

## Biology and ecology of snow algae

### 2. Formation of aplanospores in *Chlamydomonas nivalis* (Bauer) Wille (Chlorophyta, Volvocales)

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**Abstract** — In *Chlamydomonas nivalis* aplanospores are formed by cells with protoplasts elongated in a conical papilla at the apical pole, and the cells walls are detached from the protoplasts. In the initial phase, papilla, and with it the basic part of flagellar apparatus are removed from the protoplast, and the cell loses its motility. Later a cell wall is formed around the protoplast. After a time, the detached cell wall is ruptured and a aplanospore is released. Aplanospores are resistant to long periods without light and to freezing.

**Key words:** Snow algae, vegetative reproduction of *Chlamydomonas nivalis*, aplanospores.

#### 1. Introduction

Red coloration on snow and ice fields is often caused by the mass development of species of the genus *Chlamydomonas*. The most common alga of this genus is *Chlamydomonas nivalis* (Bauer) Wille, whose taxonomy, biology and ecology has not been fully investigated so far (Kawecka 1978).

In recent years Kawecka and Drake (1978), and Kawecka et al. (1979) carried out investigations on sexual reproduction, structure

of proteins, and ecology of *Chlamydomonas nivalis*. The present work contains results of further studies on this alga.

## 2. Material and method

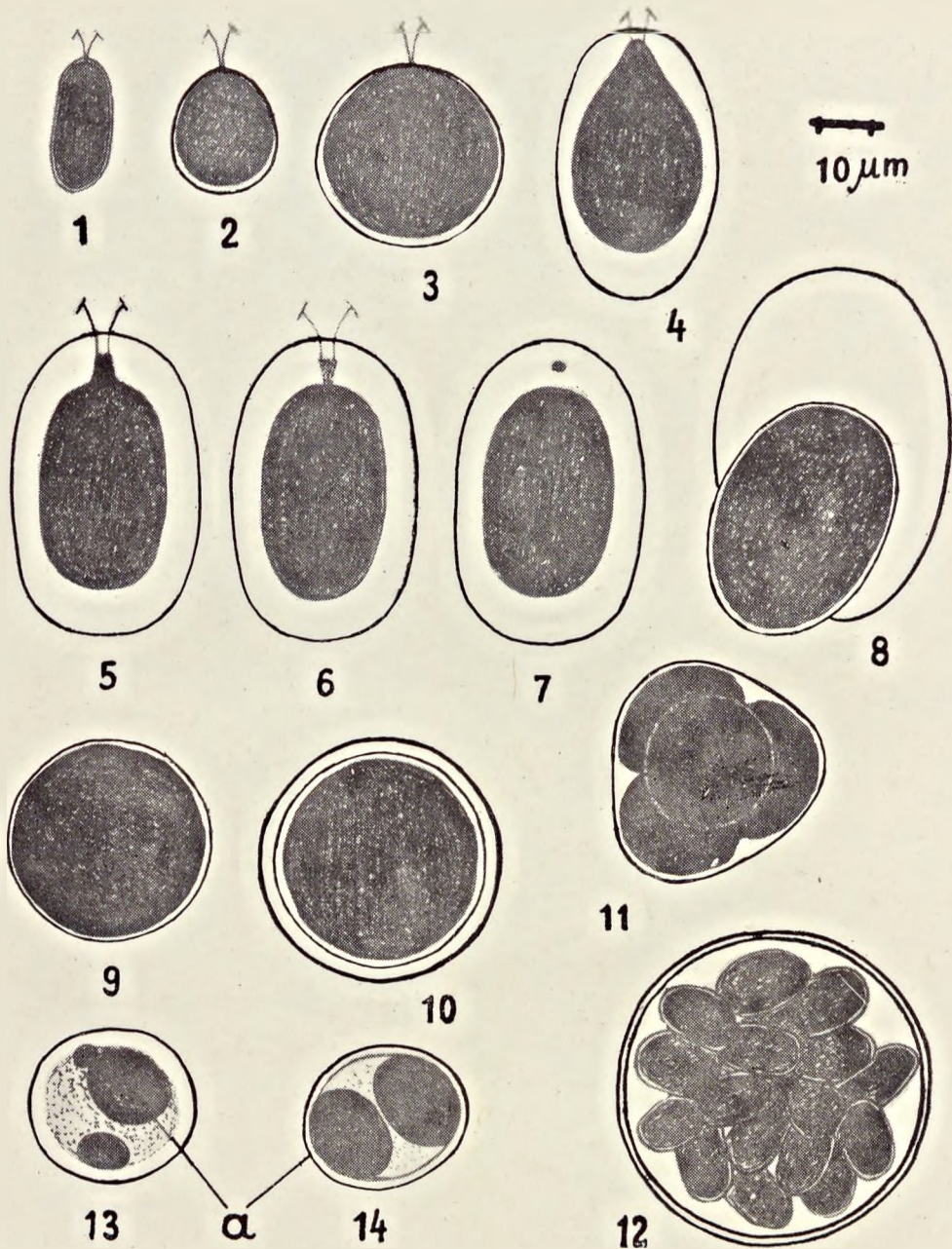
The investigated material was collected in the valley „Za Mnichem” in the Polish part of the High Tatra Mountains. In the upper part of the valley at an altitude of 2.080 m a snow-bank remains throughout the entire year. In the summer-autumn period snow algae with the most numerous *Chlamydomonas nivalis* develop on the snow.

Observations were carried out in 1978 and 1979, the collections being made on 18th August, 1978, 25th September, 1978, and 24th August, 1979. Algae were transported to the Laboratory of Water Biology of the Polish Academy of Sciences in Cracow, in thermos bottles. Here the material was divided into 100 ml flasks which were kept on ice at about 2°C, and exposed to sunlight for a few hours every day. Under these conditions algae lived for a couple of months. A part of the fresh material was freezeed in a refrigerator and incubated there in the dark during one month. After thawing, the samples were kept under the above described conditions. Cells were observed on microscopic „drop” slides filled with ice crystal.

## 3. Results

The population of *Chlamydomonas nivalis* was composed of several types of cells which distinctly varied in size and shape (Table I). Bean shaped, oval and spherical cells constituted 90% of the population (figs 1—3). Cells with a protoplast elongated into a beaked or conical papilla at the apical pole and having a cell wall more or less detached from the protoplast reached 5% of the population (figs 4, 5). Under favourable light and temperature conditions most cells were motile. They moved about actively or oscillated while remaining in place. A small percent of the population was composed of nonmotile cells covered with a thick cell wall which often had a few layers (figs 9, 10), and of groups of 2—16 daughter cells within mother cell wall (figs 11, 12).

Cells with the protoplast elongated into a conical papilla and with the cell wall detached from the protoplast were transformed into aplanospores. The basic part of the protoplasmatic papilla gradually lost its intense red colour; then it was narrowed and finally separated from the protoplast (fig. 6). Thus, the basic part of the flagellar apparatus, located in the apical part of the papilla, was also separated and the cell passed



Figs 1—14. *Chlamydomonas nivalis*: 1—3 — vegetative cells; 4—8 — formation of an aplanospore: 4 — cell with detached cell wall and protoplast elongated into beaked papilla, 5 — cell with detached cell wall and protoplast elongated into conical papilla, 6—7 — removing of papilla from the protoplast, 8 — the aplanospore liberation; 9—10 — nonmotile cells; 11—12 — daughter cells closed in the mother cell wall; 13—14 — vegetative cells of alga after freezing and dark storage; a — aggregated pigment

Table I. Morphological characteristics of *Chlamydomonas nivalis* population

Characteristic features of cells	Size of cells	Size of protoplast	Width of area between the protoplast and detached cell wall
Motile cells: from bean-shaped to oval spherical	17.5-25 $\mu\text{m}$ x 10-20 $\mu\text{m}$ diameter 20-35 $\mu\text{m}$	Cell wall envelopes the protoplast	
Motile cells: oval, protoplast elongated into a beaked papilla	32-50 $\mu\text{m}$ x 30-40 $\mu\text{m}$	30-45 $\mu\text{m}$ x 22-35 $\mu\text{m}$	2-8 $\mu\text{m}$
Motile cells: oval, protoplast elongated into a conical papilla	33-60 $\mu\text{m}$ x 30-48 $\mu\text{m}$	30-55 $\mu\text{m}$ x 23-40 $\mu\text{m}$	2.5-15 $\mu\text{m}$
Nonmotile cells: oval spherical	45-75 $\mu\text{m}$ x 40-52.5 $\mu\text{m}$ diameter 42.5-62.5 $\mu\text{m}$	32-55 $\mu\text{m}$ x 32-40 $\mu\text{m}$ 40-50 $\mu\text{m}$	2.5-15.5 $\mu\text{m}$
Mother cell closed in the mother cell wall: oval spherical	40-48.5 $\mu\text{m}$ x 30-32.5 $\mu\text{m}$ diameter 25-65 $\mu\text{m}$		

into a nonmotile stage. The rest of the separated protoplast remained suspended in the area between the protoplast and the detached cell wall under form of a red drop (fig. 7). Then, the cell wall was formed around the protoplast. Later, the detached cell wall was ruptured and the aplanospore was liberated (fig. 8). The cell maintained its oval shape formed in the course of its development, or became spherical.

Aplanospores well tolerated the experimental freezing and storage in the dark. The cells remained spherical and intensely red. Under conditions favouring their development, they soon began to reproduce, giving 16 new cells. Soon, motile bean shaped cells liberated from the mother cell wall were observed in the sample. Other cells did not tolerate freezing and storage in the dark. The cell walls corrugated, while the pigment gathered on the peripheries of the cell, this partially exposing the chloroplast (figs 13, 14). Under favourable conditions, no regeneration of these cells was observed.

#### 4. Discussion

In the investigated material, several types of cells were found, most of them corresponding to the description of *Chlamydomonas nivalis* given by Ettl (1976). However, some cells differed from this type, the subject of discussion being whether their classification into *Ch. nivalis* population was justified or not. The group in question is composed of motile cells, larger than the typical ones; some of them showing a more or less detached cell wall and, at the apical pole, the protoplast elongated in a beaked or conical papilla.

Motile cells which are 26—35  $\mu\text{m}$  in diameter, thus exceeding the dimensions given in the description of this species (according to Ettl, 1976, the length of cell is 12—26  $\mu\text{m}$ , the width 8—20  $\mu\text{m}$ ), cannot be

excluded from the *Chlamydomonas nivalis* population. I observed that most nonmotile cells change into motile forms when conditions are favourable (aquatic environment, good insulation, low temperature). According to Ettl (1976), the nonmotile cells are 12—38—(50)  $\mu\text{m}$  in diameter. Garric (1965) reported even larger forms:  $45 \times 60$ —75  $\mu\text{m}$ . Thus, it seems that the range of dimensions of motile and nonmotile cells of *Ch. nivalis*, which is chiefly known in the nonmotile stage, is not accurate and should be corrected.

Cells with detached cell walls and elongated plasmatic papilla resemble *Chloromonas bolyaiana* (Kol) Gerloff, Ettl; Ettl (1970), though they are too small as compared to this species. The similarity in the morphology and transition of cells in the nonmotile stage is also noted between *Chlamydomonas nivalis* and *Chlainomonas rubra* (Stein, Brooke) Hoham (Hoham 1974). However, the appearance of two instead of four flagella excludes the possibility of classifying these cells into the latter species. The cells can also represent a species of the genus *Sphaerellopsis* not described so far, differing from the genus *Chlainomonas* by the presence of two flagella.

It should be stressed that the discussed cells always lived for a short period and in spite of numerous observations no cells in the division stage were noted. Hence, it seems that they appear periodically, being a developmental stage in the life cycle of the alga, and do not represent separate species.

It seems that the discussed cells belong to the *Chlamydomonas nivalis* population and constitute successive stages in the formation of aplanospores (figs 4—8). They originate from some motile cells, probably through the gradual detachment of cell walls from protoplasts, while the protoplasts gradually elongate into conical papillae. As a result of further transformations the cells lose their motility and walls are formed around the protoplasts. Aplanospores formed by this process are markedly resistant to environmental conditions. Under favourable conditions, the cell contents divides and the liberated cell originate a new population.

However, it should be stressed that the presented description of aplanospores is hypothetical since no clonal culture of the algae has been obtained so far. The culture of this alga is difficult and under laboratory conditions considerable trouble arose in keeping it alive.

## 5. Polish summary

### Biologia i ekologia glonów naśnieżnych

#### 2. Tworzenie się aplanospor u *Chlamydomonas nivalis* (Bauer) Wille (Chlorophyta, Volvocales)

W Dolinie Za Mnichem (Tatry Wysokie, wysokość 2080 m), w okresie letnio-jesiennym, na płatach śniegu rozwijał się *Chlamydomonas nivalis*. Obserwacje rozwoju

tego glonu prowadzono w latach 1978 i 1979. Głony przewożono w termosach do laboratorium Zakładu Biologii Wód PAN w Krakowie, gdzie przetrzymywano je w wodzie z roztopionego śniegu w temperaturze ok. 2°C, eksponując kilka godzin dziennie na słońcu.

Populacja *Ch. nivalis* składała się z kilku rodzajów komórek zróżnicowanych w wielkości i kształcie (tabela I, ryc. 1—5, 9, 10). Większość z nich odpowiadała opisowi gatunku, część jednak odbiegała od typu i ona była obiektem szczególnej obserwacji. Były to komórki z odstającymi błonami komórkowymi od protoplastów, a same protoplasty posiadały na biegunach apikalnych charakterystyczne dziobkowate lub stożkowate papille (ryc. 4, 5). Najprawdopodobniej komórki te należą do populacji *Ch. nivalis* i stanowią stadia rozwojowe w cyklu życiowym tego glonu. Komórki z dziobkowatą protoplazmatyczną papillą są prawdopodobnie młodszymi formami komórek z papillą stożkowatą, z których w drodze dalszych przemian powstają aplanospory. Mianowicie w komórkach tych stożkowata papilla, a wraz z nią podstawowa część aparatu ruchu odcinają się stopniowo od reszty protoplastu, wskutek czego komórka traci zdolność ruchu (ryc. 6, 7). Następnie wokół protoplastu tworzy się błona komórkowa i tak uformowana aplanospora wydostaje się na zewnątrz po pęknięciu odstającej błony komórkowej (ryc. 8).

Populację *Chlamydomonas nivalis* zamrożono i trzymano w ciemności przez okres jednego miesiąca. Aplanospory zniosły ten eksperyment dobrze. Przeniesione w dogodne warunki światła i temperatury kiełkowały, a uwolnione komórki dawały początek nowej populacji.

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