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Unsolved problems in the classification of the non-motile *Chrysophyceae* with references to those in parallel groups

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This anniversary volume is published in a period of great changes in the taxonomy of algae, particularly at the generic level. The classical morphological and anatomical methods, based on the direct observation of living material from nature, reached their zenith in the series of monographs published or prepared between the world wars for the "Süßwasserflora" and RABENHORST's Kryptogamenflora. They were being supplemented already by the use of cultivated populations (e.g. PASCHER 1937—39). Although this was not a new approach (e.g. KLEBS 1896, RICHTER 1911, CHODAT 1913) some of the earlier work had fallen into disrepute because of the imperfections inherent in such pioneer studies. Since the last world war the use of cultures has increased greatly and the culture collections, stemming largely from those in Prague, have become more numerous and much bigger. This extended use of cultures has made possible many careful, exact studies on the anatomical and morphological characters used in taxonomy as well as some consideration of the implication of physiological features. The development of the electron microscope has opened up a new era in anatomy, comparable to that which the compound light microscope made possible. Light microscopy itself has also benefited from the use of positive and negative phase contrast, fluorescent dyes and other advances in technique. In electron microscopy great care is necessary to ensure that the cells examined are those of the alga studied with the light microscope. Therefore modern techniques for isolating cells and growing them are often essential.

These developments do not mean that the classical methods should be neglected. The careful examination of natural populations will always be very important. However, a number of problems will be solved more easily or only solved if modern methods are also used, and this is the case with the matters considered here. These concern the development and nature of the membranes surrounding the cells of non-motile *Chrysophyceae* and the flagellation of those species with motile reproductive bodies. It is also the case that the taxonomy of the *Xanthophyceae*, *Chlorophyceae* and *Dinophyceae* will be affected by such new knowledge.

BOURRELLY'S (1957) Monograph is a valuable oversight of the *Chrysophyceae* and is used as a basis for discussion and, unless stated otherwise, the page references given here refer to this work. When his classification is criticised it should be remembered that no satisfactory alternative is yet possible.

BOURRELLY divides the "coccoid" and "palmelloid" forms without known motile stages into *Stichogloeales*, in which the cells have "une membrane ferme bien définie" (p. 117), and *Chrysosaccates*, in which they "ne présentant pas de membrane définie" (p. 291). What, then is this distinction between definite and indefinite?

The *Stichogloeales* multiply by vegetative division or autospores and *Stichogloea* itself he cites as a "type d'état coccoidale" (p. 30). *Phacoschizochlamys* on the other hand he considers to be transitional between coccoid and palmelloid, because the vegetative divisions are accompanied by the throwing off of the mother-cell membrane as in the Green Alga, *Schizochlamys*. *Schizochlamys delicatula* was removed from the *Chlorophyta* to the *Chrysophyta* by SKUJA (1956) as *Stichogloea delicatula* (WEST), and BOURRELLY, who keeps it in this group, places it in *Phacoschizochlamys delicatula* (WEST) nom. nov. We are, therefore, concerned with a species which is rightly removed from *Schizochlamys* because it does not have the pseudocilia possessed by the type species *S. gelatinosa* A. Br. Though it is not directly material to the present discussion it should be noted that KORSHIKOV (1953) keeps *S. delicatula* in the *Chlorophyta* but in a new genus *Schizochlamydella*. I have seen algae agreeing with WEST's (1892) description in the neighbourhood of the type locality (Bowness, English Lake District) and agree with KORSHIKOV's view. If this is so, SKUJA's alga is a Chrysophyceean parallel to *S. delicatula*. From all these viewpoints it does not seem that there is any fundamental structural difference between *Stichogloea* and *Phacoschizochlamys sensu* BOURRELLY and SKUJA. Both are coccoid algae.

It seems that BOURRELLY means by palmelloid something different from many other authors. In *Chrysophyceae* they are non-motile naked forms or such stages of naked motile ones (p. 27), while coccoid forms are those which "ont acquis la membrane définie . . . qui caractérisé la phase coccoidale" (p. 30). FRITSCH (1935 pp. 15—16) on the other hand, means by palmelloid an intermediate state between motile forms and those in which "motility has disappeared during the vegetative phase and is resorted to at times of reproduction". He gives as examples of palmelloid stages species of *Chlamydomonas* and *Chromulina* which sometimes produce non-motile aggregates of cells with much mucilage. Here then we have one genus whose cells have a wall and another whose cells do not. This is the sense in which the term is often used. Sometimes it is used to cover the breakdown of some relatively highly organised plant body into a more or less irregular mass of cells enveloped in mucilage (e.g. in PASCHER 1925, 1937—39). Although, at first sight, this may seem a different usage of the word it is clearly not so in PASCHER's (1937—39 p. 30) case because he says such palmellae consist of protoplasts which ". . . stellen ebenfalls in ihrer Entwicklung gehemmte Schwärmer". The cells may be walled or naked but often possess contractile vacuoles and, sometimes, a stigma too.

Therefore the problem concerning the presence or absence of a wall has become confused with that of palmelloid states or stages. PASCHER's capsal organisation (Kapsal in FOTT 1959a) is similar to FRITSCH's palmelloid state while the coccal organisation (Kokkal in FOTT 1959a) here refers to walled cells ("behäuteten Organisationen", PASCHER 1937—39 p. 33). However, as FOTT (1957a p. 7) points out, the well known comparisons of parallel organisation between algal groups involve, on the one side, numerous naked genera in several classes and, on the other, walled genera in the *Chlorophyceae*. Further, as we shall see, no satisfactory proof of the presence or absence of a wall exists for many genera. ETTL (1956) considers the capsal structure to be that in which the cells retain the contractile vacuoles or stigma, and coccal that in which such organs are absent.

It is also not clear what BOURRELLY means by vegetative division. It would seem to cover two states. First a division in which the whole cell takes part, for on p. 27 he says in reference to the assumption of the palmelloid state by naked motile forms — "La division purement végétative continue à se produire . . .". This, then, is equivalent to the longitudinal division of naked flagellates (binary fission). SCHUSSNIG (1960, chapter 8), with his emphasis on polarity, might disagree, but it is a common feature that polarity is lost as the sedentary state becomes more predominant. This is because the organs determining such views on polarity are those characteristic of flagellate cells, namely flagella, contractile vacuoles and a stigma. BOURRELLY's second type

of vegetative division is seen in filamentous forms. In the *Sphaeridiothraceae*, which include *Chrysonema* and *Nematochrysopsis*, "La multiplication observée est purement végétative (p. 114). In the *Phaeothamniales* (e.g. *Phaeothamnion*, *Apistonema*, *Nematochrysis* etc.) "Multiplication se fait par division végétative et par zoospores". The words "vegetative division" are used by FRITSCH (1935 p. 17) in relation to the cell division of filamentous algae, but he also refers to PASCHER's (1924, 1931a) view that in many (? all) filamentous *Chrysophyceae* cell division is really autosporic. FRITSCH is concerned with algae in which, in cell division, the wall neither divides nor is thrown off. Instead, a new piece of wall is formed at an angle to the longitudinal axis of the thread (e.g. a transverse septum) or parallel to it. Whatever view is taken of cell division in diverse filamentous algae, we are concerned with walled cells so that the process differs from the complete fission of naked cells. PASCHER's (1924, 1931a) view is that, in filamentous *Chrysophyceae*, cell division involves only the protoplasts, which form their own new walls within that of the mother-cell. The extension of this view to algae in general is criticised by FRITSCH (1935 p. 18) and he would appear to be right in saying that "it is difficult to harmonise it with the many accounts . . . of the gradual ingrowth of a dividing septum during cell-division in green filamentous algae". Nevertheless the well-known mixture of coccoid and filamentous stages commonly seen in some "filamentous" *Chrysophyceae* and *Xanthophyceae* (e.g. BOURRELLY 1957 p. 31, VISCHER 1936, 1945) and the production of short threads through clearly autosporic reproduction in *Bumilleriopsis* (VISCHER 1945, Abb. 9—11, here called aplanospores) lend strong support to PASCHER's view so far as these classes are concerned. Since this is the production of autospores in successive pairs or in uniseriate groups, BOURRELLY's (1957) statement that in *Chrysophyceae* "multiplication se fait par division végétative" (*Phaeothamniales*, p. 123) presumably means that he does not agree with PASCHER's view, since, for the same process in coccoid *Chrysophyceae*, he uses the word "autosporulation" (e.g. *Stichoglocales*).

SCHUSSNIG (1960) has produced a complete, albeit complicated, morphological classification for the methods of multiplication and reproduction discussed, as well as for other types not considered here. He (p. 397) includes in schizotomy the processes leading to the production by flagellates of "gloeomorphen" or "palmelloiden" (*Gloeocystis* or palmelloid stages). Here he specifically mentions the pseudociliate green algae, but these do not undergo binary fission like naked flagellates but autospore formation, as indeed can be seen in his own figure 121 on p. 140. It is difficult to follow all the discussion of schizotomy. *Platymonas* and *Haematococcus* are mentioned under schizotomy but *Chlamydomonas* under schizogony and *Stappia* (*Tetraspora*) under both categories. The essential difference is that in schizotomy fission is binary, the two cells . . . "gleich nach vollzogenem Teilungsakt ihre Freiheit erlangen und als solitäre Schwärmzellen umherschwimmen" SCHUSSNIG 1960 p. 407). In schizogony, two to many such schizotomous divisions take place successively and only when all are complete do the daughter cells (schizites) become free. Schizogony then is repeated schizotomy and the difference between the two has nothing to do with the presence or absence of a wall ("innerhalb einer Hülle oder auch ohne seine solche" — SCHUSSNIG 1960 p. 404). However, in the taxonomy of the non-motile *Chrysophyceae*, this is a crucial question and there is no fundamental difference, in walled forms, between the production of two schizites or multiples thereof. Indeed this is true of coccoid algae in general. SCHUSSNIG (1960 p. 408) considers the filamentous *Chrysophyceae* to divide by what may either be called "intrachlamydeische schizotomie" or schizogony of a reduced type resulting in the formation of only two schizites. This is a restatement, in different terms, of PASCHER's (1924, 1931a) views.

It is this absence of agreed and exact terminology and knowledge that makes the classification of non-motile *Chrysophyceae*, and also of some *Xanthophyceae*,

so uncertain. Thus BOURRELLY's (1957) *Chrysosaccles* include four genera of which only one, *Chalkopyxis*, certainly seems to have naked cells, though even here the picture is complicated by the development from a type of cyst. *Phaeosphaera* probably has a wall and so produces autospores (LUND, 1960a), while in *Chrysosaccus* the arrangement of the cells in fours within a mucilaginous investment also suggests the formation of autospores. The reproduction of *Heimiochrysis* (see also BOURRELLY 1949), a pseudociliate genus, is said to be "une division végétative" but here again the cells are arranged somewhat irregularly in fours or eights. Until there is exact knowledge about the cell membranes and early stages in division no decision is possible. It may be that the cells are naked and simply produce mucilage of varying consistency so that they appear to lie within a wall. Such mucilage investments are common in *Chlorophyceae* but there they are commonly derived from the mother-cell walls (e.g. *Paulschulzia*, LUND 1956). The forms belonging to the gloecocystoid group also apparently have walls (but not necessarily the zoospores). BOURRELLY (1957, 1958) says that all such palmelloid forms, which he groups in the *Tetrasporales*, multiply by "division végétative sous forme immobile" as well as by zoospores. This is certainly not so in many of these genera, a striking example being *Schizochlamys gelatinosa* A. BR. In some genera it is true that the mode of subdivision is unclear, for example *Coccomyxa* SCHMIDLE and *Elakatothrix* WILLE, though in *Coccomyxa dispar* SCHMIDLE my opinion, based on personal observations, is that two autospores are produced. Here again a re-examination would be valuable.

Whether the detailed history of these various methods of propagation is known or not, they are so varied that they must involve marked differences in genetic potentialities and are therefore of taxonomic importance. Until they are understood no satisfactory classification of the non-motile genera is possible. Such an understanding may be obtained with the aid of rich, pure natural populations, clone cultures, modern refinements of light microscopy and examination with the electron microscope.

The following terminology, which is not new, is suggested as a preliminary guide to the types of multiplication considered here. The various types whether called vegetative reproduction, vegetative division, asexual reproduction or by other terms all lead to an increase in the population.

In binary fission the cell divides *in toto*, that is any externally differentiated membrane also takes part in the division, irrespective of whether the cell is motile or non-motile.

In zoospore or autospore formation the new protoplasts are either naked or form new walls of their own, but the parental wall is not included in the division stages, although it may fracture, turn into mucilage or disintegrate. Aplano-spores, hemi-autospores (ETTL 1956) or hemizoo-spores (KORSHIKOV 1953) are included here as they are autospores in which structures such as contractile vacuoles and a stigma are present, suggesting that they are arrested zoospores or spores which still have features of the zoosporic ancestor. If these are produced in a row and remain attached a filament is produced. It should be mentioned that the word autospore is used here for all the non-motile endospores of coccoid algae and not in the original sense of CHODAT (1897). This is in accordance with current usage. CHODAT invented the word for the spores of *Lagerheimia genevensis* CHOD., which develop the spines and other characteris-

tics of the adult cell while still within the parental wall, but he himself used it later in the modern sense, for example in *Scenedesmus* (CHODAT 1926) where the daughter cells may or may not have reached the adult form before liberation (LUND 1960b).

It would appear that in all *Chrysophyceae* these spores are produced by successive divisions of the protoplast, that is by schizogony. SCHUSSNIG (1960) uses the term cytogony for their production by simultaneous divisions of the protoplast, citing the production of zoospores in the sporangia of many chytridiaceous fungi as a typical example. Even if simultaneous division is found to occur in some *Chrysophyceae* it must be uncertain what taxonomic emphasis should be laid on it. In coccoid *Chlorophyceae* successive or simultaneous divisions may be found in auto- or zoosporangia of a single genus (e.g. STARR 1955, AHMADJIAN 1960).

In septate division a new piece of wall is produced which, by various methods, forms a septum across the cell and fuses at its ends with the wall of the parent cell. Thus the daughter cells have walls which are partly parental and partly formed *de novo*.

On this view, filaments arise in various ways, by uniseriate autosporeulation septate division, siphonaceous elongation or by the special methods seen in diatoms and desmids. The term vegetative reproduction is not used because it has other meanings in botany.

Before summarising the present position in *Chrysophyceae* the question of the number and types of flagella present in motile stages must be referred to briefly. This, incidentally, is a subject which has been reconsidered as a result of observations with the electron microscope. BURRELLY (1957) separates his orders according to the number and types of flagella present. Thus there may be one flagellum (*Chromulinales*, *Chrysosphaerales*, *Thallochrysidales*), two differing in size and structure (*Ochromonadales*, *Phaeothamniales*, *Chrysapionales*), two of the same size and structure, sometimes with a haptonema between them, (*Isochrysidales*), or one (*Chrysosaccales*, *Phaeoplacales*, *Stichogloeales*). Modern work, much of which is described in his monograph, suggests that several supposedly unflagellate genera are biflagellate and also heteromorphic, while among those which have two flagella of equal length and similar structure are some with heterodynamic flagella. Flagella are described or discussed in, for example, FAURÉ-FRÉMIET et ROUILLER (1957), FOTT (1959b), PARKE, MANTON and CLARKE (1955 to 1959), VON STOSCH (1958), PRAUSER (1958), KORNMAN (1955). If there are only two basic types of flagellation, then PASCHER's (1925, see also FOTT 1959a) preliminary classification of the non-motile *Chrysophyceae* is the best base on which to build.

In table 1 the non-motile *Chrysophyceae* are divided into supposedly walled and naked genera. Rhizopodial, pseudopodial, and certain sessile flagellate genera are obviously naked and so are excluded. Similarly the filamentous genera clearly have cell walls; *Sphaeridiothrix*, however, might be excluded. *Geochrysis* PASCHER appears to have both naked cells and walled ones which may reproduce their kind. Forms which have naked cells within a case or envelope are considered as naked, though this view may not always be correct (e.g. *Chalkopyxis* PASCHER 1931b). Several genera are so doubtful that no proposal can be made, while the *Coccolithophoridae* can be considered later when the frequency of filamentous and other stages in the group has become more apparent. BURRELLY's (1957) nomenclature is used.

Naked

Celloniella
Chalkopyxis
Chrysocapsa p. p.
Chrysocapsella p. p.
Chrysochaete
Chrysonebula
Chrysospora
Chrysotilos
Geochrysis

Gloeochrysis
Hydrurus
Kremastochrysis
Kremastochrysopsis
Naegeliella
Phaeaster
Phaeocystis
Ruttnera
Tetrasporopsis

Walled

Arthroglaea
Aurospira
Chrysocapsa p. p.
Chrysocapsella p. p.
Chrysapion
Chrysosaccus
Chrysosphaera
Chrysotila
Entodesmis

Epicystis
Heimiochrysis
Koinopodium
Nannochrysis
Phaeogloea
Phaeoschizochlamys
Phaeosphaera
Pterococcus
Pterocystis

Pterosphaera
Pterosperma
Pulvinaria
Sarcinochrysis
Selenophaea
Stichogloea
Sphaeridiothrix
Tetrapion

Table 1. Tentative division of certain genera of non-motile *Chrysophyceae* into those with naked and those with walled cells. Nomenclature and generic limits as in BOURRELLY (1957).

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