notes on the species, JOHN et al. (2002: 536) note: "semicell shape and arrangement of granules very variable".

***Cosmarium laeve* RABENHORST**

According to PETKOFF (1925: 74-75) in the floods below the spring of Bansko the cells were "small, with a deep linear constriction in the central part. The semi-cells hemispherical, widely rounded at upper side and slightly blunted or bended, each with one chloroplast. The cell wall was smooth or punctate. The cells were 16-22 μm long (rarely up to 28) and 12-15 μm wide (rarely up to 6 - ?err. typogr). The isthmus was with a width of 4-5 μm (rarely up to 6 μm)... The specimens found correspond particularly to fig. 11 on plate LXXIII in WEST & WEST (1908: 99)".

***Cosmarium subtumidum* NORDSTEDT**

According to PETKOFF (1925: 71) in the floods below the spring of Bansko (where the species occurred only rarely) the cells were "typical for the species (particularly close to fig. 19 on plate LXIII in WEST & WEST 1905:192), 36 μm wide and 42 μm long with an isthmus with a width of 10 μm". However, according to PALAMAR-MORDVINTZEVA (1982) and LENZENWEGER (1999) the dimensions of *Cosmarium subtumidum* NORDSTEDT var. *subtumidum* are 26-33x30-40 with isthmus 7-12 μm and 28-35x30-38 μm with isthmus 10-11 μm, respectively. WEST & WEST (1905) and PALAMAR-MORDVINTZEVA (1982) pointed out the following dimensions for the cells of *C. subtumidum* NORDSTEDT var. *klebsii* (GUTWINSKI) W. et G. S. WEST: 29-35 μm width and 32-41 μm length with isthmus 7-11 μm.

***Cosmarium sexnotatum* GUTWINSKI ad var. *tristriatum* (LÜTKEMÜLLER) SCHMIDLE (WEST & WEST** 1908: 228, pl. LXXXVI, Fig. 8-9)

According to PETKOFF (1925: 77-78) in the floods below the thermal bath of Simitli

"the hemicells were pyramidal-trapeziform, with flat apex and waved-angular margins, at the basis from isthmus in wide profile there were 3 elongated granules, but often they also were grain-shaped. The cell wall was punctate. Cells were 25-26 μm long and 20-21 μm wide; isthmus was 6-7 μm, rarely – 10 μm. This variety in our localities varied strongly in terms of isthmus granules and cell wall punctae..."

According to PALAMAR-MORDVINTZEVA (1982) and LENZENWEGER(1999) the dimensions are: 14-22 μm width, 16-26 μm long and isthmus 4-8 μm wide, and 13-23 μm width, 15-25 μm long and isthmus 5-6 μm wide, respectively.

***Cosmarium turgidum* BRÉBISSON**

According to PETKOFF (1925: 75) in the floods below the spring of Bansko (where it was abundantly developed) the cells were "elongated, with semicells which were widely taped at the ends or extremely narrowed there. The cells at the middle part widely and shallowly constricted and with parietal band-shaped chloroplasts. The cell wall smooth or with small punctae. The cells were 220-228 μm long, 82-91 μm wide; the isthmus was 72-78 μm wide".

***Cosmarium venustum* (BRÉBISSON) ARCHER**

According to PETKOFF (1925: 72) in the floods below the spring of Bansko "the cells were typical in shape, but characterized by the smallest dimensions, pointed out for the species and even with 2-3 μm shorter. Generally, the dimensions were 22-24 μm width, 30-31 μm length with an isthmus with a width of 6-8 μm".

According to PALAMAR-MORDVINTZEVA (1982), LENZENWEGER(1999) and JOHN et al. (2002) the dimensions are 22-32.5x32.6-42 μm with isthmus 5.7-13.4 μm wide, 20-35x30-45 μm with isthmus 5-10 μm wide and 17-38x25-48 μm, respectively.

***Euastrum binale* (TURPIN) EHRENBERG ex RALFS f. *secta* TURN.**

According to PETKOFF (1925: 66) in the floods below the spring of Bansko "the cells were with the typical shape but with a little bit larger dimensions: the cells were 32 μm long and 22 μm wide; the isthmus was 5-6 μm wide".

According to LENZENWEGER(1996) this form belongs to a group of unclear taxa or with illegitimate names.

Euastrum insulare* (WITTROCK) J. ROY var. *insulare

According to PETKOFF (1925: 68) in the floods below the spring of Bansko "the hemicells were with wide apex, slightly concave, the lateral parts were also slightly concave and with narrow isthmus. The cells were 28-30 μm long, 20-22 μm wide; the isthmus was 5-6 μm wide".

***Gloeocystis ampla* KÜTZING**

According to PETKOFF (1925: 89) in the floods below the Bansko spring "the "fami-

lies" were with different dimensions, up to 50 μm. The cells were spherical or very elongated (in the diagnosis – commonly elongated) with diameter of 8-16 μm (in the diagnosis 9-15 μm long and 12 μm wide), forming spherical or almost spherical green masses. The cell wall was clearly layered."

Perhaps a *Chlamydocapsa* species, following ETTL & GÄRTNER (1988).

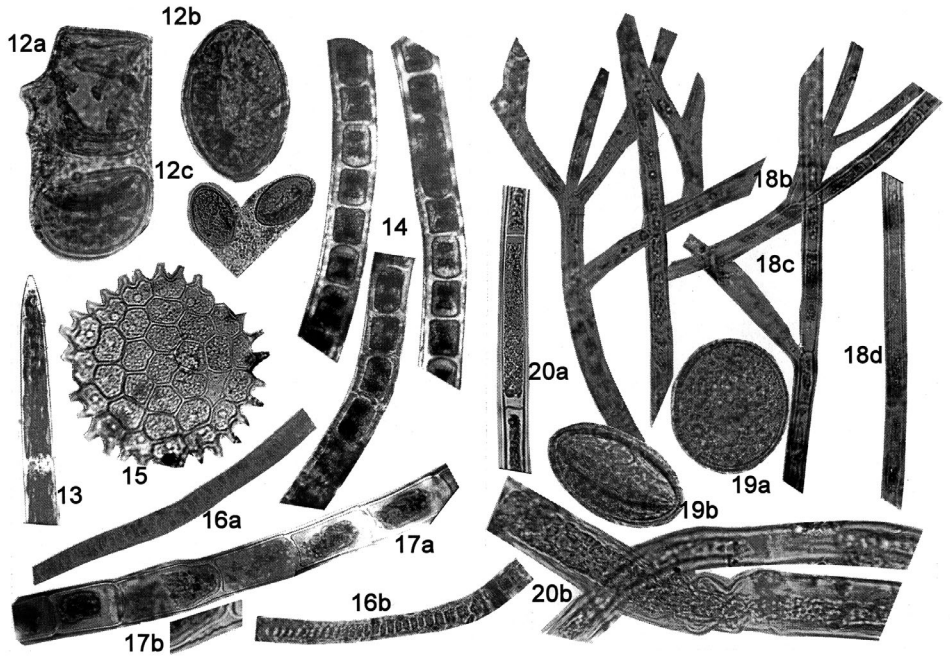


Fig. 3: 12 - *Spirogyra columbiana* CZURDA from Ovcha Kupel (2002): **a** – two-celled fragment: cell (48 μm wide at the upper cell wall) with conjugation canal and cell with zygote (64 μm long); **b, c** – mature zygotes at different magnifications (51.8 x 94-96 μm); **13** - *Closterium pritchardianum* ARCHER var. *pritchardianum* from Ovcha Kupel (2002) – cell 38.8 μm wide with 3.8 μm wide apex; **14** - *Zygnema* sp. ster. (? *Zygogonium* sp. ster.) from Opitsvet (2002) - sterile filaments with vegetative cells 44.4-48 μm wide and 37-48-63 μm long; more dark cells and chloroplasts were with red colour; **15** – *Pediastrum boryanum* (TURPIN) MENEGHINI from Ovcha Kupel (2002) – cell diameter 18 μm; **16** – *Ulothrix zonata* KÜTZING from Opitsvet (2002) – filament fragments: **a** – cells 7x18 μm; **b** – cells 7-9x17.8 μm; **17** – *Oedogonium* sp. ster. from Opitsvet (2002): **a** – filament fragment with cells up to 14. 8 μm wide; **b** – magnificated cell part with characteristic cell wall rings; **18** - *Stigeoclonium thermale* A. BRAUN from Zheleznitsa (2002): **a-c** - thalli with 3 (**a**) and 2 branchings (**b, c**) with cells up to 12.8 μm wide; filament with double branching and cells 5.5 μm wide; **d** - unbranched filament with cells 7x84 μm; **19** – *Spirogyra crassa* (KÜTZING) CZURDA emend. (photo from PETKOFFS' material): **a, b** – zygotes (167 μm long); **20** - *Rhizoclonium hieroglyphicum* (AGARDH) KÜTZING from Opitsvet (2002): **a** – thinwalled filament with cells 25.6 μm wide; **b** - filaments with curved thick walls 29 μm wide and with straight walls 26 μm wide.

***Gloeocystis vesiculosa* NÄGELI**

According to PETKOFF (1925: 89) in the floods below the Bansko spring "the thallus consisted of spherical and more rare – of elongated cells, up to 6 μm in diameter (in the diagnosis 2, 4-12 μm), which formed green, soft vesicle shaped mass. The cell wall was clearly layered. The zoospores were formed from the vegetative cells. The whole "family" was 20-64 μm in diameter".

According to HINDÁK (1978), KOMÁREK & FOTT (1983) and ETTL & GÄRTNER (1995) cells are widely oval to spherical, 6-8 μm in diameter and colonies – up to 50 μm.

***Hydrodictyon reticulatum* LAGERHEIM**

According to LUKOV (1964: 21) the "cylindrical cells were up 1.5 cm long and the whole coenobium was about 20 cm long." The species was abundantly developed on the bottom of insolated floods of Hisarya springs with depth up to 30 cm at temperature ranges 24-41 °C.

PETKOFF (1925) noted its abundant development in the floods below Ognyanovski Bani.

***Mesotaenium endlicherianum* NÄGELI var. *grande* NORDSTEDT f. *brevior* PETKOFF 1925**

According to PETKOFF (1925: 55) "the cells were cylindrical, widely rounded at their ends, more wide than in the typical species, but by 12-16 μm shorter than in the typical variety. The wall was smooth." This taxon "occurred rarely in the floods of Bansko spring in combination with other conjugates" (PETKOFF 1925: 55).

***Mougeotia angusta* HASSALL (Syn.: *Mougeotia parvula* HASSALL var. *angusta* (HASSALL) KIRCHNER)**

According to PETKOFF (1925: 87) the species was abundantly developed in the floods below the Bansko spring; vegetative cells were 5 μm wide; zygotes were 7-8 μm long.

***Mougeotia* sp. ster.**

In the notes by PETKOFF (1908) the species was abundantly developed in the floods of Narechenski Bani; vegetative cells were 22 μm in diameter.

Single filaments 18-20-(22) μm wide were found in the effluents of Zheleznitsa springs. Filaments with vegetative cells 38 μm wide and 111 μm long were found in Bistritsa springs near to the village of Opitsvet in 2002.

***Netrium digitus* (EHRENBERG ex RALFS) ITZIGSOHN et ROTHE**

According to PETKOFF (1925: 56) in the floods below the Bansko spring "the cells were with typical for the species shape, 164-205 μm long and 48-50 μm wide, at the ends 18 μm wide".

***Oedogonium intermedium* WITTROCK in WITTROCK et NORDSTEDT** – Fig. 2: 9a, b

According to LUKOV (1964: 21-22, Fig. 5) "monoecious...Oogonia single, ovoid or rounded with an upper/apical pore. The oospore was rounded and filled-up the oogonium. The wall of the oospore was dense and smooth. The length of vegetative cells was 47-70 μm, the wide was 12-18.8 μm; the length of oogonia – 33-38 μm and width – 37-38 μm; the oospores spherical, 33 μm in diameter ". It developed in the Hisariya spring Samodivsko Kladenche at temperature 41 °C together with *P. oedogonia* (see below).

There are very insignificant, in our opinion, differences with the dimensions provided by MROZINSKA-WEBB (1969) and MROZINSKA (1985): the maximum width of vegetative cells - 18 μm and of the oogonia - 37 μm, the minimum length of oogonia –34 μm.

***Oedogonium* spp. ster.** – Fig. 3: 17a, b

In the material from the floods of Bistritsa springs near to Opitsvet village (water temperature 15 °C) sterile filaments with cells wide 14.8-15 μm were found. In the same material filaments with short cells (7 μm long and 17.8-18 μm wide), which resembled male filaments with antheridia were found also.

In the material from the floods below Zheleznitsa springs with temperature 30 °C single sterile filaments with cells up to 14.4 μm wide were found.

***Oocystella solitaria* (WITTROCK in WITTROCK et NORDSTEDT) HINDÁK. (Syn. *Oocystis solitaria* WITTROCK in WITTROCK et NORDSTEDT)**

According to PETKOFF (1925: 91) this species "occurred rarely in the floods below the Bansko spring; the cells were 23 μm in length and 18 μm in width. The cell wall was thickened at both cell ends. "

***Palmella mucosa* KÜTZING**

According to PETKOFF (1925: 88) the species was abundantly developed as attached to the submerged stones in the floods below the Bansko spring. "The thallus was olive-green vesicle-shaped and soft, formed by spherical cells, with a thin cell walls and pale green color, 6-13 μm in diameter (in the diagnosis 6-14 μm)".

Following ETTL & GÄRTNER (1988), most probably, this taxon has to be referred to *Palmellopsis gelatinosa* KORSCHIKOFF, but it is questionable without drawings and data on zoospores.

***Pediastrum boryanum* (TURPIN) MENEGHINI var. *vagum* (A. BRAUN) CHODAT (*P. vagum* A. BRAUN)**

According to PETKOFF (1925: 90) this alga occurred rarely in the floods below the Bansko spring and was characterized by "elongated in tangential direction peripheral cells (24 up to 25 μm) and cells of in radial direction with dimensions 16 up to 20 μm. Coenobia were formed by 64 up to 128 cells, with maximum dimensions 264 μm in length and 180 μm in width...In our specimens both peripheral layers were regular; however, the inner lay-

ers are irregular and between some of the cells there are perforations. Otherwise, by their shape and by granulose cell wall they correspond completely to the variety on the figures by A. BRAUN". CHODAT (1902: 229) listed *P. boryanum* var. *vagum* among some "formes" of *P. boryanum* (TURPIN) MENEGHINI. Therefore, it could be accepted that CHODAT gave P. *vagum* A. BRAUN only synonymy status. After SULEK (1969) this taxon is doubtful and, most probably, at the moment has to be listed together with *P. boryanum* (TURPIN) MENEGHINI, until the original material is checked.

***Pithophora oedogonia* (MONT) WITTROCK (Syn. *P. kewensis* WITTROCK)** - Fig. 2: 5, 10 (1-9)

According to VALKANOV (1955: 119) the collected material "corresponded almost completely to the description by HEERING (1921: 62). Thallus is filamentous, branched, multicellular; cells were cylindrical, generally 200-300 μm long (only rarely they reached up 1 200 μm in length) and 50-90 μm wide; the branching started sub-terminally; commonly there were branches of 1st order and rarely – of 2nd order. The chloroplast was net-shaped with many pyrenoids..." Helikoids were developed with the exception of filaments, which laid on muddy bottom. An autotropism was detected. All cells had the ability to form akinetes. They were generally cylindrical to ovoid, 200-300 μm long and 65-100 μm wide. However, this author observed also some other shapes of akinetes. The cell wall of vegetative cells was 2 μm, of young akinetes – 4-6 μm and of the μm developed akinetes – up to 18 μm. The notes mentioned above concerned the filaments found in one Hisarya spring (Samodivski Kladenets) with water temperature of 30 °C, The filaments were developed on the bottom of this spring and in its outflow canal, where they were attached to different submerged objects.

LUKOV (1964:23-24) described "filaments with akinetes; at the base the width of cells was 81 μm in average; there were some branches of 1st order and only rarely –branches of 2nd order. Akinetes were singular, intercalar or terminal. The intercalar akinetes are barrel-shaped, 81-117 μm long and 210-269 μm wide. Terminal akinetes are barrel-shaped but with bottle-lie top, 82 μm long and 245 μm long. According to LUKOV (1964: 24) "it differs from the WITTROCKs' diagnosis by bigger dimensions: 81 μm at the filament basis (and 59 μm according to WITTROCK). Intercalar akinetes – 81-117x210-260 μm while according to WITTROCK they were 81x205 μm. Terminal akinetes were 82x245 μm, which is relatively close to WITTROCKs' data: 88x219 μm". These notes concerned material found in the Hisarya spring Samodivski Izvor at temperature 41 °C.

In the description provided by ZAUER in VINOGRADOVA et al. (1980) in *P. oedogonia* the vegetative cells of main filaments are (38) 45-85 (117) μm wide; cells of the branches of 1st order (25) 30-50 (71) μm wide; the akinetes of main filaments are intercalar (with dimensions 71-150 x107-210 μm and those in branches – 58-118x117-190 μm) or terminal (54-74 x 107-236 μm) with cell wall 4-14 μm.

***Pithophora* sp. (*Pithophora* aff. *oedogonia* (MONT) WITTROCK)**

According to VALKANOV (1955) the vegetative cells were cylindrical and reached up

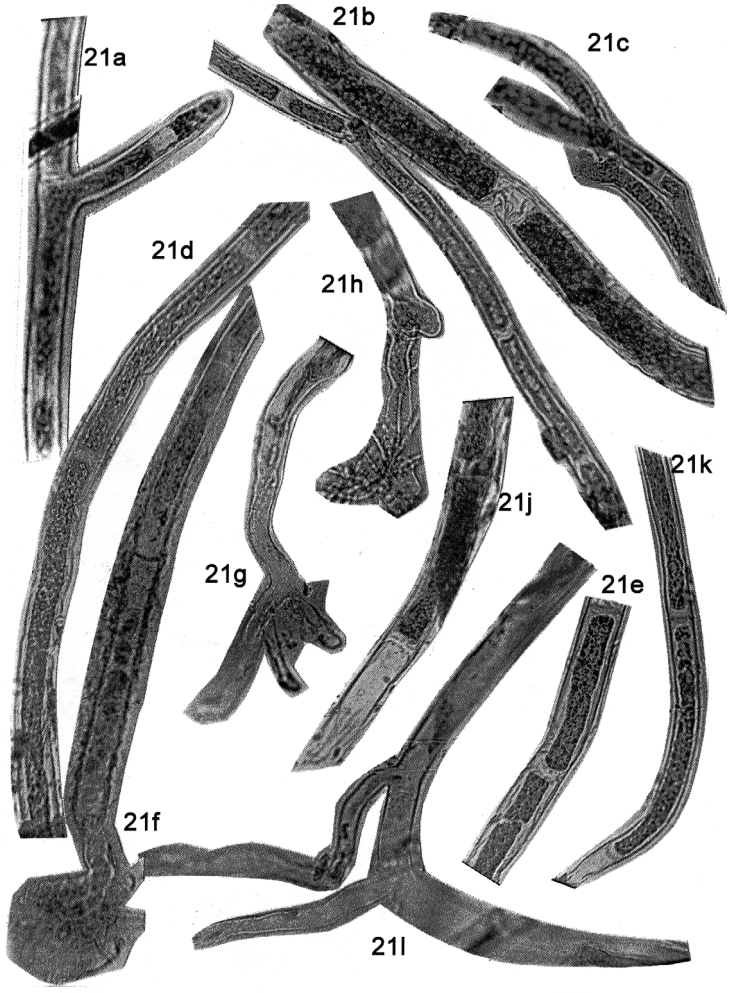


Fig. 4: 21- *Rhizoclonium hieroglyphicum* (AGARDH) KÜTZING from Zheleznitsa (2002 – a-i, l) and from Opitsvet (2002 – b, k): **a** – fragment of filament (26 μm wide) with branching; **b** - filaments with different width (18 μm and 38 μm); **c** – unbranched filament (18 μm) and filament with initial branching (20 wide); **d, e** – fragments of filaments 26 μm wide; **h, l** – fragments with both basal and lateral rhizoidal branching (above the lateral branching filaments are 26 μm wide); **j, k** - basal part of the filament (j – 26 and k - 18 μm wide); **g, f** - filaments with basal rhizoidal branches (cells above the rhizoids - 30x242- 244 μm).

5 000 μm in length; their average width was 100-120 μm with maximum of 160 μm. The material collected at temperatures 30-36 °C was without akinetes. Akinetes developed only in culture after 3-4 weeks in the laboratory and were in average 200-220 μm long and 100-120 μm wide. In the opinion of VALKANOV (1955:123) "this form is identical with the described above (as *P. kewensis* WITTR.), but now I left it unidentified until the final clarifying of the genus classification".

Pleurotaenium trabecula* (EHRENBERG) NÄGELI var. *trabecula

According to PETKOFF (1925: 62-63) "cells were cylindrical, tapering to the ends and with thickened wall there, with two inflations in the constricted central part, with widely cut ends. The cell wall was thin. The cells were wide, about 28 μm and about 5-6 times longer (according to DE TONI 1889: 895), however, in our material they varied to a great extent."

According to LENZENWEGER(1996) cells of *P. trabecula* var. *trabecula* are 10-30 times longer than wide.

***Rhizoclonium hieroglyphicum* (AGARDH) KÜTZING** – Figs. 3: 20a, b; 4: 21 a-l

The species was abundantly developed in green masses in two of Zheleznitsa thermal springs with temperature of 30 °C, where it was attached to submerged stones, as well as to the tubes, only moistened by water spatters. There most of the filaments were rarely branched and with basal rhizoids; cell dimensions were 25.9-29-29.6-30-(38)x220-222-244 μm. More narrow filaments were thincelled, while more wide had more thick and sometimes layered cell walls. In several filaments, separated rhizoidal branchlets have been observed, which resembled these described for *R. riparium* (ROTH) HARVEY and were similar to these on Fig. 32 (1) in VINOGRADOVA et al. (1980: 72).

The species was found also in floating masses in the floods of Bistritsa springs near to the village of Opitsvet. There the filaments were almost unbranched, with dimensions (18)-22-26-29-(30) x 111-222 μm and basal rhizoids.

In JOHN et al. (2002: 470) it was noted "rhizoid branchlets rare or absent".

***Spirogyra columbiana* CZURDA** – Fig. 3: 12a-c

In 2002, in the material from the side-floods of Ovcha Kupel springs (with temperature 28 °C) vegetative cells were (37)-41.8-48-51.2-55.5 μm wide; the conjugation was mainly scalar; zygotes were ellipsoid, up to 96 μm long, 43-51.8-56 μm wide with smooth exospore and smooth, thick yellow-brownish mesospore. The width of cells containing zygotes was up to 62 μm.

***Spirogyra jugalis* (DILLWYN) KÜTZING**

In the opinion of KOLKWITZ & KRIEGER (1944: 354) *Conferva jugalis* DILLWYN is a synonym of *S. setiformis* (ROTH) KÜTZING, whereas the taxon *S. jugalis* (DILLWYN) KÜTZING is included in the list of unsolved taxa (KOLKWITZ & KRIEGER 1944: 475). Accor-

ding to JOHN et al. (2002) *Conferva jugalis* DILLWYN is a basionym of *S. jugalis* KÜTZING.

This taxon is included in the list of species, provided by GEORGIEV (1948) for the floods below the springs of Marikostinovo (with temperature range 40-58 °C and pH=7.7) without any description, note or figure. Due to the peculiar ecology, we included it under this name until more or/and original materials would be found.

***Spirogyra reticulata* NORDSTEDT forma PETKOFF 1934/35** – Fig. 2: 4a-c

In the opinion of KOLKWITZ & KRIEGER (1944) the form of *S. reticulata* described by PETKOFF (1934/35) from the floods of Vurshets Mineral Baths without name is a synonym of *S. willei* CZURDA. Taking in mind the particular ecology of this taxon, which was not discussed in the text of their flora, there is need to outline the difference in cell wall sculpture pointed out by PETKOFF (1934/35: 2) in the text as "membranne moyenne fortement et irrégulièrment plissée (fig. 2) d'un aspect particulier " and this provided by KOLKWITZ & KRIEGER (1944: 470-471) according to CZURDAS' drawing at their Fig. 772-773 and described as "Exospor dünn, glatt und farblos". In the description by PETKOFF (1934/35: 2-3) the dimensions of vegetative cells were 32-35 μm in diameter, of reproductive cells with ellipsoid shape – 60-61 μm and of zygospores were 54-60 x 92-108 μm. The chloroplast was one, with no more than 10 folds and the transversal walls were smooth, without ring. KADLUBOWSKA (1984) did not discuss this form, writing about *S. reticulata* NORDST. and included *S. willei* SKUJA 1928 and not of *S. willei* CZURDA. Doubtless, more precise taxonomic conclusion needs the original material to be checked.

***Spirogyra varians* (HASSALL) KÜTZING.**

According to PETKOFF (1925: 87) the material from the floods below the Bansko spring (where the species was abundantly developed) corresponded completely to diagnosis and to the other specimens found in other localities in Bulgaria: vegetative cells were 28-30 μm wide, zygotes – 32-36 μm wide and 44-64 μm long.

***Spirogyra* spp. ster.**

According to PETKOFF (1908), sterile filaments of *Spirogyra* were abundant in the hot springs Svetata Voda and Novata Voda (still not identified due to lack of description of their location, but most probably situated in the region of Kyustendil), in the spring near the village Yanensko and in the floods below Narechen Mineral Baths.

The filaments found by LUKOV (1964: 23-24) in Hisarya springs consisted of cells 48 μm wide and 117-176 μm long.

Among the free-floating algal mats in the floods of Bistritsa springs near to Opitsvet village sterile filaments with vegetative cells up to 74 μm wide were found.

In the effluents of Zheleznitsa springs sterile filaments with 25-30 μm wide cells were found.

***Stigeoclonium thermale* A. BRAUN** – Fig. 3: 18a-d

According to LUKOV (1964: 23, Fig. VII-2) the filaments from Hisarya springs consisted of basal cells 8-10 μm wide and 21-23 μm long, while in the upper parts of the thalli cells were 8-9 μm wide and 21-59 μm long.

In Zheleznitsa this species was developed abundantly as attached to the stones in the floods of upper non-captured and insolated springs (with temperature 30 °C), as well as in single filaments in the effluents of lower captured springs and in the shadowed effluents below the small laundry (with temperature 28-30 °C). Most of the free floating filaments were long, unbranched and more thin than described in diagnosis: (4.8)-6.4-(7-7.7) μm wide (while according to the diagnosis they have to be 7-12 μm). In the upper non-captured spring, these filaments formed visible yellow-green masses on both submerged and moistened stones. There they were rarely branched (with 2-3 branches) and were (5)-7.2-8-12.8 μm wide with no obvious difference between different parts of the thalli. The cell length in all filaments exceeded the width 4-5–5.5-6-(8-10-12) times.

Single floating filaments were found also among the masses of *Rhizoclonium hieroglyphicum* in Bistritsa springs near to Opitsvet village as unbranched fragments, with 5-7 μm wide cells with length exceeding the width 4-5-5.5-6 -(8-10) times.

Such a ratio between cell width and length is typical for *S. subsecundum* KÜTZING and for *S. aestivale* (HAZEN) COLLINS, but the cell wide in these species is bigger - 10-20 μm and 7-9-11 μm, respectively. The close relations between these species were discussed by ISLAM (1963) and were mentioned by JOHN et al. (2002).

***Ulothrix zonata* KÜTZING** – Fig. 3: 16a, b

According to PETKOFF (1922) the thalli found in the floods of Ovcha Kupel springs were dark-green or yellow-green, attached to the submerged objects (mainly to *Chara foetida* f. *thermalis*) and after that floating, 5-30 cm long; filaments were 10-72 μm wide, equal in the whole length, with a thick, layered wall.

Among the floating algal masses in the floods of Bistritsa springs near to Opitsvet village single filaments up to 18 μm wide were found in July 2002.

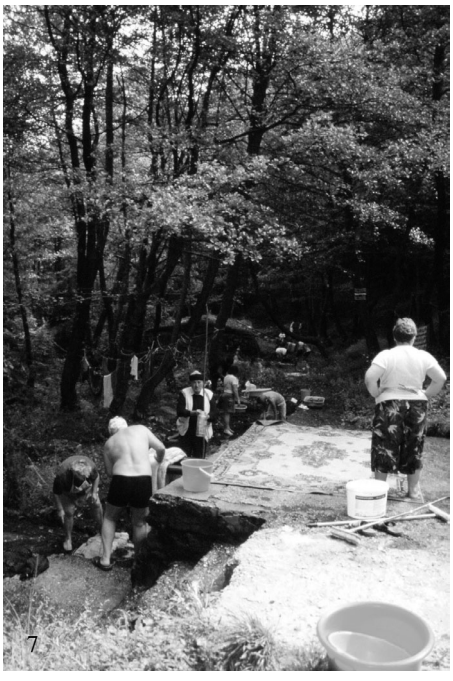
***Zygnema* sp. ster. (? *Zygogonium* sp. ster.)** – Fig. 3: 14

Among the free-floating algal mats in the floods of Bistritsa springs near to Opitsvet village (water temperature 15 °C) sterile filaments consisting of vegetative cells 44.4-48 μm wide and 37-48-63 μm long with two weakly star-shaped chloroplasts were found. The cell wall was 4.8-5 μm thick. Some of these cells were of green color, but most were with purple cell content. These sterile filaments are close to the description of *Zygogonium ericetorum* (KÜTZING) HANSGIRG, but the absence of reproductive structures together with the finding of the material in alkaline conditions prevented us from referring of this material to *Z. ericetorum*.

"Representatives of Chlorococcales"

They were pointed out as co-dominants in the algal community, which developed in the basins of Haskovski Mineralni Bani at depth down to 1 m and water temperature between 54 and 57 °C. This community was dominated by nostocalean algae and appeared as green-brownish mat on the bottom and walls of the basins (SEMERDZHIEV et al. 1980).

Figs. 5, 6: One of the thermal springs nearby the rivulet Zheleznitsa (Z on map – Fig.1, ca. 15 km south of Sofia) in a natural status and used as free mineral bath by the local people since decades.



Figs. 7, 8: One of the thermal springs nearby the village Zheleznitsa (Z on map – Fig.1, ca. 15 km south of Sofia) used as carpet-laundry by the local people since decades and recently even with detergents.

Nevertheless of the occasional character of the actual floristic and taxonomic studies on the Bulgarian thermal springs it is obvious that these habitats support a rich diversity of green algae. Some thermal spring localities in Bulgaria are still kept in natural status (Figs. 5, 6) and used by man since decades as free mineral baths. The development of balneotherapy centers and/or utilization of these springs for water-supply or even destruction by use as bathing places and laundries (Figs. 7, 8) have negative effects (e.g. from detergents) on their algal biodiversity and should be taken under control.

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