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Two scarcely known genera of the class *Dinophyceae: Bernardinium* CHODAT and *Crypthecodinium* BIECHELER

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Bernardinium CHODAT

Occurrence

In August, 1958, an interesting dinoflagellate was observed at two different localities. It was identified with the species *Bernardinium bernardinense* CHODAT described from an Alpine lake.

The first finding-place was an experimental water-proof silon tank of a cylinder-like shape, open at the bottom, suspended in a 10 m. depth in the dam reservoir on the river Želivka (near Sedlice in south-eastern Bohemia, field station of the Institute of Hygiene, Prague).

During the experiment, KH_2PO_4 was added to the reservoir water in the tank and the phosphorus circulation between the free water and the bottom studied. On the day of sampling, the following values were ascertained:

Near the level of the experimental tank — temperature 21.5° C.; pH 9.2; diluted phosphorus 530 μg/l.; alkalinity 1.4 mval/l. Near the bottom of the tank — temperature 15.8° C.; pH 6.9; diluted phosphorus 1180 μg/l. (Dr. M. Štěpánek from the Institute of Hygiene in personal communication).

B. bernardinense CHODAT was rather scarce at the top layer of the experimental tank, where an intensive vegetative colouration was effected by green flagellates: *Chlorogonium elongatum* DANG. and *Chlamydomonas* sp. div. Further species found were: *Cryptomonas curvata* EHRENB., *Katodinium vorticella* (STEIN) FOTT, *Pandorina morum* BORY and various species of *Chlorococcales*. *B. bernardinense* CHODAT did not occur at the bottom of the tank, neither other flagellates except *Chlamydomonas* sp.; *Chlorococcales* prevailed. *B. bernardinense* CHODAT was found neither in the other experimental tanks (7 in number), wherein different chemical compounds had been added to the reservoir-water, nor in the free water of the reservoir.

Two weeks later, the flagellate mentioned above was taken in the pond Žabinec near Třeboň (southern Bohemia). It was rather scantily present in the submerge growths of *Sphagnum* (pH 6.3) amid a rich assemblage of *Hemidinium nasutum* STEIN and *Peridinium umbonatum* STEIN.

Dimensions and morphology

The individuals taken at the two localities are almost alike, differing only slightly in their dimensions. Samples from the river Želivka (Tab. VII : 1), 18 μ long, 12 μ wide, 9 μ thick; samples from Třeboň (Tab. VII : 2—3), 20.5 to 21.8 μ long, to 17 μ wide, 10.5 to 11.8 μ thick. They resemble the genus *Hemidinium* by the shape of their cells. The girdle begins on the ventral side in about the place of the longitudinal axis somewhat beneath the centre, so that the epicone is a little higher and wider than the hypocone. The girdle passes but along the left side as a slightly sinking spiral. Its notch into the left side of the

cell is very distinct, however it disappears as near as on the dorsal side in the opposite place of the longitudinal axis, so that it encircles only about one half of the equatorial girth of the cell. Consequently no sinus is distinguishable on the right side of the cell, at most some slight depression in the lateral line. The sulcus extends at acute angle straight to the beginning of the girdle on the ventrum. It does not touch the epicone and it is less perceptible on the hypocone without reaching the antapex, so that it cannot be seen in ventral view in the outline of the cell. In the sulci two flagella are inserted in a way common in dinoflagellates. The transverse flagellum does not obviously overlap the length of the incomplete girdle. The longitudinal flagellum is approximately 1.5 of the cell's length.

The cell is, with regard to the total outline, widely ellipsoid with both ends rounded, dorso-ventrally flattened. The epicone somewhat overlaps the margin of the girdle, which is particularly distinct on the left side.

M e m b r a n e. So far as I could study this flagellate on several individuals, I assume that it has no firm cellulose membrane, but merely a thin periplast without any structure visible in vivo. After long-time observations under microscope, the cells have been found to lose their characteristic shape becoming spherical in form. On preserving the material with either KJ + J or formol, no identifiable rests were found.

P r o t o p l a s t. The flagellate observed did not have any chromatophores. In the colourless plasma shapeless slightly yellowish green lumps could be found at times (Tab. VII : 1), which might have been the remnants of chloroplasts of ingested algae, but certainly they could not be looked at as the chromatophores of the flagellates in question. In the epicone nearly 1 to 2 corpuscles (gu) of irregular shape and reddish brown colour could be observed. These obviously ought to be reverse materials (oil?) coloured by carotenoids, frequently occurring in *Dinophyceae*. Moreover, some minute colourless grains were found in some cells, concentrated mainly near the girth of the hypocone (Tab. VII : 1). From all these inclusions a bright red stigma distinctly contrasted in all individuals placed on the ventral side of the cell somewhat beneath the insertion of the flagella. It generally was of an irregular rectangular shape.

The nucleus was observed in one specimen only (Tab. VII : 3). It was round, comparatively large, not distinctly visible, having an undefined structure, placed in the hypocone.

R e p r o d u c t i o n. Reproduction by division at a motile stage was observed but once in individuals from the Žabinec pond (Tab. VII : 2, a—c) at the final phase closely before the splitting of the cells.

D i s c u s s i o n a n d s y s t e m a t i c c l a s s i f i c a t i o n

CHODAT (1923) described the genus *Bernardinium* on the basis of a single species *B. bernardinense* from the Grand St. Bernard, Switzerland, reported since then, as far as I know, only by THOMPSON (1950) from Cansas, U.S.A. CHODAT characterized the whole organism drawing it upside down, which was rectified by SCHILLER (1937). THOMPSON's observations as well as my own confirm SCHILLER's conception as to the orientation of the cell.

CHODAT denies the presence of the sulcus in *Bernardinium*, which is easily comprehensible, indeed, for the very indistinct formation. According to CHODAT's drawings, the longitudinal flagellum seems to insert on the dorsal side, however, this obviously is a failure connected with the error of the whole orientation.

CHODAT disputes the presence of the stigma either, yet he describes and draws the "haematochrome grains" near to the insertion of the flagella, which he supposes to be of unknown origin. He separates the new genus from the genus *Hemidinium* STEIN with which it is related, marking

out the following differences: Absence of a distinct sulcus, as well as of chromatophores, and smaller dimensions. He does not say much about the quality of the membrane, finding it rather stiff, colourless, however, he uses the Latin diagnosis, calling it "membrana plasmatica", and continues to speak about the ejection of particles from the plasma.

THOMPSON (l.c.) observed merely "a very brief suggestion of a sulcus", on the other hand a continuation of the girdle through a shallow groove into the right side. He found no stigma; he describes and pictures many "noncontractile small vacuoles" at the lower margin of the hypocone, which according to my observations correspond to the minute corpuscles described above (Tab. 7 : 1). He does not want to deliver himself definitely on the membrane quality. He says: "Other than the rigidity of the cell and the sharpness of the girdle margins there was nothing to suggest the presence of a theca". Very surprising are THOMPSON's observations on chromatophores in some specimens. He describes them as "many small, diffuse, parietal, very pale yellow-green chromatophores". The question arises of whether THOMPSON's species from Cansas is identical with the Alpine and the Czechoslovak specimens; it indisputably belongs to the same genus. According to the character of the plastids observed and with regard to that they appeared merely in some individuals, only some remains of pigments of the ingested algae as described above may be considered.

Data on dimensions (μ) resulting from three observations were put into a table. The smallest dimensions are given for the specimens observed by THOMPSON, the largest for the specimens observed by me; CHODAT's data on dimensions are between them, showing the width of variability

Specimens described by	Length	Width	Thickness
CHODAT	16.8—19	13.2—14	—
THOMPSON	15 —17	10 —12	—
The author	18 —21.8	12 —17	9—11.8

SCHILLER (1937) claims that the genus *Bernardinium* has been unsufficiently investigated and that it probably is identical with the genus *Hemidinium* STEIN. HUBER-PESTALOZZI (1950) accomplished this identification of the two genera according to the literature.

CHODAT's original description, THOMPSON's record and my own observations as well, prove the incorrectness of the above combination. *Bernardinium* CHODAT is to be looked at as an independent genus, however not with regard to CHODAT's arguments, but first of all for the absence of the structural membrane. It resembles the genus *Hemidinium* STEIN in the characteristic course of the girdle, yet it obviously does not produce a metaplasmatic cellulose membrane, but solely a structureless periplast, similarly as the typical species of the genus *Gymnodinium* STEIN. Consequently there is one and the same difference between the two similar genera as between the typical species of the genus *Gymnodinium* STEIN and the typical species of the genus *Glenodinium* (EHRENB.) STEIN with the well known structure. From this results also the systematic classification of the genus *Bernardinium* CHODAT, which is to be placed into the family *Gymnodiniaceae*. Evidently the absence of chromatophores is characteristic for this genus as well.

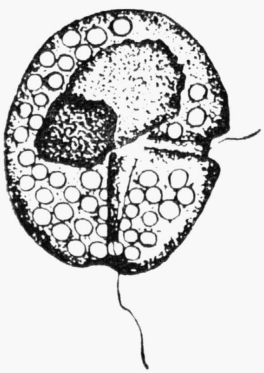

Fig. 1 : *Bernardinium salinum* (ANIS.) comb. nova — ventral view. (According to ANISIMOVA, 1926.)

Further species of the genus *Bernardinium*

ANISIMOVA (1926) describes a new species *Hemidinium salinum* ANIS. (Fig. 1) from the salt lake Srednoe in Russia (1% Cl), much resembling the species *B. bernardinense* CHODAT by its habitus and dimensions (length 15.5 to 18.5—24 μ, width 13.5 to 17 μ). The protoplast also contains the typical

reddish brown lumps. No stigma was observed. The species was, according to the authoress herself, unsufficiently examined. There is no reference to a membrane, however, the similar appearance of the two species is so much evident that they even may be looked at as identical. Yet due to both, the entire different formation of the sulcus (in the species described by ANISIMOVA this structure is more conspicuous, extending to the antepex and forming a sinus in the outline) and the ecological difference, the two above species have been considered as not identical and a new combination *Bernardinium salinum* (ANIS.) comb. nova has been established.

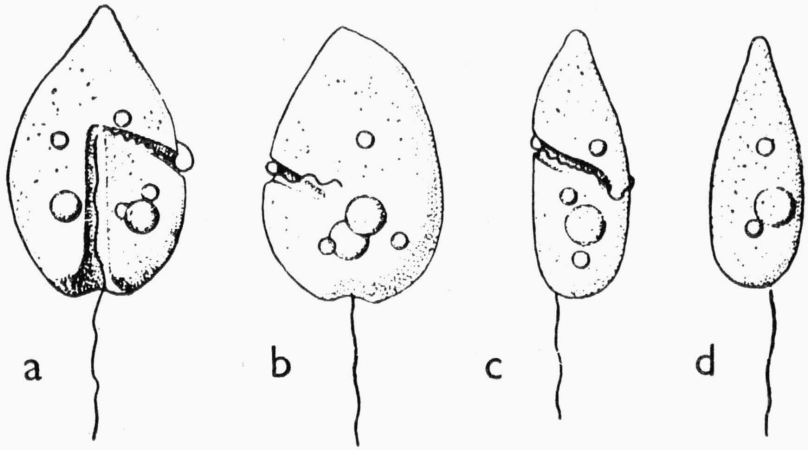

Fig. 2: *Bernardinium thiophilum* (CONRAD) comb. nova — a — ventrum, b — dorsum, c — latus sinistrum, d — latus dextrum. (According to CONRAD, 1939.)

CONRAD (1939) describes from the Belgian salt marshes (H_2S) a new species *Hemidinium thiophilum* CONRAD (Fig. 2), which according to my opinion may be a species of the genus *Bernardinium* CHODAT as well. This taxonomical position is justified not only by the absence of chromatophores, but mainly by the quality of the membrane, according to CONRAD: "La membrane est peu déformable, lisse, hyaline, je n'ai pas réussi a y découvrir une tabulation". It differs from the typical species *B. bernardinense* CHODAT by: larger dimensions (length 24—30 μ, width 15—19 μ, thickness 8—11μ), a pear-like shape of the cell (smaller, tapered epicone, larger hypocone, well cut out at the bottom), the course of the sulci (girdle above the middle of the cell, sulcus distinctly developed on the antapex, forming a sinus), its ecology (it scarcely swims, but mostly crawls on the mud surface in an environment containing H_2S besides a considerable amount of chlorides). In his description, CONRAD mentions CHODAT's species *Bernardinium*, however, he does not place his new species to it, assuming that the question is of "Hemidinium inverse" with an inverse course of the girdle. This certainly is a failure caused by CHODAT's error mentioned above. Thus a new combination results: *Bernardinium thiophilum* (CONRAD) comb. nova.

Taxonomical survey

Classis: *Dinophyceae* Subordo: *Gymnodiniineae*
Ordo: *Peridiniales* Familia: *Gymnodiniaceae**)

Bernardinium CHODAT 1923

Syn.: *Bernardinium* CHODAT, Bull. Soc. Bot. Genev. 14 : 41, 1923.
Hemidinium STEIN p.p., sensu auct.: ANISIMOVA, Russ. Gidrob. Žurnal 5 : 191, 1926; CONRAD, Bull. Mus. Hist. Nat. Belg. 15 : 8, 1939; HUBER-PESTALOZZI, Phytopl. Süsswass. 3 in Binnengewässer 16, 3, Stuttgart 1950 : 164.

Bernardinium bernardinense CHODAT 1923

Bas.: *B. bernardinense* CHODAT 1923 (type of the genus).
Syn.: *Hemidinium bernardinense* (CHODAT) HUBER-PEST. 1950.
Iconotype: CHODAT 1923, p. 40, Fig. VII.

B. salinum (ANIS.) comb. nova

Bas.: *Hemidinium salinum* ANISIMOVA, Russ. Gidrob. Žurn. 5 : 191, 1926.
Iconotype: ANISIMOVA 1926, p. 189, Fig. 1 : 8 (reproducted in the present text, Fig. 1).

B. thiophilum (CONRAD) comb. nova

Bas.: *Hemidinium thiophilum* CONRAD, Bull. Mus. Hist. Nat. Belg. 15 : 8, 1939.
Iconotype: CONRAD 1939, p. 9, Fig. 8—11 (Reproducted in the present text, Fig. 2: a—d).

Key to the determination of the species

(1) Cell ellipsoid in the outline, epicone rounded, length up to 24 μ.
  (a) Sulcus short, not marked by a sinus in the outline of the antapex. Freshwater species . . . . . *B. bernardinense*
  (b) Sulcus with a sinus in the outline of the antapex. Halophile species . . . . . *B. salinum*
(2) Cell pear-like, epicone tapered, length from 24 μ to more. Halophile species, H_2S. *B. thiophilum*

Crypthecodinium BIECHELER

The monotypical genus represented by the only species *Crypthecodinium cohnii* (SELIGO) comb. nova may be easily cultivated. No wonder that it was thoroughly studied both morphologically and cytologically in the past, being an object of physiological research nowadays as well. Hence it is by no means "scarcely known" in this sphere. Obscurities, however, have been found in its ontogenesis, membrane structure, and consequently in its taxonomy. I have studied it from this point of view, having been ever before much interested in it for its external resemblance to the genus *Bernardinium* CHODAT.

Material

The material for the present study from the culture collections of the Haskins Laboratories in New York was kindly given to my disposal by Dr. L. Provasoli. It represented strains, isolated

*) Higher taxa according to FOTT (1959) are used.

from rotting *Fucus* from two localities: Woods Hole, Massachusetts, U.S.A. (strains *a—d, 1—2*) and from Bimini, Bahama Islands (strains McLaughlin *1, 3, 5*) (Dr. L. Provasoli in personal communication). Both finding-places were on the western seaside of the Atlantic Ocean.

I did not succeed to get in reproduction the strains from the Bahamas and that is why my study is chiefly based on the material obtained from Massachusetts.

Habitus

C. cohnii (Seligo) comb. nova occurs at two ontogenetic stages, namely at a motile and at an unmotile one, as may be well seen from the original description: Seligo (1887) calls them "Cysten" and "Schwärmer", which is used by the other authors as well besides other designations for the motile stage, e.g. "die schwärmenden Individuen" (Küster, 1908), "die freien Flagellaten" (Jollos, 1910; he used the term "Schwärmer" in a different sense of meaning, see paragraph "Reproduction"), "die schwärmenden Flagellaten", "die schwärmenden Zellen" (Griessmann, 1913). From the reasons explained in paragraph "Reproduction", I call the unmotile stage "v e g e t a t i v e c e l l s", the motile flagellates "z o o s p o r e s", the unmotile reproductive cells "a u t o s p o r e s".

Morphology

The v e g e t a t i v e c e l l s are rounded or slightly ellipsoidal, single living. Out of 130 undivided cells, 62 (47.7%) were isodiametrically shaped, i.e. precisely rounded. The average ratio between the longer diameter and the shorter one in all measured cells was $1{,}046 \pm 3 \,.\, 0.0102$. In long-term cultures on agar, of course, also much more elongated and variously deformed cells could be found, which I, however, regard as teratological forms arising due to the influence of culture conditions.

The m e m b r a n e is smooth, showing without previous preparation no definite structure. It is relatively thin, growing somewhat thicker in older cells, without becoming mucous, only sometimes getting stratified presumably by a repeated division, during which always one cells stays in the mother membrane (Tab. VIII : 3). The membrane is colourless, at times, by the influence of the culture medium, a pale yellow or brown. After silver impregnation according to Chatton and Lvoff (Jírovec, 1953), using osmium acid as a fixative (Biecheler, 1952), the membrane displays a system of argentophilous lines, which form a more or less irregular plate-like structure (Tab. XI : 9—12). However, they cannot be fully treated as identical with the structure of the zoospores. Besides these sutures also a fine punctate sculpture appeared on the membrane. In this way membranes were studied in a culture reproduced for a long time-period by autospores. The membrane of the vegetative cells and of the zoospores as well, is elastic, considerably resistant even against lye and acid, which was previously observed by Seligo (l.c.). I could not achieve the typical reaction to cellulose by the use of Cl + Zn + J reagent, but only an untypical pale violet colouration reported already before by several authors.

The z o o s p o r e s are ellipsoid or ovoid, slightly dorsoventrally flattened. The epicone is widely rounded, up to a blunt conic, as a rule higher and wider than the hypocone, which is always widely rounded. Both parts are unsymmetrical, since the girdle forms a left hand downward spiral. The sulcus meets it on the top at more or less acute angle, yet it does not reach the antapex

at the bottom and does not appear as a sinus in the profile either. The girdle is rather distinct on the right side, yet it does not extend to the end of the sulcus (Tab. X: 2—4).

The distance between the two ends of the girdle on the ventrum is, in proportion to the length of the cell, ½ to $\frac{1}{2,4}$. By the formation of the girdle the zoospores resemble the dinoflagellates of the genus *Gyrodinium* KOFOID et SWEZY. The two flagella, both free, insert somewhere near the angle of the sulci. The longitudinal flagellum generally represents 1.5 of the cell's length; the longest (48 μ) was found in a cell of a 14 μ length. The transverse flagellum is not apparent as a rule on the right side of the cell in ventral view.

The membrane of the zoospores is thin, smooth, showing no structure in vivo and after the application of current reagents and dyes (KJ + J, Cl + Zn + J, methyl blue, safranin) respectively. When impregnated with silver, it displays a plate-like structure (Tab. XI: 1—8). In the material (strains from Massachusetts), only a very slight and merely partly perceptible structure was found even after the most successful preparation, so that I did not succeed to treat it as fully identical with the scheme given by BIECHELER (1952: p. 83). Especially indistinct were the surroundings of the sulcus. It was, however, possible to identify relatively well the supplemental plate between the ends of the unclosed spiral scratch of the sulci, which BIECHELER marks as "*x*", taking it for typical of the genus and the family (Tab. XI: 1—3). On the plates of some cells, large irregularly straggled points were found after silver impregnation. This sculpture, common in armoured dinoflagellates after all, is mentioned by BIECHELER as well. It differs from the finely and on the whole regularly punctate membranes of the vegetative cells.

Dimensions

130 vegetative cells were measured in two diameters upright to each other. Out of these 260 measurements, the mean value of $18.50 \pm 3 \,.\, 0.2439$ μ was obtained. The minimum diameter in the free undividing cell represented 9 μ, the maximum 31 μ (Tab. VIII: 1a—c). In eight cultivated strains I found the dimensions of vegetative cells, put into the following table, which gives the minimum and the maximum diameters measured, the arithmetical mean value of the diameters (d) and the ratios between the shorter and the longer cell diameters (r). All measurements carried through concerned free non-dividing vegetative cells from cultures (PROVASOLI's artificial medium).

Strains	Diameters in μ			
	min.	max.	d	r
a	9.0	21.5	17.46	1.07
b	15.5	24.0	18.97	1.062
c	17.0	29.0	21.37	1.013
d	10.0	27.0	19.17	1.063
1	15.0	29.0	20.35	1.067
2	14.0	31.0	21.50	1.048
Mcl. 1	10.0	27.0	18.90	1.038
Mcl. 3	14.0	21.0	17.87	1.034
Mcl. 5	11.5	19.0	15.72	1.013

Strains *a—d* represent isolations from more cells (material from Massachusetts) on an artificial agar medium. Strain *1* from the same material concerns the isolation from one single cell, strain *2*, the isolation from two cells. Strains *Mcl. 1, 3, 5* from the material from the Bahamas represent a liquid medium of a different structure (Dr. L. Provasoli in personal communication). The strains from the Bahama Islands seem to have somewhat smaller cells on the average, which, however, might have been caused by a different culture medium. It was impossible to transfer them onto an artificial agar ground. For the great variability of dimensions and the lack of some diacritical features, I consider them as the same species as are the strains from North-America.

The zoospores were at minimum 12 μ long and 9 μ wide, maximum length 27 μ, maximum width 21 μ, mean values 17.6 μ long and 14.4 μ wide (Tab. X: 2—4).

Individual authors give the following dimensions (in μ):

Authors	Vegetative cells	Zoospores	
		length	width
Seligo, 1887	8.6—25	13	8—10
Küster, 1908	56—100	(28)—65—75—(85)	60—65
Jollos, 1910	—	15—20—(40)	—
Griessmann, 1913	—	(4)—10—25—(40)	7—17
Biecheler, 1952	—	(10)—15—(20)	(8)—10—(15)
My specimens	(9)—18.5—(31)	(12)—17.6—(27)	(9)—14.4—(21)

The survey shows a considerable variability in dimensions of the vegetative cells and the zoospores, however, an obvious homogenity with regard to the material. Only the dimensions given by Küster (1908) are remarkably different from the others. However, since Jollos (1910) worked at material from the same locality (Helgoland) and even at Küster's cultures, we may infer an error in Küster's measurings.

Protoplast

The vegetative cells and zoospores are substantially alike by the structure of the protoplast. The plasma is hyaline, colourless or a pale yellow to brown (on soils with a Fucus extract the cells obviously absorb the brown colour from the medium).

The ovate nucleus (Tab. VIII: 1a,5, 6; IX: 4; X: 9—10) is placed in the centre or at the periphery of the cells, in zoospores being found in the hypocone (Tab. X: 11); it is almost imperceptible in vivo, having no distinguishable structure, being well differentially diable with methyl-green (1% solution of methyl-green in 1% acetic acid); (Tab. X: 9—11.) After preparation with osmium acid, it displays a thin pearl-like structure common in dinoflagellates. Jollos (l.c.), studying the nucleus cytologically, found caryosome and centriole. On the basis of his investigations, Hartman (1911) placed this nucleus to the group which he described as "massige Kerne" according to Doflein, taking it for typical of dinoflagellates.

The chromatophores are always missing and neither do any corpuscles appear, differentially coloured from the adjacent plasma. The zoospores were found to have no stigma. In vegetative cells, the protoplast is easily separatable from the membrane (Tab. VIII : 7—9; IX : 1). This process is characteristic for older undividing cells, in which also large vacuoles appear as a rule (Tab. VIII :2). Artificially, e.g. by the action of KJ + J, the plasmolysis may be achieved in vegetative cells and zoospores as well (Tab. X : 8).

Nutrition

The nutrition is merely heterotrophic for the lack of assimilatory pigments. No consumed corpuscles are to be seen, nor any adaptability to animal nutrition. From the fact that *Crypthecodinium* may be cultivated in bacteria-free cultures (PRINGSHEIM, 1956; PROVASOLI and GOLD, 1957), follows that it feeds osmotically. Studies of PROVASOLI and GOLD (1957 and 1959) deal with the investigation on its metabolism. The reserve materials are of two kinds: small, differently shaped grains dispersed all over the protoplast, occurring in all cells, and larger, more spherical or ovoid, more light-breaking corpuscles, which, too, are very common in cells, but may be absent at times. This concerns the vegetative cells and zoospores as follows from a series of pictures in tables VIII, IX, X. None of these corpuscles gives when using iodine a typical reaction of starch; SELIGO studied them using different reagents and assumed that they were neither starch nor oil. GRIESSMANN considered them as oil and as a substance similar to starch; BIECHELER thinks them to be glucides, PRINGSHEIM regards them as starch. PROVASOLI and GOLD did not determine the reserve materials (in personal communication).

Cultivation

The isolation is commonly carried out from rotting *Fucus* material. KÜSTER cultivated *Crypthecodinium* both on liquid and solid media (agar, gelatine) from artificial sea-water and *Fucus*-decoctum. GRIESSMANN using this way of cultivation found a decrease in dimensions and added in order to retain the normal state cane suggar, starch, and wine acid. PRINGSHEIM isolated *Crypthecodinium* in a medium, containing yeast digest, sodium acetate, and peptone in sea-water. PROVASOLI and GOLD (1959) prepared a fully artifical culture medium for that purpose. I tried to cultivate North-American strains on all these media. PROVASOLI-GOLD's artificial medium proved to be the most suitable. In that medium in a liquid state, *Crypthecodinium* multiplied by zoospores at a maximum rate (Fig. 3 : A—B); on media solidified with agar it reproduced unlimitedly by autospores.

Bionomy and reproduction

Authors working on bionomy of this species describe the motile and the unmotile stages, except for BIECHELER (l.c.) who speaks only of flagellates. The unmotile stage is defined as cysts by all authors; various definitions are used for the motile stage, which is, however, considered to play the leading part in the bionomy of the species. I could not observe the organism in question under its natural conditions. As to cultures, I am sure that it may live under certain conditions solely in the stage of "cysts" with the full exclusion of the motile stage, however, that it can never permanently vegetate in the form

of a flagellate only. On the basis of my own studies, I do not hold the unmotile stage for resting or multiplying cysts according to other authors, but I attach the greatest importance to it in the bionomy of the species and the genus as well. Whether the organism vegetates merely in the form of vegetative cells or whether it also produces zoospores, obviously depends on physiological factors. Thus, in PRINGSHEIM's medium with yeast decoctum the cells had a tendency to multiply at all times by zoospores even on a medium solidified

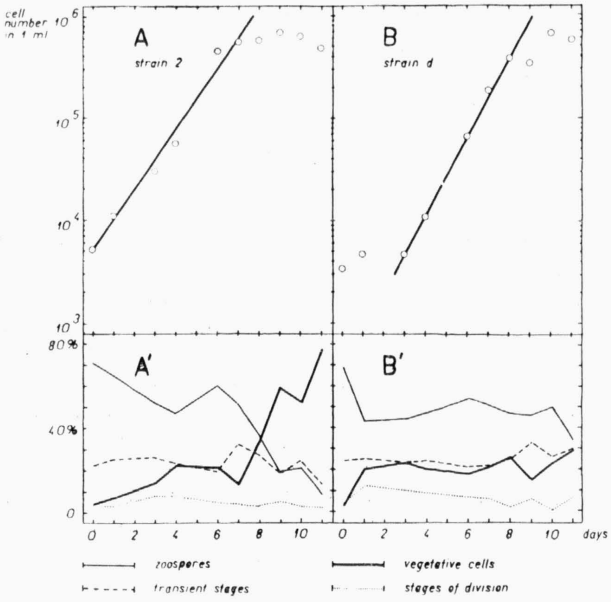

Fig. 3: *Crypthecodinium cohnii* (SELIGO) comb. nova — A — growth of Provasoli's strain 2 in artificial liquid medium, B — growth of Provasoli's strain d in artificial liquid medium, A', B' — procentual representation of ontogenetic stages in the two strains.

with agar, building up teratological forms (reported also by KÜSTER and BIECHELER). In PROVASOLI-GOLD's medium, which obviously fully suited the organism as to its physiological demands, the way of multiplying by autospores or by zoospores changed according to the passage on the solid and in the liquid medium; autospores, too, may be built up in a liquid medium, though rather seldom.

Reproduction proceeds asexually; sexual processes have never been observed. Only vegetative cells, the so-called "cysts" were found to divide. There are two products of division (Tab. VIII:4–7), frequently four (Tab. IX:2–3), and exceptionally eight in one cell. JOLLOS (1910) describes during the formation of four and more products the division of the nucleus as succedaneous, the division of the protoplast as succedaneous or simultaneous. I did not study the caryology of the species, but following external morphology, I found the division of the cells always to appear as simultaneous, the products of division being always placed spacially in even numbers (Tab. XII:f, g, i). I do not exclude anomalies during intensive culture growth.

The reproduction of *C. cohnii* generally proceeds by zoospores. The products of division change inside the mother membrane into zoospores of the above mentioned type, forming flagella and their own wall, and they escape by breaking through the mother-membrane. Sometimes they remain for quite a short time-period mechanically attached by the flagella, even during motion. Frequently only one zoospore is found in a single cell, i.e. no previous division of the protoplast has occurred (Tab. IX : 5—7; X : 1; XII : k).

Division of a motile flagellate never comes about. Neither was any observed by former authors. JOLLOS' dividing "Schwärmer" certainly were of foreign origin in cultures, reported already by KOFOID and SWEZY (1921), who took them for flagellates of the genus *Bodo* or *Prowazekia*. I frequently observed the cultures in various phases of development, also in a wet chamber at day and night hours, however, I never noticed any division of the flagellates. Also in the logarithmic phase of growth, *C. cohnii* always produces vegetative cells and multiplies solely inside them. Figure 3 : A, B illustrates the logarithmical growth of cultures of PROVASOLI's strains *2*(A) and *d*(B) in an artificial liquid medium. Data concerning the number of cells were obtained by counting in an adaptated haemacytometer after fixation with $OsSO_4$. Graphs Fig. 3 : A', B' give the procentual representation of the individual ontogenetic stages during the development of both cultures. I followed the vegetative cells, the stages of their division respectively, further the cells of uncertain shape (passing from zoospores to vegetative cells, however, sometimes obviously also zoospores, largely deformed by preservation), and the zoospores as well. The material has been inoculated from the supernatant of the culture in solution, i.e. almost only zoospores. Graph A' brings the representation of the vegetative cells even during the strongest culture growth and the progressive increase of their share in the total quantity. Graph B' expresses this tendency less marked, however, a well evident representation of the vegetative cells in all phases of culture development.

After several hours of steering, the zoospores settle down, loosing their flagella and characteristic shape, changing into round vegetative cells (Tab. X: 5—7). The membrane of the zoospores simultaneously adapts in a pliant way to the shape obviously retaining the characteristic structure (Tab. XI: 9—12).

Multiplication by zoospores is not the only way of reproduction observed in *C. cohnii*. On solid culture media almost solely, but rather in liquid media as well, the reproduction products of vegetative cells do not change into zoospores, but grow spherical, enveloping themselves with their own wall inside the mother membrane (Tab. VIII : 6; IX : 3—4; XII : a—c, h). Such fully built up cells seldom stay in the mother membrane (Tab. VIII : 7; XII : c). As a rule they very soon break through and free themselves (Tab. VIII : 8—9; IX : 1, 4; XII : d—e). No colonies are being formed, but free groups of single-living cells (Tab. XII : i). This way of reproducing cells rather similar to the mother cell are defined as autospores.

The following explanation concerns the reason, why instead of flagellates and their resting and multiplying cysts, coccoid algae multiplying by zoospores and autospores are being considered. In many flagellates, also of other algae groups (*Volvocales*, *Euglenales*, *Cryptophyceae*), this kind of reproduction in cysts has been recorded. At these resting stages of flagellates, it certainly is possible to seek for the phylogenetic origin of capsal and coccoid organis-

ations of algae. The question arises of where to place the limiting line. The cysts of flagellates are usually kept in mucus or the membrane gets mucous or at least very thick after some time. In *C. cohnii* repeatedly evident, structured and rather thin membranes are formed. The way of multiplication, however, must be considered as the most important criterium. *Crypthecodinium* entirely lacks the characteristic reproductive process common in flagellates, namely the division in a motile stage, neither are any indications of it left behind. In this case the flagellate is a spore, indeed, lacking the ability of reproducing by itself, which lasts only a limited time-period, and always changing in a single vegetative cell; it obviously does not often serve for reproduction, however, rather for an expansion of the species, which is evident from the frequent formation of the single zoospore only (Tab. IX : 5; XII : k). The vegetative unmotile cells, however, are capable of an independent existence; they may reproduce not only by zoospores, but even by formations rather similar to them, which are to be taken for autospores. While these are built up, no indication of flagellate stages (sulcus, flagella) appears. It may be objected that in a certain environment (fresh liquid medium) great numbers of zoospores predominate above the vegetative cells. This way, however, may also be brought about in other algae, reproducing by zoospores or at times in filamentous algae as well. It may further be pointed to the fact that in a different environment (solid medium) merely vegetative cells exist, zoospores being entirely absent. It would certainly be interesting to pay attention to these relationships at natural localities as well. Yet it is assumed that neither here would the predominance of flagellates give any evidence for the taxonomical position of *C. cohnii* to *Peridiniales*. The ability of reproduction must be considered as a deciding limit, distinctly according to which *C. cohnii* must be placed to *Dinococcales**).

Systematic classification and nomenclature

Seligo (1887) described the organism dealt with in the present study as a species of *Glenodinium* (Ehrenb.) Stein for its apparent structureless membrane. Independently of that, Küster (1908) described it as a species of the genus *Gymnodinium* Stein, since he did not notice the membrane of the flagellate. His conception is taken over by Jollos (1910) in his cytological study. Griessmann (1913) identified well Küster's species with the one of Seligo and kept to its placing to the genus *Glenodinium*. Kofoid and Swezy (1921) while describing the new genus *Gyrodinium*, place also Küster's species to it, yet on the basis of literary statements only; they knew neither Seligo's original description nor Griessmann's study. Schiller (1933) rectified this conception by setting up a new combination of Seligo's species and the genus *Gyrodinium* Kofoid et Swezy. Biecheler (1952) described from the French sea-side of the Mediterranean a new genus and species of the dinoflagellate *Crypthecodinium setense* Biech. on the basis of the argentophilous plate structure of the membrane and established even a new family for it, which she calls *Crypthecodinidae* Biech. She thinks it possible to combine the species *Crypthecodinium setense* Biech. and *Gyrodinium cohnii* (Seligo) Schill., if the same tabulation is proved in the latter.

On the basis of my studies, I am of the opinion, indeed, that all descriptions and combinations mentioned above relate to the same species. I consider it as a coccoid alga and this is why I exclude it from the genera *Glenodinium*, *Gymnodinium*, and *Gyrodinium* as well. By the formation of autospores, it mostly resembles the fresh-water genus *Phytodinium* Klebs (1912), which,

*) The structure membrane of zoospores and vegetative cells as well, is known in these algae, e.g. in the species *Stylodinium tarnum* Baumeister (1943).

however, is known to possess no zoospores. Perhaps there exists a closer relation to the unsufficiently studied fresh-water genus *Gloeodinium* KLEBS (1912) in which KILLIAN (1924) discovered zoospores very much suggesting *Crypthecodinium* by the shape of the cells. The vegetative cells of the genus *Gloeodinium* KLEBS, however, form gallerts with a stratified mucus, and conspicuously divide in a succedaneous way. PASCHER (1927) takes them therefore for the capsal organisation of *Dinophyceae*. WOLOSZYŃSKA (1925) discovered dividing cysts of the genus *Hemidinium* STEIN, taking them for similar, later on for identical with the genus *Gloeodinium* KLEBS. She simultaneously describes the plate structure in the genus *Hemidinium*, which, however, cannot be identified with the structure of the genus *Crypthecodinium* BIECH. (BIECHELER, l.c.). As far as I know, there does not exist any observation on the division of flagellates in the genus *Hemidinium*, except for the original rather indistinct picture by STEIN (1883; Fig. II : 26), which probably represents a mechanical connection of two individuals. In case that the identification of the genera *Gloeodinium* KLEBS and *Hemidinium* STEIN is proved, as well as the reproduction merely at the stage of vegetative cells, it will be possible to take *Hemidinium* for a capsal genus or a coccoid alga, very close to the genus *Crypthecodinium* BIECHELER, yet not identical with it.

On the basis of the mentioned observations and comparisons it proves essential to separate the organism investigated and place it into an independent genus. Although my conception of this genus is substantially different from BIECHELER's conception, I must respect the International Code of Botanical Nomenclature and use the name of the genus *Crypthecodinium*, even though I adapt the description of the genus. Before the affinities of the genus are put clear, it may be considered as right to separate it into the independent family *Crypthecodiniaceae*, the conception of which has been naturally altered contrarily to BIECHELER. The systematic position as well as the nomenclature of the investigated organism is as follows.

Taxonomical survey

Classis: *Dinophyceae*
Ordo: *Dinococcales*
Familia: *Crypthecodiniaceae* BIECHELER 1952

Crypthecodinidae BIECHELER, Bull. Biol. Franc. et Belg., suppl. 36 : 81, 1952; orthographic variant.

Description: Single-living coccoid cells reproducing by autospores and zoospores of the *Crypthecodinium* type.

Crypthecodinium BIECHELER 1952

Crypthecodinium BIECHELER, Bull. Biol. Franc. et Belg., suppl. 36 : 81, 1952 (type of the family).

Syn.: *Glenodinium* (EHRENB.) STEIN p.p., sensu auct.: SELIGO in COHN, Beitr. Biol. Pflanz. 4 : 156, 1887; GRIESSMANN, Arch. Protistenk. 32 : 4, 1913.

Gymnodinium STEIN p.p., sensu auct.: KÜSTER, Arch. Protistenk. 11 : 352, 1908; JOLLOS, Arch. Protistenk. 19 : 178, 1910; SENN, Zeitschr. wiss. Zool. 97 : 639, 1911;

Gyrodinium KOFOID et SWEZY, Mem. Univ. Calif. 5 : 273, 1921 p.p.; SCHILLER, Dinoflag. 1, Leipzig 1933 : 467.

Description: Vegetative cells, spherical or slightly ellipsoidal with uneasily visible plate tructure (after preparation), reproducing by autospores and zoospores. The zoospores have

an incomplete left-hand spiral girdle and plate membrane (visible after preparation only) with a supplemental plate inserted between the two ends of the sulci, where the hypocone and epicone are in touch.

Type of the genus: *Crypthecodinium setense* BIECHELER 1952 (= *Crypthecodinium cohnii* (SELIGO) comb. nova).

Crypthecodinium cohnii (SELIGO) comb. nova.

Bas.: *Glenodinium cohnii* SELIGO in COHN, Beitr. Biol. Pflanz. **4** : 156, 1887.
Syn.: *Gymnodinium fucorum* KÜSTER 1908 (false *P. fucorum*, p. 352)
Gyrodinium fucorum (KÜSTER) KOFOID et SWEZY 1921
Gyrodinium cohnii (SELIGO) SCHILLER 1933
Crypthecodinium setense BIECHELER 1952

Ikonotype: SELIGO 1887, Figs. 22—26.

Description: Plasma and membrane colourless or a light yellow to a light brown No chromatophores, no stigma and no coloured corpuscles in the plasma. Vegetative cells measure in diameter (9) — 18.5 — (31) μ, the average ratio of the longer diameter to the shorter one is 1.046. The zoospores are (10) — 17.6 — (27) μ long, (8) — 14.4 — (21) μ wide. Scheme of the membrane plate structure: epitheca 4' — 3a — 5''; supplemental plate *x* between the two ends of the sulci, where the epicone and hypocone are meeting; hypotheca 5'''—3''''. Sea species.

Occurrence: In the *Fucus* growths, littoral regions of the Baltic Sea (Germany), the Atlantic Ocean (France, U.S.A., Bahama Islands), the Mediterranean Sea (Italy, France).

Summary

1. The present paper deals first with two records of the dinoflagellate *Bernardinium bernardinense* CHODAT from Czechoslovakia. On the basis of personal observations, the genus *Bernardinium* CHODAT is regarded as different from the cognate genus *Hemidinium* STEIN (which it is generally connected with) for the absence of the structural cellulose membrane. Consequently two new combinations have been established: *Bernardinium salinum* (ANIS.) comb. nova and *B. thiophilum* (CONRAD) comb. nova.

2. Culture studies and membrane preparations helped to accomplish the identification of the species *Gyrodinium cohnii* (SELIGO) SCHILL. with *Crypthecodinium setense* BIECH. and to set up the new combination *Crypthecodinium cohnii* (SELIGO) comb. nova. Also studies on bionomy and reproduction have well explained that *Crypthecodinium cohnii* (SELIGO) comb. nova is a coccoid alga, which in consequence of this has been placed in the family *Crypthecodiniaceae* to the order *Dinococcales*.

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Explanations of plates

Tab. VII : *Bernardinium bernardinense* Chodat

1 — specimens from the Želivka, 2, 3 — specimens from Třeboň: 1a — ventrum, 1b — dorsum, 1c — latus sinistrum, 2a—c — cells closely before division in different views, 3a — cell after division, ventrum, 3b — antapex.
(Original; nu = nucleus, gu = reddish-brown lumps, st = stigma)

Tab. VIII: *Crypthecodinium cohnii* (Seligo) comb. nova

1, 2, 3 — vegetative cells: 1a — average size, 1b — minimum size, 1c — maximum size, 2 — old plasmolized and vacuolized cell, 3 — cell with stratified membrane;
4 to 9 — reproduction: 4, 5 — division of one cell into two cells, 6, 7 — formation of two autospores, 8, 9 — their release.
(Original; nu = nucleus)

Tab. IX: *Crypthecodinium cohnii* (Seligo) comb. nova

1 to 7 — reproduction: 1 — released autospores with empty mother-membrane, 2a—b — division of one cell into four cells, 3 — formation of four autospores, 4 — their release, 5 — formation of one single zoospore, 6 — formation of two zoospores, 7 — their release.
(Original; nu = nucleus)

Tab. X: *Crypthecodinium cohnii* (SELIGO) comb. nova

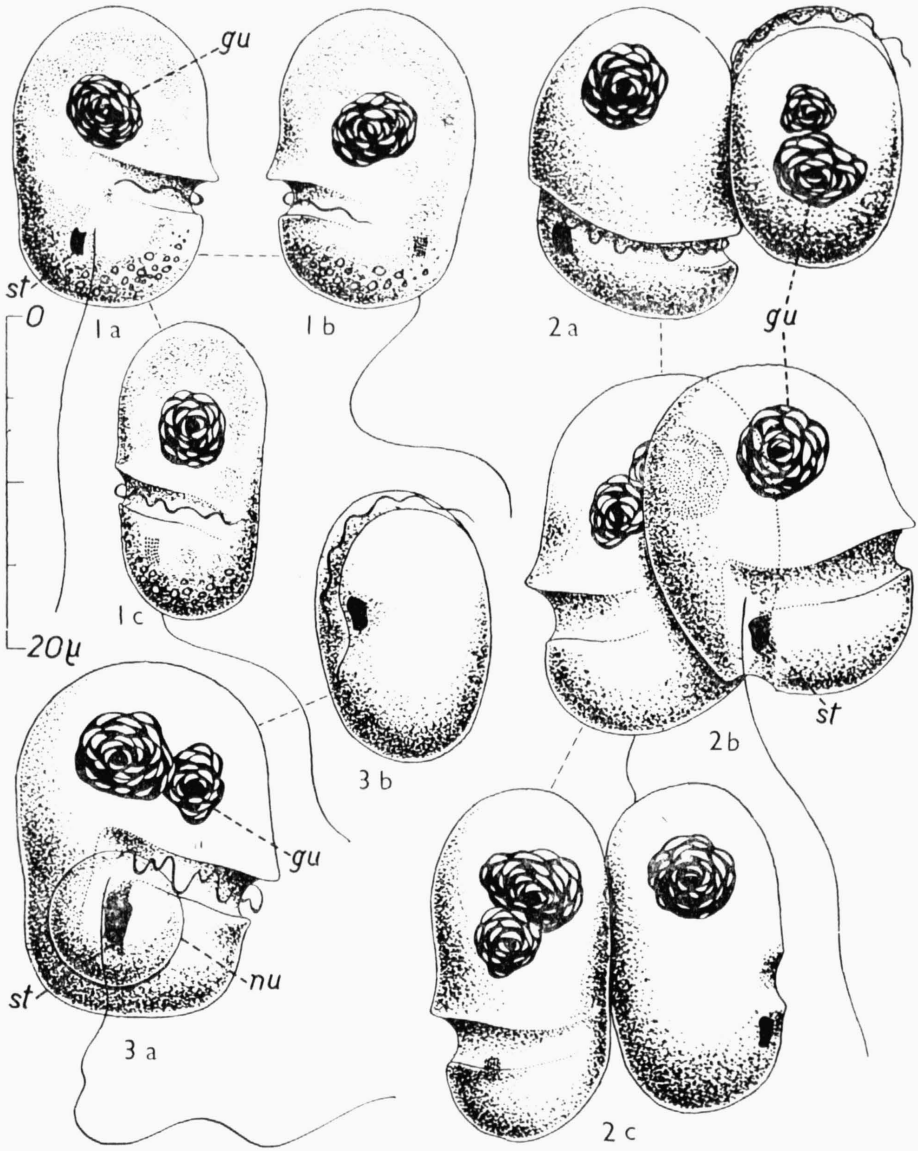
1 — zoospore with built up flagella still in the mother-membrane; 2, 3, 4 — zoospores: 2 — average size, 2a — ventrum, 2b — dorsum, 2c — antapex, 3 — minimum size, 4 — maximum size; 5, 6, 7 — change of zoospores into vegetative cells, 8 — plasmolysis of a zoospore called forth by KJ+J, 9, 10, 11 — nuclei coloured differentially with methyl-green: 9 — vegetative cell, 10 — forming autospores, 11 — zoospore.
(Original; nu = nucleus)

Tab. XI: *Crypthecodinium cohnii* (SELIGO) comb. nova

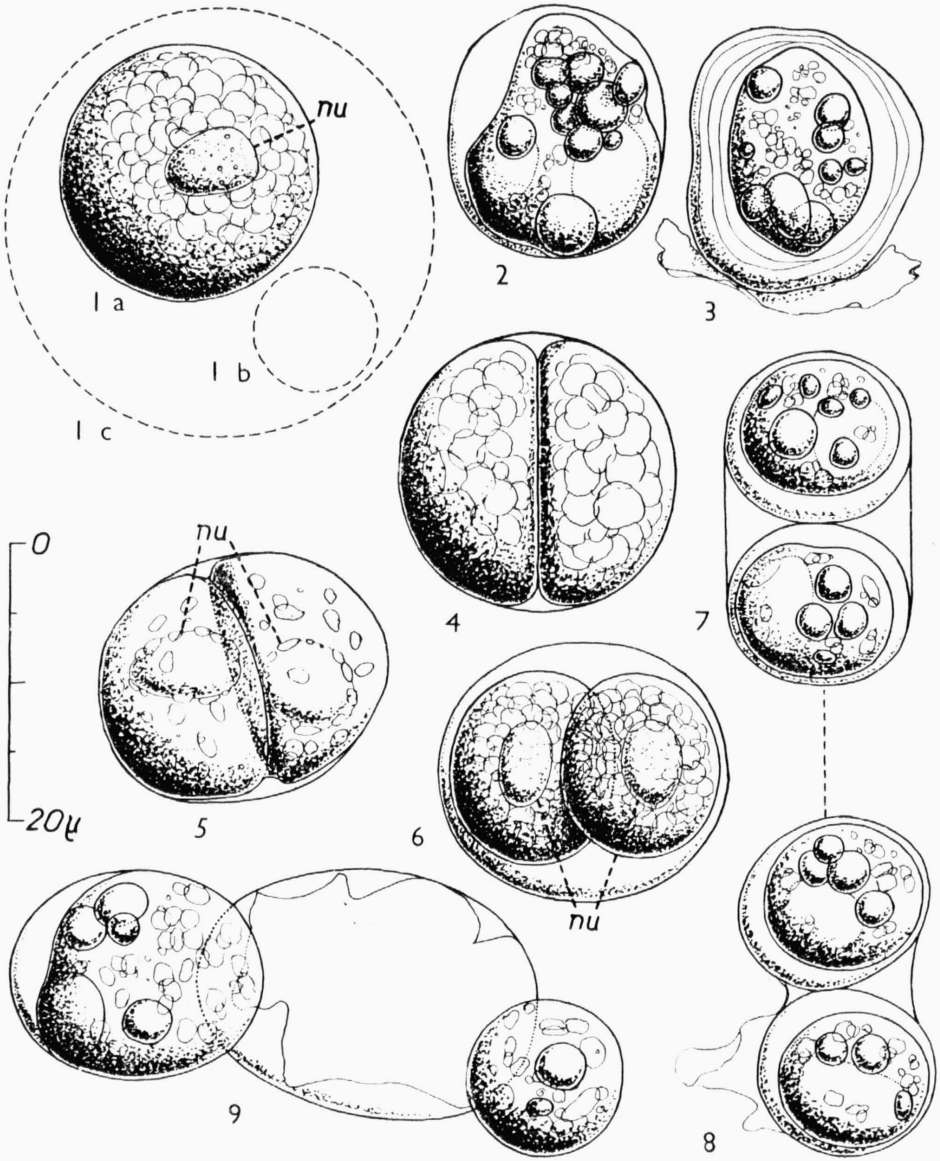
1 to 8 — plate-structure of zoospore membranes after silver impregnation: 1 — ventrum, 2 — antapico-ventrum, 3 — latus dextrum, 4 — antapex, 5 — latus sinistrum, 6 — dorsum hypothecae, 7 — dorsum, 8 — latus sinistrum epithecae; 9 to 12 — plate-structure of membranes of vegetative cells after silver impregnation.
(Original; marking of plates according to BIECHELER's scheme 1952)

Tab. XII: *Crypthecodinium cohnii* (SELIGO) comb. nova

a, b, c — forming of two autospores, d, e — their release, f, g — division of one cell into four cells, h — cells with two and four autospores, i — group of vegetative cells with formation of autospores, k — forming of one zoospore.
(Original; various magnifications)



P. Javornický: Two scarcely known genera of the class *Dinophyceae: Bernardinium* Chodat and *Crypthecodinium* Biecheler



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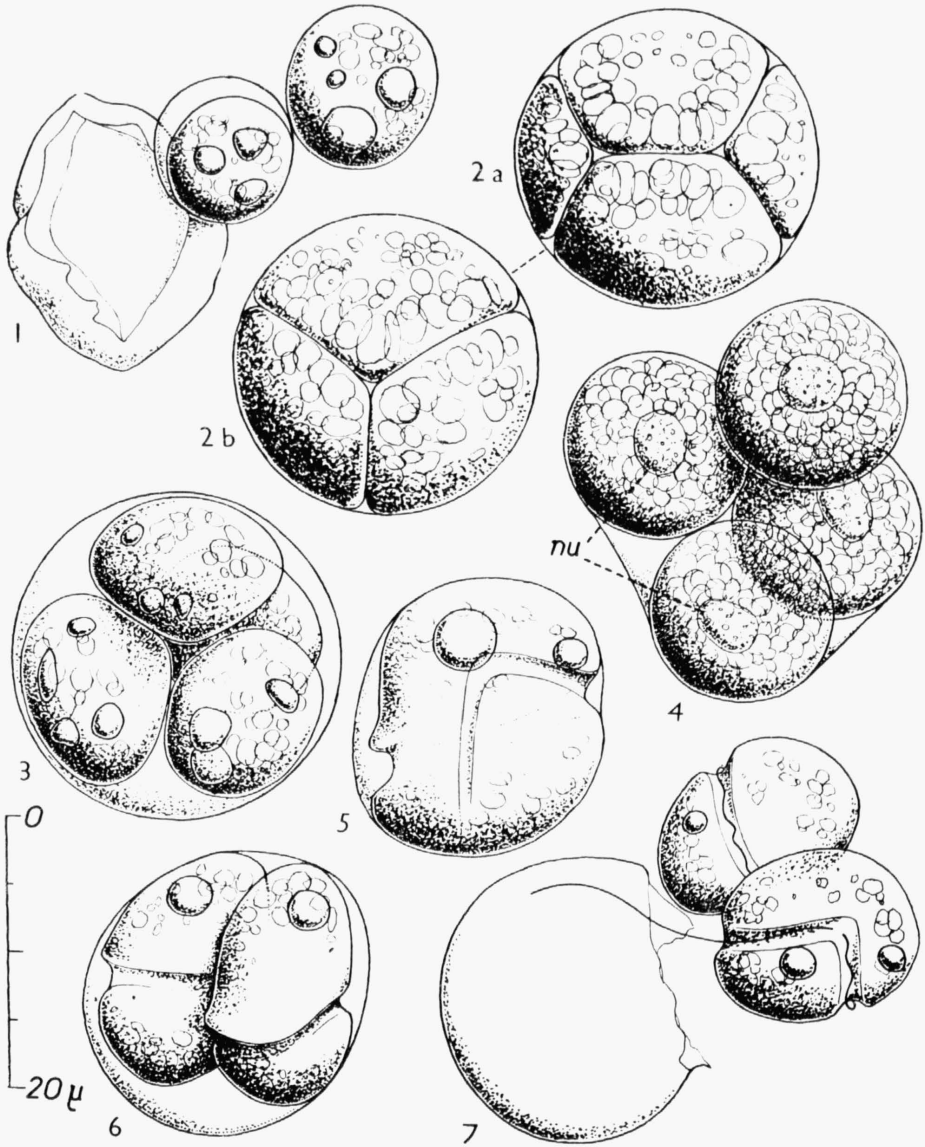

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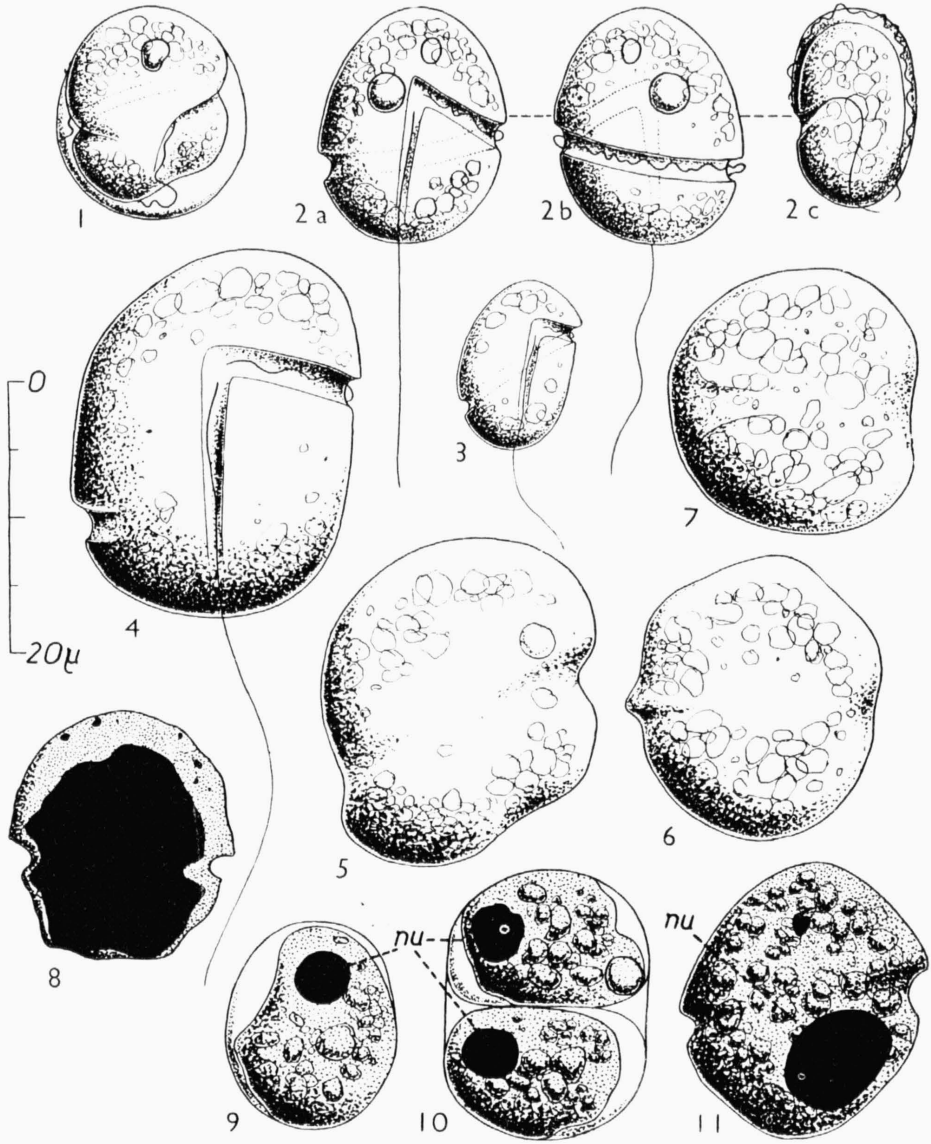

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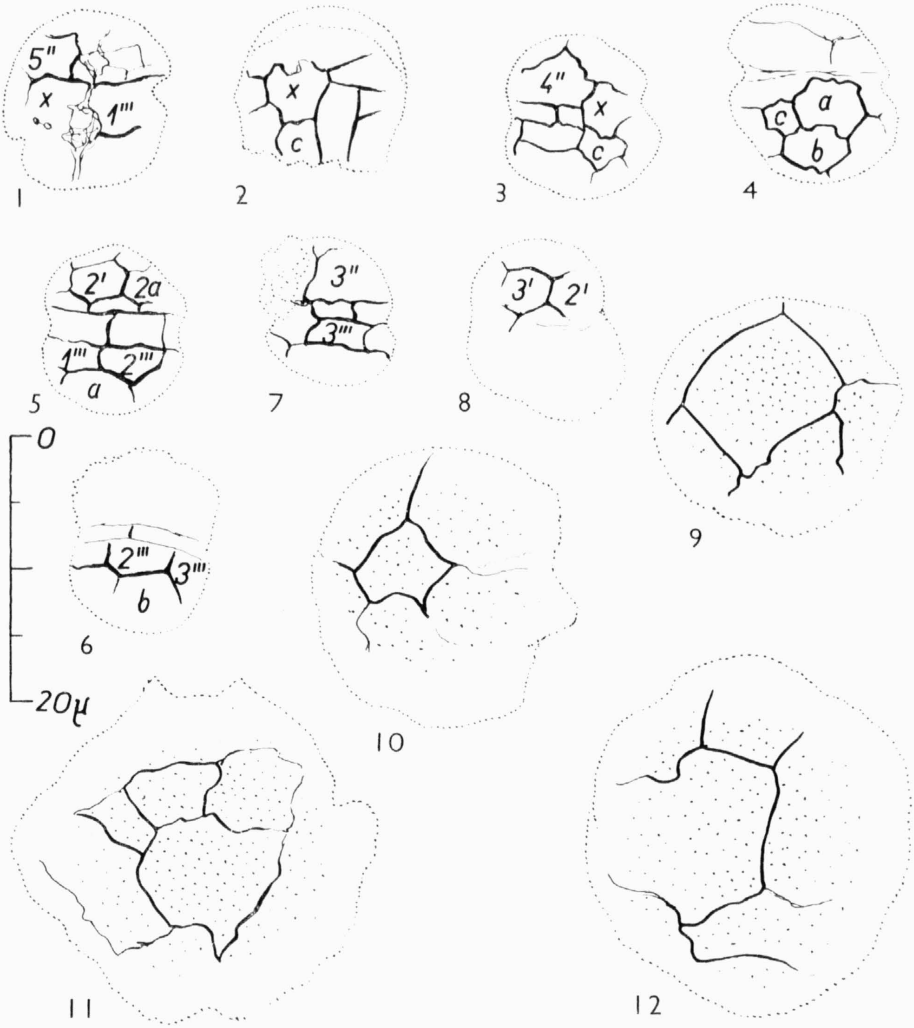

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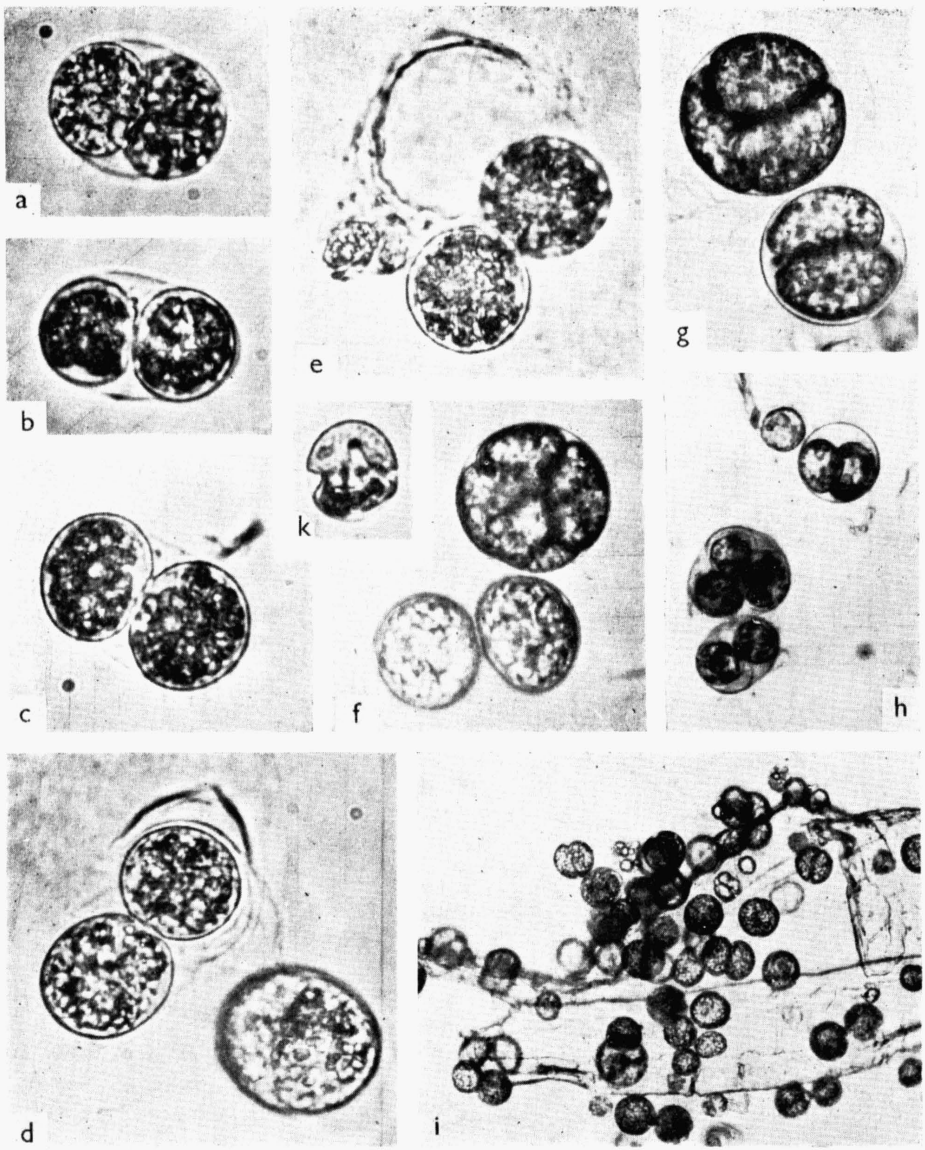

P. Javornický: Two scarcely known genera of the class *Dinophyceae: Bernardinium* Chodat and *Crypthecodinium* Biecheler