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# Cryoseston in Stara Planina (Balkan) Mountains, Bulgaria

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Numerous snow fields persisted in the Stara Planina (Central Balkan) Mountains, some with a very light tint, but with dirty surfaces, at the end of May 2009. The cryoseston community was quite different in individual snow fields. We found Chlorophyta: zygospores of *Chloromonas nivalis* and *Chloromonas rostafiński*, Chromophyta: *Chromulina elegans*, Bacillariophyceae: *Aulacoseira granulata* var. *angustissima*, *Aulacoseira italica, Cyclotella menenghiniana, Navicula nivalis*, *Hantzschia amphioxys*, Fungi: *Chionaster nivalis* and *Selenotila nivalis*. Alpine species such as *Chlamydomonas nivalis* were not found in the snow fields studied, even though they were located in the alpine zone.

**Key words**: Cryoseston, Stara Planina (Balkan), Bulgaria

## Introduction

A luminary of cryoseston research, KOL (1968) described 78 taxa classified as cryoseston, 63 of which were Chlorophyta, 6 Cyanobacteria, 1 Xanthophyceae, 1 Dinophyceae and 7 Fungi. Many of the described taxa were synonymous, representing different stages of a single life cycle, or were observed incompletely, and they have been progressively eliminated through cultivation and molecular biology (HOHAM 1973, 1979; HOHAM and MULLET 1977; HOHAM and BLIN 1979; KOMÁREK and NEDBALOVÁ 2007). The last authors listed 47 Chlorophyta in the cryoseston.

In Bulgaria, cryoseston was recorded for the first time by KOL (1956) in the Rila and the Pirin Mts. These reports were confirmed by WODENITSCHAROFF (1962) in the Rila Mts., and a new locality was found by LUKAVSKÝ et al. (2009) in the Vitosha Mountains. Although the Stara Planina (Balkan) Mountains have the same geographic attributes that enable the occurrence of cryoseston in other mountains in Bulgaria, snow algae have not previously been recorded there.

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In memory of Stefan Petkov 1866–1951, luminary of Bulgarian phycology
Stefan Petkov, Bulgarian botanist, born on 5th of June 1866 in Lovech, Ottoman Empire; died on 8th of December 1951 in Sofia, Bulgaria. He was a lecturer in Sofia University »St. Kliment Ohridski«, the author of the first bibliography of the Bulgarian flora. From 1907, he was a member of Bulgarian Literary Society, today the Bulgarian Academy of Sciences.

Recently, extreme habitats (e.g. snow, polar soils, thermal springs) have received increasing attention as a potential source of algae with unusual physiological characteristics and biochemical pathways and products (ŘEZANKA et al. 2007, 2008). The isolation of new algal strains with such characteristics is the goal of this project.

## Material and methods

### Localities

Snow fields were sampled in the central Stara Planina (Balkan) Mts., between the Mazalat chalet to the Dermenka chalet, during the last week of May 2009. The snow fields were tens of square meters in size. GPS positions and altitudes of the sites are given in table 1.

**Tab. 1.** Localities studied in central Stara Planina (Balkan) Mountains at end of May 2009.

| No | Location | GPS | Altitude, m a.s.l. |
|---|---|---|---|
| 1 | near Chalet Taza | N 42° 44' 19.6" E 025° 04' 16.3" | 1994 |
| 2 | main chain | N 42° 44' 02.8" E 025° 03' 77.4" | 2037 |
| 3 | main chain | N 42° 44' 04.6" E 025° 03' 17.5" | 1993 |
| 4 | main chain | N 42° 44' 06.4" E 024° 59' 02.1" | 1882 |
| 5 | main chain | N 42° 43' 64.3" E 024° 56' 35.1" | 2133 |
| 6 | under peak Botev | N 42° 43' 02.0" E 024° 56' 30.4" | 2201 |
| 7 | Peak Botev | N 42° 43' 02.8" E 024° 55' 05.3" | 2376 |
| 8 | Botev Shelter | N 42° 43' 21.0" E 024° 54' 32.8" | 2057 |
| 9 | Near peak Zhaltets | N 42° 43' 45.4" E 024° 53' 02.3" | 2108 |

### Sampling and sample processing

Snow samples were collected in plastic bags of volume ca 50 mL and transported in thermos bottles to the laboratory of the Institute of Plant Physiology in Sofia. Samples were melted and centrifuged 20 min. at 3000 g, and living material was examined using a NU2 microscope (C. Zeiss, Jena, D) with 63/0.8 and HI 100/1.35 objectives, and photographed with a DP10 digital camera (Olympus, Japan). The samples were also inoculated into Z nutrient solution, according to Zehnder in STAUB (1961), to be isolated into unialgal culture. All data in this paper were derived only from samples collected in the field.

## Results and discussion

In snow samples from the Stara Planina Mts., we found and determined the following organisms, (Figs. 1–55):

### Chlorophyta, Chlorophyceae

***Chloromonas nivalis*** (Chodat) Hoham and Mullet 1978 (syn. ***Scotiella nivalis, Chloromonas cryophila***), Figs. 1–4, 10, 11.

Cysts ellipsoid, 26–31 × 12–15 μm, a smooth, thick cell wall, with 6 longitudinal, twisted ribs on surface, joined into prominent thickenings at each pole (Fig. 2). Cell volume filled with amorphous green chloroplast, grains, and yellow-red oil drops; green cells were seen rarely. Dominated in fields 5 and 8. Flagellates not observed.

The characteristic big oil drops at each cell pole (LUKAVSKY et al. 2009) were lacking but the size range and external ornamentations of the cells confirm our identification. Resting stages of several snow algae in the genus *Chloromonas* were formerly described as vegetative cells, and given the genus name *Scotiella* Fritsch. The life history of *Chloromonas nivalis* was described in detail by HOHAM and MULLET (1977, 1978) and KAWECKA (1981). A characteristic feature of the cysts of some biflagellate volvocalean algae, which allows identification to species level, is the number and shape of ribs, or shape and length of spines. KOMÁROMY (1982) with respect to cyst morphology distinguished five groups, where *Chloromonas nivalis* stands apart. This alga dominated in spring as well as in summer snow collected in the Vitosha Mts. (LUKAVSKÝ et al. 2009). It is recognised as a characteristic organism of middle altitude, open localities (HOHAM and MULLET 1977, KOMÁREK and NEDBALOVÁ 2007).

***Chloromonas rostafiński*** (Starmach et Kawecka) Gerloff et Ettl (nom. dubium *Chlamydomonas flavo-virens*, syn. *Chlamydomonas rostafiński*), Figs. 5–9, 35, 36, 41–55

Cysts ellipsoidal, 15 × 25 µm, cell wall thick, uniformly covered with blunt protrusions or spines (Figs. 52, 53), cell content green – orange (Fig. 43), chloroplast without pyrenoid. Flagellates egg-shaped 12 × 8 µm, papilla lacking, chloroplast cup-shaped without pyrenoid (Figs. 5, 36), flagella not observed. Dominant in fields 5 and 6.

*Chloromonas rostafiński* was discovered by Rostafiński and published as *Chlamydomonas flavo-virens*, but without the diagnosis and description required by the Botanical Code. A valid description of this organism (but from a different locality) was provided by STARMACH and KAWECKA (1965) as *Chlamydomonas rostafiński*, from yellowish-green snow in the alpine zone of the Polish Tatra Mts. It has been recorded in the High Tatra Mts. (KAWECKA et al. 1979, KAWECKA 1984, LUKAVSKÝ 1994) and Vitosha Mts. (LUKAVSKÝ et al. 2009). The cell wall of resting cells is covered by numerous longitudinal ribs which show indentations of different length, and hence the papillae-like structures visible by light microscopy are formed (Figs. 45, 49, 52, 53, 55). These non-motile cells are believed to be formed from vegetative cells through gradual development of cell wall ornamentation (KAWECKA and ELORANTA 1986). Sexual reproduction was described by KAWECKA (1984) as isogamy. A small joined cell, and probably the sporangium of a parasitic Chytrid attached to a big spore can be seen in figure 51. More research on this species in the Stara Planina Mts. is clearly necessary.

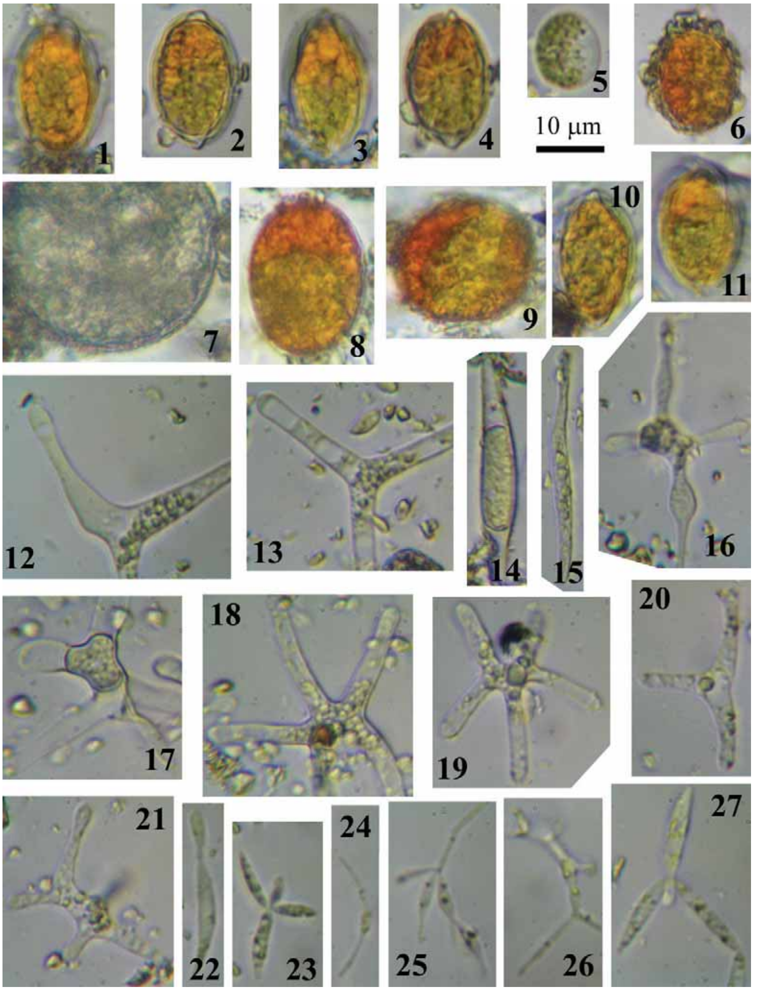
**Chromophyta,**

**Chromophyceae**

***Chromulina elegans*** Doflein

Cells spherical (rarely with pointed posterior), diameter 6–8 µm, 1 flagellum 6–8 µm in length. One cup-shaped chromatophore, yellow-orange, without pyrenoid or stigma, a few small drops or grains in protoplasm. Present in field 9.

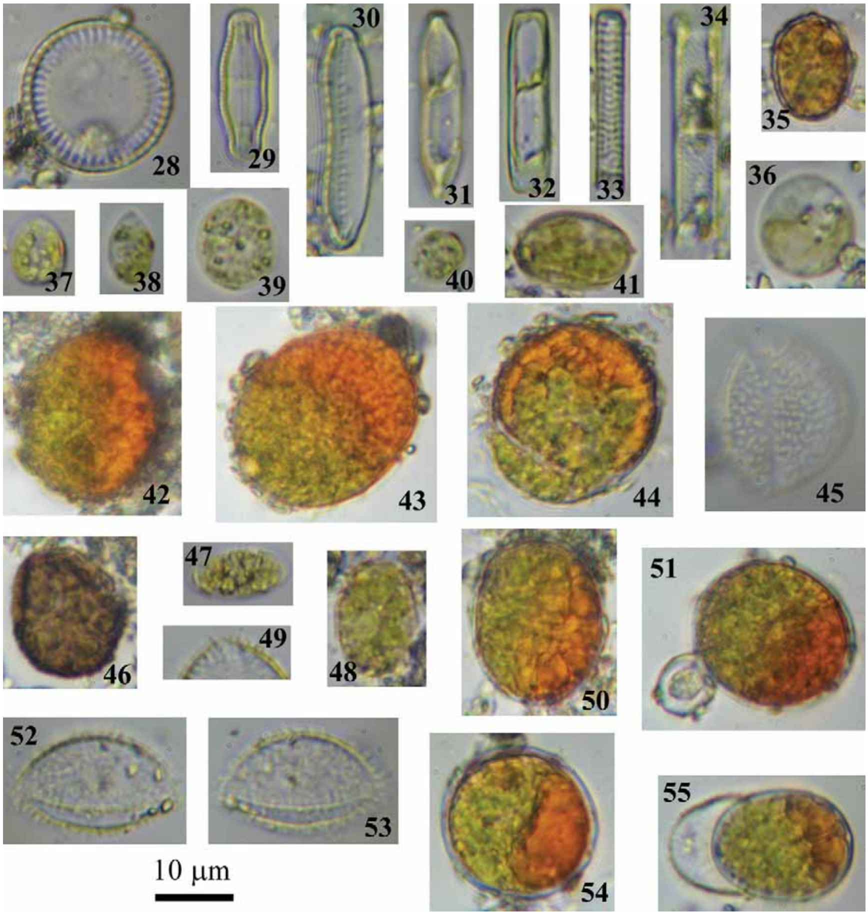
This alga was described by Doflein from a peat-bog in Germany. NOVIS (2002) tentatively identified it in cryoseston collected on Mt. Philistine, Arthur´s Pass national Park,




**Figs. 1–27.** Cryoseston of snow fields in the Stara Planina Mountains, collected in May 2009. **1–4, 10, 11** – zygospores of *Chloromonas nivalis*; **5** – zoospore, **6–9** – cysts of *Chloromonas rostafiński*; **12–21 –** *Chionaster nivalis*, variability of spores, **14, 15** – cf. *C. bicornis*; **22–27** – *Selenotila nivalis*, variability of thalli. Origins of samples: snow field No. 1. (12, 22–24, 27); No. 2. (13, 21); No. 4. (1–4); No. 5. (25, 26); No. 6. (7–11); No.7. (5, 6).

New Zealand, at 1600–1700 m. a.s.l. LUKAVSKÝ et al. (2009) reported the species from snowfields in the Vitosha Mountains.

It is similar, also to *Chromulina ettlii* Hindák, which was described from brownish snow in the High Tatra Mts (HINDÁK 1969). The last had cells spherical, 7–9 µm in diameter, flagellum of 12–15 µm long, chromatophore one, cup-shaped, yellow-brown, lacking pyrenoid, contaractile vacuoles 1–2. Another similar species, *Chromulina chionophila* Stein was reported from NY USA, in coniferous canopy as yellow-green snow (HOHAM 1975), but this species has 2–3 chromatophores per cell and it is flattened in cross section. Some species ascribed to the genus *Chromulina*, originally defined as uniflagellate, have




**Figs. 28–55.** Cryoseston of snow fields in the Stara Planina Mountains, collected in May 2009. **28** – *Cyclotella meneghiniana*; **29 –** cf. *Navicula nivalis*; **30–32** – *Hantzchia amphioxys*; **33** – *Aulacoseira granulata* var. angustissima; **34** – *Aulacoseira italica*; **35, 36** – zoospores of *Chloromonas rostafiński*; **37–40** – zoospores »small«; **41–44**, **46–48, 50, 51, 54, 55 –** cysts of *Chloromonas rostafiński*; **49, 52, 53** – empty cell walls, **51** – sporangium of a Chytridiomycet?. Scale bar = 10 µm. Origins of samples: snow field No. 1. 49–55; No. 2. 29, 30; No. 3. 31, 32, 37–40; No. 5. 41–48; No. 6. 33, 34; No. 8. 28, 35, 36.

since been shown to possess a second smaller flagellum not visible by LM (Novis 2002). Ultrastructural studies would be required to determine this material.

## Bacillariophyceae

The centric diatoms *Cyclotella menenghiniana* (Fig. 28), *Aulacoseira italica* and *A. granulata* var. *angustissima* (Figs. 33, 34) were found in fields 1 and 8 (Tab. 2), but only as empty frustules. Similarly, the pennate diatom cf. *Navicula nivalis* (Fig. 29, cf. also *Achnanthes coarctata*) was also only observed as empty frustules. The only living diatom species was *Hantzschia amphioxys* (Figs. 30–32) from snow fields 2 and 3.

**Tab. 2.** Cryoseston of central Stara Planina (Balkan) collected at end of May 2009. Species distributions in individual snow fields. GPS positions and altitudes see Tab. 1. d = dominate, x = present, r = rare, ZSP = zoospores.

| Species | No of locality | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 8 | 9 |
| **Chromophyceae** | | | | | | | | |
| *Chromulina elegans* | | | | | | | | x |
| **Bacillariophyceae, Centrales** | | | | | | | | x |
| *Cyclotella meneghiniana* | | | | | | | x | |
| *Aulacoseira granulata* var. *angustissima* | | | | | | x | | |
| *Aulacoseira italica* | | | | | | x | | |
| **Bacillariophyceae, Pennales** | | | | | | | | |
| *Hantzschia amphioxys* | | x | x | | | | | |
| cf. *Navicula nivalis* | | x | | | | | | |
| **Chlorophyceae** | | | | | | | | |
| *Chloromonas nivalis*, | | | x | x | x | d | | |
| *Chloromonas rostafiński* | d | | | d | x | | | |
| ZSP- *Chl. rostafinski* | | | | | x | | | d |
| ZSP »small«, diameter 8–10 µm | | | x | x | x | | | x |
| **Fungi** | | | | | | | | |
| *Chionaster nivalis* | x | d | | | x | x | | |
| *Selenotila nivalis* | x | r | x | x | x | x | | x |

*Navicula nivalis* Ehr. 1854 (syn. *Navicula mutica* var. *nivalis* (Ehr.) Hustedt 1911 is a cosmopolitan of high mountains from aerial biotopes such as moss mats, wet rocks (KRAMMER and LANGE-BERTALOT 1986). The other species, from our samples can be ubiquists transported by wind.

Samples were not sufficient for the creation of permanent preparations, and further studies and determinations are necessary to distinguish the genera *Navicula* and *Achnanthes* (the last has raphae only on one valve). Surprisingly no Bacillariophyceae were listed by KOL (1968), but later 33 taxa were determined in Antarctic cryoseston (ZIDAROVA 2008).

## Fungi

***Chionaster nivalis*** (Bohl.) Wille, Figs. 12–21.

Cells colourless, 2–3 (rarely more) projections up to 25 µm long arise from a triangular centre (diameter 12–14 µm; e.g. Fig. 17) filled with numerous small spheres. Rarely, there were only 2 projections (Figs. 14–15); this form has been described as *Chionaster bicornis* Kol. The variability in morphology and the number of projections is very wide in our material; the specimen closest to typical *Chionaster* (*sensu* Kol) is Fig. 17. Reproduction was not observed. Dominant in field 2.

Notes: This species was already recorded in Bulgaria, in the Pirin Mts. by KOL (1956), the Vitosha Mts. by LUKAVSKÝ et al. (2009), and the Rila and Pirin by LUKAVSKÝ (unpublished). According to the list of genera of fungi (KIRK et al. 2001), *Chionaster* Wille (1903) is a »nomen dubium«, and its reproduction was never observed. It is probably a spore of some yet unidentified fungus from Hyphomycetes.

***Selenotila nivalis*** Lagerh. (syn. ***Selenozyma***), Figs. 22–27**.**

Cells cylindrical-clavate, 30 × 3–5 µm, arranged into irregular clusters, colourless, filled by small globes, reproducing through budding. Dominant in fields 7 and 9, present in all sampled snow fields. There is a wide variability in morphology of thalli.

Notes: *Selenotila nivalis* is an invalid taxon according to KIRK et al. (2001). Sometimes it is included in the genus *Selenozyma* (ARX et al. 1977). Similarly to *Chionaster nivalis*, this species often occurs with snow algal populations.

### Species richness and composition

We have determined 13 taxa together, including Fungi. The species composition and general distribution pattern of snow algae in the Stara Planina Mts. was similar to that in the Bohemian Forest (LUKAVSKÝ 1993). Only low–moderate altitudes species (*Chloromonas nivalis* and *C. rostafiński*) were found, despite the large areas of snow habitats with open exposure. In contrast, the cryosestons of the Rila and Pirin Mts. were dominated by giant cells of cf. *Chlamydomonas nivalis*, accompanied by only a few other species, e.g., *Koliella viretii* (Chod.) Hind. (KOL 1956, WODENITSCHAROFF 1962, LUKAVSKÝ unpublished data). The Vitosha and the Giant Mts, however, contained a combination of ecotypes: *Chlamydomonas nivalis* was present but not dominant (KOCIÁNOVÁ et al. 1989, LUKAVSKÝ et al. 2009). In the Stara Planina we found a single »giant spore« resembling *Chlamydomonas nivalis* (Fig. 7), but it was brown rather than the typical brick-red and we believe that this is a cell of *Chloromonas rostafiński*.

Surprisingly, some common genera such as *Koliella*, *Raphidonema*, *Stichococcus* (KOMÁREK et al. 1973, HOHAM 1973, HOHAM and DUVAL 2001, NOVIS 2002, LUKAVSKÝ et al. 2009) were absent from our samples. All our snow samples were heavily contaminated by dust and pollen grains, so nutrients should be present in excess. Perhaps the reason for the absence of the species is that Stara planina Mts. are high and long, but narrow and chain-like mountains, not huge and compact complexes as the Rila, the Pirin or the Vitosha Mts. The former are accordingly more influenced by their surrounding, and the snow fields melted too early.

Bacillariophyceae were present in a few fields (Tab. 2.) but usually as dead valves. Living cells of *Hantzschia amphioxys* (Figs. 31–32) were found only in fields 3 and 4. Living diatoms are known as components of the cryoseston, for example on Livingstone Island, Antarctica, where ZIDAROVA (2008) determined 33 taxa of Bacillariophyceae, 2 Ochrophyceae (Chromophyta) and 4 taxa of Chlorophyta.

We can conclude that the Stara Planina Mts. provide suitable localities for cryoseston, particularly Volvocales, and Fungi, but so far not in such density as to prominently colour the snow.

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## References

ARX, J. A., MIRANDA, R., SMITH, M. T., YARROW, D., 1977: The genera of yeasts and the yeast-like fungi. Studies in Mycology 14, 12–78.

HINDÁK, F., 1969: Brownish snow in the High Tatras. Biologia (Bratislava) 24, 80–85.

HOHAM, R. W., 1973: Pleiomorphism in the snow alga, *Raphidonema nivale* Lagerh. (Chlorophyta) and a revision of the genus *Raphidonema* Lagerh. Syesis 6, 255–263.

HOHAM, R. W., 1975: Optimum temperatures and temperature ranges for growth of snow algae. Arctic and Alpine Research 7, 13–24.

HOHAM, R. W., 1979: The life history and ecology of the snow alga *Chloromonas brevispina* comb. nov. (Chlorophyta, Volvocales). Phycologia 18, 55–70.

HOHAM, R. W., MULLET, J. E., 1977: The life history and ecology of the snow alga *Chloromonas cryophila* sp. nov. (Chlorophyta, Volvocales). Phycologia 16, 53–68.

HOHAM, R. W., MULLET, J. E., 1978: *Chloromonas nivalis* (Chod.) Hoh. et Mull. comb. nov. and additional comments on snow alga, *Scotiella*. Phycologia 17, 106–107.

HOHAM, R. W., BLINN, D. W., 1979: Distribution of cryophilic algae in an arid region, the American Southwest. Phycologia 18, 133–145.

HOHAM, R. W., DUVAL, B., 2001: Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: JONES, H. G., POMEROY, J. W., WALKER, D. A., HOHAM, R. W. (eds.), Snow ecology, 168–228. Cambridge University Press.

KAWECKA, B., 1981: Biology and ecology of snow algae. 2. Formation of aplanospores in *Chlamydomonas nivalis* (Bauer) Wille (Chlorophyta, Volvocales). Acta Hydrobiologica 23, 211–215.

KAWECKA, B., 1984: Biology and ecology of snow algae. 3. Sexual reproduction in *Chloromonas rostafiński* (Starmach et Kawecka) Gerloff et Ettl (Chlorophyta, Volvocales). Acta Hydrobiologica 25/26, 281–285.

KAWECKA, B., STARMACH, K., ZMYSLONY, J., 1979: Electrophoretic analysis and ecological observations of *Chlamydomonas nivalis* (Bauer) Wille and *Chloromonas rostafinski* (Starmach et Kawecka) Ettl in the Walley »Za Mnichem«, High Tatra Mts. Poland. Annuaire de l'Universite de Sofia, Faculte de Biologie 2, 285–288.

KAWECKA, B., ELORANTA, P., 1986: Biology and ecology of snow algae. 4. SEM studies on the cell wall structure of »resting cells« of *Chloromonas rostafiński* (Starmach et Kawecka) Gerloff et Ettl (Chlorophyta, Volvocales). Acta Hydrobiologica 28, 387–391.

KIRK, P. M., CANNON, P. F., DAVID, J. C., STALPERS, J. A., 2001: Dictionary of the fungi. CAB International, Wallingford.

KOCIÁNOVÁ, M., ŠTURSOVÁ, H., ŠTURSA, J., VANĚK, J., VÁVRA, V., 1989: New records of red snow in the Giant Mountains (in Czech). Opera Corcontica 26, 151–158.

KOL, E., 1956: Sur la microvégetation de la neige des montagnes de la Bulgarie I. Bulletin de l'Institut Botanique, Academie des Science de Bulgarie 5, 377–384.

KOL, E., 1968: Kryobiologie I. Kryovegetation. In: ELSTER, J., OHLE, W. (eds.), Die Binnengewasser 24, 1–216. Schweizerbart, Stuttgart.

KOMÁREK, J., HINDÁK, F., JAVORNICKÝ, P., 1973: Ecology of the green kryophilic algae from Belanské Tatry Mountains (Czechoslovakia). Archiv für Hydrobiologie/Supplement 41, Algological Studies 9, 427–449.

KOMÁREK, J., NEDBALOVÁ, L., 2007: Green cryosestic algae. In: SECKBACH, J. (ed.), Algae and cyanobacteria in extreme environments, 321–342. Springer, Netherlands.

KOMÁROMY, Z. P., 1982: Application of cluster analysis in the taxonomy of *Scotiella* species (Chlorophyceae). Archiv für Hydrobiologie/Supplement 60, Algological Studies 29, 432–438.

KRAMMER, K., LANGE-BERTALOT, H., 1986: Bacillariophyceae, 1: Naviculaceae. In: ETTL, H., GERLOFF, J., HEYNIG, H., MOLLENHAUER, D. (eds.), Süsswasserflora von Mitteleuropa 2/1. Gustav Fischer, Jena.

LUKAVSKÝ, J., 1993: First record of cryoseston in the Bohemian Forest Mts. (Šumava). Algological Studies 69, 83–89.

LUKAVSKÝ, J., 1994: Algal flora of lakes in the High Tatra Mountains (Slovakia). Hydrobiologia 274, 65–74.

LUKAVSKÝ, J., FURNADZHIEVA, S., NEDBALOVÁ, L., 2009: First record of cryoseston in the Vitosha Mts., Bulgaria. Nova Hedwigia 88, 97–109.

NOVIS, P. M., 2002: New records of snow algae for New Zealand, from Mt Philistine, Arthur´s Pass national Park. New Zealand Journal of Botany 40, 297–312.

ŘEZANKA, T., NEDBALOVÁ, L., SIGLER, K., CEPÁK, V., 2007: Identification of astaxanthin diglucoside diesters from snow alga *Chlamydomonas nivalis* by liquid chromatografy – atmospheric pressure chemical ionization mass spectrometry. Phytochemistry 69, 479–490.

ŘEZANKA, T., NEDBALOVÁ, L., SIGLER, K., 2008: Unusual medium-chain polyunsaturated fatty acids from snow alga *Chloromonas brevispina*. Microbiological Research 163, 373–379.

STARMACH, K., Kawecka, B., 1965: The yellowish-green snow in the valley (In Czech). Limnological Investigations in the Tatra Mountains and Dunajec River Basin. Komitet Zgospod. Ziem Gorskich PAN, Kraków 11, 75–80.

STAUB, R., 1961: Ernährungsphysiologische Untersuchungen an der planktonischen Blaualge *Oscillatoria rubescens* DC. Schweizerische Zeitung für Hydrologie 23, 82–198.

WODENITSCHAROFF, D. G., 1962: Hydrobotanische Untersuchungen über das Urdino-Kar (N-W Rila-Gebirge), Annuaire de l'Universite de Sofia, Faculte de Biologie (Botanique) 54/55, 141–209.

ZIDAROVA, R.P., 2008: Algae from Livingston Island (S Shetland Islands): a checklist. Phytologica Balcanica 14, 19–35.