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**Nowe gatunki z rzędu *Dinococcales* (*Pyrrhophyta*) z Groty Twardowskiego w Krakowie — New species of the order *Dinococcales* (*Pyrrhophyta*) from the Twardowski Cave in Kraków**

Mémoire présenté le 5 novembre 1962 dans la séance de la Commission Biologique de l'Académie Polonaise des Sciences, Cracovie

A fairly large cave, washed out in limestone and situated on the right bank of the Wisła (Vistula) river, has been for a long time known under the name of the Twardowski\* cave. This cave has a big opening towards the south, a large antechamber about 10 m long, descending downwards and passing into narrowing corridors. The roof and the walls of the antechamber are formed by overhanging rocks, dripping with water to a greater or lesser degree. On the collecting day, September 9, 1962, they were fairly damp and covered with a coating of olive-gray or yellowish-gray algae, partly greenish or bluish-green in places. The algae integuments were mostly crusty owing to a relatively slight dampness and chipped off easily. On the side pillar of the antechamber only, about 10 m from the entrance, where the dampness was relatively great, gelatinous, skiny and slippery integuments were formed, of a dark bluish-green colour, occupying about 0.5 m<sup>2</sup> of the surface. The surface of the wall was directed here towards the entrance of the cave and thus illuminated by a diffused light, with an intensity of about 10 per cent as compared with the open space. (An approximate measurement by means of a graded photographic photometer).

The gelatinous, bluish-green integuments on the wall of the cave were mostly formed by the blue-green alga *Microcystis muscicola* (M e n e g h.) E l e n k i n, with an addition of *Gloeocapsa montana* K ü t z i n g, *G. varia* (A. Br.) H o l l e r b a c h, *Synechocystis Pevalekii* E r c e g o v i c, *Aphanothece saxicola* N ä g e l i and of *Chloroglossa microcystoides* G e i t l e r. Besides these, groups of *Botryochloris cumulata* P a s c h e r

\* Twardowski, legendary figure of the XVIth century, a magician, counterpart of the German Dr. Faust.

(*Xanthophyceae*) appeared between the blue-green algae and separate scattered cells or small irregular agglomerations of two characteristic species of the *Dinococcales* order Phylum *Pyrrhophyta*, Class *Dinophyceae*) the description of which is presented below; they are considered as species not described hitherto.

### 1. *Phytodinium aureum* sp. nov. (Fig. 1, Table I)

The cells are of an elliptical shape, sometimes curved, bean-like, after division spherical or broadly elliptic, surrounded by a distinct, colourless membrane adhering closely to the protoplast, unstratified or having 2—4 layers in older cells, before division. The protoplast has no eyespot and no furrows and certain numerous discoid parietal chromatophores of a golden-yellow or, more rarely, of a greenish-yellow colour, a large nucleus, in which the contours of chromatine threads can be seen, numerous, skiny, elliptic grains of volutine grouping themselves especially around the nucleus, and scattered grains of starch. The nucleus is usually situated excentrically in the cell.

Reproduction occurs by means of a division running vertically to the longitudinal axis of the cell. The daughter cells develop their own membranes while still in the interior of the maternal cell and have



Fig. 1. *Phytodinium aureum* sp. nov. Typical cell shapes and dividing cells.



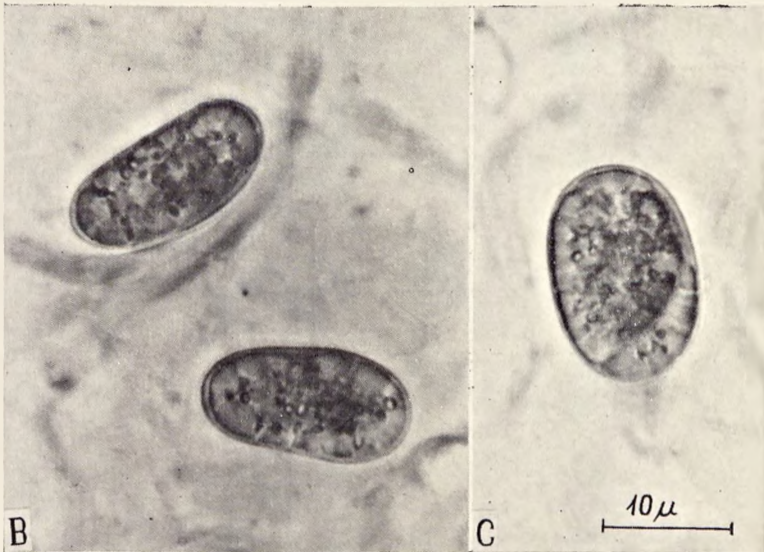


Table I. *Phytodinium aureum* sp. nov. A — Group of cells among *Gloeocapsa* colonies, B—C — Separate cells more strongly magnified



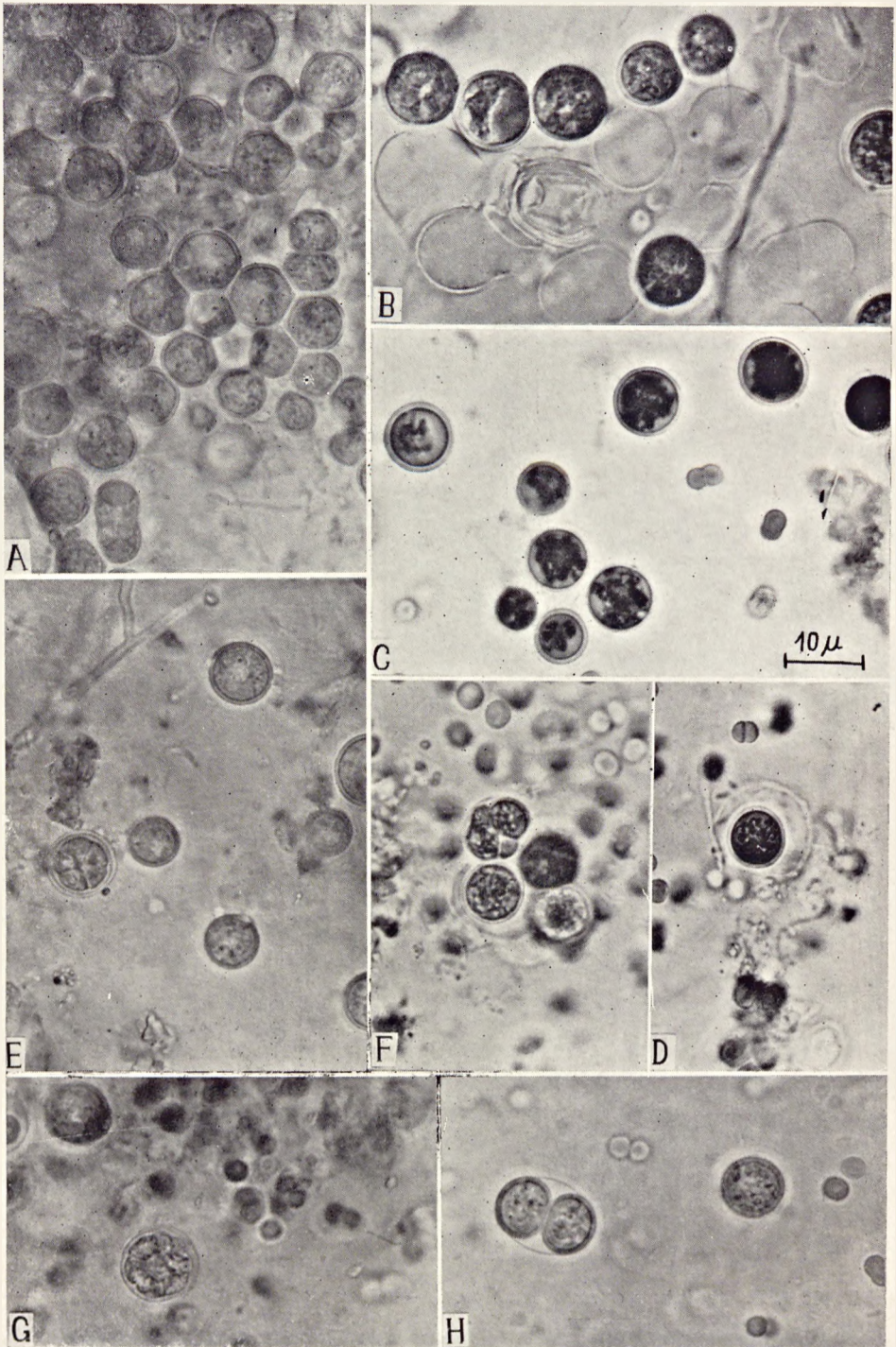


Table II. *Gloeodinium cracoviense* sp. nov. A — Group of cells on the surface of a colony of blue-green algae, B — Cells and empty membranes after liberation of autospores, C — Cells to which J + KJ has been added; small dark grains of starch are visible, D — Cell with a swollen membrane, E, F, G — Formation of 4, 8 and 16 autospores, H — Division of the cell; two offspring cells are surrounded by a common membrane



therefore the character of autospores. The membranes of the maternal cells during a certain period of time surround daughter cells (autospores), which have then an almost spherical or elliptical, sometimes irregular, shape.

When liberated after the rupture of the maternal membrane they assume an oval, elliptical shape, often slightly curved and asymmetric.

Adult cells are usually elongated and attain dimensions of 8—12  $\mu$  in length. In preparations, cells 9  $\mu$  broad and 16  $\mu$  long occur most frequently.

The cells appear separately or in small groups of ten or more amidst gelatinous integuments of blue-green algae (mostly *Microcystis muscicola* (M e n e g h.) E l e n k i n and *Gloeocapsa montana* K ü t z.) on the damp walls of the Twardowski cave in Kraków, in a faint and diffused light.

Of the genus *Phytodinium*, described by K l e b s (1912), two species are known: *Ph. simplex* K l e b s has spherical or broad oval cells 42—50  $\mu$  long and 30—45  $\mu$  broad and was found in marshes in Germany (near Tübingen) — and *Ph. globosum* P a s c h e r, with spherical cells 25—30  $\mu$  in diameter, appearing in pools containing peat on meadows in Czechoslovakia. *Phytodinium aureum* from the Twardowski cave resembles *Ph. simplex* in shape, but differs considerably in its greater dimensions, larger and differently coloured chromatophores and in the localities in which it is found. It does not in the least resemble *Ph. globosum*.

#### Diagnosis

*Cellulae immobiles, ellipticae, nonnumquam leviter curvatae, divisione in formam globosam vel late ellipticam abeunt, circumdatae membrana achroa contentu cellulae arte adhaerenti, quae in cellulis statu iuvenili simplex, in aetate maioribus lamellosae duabus vel quattuor stratis composita est. Cellulae contentus sine stigmatate et sulcis, chromatophoris flavo-aureis ad parietos positus, magnus nucleus in cellula excentrice positus, granis volutinae et amyli conspicuis. Propagatio fit divisione in duo autospora forma fere globosa, elliptica vel nonnumquam irregulari. Cellulae maturae 8—12  $\mu$  latae, 14—24  $\mu$  longae, saepissime 9  $\mu$  latae, 16  $\mu$  longae.*

*Habitat solitarie vel parvis aggregatis inter gelatinosa strata Cyanophycearum in humidis rupibus in antris Twardowski dictis Cracoviae.*

## 2. *Gloeodinium cracoviense* n. sp. (Fig. 2, Table II)

The cells are immobile, spherical or broadly elliptical and appear in groups composed of a few or more cells among the blue-green algae forming integuments on the damp rocks in the faintly illuminated

limestone cave. Adult cells are surrounded by many membranes (4 at least) of which the internal one, durable and relatively thick, adheres closely to the protoplast, and the external ones, more or less detached, do not swell or peel away.

The interior of the cell is of a golden-yellow colour and has a granular content with indistinct, weakly differentiating chromatophores. Their contours are blurred, they are goldish-brown in colour, sometimes with

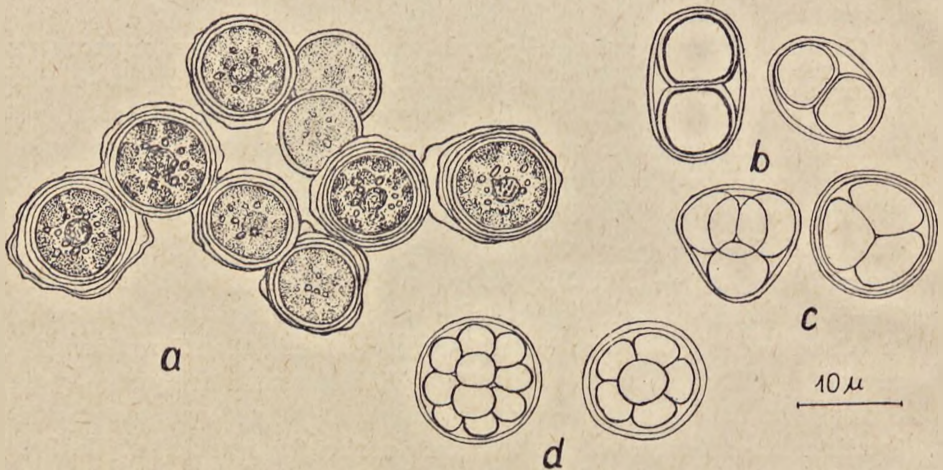


Fig. 2 *Gloeodinium cracoviense* sp. nov. a — Group of cells; cells covered by membranes of which the oldest become detached and peel off, b—d — Autospores forming in the number of 2—18

a greenish hue. The chromatophores become slightly more distinct when stained with iodine. Near the chromatophores fairly numerous shiny oval grains are seen, sometimes short and goblet like in shape (volutine), and larger globules strongly refracting light on their edges (oil). After J + KJ staining starch grains with indistinctly marked contours can be observed. A large nucleus is situated in the centre or at the side of the cell. Its structure is filiform and not very distinct, only visible under immersion. Sometimes the plasmatic content accumulates at the side of the cell and then the remaining space is filled with an orange-brown oily mass.

A characteristic and constant phenomenon is the inflation and peeling off of cell membranes, but only of the external ones. The remains of broken membranes are always visible near cell agglomerations. They appear not only at the time of autospore liberation but also during the growth of cells. The membranes are colourless and only the two external strata stain more distinctly, although weakly, in zinc chloride with iodine. The other membranes remain colourless or pale yellow. No inflation peeling away or jellifying of membranes takes place. Their remains have sharp outlines and stay for a long time close to live and growing cells.



Cells with membranes have a diameter of 9—14  $\mu$  and 7—10,5  $\mu$  without the peeling membranes.

Reproduction takes place by means of cell division and by the formation of 2—8 autospores. Differences between the vegetative division and the formation of autospores are numerical. At the time of division two daughter cells are formed which are surrounded by their own membranes when they are still in the interior of the maternal cell. They then grow and rupture the membranes of the maternal cell.

Autospores form by means of the division of the protoplast into 2—8, or, more rarely, 16 parts, which become spherical, produce their own membranes and grow while under the membrane of the maternal cell. The size of the autospores depends on their number in the cell. When the autospores are 8—16 in number they are small. Liberated after the splitting or rupture of the maternal membrane they begin to grow and obtain new layers on the periphery of the membrane adhering to the protoplast. Usually after attaining 6 layers a new division of the cell takes place or autospores are formed. When autospores develop here multicellular colonies arise in the form of an irregular, shapeless cell agglomeration. They form nests among the blue-green algae which cover the damp and shady walls of the Twardowski cave in Kraków.

Motile stages were not observed. Algae scraped off from the walls of the cave and placed in Petri dishes in a damp atmosphere grew within a few weeks and produced autospores, but no motile cells.

The only known species *Gloeodinium montanum* Klebs (1912) appears commonly on peat bogs or in small pools containing peat in meadows. The cells are spherical or slightly elliptical, 19—25  $\mu$  broad, 19—25  $\mu$  long. The motile stage resembles that of *Hemidinium* cells. *G. cracoviense* has cells of a similar type, but is considerably smaller. It also differs as to the places of occurrence.

#### Diagnosis

*Cellulae immobiles, globosae vel late ellipticae, lamellosis membranis circumdatae. Cellulae contentus membranae apte odhaerens, granulosus, flavo aureus, chromatophoris vix conspicuis, magnus nucleus filis parum conspicuis constans, multa granula lucem refingentia, volutinae, guttulas olei amyllum continens. Propagatio fit cellularum divisione in 2—8 rarius in 16 autospora. Autospora membrana maternae cellulae confracta evadunt, ipsa membrana non lamellosa praedita quae simul atquae crescunt pluribus laminis circumdatur. In cellula matura membrana lamellosa sex stratis constat, quorum exteriora deiciuntur, sed non intumescunt nequae gelatinosa fiunt. Cellulae cum membranis diametro 9—14  $\mu$ , cum membrana simplici 7—10,5  $\mu$ .*

*Habitat in humidis rupibus antri Twardowski dicti Cracoviae, inter gelatinosa strata Cyanophycearum, praecipue Microcystis muscicolae (Menegh.) Elenkin in familias non magnas indefinitas congregatus.*

## STRESZCZENIE

Wśród nalotów glonów, głównie sinic, pokrywających wilgotne ściany Groty Twardowskiego na Krzemionkach w Krakowie, spotkano dwa nie opisane dotąd gatunki dinokokków. Jeden z nich o komórkach eliptycznych, o wymiarach  $8-12 \times 14-24 \mu$  należy do rodzaju *Phytodinium* i został opisany pod nazwą *Ph. aureum* sp. nov. Drugi o kształcie kulistym, średnicy  $9-14 \mu$ , należy do rodzaju *Gloeodinium* i został nazwany *Gl. cracoviense* sp. nov. Podano opisy i diagnozy łacińskie, jak również mikrofotografie i rysunki obu gatunków.

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