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Studies on micro algal fine-structure, taxonomy and systematics : cryptophyceae and bacillariophyceae.

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Studies on microalgal fine-structure, taxonomy, and systematics: Cryptophyceae and Bacillariophyceae

In Two Volumes

Volume I (Text)

by

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Abstract

Part I deals with the fine-structure of 25 marine and 3 freshwater strains of cryptomonads, and 2 species observed in fixed marine plankton samples. Emphasis is placed on scanning electron microscope observations, which yield valuable taxonomic data, providing that cell shrinkage (up to about 25 % linear), and other undesirable effects of preparation schedules, are taken into account. The fine-structural information is used for judging the taxonomic value of some cell features. Characters having taxonomic significance at various levels include the cell shape, periplast features (especially the shape and size of the periplast areas), chloroplast number, nucleomorph position, presence of a 'tail', and the presence of a true, non-artifactual furrow on the ventral cell face. A new classification system is proposed. This allows for the existence of three orders, four families, seven pigmented genera, and two colourless ones. Most diagnosis or descriptions of taxa above the generic level are based on only one character. This should make the system capable of accomodating new taxa with little difficulty. The following new taxa are described: *Rhinomonas reticulata* comb. nov., *R. reticulata* var. *atrorosea* comb. et stat. nov., *R. reticulata* var. *compressa* var. nov., *R. reticulata* var. *eleniana* var. nov., *Proteomonas pseudobaltica* comb. nov., *P. pseudobaltica* var. *leonardiana* var. nov., *Pyrenomonas salina* var. *curvata* var. nov., *Pyrenomonadales* ord. nov., *Pyrenomonadaceae* fam. nov., and *Cryptomonadales* ord. nov.

Part II deals with the diatom genus *Mastogloia*. An update of the taxa assigned to this genus is given, together with a 'resemblance list' useful for identifying taxa not included in the main reference work for the genus. The frustule architecture of *Mastogloia smithii* is examined with particular reference to the valvocopula, especially the features involved in its integration with the valve.

Foreword

Whether the facts of nature are only awaiting to be discovered, or whether they are revealed to us with parsimony, is a matter which may be adequately discussed by philosophers alone. In the case of the cryptomonads, however, it is unnecessary to be versed in philosophy for agreeing fully with the second point of view. These organisms have been studied for more than a century and a half, and yet remain an intriguing and mysterious group of algae, protozoans, protists, protoctists, chromists, or ... (?). The first studies on their fine-structure brought about the feeling that their secrets would soon be unlocked. It appears that this promise has not yet been fulfilled: the cryptomonads, which have cast new light on the origin and evolution of the eukaryotic cell, have been extremely reticent about themselves. Have they given all that they can give, or can they tell us more? Could it not be that the notorious difficulties of their taxonomy and systematics are due, at least in part, to some kind of myth? It is hoped that Part I of this work may provide a clue, however modest, towards the answer.

The diatoms are probably the most intensively studied group of microalgae, and yet they still raise numerous questions: for instances, the process of identifying species within the large genera; the structure of the frustule, especially the architecture and integration of the various parts; and the phylogenetic relations between genera. It is hoped that Part II of this work may contribute some information of interest.

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1. THE FATE OF TAXONOMY AND SYSTEMATICS

On 7th December 1989, Professor Arnold Kluge (University of Michigan), gave the Annual Address talk of the Systematics Association at the Linnean Society, London, entitled

Will systematics die in spite of itself?

Perhaps, this was (erroneously) interpreted by the management of the British Museum (Natural History), as recommending that systematics *should* die - at least as far as can be judged by their decision, made during the spring of 1990, to cut down research in many areas where the Museum had gained its international reputation of excellency in the study of systematics. From an analysis of comments made by the scientific community, and reported in journals such as *Nature* and *New Scientist*, it appears that the overall popularity of systematics is at its all-time low. This appears to apply in particular to botanical systematics, which Professor Dr Friedrich Ehrendorfer (Vienna University), will interrogate during the forthcoming (1990) Annual

Address of the Systematics Association, entitled

Quo vadis systematic botany?

A question which comes spontaneously to mind is: *why* is systematics becoming increasingly unpopular? If we accept the point of view of Heracleitos:

> *One can only understand the essence of things when one knows their origin and development*

then that of Theodosius Dobzhansky is wholly justified:

> *Nothing in biology makes sense, except in the light of evolution.*

Also, if we agree with J.P. Thorpe, who stated (Thorpe 1982):

> *Studies of evolution and systematics are so intimately linked that there should not be any clearcut borderline between them*

then we could perhaps replace 'evolution' with 'systematics' in Dobzhansky's phrase. The resulting sentence, which may sound excessively radical, could perhaps be rewritten as follows:

> *(Nearly) nothing in biology makes sense, except in the light of systematics.*

If this is correct, then why is systematics so unpopular among those people who finance scientific

research? One reason could be that the present-day trend in financial investments as a whole is that of favouring all that which is cost-effective, and pays dividends over a short period of time. From that philosophical viewpoint, it is usually believed that science is (or should be) a business-like activity, whose results are judged on the basis of their greater or lesser potential of becoming 'useful', i.e., capable of generating economic wealth. Systematics, on the other hand, despite being a very frugal area of biological research, is time-consuming, and has little economic potential in the sense outlined above.

An alternative explanation could be embedded within systematics itself. It is frequently pointed out that systematics, in its traditional, most widespread approach, has an ill-defined methodology, and subjective opinion is present at virtually every stage of research. (This lies at the very heart of the problem of the instability of scientific names, probably the single aspect of systematics which non-systematists find most irritating: see for instance Crisp & Fogg 1988). As I underlined in a previous thesis (Novarino 1985), this lack of objectiveness has led many investigators to deny that systematics is science at all. Lipscomb (1984) wrote that it is 'not science but learned opinion'. To make things even worse, personal opinion is officially recognized as a valid principle of operative taxonomy: the International Code of Botanical Nomenclature states (Article 32.2):

> *A diagnosis of a taxon is a statement of that which in the opinion of its author distinguishes the taxon from others.*

Deciding what science is and is not goes far beyond the scope of the present work. However, being excessively categorical on the subject could be an unfruitful attitude *per se*. Whatever the reader's opinion on the matter, it should be noticed that classification systems, the principal products of systematic research, will always have some kind of *predictive value*, that is, the property of predicting the features of hitherto unknown organisms. The scientific community may accept, reject, or modify a particular system on the basis of the degree of its predictive value. In other words, we do have a method by which classification systems can be tested, whose general principle does not differ in substance from that used for verifying formal scientific theories.

2. TAXONOMY AND SYSTEMATICS

The words 'taxonomy' and 'systematics' are frequently considered synonyms and, indeed, for sake of simplicity they have been in the discussion made above. This, in fact, is not entirely correct. *Taxonomy*, in its etymological sense, deals with the process of recognizing, delimiting, and naming categories (taxa) of objects of any sort. In the field of biology, its objects are the living organisms; its tools include the rules of biological nomenclature and the various methods of biological identification, for instances, direct comparisons with type-materials, taxonomic keys, and computer databases. The scope of *systematics*, on the other hand, is that of arranging taxa in an orderly scheme that:

1. reflects as much as possible the supposed

phylogenetic affinities between taxa; and

2. is capable of accomodating new information as it becomes available, and new taxa as they are described.

It could be argued that striving for a phylogenetic content within biological systematics is merely a matter of taste, leading to totally arbitrary classification schemes in that phylogenies can never be demonstrated but merely inferred. This seems to be the argument of the phenetic school, at least as far as can be judged on the basis of Minkoff's (1983) comments. Phylogeny is an historical process. Thus, it is true that it can never be known with absolute certainty whether our mental picture of it faithfully reflects what actually happened during its course. Nevertheless, we hardly have any doubts on other historical events, for instances, that in 1789 the French monarchy was overturned by the republican revolution, or that in 1870 Rome was taken over by the Italian unionists. Nothing about those events can be known with absolute certainty, and yet we firmly believe that their being 'true' can undeniably be demonstrated using appropriate methods. Why, then, should our mental attitude towards phylogenetic history be any different?

3. WHY IS THIS THESIS ABOUT MICROALGAL TAXONOMY AND SYSTEMATICS?

It could be asked if writing a taxonomic thesis is something worthwhile in a period when taxonomy and systematics are so unpopular. The main part of this thesis deals with the cryptomonads (Part I). Little is known about the taxonomy of cryptomonads in

particular, and that of phytoflagellates in general. This is rather unfortunate, at a time when the eutrophication of our coasts, lakes, and rivers, results all too often in massive, sometimes dramatic blooms of phytoflagellates, whose precise taxonomic identity may remain unknown even to the specialists.

In addition, the cryptomonads appear to be an excellent source of food for rearing different sorts of animals (e.g., crustacean larvae), both on the experimental and mass (aquaculture) scales (Amjad, pers. comm., Budd, pers. comm.). Here, a better knowledge of cryptomonad taxonomy might eventuallly become useful for choosing the most appropriate diet for a given animal species.

Part II of this thesis deals with diatoms, and almost entirely with a single, large naviculoid genus (*Nastogloia*). The members of this genus may be ecologically important in subtropical or tropical, coastal marine ecosystems, but are notoriously difficult to identify to the species level. The structure of the specialized girdle band characteristic of *Nastogloia* is so variable, that it appears reasonable to cast a doubt on the idea that the genus, as presently conceived, is a monophyletic assemblage. *Nastogloia* is also a 'critical' genus in the context of the evolution of the monoraphid diatoms.

The reasons given above might, alone, justify the present thesis. However, there is a further one, perhaps of equal importance: the microalgae awaken our curiosity, and never cease to provoke admiration for so much beauty at such a small scale.

Part I

Cryptophyceae

Chapter 1

Introduction to Part I (Cryptophyceae)

Chapter outline: 1. General features of the cryptomonads. 2. High-level systematics. 3. Aims of Part I.

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1. GENERAL FEATURES OF THE CRYPTOMONADS

A review of the literature on the fine-structure, taxonomy, and systematics of the cryptomonads from the time of Ehrenberg (1831), up to and including 1974, was made by Santore (1975). The most up-to-date review of the group is that of Gantt (1980). The notes which follow highlight some later, significant advances, which appeared in the literature until early 1990. They also summarize our current concepts on the general features of the group.

The cryptomonads are eukaryotic, mostly photosynthetic organisms. The motile unicell is the most frequent cell type, but palmelloid cells (single, paired or forming irregular colonies), and resting stages (cysts), may also occur. Motile cells have two flagella, both of which are usually pantonematic; apparently, the flagellar hairs may be arranged according to a multitude of patterns (Kugrens *et al.* 1987). The flagella arise from a depression in the anterior or lateral part of the cell. The nature of the depression is a matter of much controversy. Some recent studies (e.g., Hill &

Wetherbee 1986, 1989) support the view of several early light microscopists that the depression may give rise to a groove (furrow) running on the ventral cell face towards the cell posterior. In contrast, other investigations (Santore 1977, 1984) point out that a tubular depression (gullet) is always present, the furrow being an artefact caused by inadequate preparation of the cells for microscopical observation. Large ejectile organelles, which have been variously termed trichocysts, ejectosomes, or ejectisomes, are found underneath the plasma membrane (PM) lining the depression; smaller ones underlie the PM in the remaining regions of the cell.

The features of cell covering, or periplast, are rather complex. Since the first fine-structural observations (Lucas 1970a, Gantt 1971, Hibberd *et al.* 1971, Faust 1974), our knowledge of its structure has progressed considerably. Until it was studied using freeze-fracture methods, it was generally conceived as follows. In one type of periplast, a layer of proteinaceous material, appearing in TEM sections either as a series of discrete 'plates', or a single, sheet-like component, was present underneath the plasma membrane (PM); a 'fuzz' of unknown composition was found outside the PM. The 'fuzz' often appeared to be composed of heptagonal rosette scales (e.g., Santore 1983). In a second type of periplast, both the internal and external layers were composed of discrete 'plates'.

Freeze-fracture studies have revealed more details of the periplast structure (Brett & Wetherbee 1986, Hill & Wetherbee 1986, Wetherbee *et al.* 1986, Kugrens & Lee 1987, Hill & Wetherbee 1988,

1989). The external periplast component appears to be very variable: for instance, it may have a crystalline or fibrillar substructure. The periplast 'plates' are apparently attached to the PM via intramembrane particles (IMPs), presumably protein complexes embedded in the lipid bilayer of the PM.

Photosynthetic cryptomonads have one or two chloroplasts; heterotrophic forms such as *Chilomonas* bear modified plastids (leucoplasts), whereas *Cyathomonas* lacks plastids altogether. Chloroplasts contain chlorophylls *a* and c_2, and phycobiliproteins as the main accessory pigment. Contrary to the red and blue-green algae, where the phycobilins occur in aggregates (phycobilisomes) on the external face of the thylakoid membranes (Bold & Wynne 1985, and references therein), in the cryptomonads these are found within the lumen of the thylakoids, which are frequently arranged in loose pairs (see for instance Dwarte & Vesk 1983). Either a phycoerythrin or a phycocyanin is present in a single cryptomonad species. The spectral characteristics of cryptomonad phycobilins, reviewed by Hill & Rowan (1989), differ from those found in the red and blue-green algae (see for instance Goodwin 1974).

There may be 1, 2, 4, or multiple pyrenoids within the chloroplasts. The double envelope of the chloroplast is enclosed by a further pair of membranes of the endoplasmic reticulum (chloroplast endoplasmic reticulum, CER). Granular starch is present in the space between the chloroplast envelope and the CER (periplastidial compartment).

A peculiar nucleus-like organelle termed 'nucleomorph' also occurs in the periplastidial compartment. One nucleomorph is associated with each chloroplast. It has been shown to contain DNA

(Hansmann *et al.* 1985, 1986, Ludwig & Gibbs 1985), and a recent *in situ* hybridization study (McFadden 1990), showed that there are two types of genes coding for the 5.8S rRNA in cryptomonads; it was inferred that one of these occurs in the nucleomorph. The detection and interpretation of the nucleomorph was probably the greatest single contribution to the knowledge of cryptomonad fine-structure made by Santore and Greenwood in their intensive study of the group (Santore & Greenwood 1976, 1977, Greenwood *et al.* 1977, Santore 1977, 1978, 1982a, b, c, Oakley & Santore 1982, Santore 1983, 1984, 1985, Santore & Leedale 1985, Santore 1986, 1987). Although the nucleomorph had already been noticed in some studies preceding those by these authors, different interpretations were given of its structure. For instance, Lucas (1970a, b), noticed a 'cytoplasmic tongue' in a groove in the pyrenoid of some species. This is now known to represent a nucleomorph. The organelle has been of paramount importance in supporting Lynn Margulis's theory on the origin of eukaryotic cells by serial endosymbiosis (see for instance Margulis 1981), and its extension to include eukaryotes themselves as possible endosymbionts (see for instances Gillot & Gibbs 1980, and Ludwig & Gibbs 1985). There is still some uncertainty on the true paternity of the discovery of the nucleomorph; however, Greenwood (1974) was the first to use the term in the literature.

There is a single, large mitochondrion (Santore & Greenwood 1977, Hill & Wetherbee 1986). This is variously ramified, and bears flattened, finger-like cristae.

The Golgi apparatus is found towards the cell

anterior. In the forms where palmelloid cells occur, it may produce the mucilage which covers the cell surface. The transport of mucilage towards the cell exterior takes place via the anterior depression of the cell.

The nucleus is located towards the cell posterior. In the interphase nucleus, the chromatin is loosely condensed, with scattered masses of tightly condensed heterocromatin occurring here and there in the nucleoplasm.

Reproduction takes place by simple longitudinal division in the motile or non-motile state. Mitosis has been investigated in detail by Oakley (1978), Oakley & Bisalputra (1977), Oakley & Dodge (1973, 1976), and Oakley & Heath (1978). Its salient features are the apparent involvement of the flagellar bases in the initial formation of the mitotic spindle, the breakdown of the nuclear membrane, and the presence of a single chromatin mass at metaphase. There are a few reports on the occurrence of sexual reproduction. Wawrik (1969, 1971), observed gametic fusion in *Chroomonas acuta* and *Cryptomonas* sp.. Hill & Wetherbee (1986), observed two morphological phases of *Proteomonas sulcata*, believed to be diploid and haploid, respectively. Kugrens & Lee (1988), made some fine-structural observations on cellular and nuclear fusion in *Chroomonas acuta*.

The cryptomonads are widely distributed. Although they may occur in subaerial habitats (e.g., snow: Javornicky & Hindak 1970), their ecological importance is greatest in freshwater ecosystems and the sea, especially in the planktonic communities (Beers *et al.* 1980, Booth *et al.* 1982, Chang 1983, Droop 1953, Eriksson *et al.* 1977, Findenegg 1971,

Hecky & Kling 1981, Ilmavirta 1975, Lack 1971, Lackey & Lackey 1963, Pratt 1959, Reynolds 1978, Ruttner 1959, Schanz 1985, Wailes 1939).

2. HIGH-LEVEL SYSTEMATICS

In the past, the position of the cryptomonads within the general systematic framework of living organisms has generated considerable debate. Much of the controversy still exists, although on grounds altogether different from those of past times. The various ideas on the subject can be divided, for sake of clarity, into two groups: 'traditional' and 'modern'. In the traditional approach, the cryptomonads are regarded either as 'animals' or 'plants', while modernists do not consider them as either.

Traditional approaches: zoological systems

In numerous zoological classification systems (Doflein & Reichenow 1928, Borradaile & Potts 1935, Hyman 1940, Kudo 1950, Mackinnon & Hawes 1961, Grell 1973, and Lee *et al.* 1985), the cryptomonads were considered as an order (Cryptomonadina) of the 'protozoan' class Flagellata (Mastigophora) or Phytomastigophorea. There are at least two cases, however, in which the cryptomonads were given a different status: the system adopted by Claus, Grobben & Kuhn (1932), where they were regarded as a family (Cryptomonadidae) of the order Chromomonadina, which included all those members of the class Flagellata 'mit gelben bis braunen Chromatophoren'; and the system of Grassé (1952), where they were raised to the rank of a class ('Cryptomonadines') belonging to the superclass

Flagellata.

Traditional approaches: botanical systems

Pascher (1914) considered the cryptomonads as a class (Cryptophyceae) of the division Pyrrophyta, which also included the Desmokontae and the Dinophyceae. Pascher's scheme was followed in substance by several authors (Fritsch 1935, 1948, Huber-Pestalozzi 1950, Chapman 1962, Klein & Cronquist 1967, and Bourrelly 1970). As early as the late 1930s and the mid-1940s, however, different views were being put forward. Tilden (1937) considered the cryptomonads as a subclass (Cryptomonadineae) of the class Chrysophyceae (division not mentioned), which also included the Diatomeae, Peridineae, Chrysomonadineae, Chloromonadineae, and Euglenineae. Pringsheim (1944) expressed some doubts on the idea that the Cryptophyceae were closely allied to the Dinophyceae ('Although there is possibly some relation to Cryptophyceae, the lines of demarcation are perfectly clear'); however, he did not propose an alternative system, and regarded the Cryptophyceae simply as a class of 'Flagellata'. The substance of Pringsheim's viewpoint seems to have been followed in recent years by Fott (1974), who also commented on the lack of common features between the Dinophyceae and the cryptomonads; like Pringsheim, he avoided assigning the latter to a division, and considered them among his 'residual flagellates', class Cryptophyceae, order Cryptomonadales. Christensen (1962, 1980), by assigning the Cryptophyceae to the division Chromophyta, implied that the class is no more related to the Dinophyceae

than it is to all the other algae containing chlorophylls *a* and *c*. Lee (1980) adopted a system modified from Christensen: the Cryptophyceae were regarded as a class of the Chromophyta but, unlike Christensen, his definition of the division did not take into account the chlorophyll types and was based exclusively on fine-structural features (presence of chloroplast endoplasmic reticulum, and starch outside the chloroplast envelope). Round (1965, 1977), Kimura (1979), and Bold & Wynne (1985), assigned the cryptomonads to the Cryptophyta, a division comprising only the class Cryptophyceae, implying that the cryptomonads are isolated from the Dinophyceae and all other groups of algae alike.

Modern approaches: the systems of Margulis and Cavalier-Smith

The modern approaches are substantially different from the traditional ones, in that little importance is given to the 'plant' or 'animal' character of the organisms. The system of Margulis (1981), can be referred to as the 'protoctistan' one. Here, the cryptomonads are assigned to a phylum (Cryptophyta) of the Kingdom Protoctista. This includes all of the groups of unicellular eukaryotes (*Protista*), as well as closely related groups of multicellular organisms - in practice, the 'algae', 'protozoa', and several groups of 'fungi'. The 'chromistan' scheme (Cavalier-Smith 1986), of which some aspects were further discussed on a later occasion (Cavalier-Smith 1988), considers the Cryptophyta as a subkingdom of the Kingdom Chromista, which 'comprises the brown algae and all

protista having either tubulary ciliary mastigonemes or chloroplast endoplasmic reticulum, or both' (Cavalier-Smith 1986, p. 309). The main features defining the subkingdom are the presence of ribosomes and one or two nucleomorphs in the periplastidial compartment, the bipartite flagellar hairs, and the flattened, finger-like mitochondrial cristae.

The present work follows an established botanical scheme, viewing the cryptomonads as the only class (Cryptophyceae) of the division Cryptophyta, as defined, for instance, by Bold & Wynne (1985), and Lee (1989). This does not necessarily imply that the 'modern' approaches are unsustainable. The choice depends mostly on the fact that following any one of those would mean accepting their numerous implications on the evolution and systematics of living organisms as a whole. It goes without saying that any discussion thereupon would be beyond the scope of this thesis.

The systematics of the cryptomonads below the class level will be discussed in Chapter 8.

3. AIMS OF PART I

The aims of Part I of this thesis are the following:

1. To investigate some aspects of the fine-structure of 25 marine and 3 freshwater strains of cryptomonads, and 2 species observed in fixed marine phytoplankton samples. Emphasis is placed on scanning electron microscope (SEM) observations.
2. To use the fine-structural information for judging the taxonomic value of some cell features, and consequently making some taxonomic proposals.
3. To propose a new classification system for the group.

Chapter 2

Material and methods

Chapter outline: 1. Cultures. 2. Plankton samples from the North Sea. 3. Light microscopy. 4. Scanning electron microscopy. 5. Transmission electron microscopy. 6. Photomicrography. 7. Pigments.

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1. CULTURES

At the inception of this work, as wide a documentation as possible was sought on the cryptomonad strains currently available in culture, with a view to compiling a comprehensive computer database. Much information was obtained from the catalogues of the large culture collections, whereas, on the whole, little input was received from individual workers. Nevertheless, it has been possible to create a database of more than 150 strains. This was done on the author's personal computer, an Amstrad PCW 8512 running dBase II (TM), Masterfile 8000 (TM), and ARC-II (TM) database management programs. A print-out version of the database is given in the Appendix.

Table 2.1 summarizes the collection numbers and original names of the strains investigated. These originated from the Culture Collection of Algae and Protozoa (CCAP), the Plymouth collection (PLY), the Culture Collection at the University of Texas at Austin, U.S.A. (UTEX), the Centre for the Culture of

Marine Phytoplankton, Bigelow Laboratories, West Boothbay Harbor, Maine, U.S.A. (CCMP), the *Sammlung von Algenkulturen*, Gottingen, F.R.G. (SAG), the collection of Dr. R.W. Butcher prior to its relocation at Westfield College, London (BUTCH), the personal collections of Drs I.A.N. Lucas, Marine Science Laboratories, Menai Bridge (IANL), and D. Klaveness, University of Oslo, Norway (DK). A number of strains were also isolated anew by the author (GN) from enrichment cultures started from marine rock-pool sediments, collected at Ynys Faelog, Isle of Anglesey, U.K., on 10th January 1989 (strain GN A), and 14th April 1990 (strain GN Beta). For this purpose, the material (collected in sterile polypropylene 10 ml tubes) was left undisturbed for one week on a laboratory bench, well lit but receiving no direct sunlight. Then, it was diluted approximately one hundred times using sterile culture medium (see below). This was followed by a series of ten dilutions, where the material was diluted ten times at each passage. As soon as cryptomonads were seen growing in large numbers in a given culture, this was used for starting a new dilution series. Following one or more reiterations of the process, several unialgal cultures were established. As an alternative, single-cell isolation was used, alone or in conjunction with the dilution method. This was performed under an inverted microscope at a magnification of x250, using a micropipette fitted to a general use laboratory micromanipulator, slightly modified so that the micropipette could be brought at the height of the microscope stage. (For information on micromanipulator methods in single-cell isolation, see Haller Nielsen 1950, Nicholson 1957, and

Throndsen 1969b.) Apart from the cryptomonad strains examined here, several other interesting phytoflagellates were isolated, and are still being maintained by the author along with the cryptomonads. These include four *Tetraselmis* spp. (strains EPD, PD2, PRA, RPP), one *Pyramimonas* sp. (strain MSRP), one *Chlamydomonas* sp. (strain CHLA), and one *Brachiomonas* sp. (strain BRACHIO). During a visit to Italy in December 1989, a (nearly unialgal) sample of *Haematococcus* sp. was collected from a cattle trough at Podere Santa Maria, Scrofiano, Siena; this is also being grown along with the other strains (strain HAEMA).

All of the marine strains were grown in enriched seawater medium (Erdschreiber). Initially, this was prepared according to the indications of Starr (1978); it gave good results with many strains, but by no means all of them. Strains PLY 403 and 409, and CCAP 978/8, for instance, did not grow at all, whereas strain CCAP 978/11 remained in a permanent non-motile state. By trial and error it was found that all of the 'reluctant' strains grew well when thiamin (0.1 mg/l) and biotin (1 µg/l) were added to Starr's recipe. The freshwater strains were grown in WC medium (Guillard & Lorenzen 1972), modified according to Dr D. Klaveness (personal communication): no buffer, no sodium silicate, and with dibasic potassium phosphate and sodium nitrate at half-strength. As a rule, cultures were maintained in screw-cap Pyrex tubes (10 - 20 ml, half-filled with medium), in an incubator set at 18° C, under a 14:10 photoperiod and with an average irradiance at the surface of the culture vessels of $35 \cdot \mu E \cdot m^{-2} \cdot sec^{-1}$. When larger volumes of culture were required, conical flasks plugged with non-

absorbent cotton wool bungs were used instead (250 ml capacity, with 100 - 125 ml of medium). Routine subculturing was usually carried out monthly, although it was performed more frequently (fortnightly) in the case of rapidly growing or newly established strains, and less frequently (every two months) where the growth rate was slow. All culture media, vessels, and implements, were sterilized prior to use, in an autoclave at 15 p.s.i. (corresponding to a temperature of 120° C) for 20 minutes; vitamin solutions were filter-sterilized through 0.22 µm cellulose acetate filters. Inoculation of cultures and the preparation and handling of media took place under a laminar-flow cabinet. Although the cultures were not axenic, the sterile techniques used ensured the perpetuation of the unialgal condition, and an adequate control of the levels of bacterial contamination.

2. PLANKTON SAMPLES FROM THE NORTH SEA

In addition to the cultured material, Lugol-fixed bottle samples from the North Sea plankton were examined. These were collected during the Natural Environment Research Council (NERC) North Sea Project 1988/89. General information on the project can be found in Howarth (1988). As far as the Cryptophyceae are concerned, interesting samples were those from the southern North Sea (German bight coastal region). Within the framework of contract research work undertaken by the author, routine phytoplankton counts were made using the inverted microscope (Utermöhl) method. While the figures obtained and their seasonal and spatial distribution

are outside the scope of this work, it may be mentioned that estimates of up to 1.67 . 10 ⁶ cells per litre were made for individual species of Cryptophyceae. Using a fixation schedule slightly different from that used for cultures (cf. this chapter, and Chapter 6), a number of samples rich in Cryptophyceae were examined with the scanning electron microscope (see below).

3. LIGHT MICROSCOPY

All observations were done using a Zeiss Standard Universal microscope, fitted with Neofluar or Planapochromatic objectives, using bright-field, polarized, or phase-contrast illumination.

Cells were observed either in the living condition, or after fixation in 10 % Lugol's solution, 2.5 % glutaraldehyde in seawater (marine forms), or 1 % glutaraldehyde in distilled water (freshwater forms). Fixation lasted a few seconds, and was carried out in a pipette previously rinsed several times with fixative.

Measurements were taken using a calibrated eyepiece, at a magnification of x625. Measuring live material required waiting for cells to slow down, and finally come to a halt. This process seemed to occur frequently during light-microscopical observation, although a little time was usually required for it to develop; it was probably related to the evaporation of liquid from underneath the cover-slip. Unfortunately, comparatively few cells were measured. This was because many cells swam away or collapsed shortly after settling down. A simple perfusion chamber was constructed and tested, in the hope that it might eliminate the

problem. In the chamber, evaporation was indeed reduced, but to such an extent that very few cells came to a halt at all.

4. SCANNING ELECTRON MICROSCOPY (SEM)

The standard procedure was as follows:

a) collection of the material using a centrifuge operating at ca. 300 - 400 *g*;

b) primary fixation of the pellet in 3 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, osmotically balanced with 0.25 M sucrose (overnight at 4° C);

c) 3 rinsees (10 minutes each) in buffer with decreasing amounts of sucrose (0.125 M, 0.0625 M, no sucrose);

d) post-fixation in 1 % osmium tetroxide in buffer (1 - 2 hours at room temperature);

e) 3 rinses (15 minutes each) in large volumes of distilled water;

f) dehydration in a graded acetone series (30% - 50% - 70% - 90% - 100% - 100%), 5 - 10 minutes at each concentration (except for the two final steps, where dehydration time was doubled);

g) critical point-drying using a Polaron apparatus, with CO_2 as the transition fluid;

h) 'cool' sputter-coating at 2.5 kV and 18 mA, with a platinum target placed at 5 cm from the specimen stage (10 minutes).

All fluids (fixatives, etc.) were filtered through a 0.22 µm membrane filter immediately prior to use. Alternative fixation schedules were used, as already mentioned, for the North Sea samples (Chapter 6), and also for *Hemiselmis virescens* (Chapter 3).

Critical point-drying was carried out on Nucleopore or Millipore 'Isopore' 13 mm polycarbonate filters (pore diameter = 2 μm). A container based on that of Marchant (1973) was used. 'Beem' capsules, normally used for inclusion of specimens in resin for transmission electron microscopy, were modified as follows. The pointed end was removed. A filter was placed at one end of the capsule, where it was held firmly in place by a capsule top, previously pierced using a thin soldering iron. After introducing the cell suspension (in absolute acetone) inside the capsule, this was sealed at the other end with another filter and pierced top. After critical point-drying, the filters were carefully removed and placed on aluminium stubs using double-sided sticky tape, for sputter-coating and subsequent observation.

Viewing was with a Cambridge Stereoscan 120, operated at 15 kV, with the specimen stage untilted, a working distance of about 8 - 10 mm, a 100 nm final aperture, and a small beam size. Under those conditions, a maximum resolution of ca. 20 nm was achieved on appropriately fixed, dehydrated, critical point-dried, and sputter-coated material; this is roughly 2 - 2.5 times greater than the maximum theoretical limit indicated by the manufacturer. Extensive use was made of differential contrast correction, especially for observing the details of the periplast pattern at high magnification. The magnification range for taking micrographs was roughly between x5,000 - x45,000. Micrographs were usually taken using the slowest scan speed available on the microscope (200 seconds); although time-consuming, this greatly improved the signal-to-noise ratio on the

photographic negatives.

Measurements of cell length, thickness, and width were made using the point-to-point measuring facility of the microscope. Thanks to the latter it was also possible to make rough estimates of the flagellar length. This was done using a length of flexible, non-elastic wire, which was first superimposed as accurately as possible on the image of the flagellum on the SEM screen, then straightened, and finally measured using the microscope facility. Although the method sufferred from systematic error, introduced mainly by the curvature of the SEM screen, it was considered acceptable for the purposes of the thesis; in any case, the amount of error is probably less than that made whilst estimating flagellar length with the light microscope. Periplast features were measured on enlarged photographic negatives (final magnification: between x50,000 - x100,000), using a caliper calibrated against an enlarged scale of known length. Owing to the fact that the specimen stage was always kept untilted, measurements were not corrected to compensate for tilt angle.

5. TRANSMISSION ELECTRON MICROSCOPY (TEM)

The standard preparation procedure was as for SEM up to stage f (dehydration) included. Following this, dehydrated cells were:

a) infiltrated with a mixture of 100 % acetone and Spurr's (1969) resin, in equal proportions (24 hours at room temperature);

b) infiltrated with pure resin (24 hours at room temperature); and

c) embedded in pure resin (24 hours at 70° C,

followed by 48 - 72 hours at room temperature). Throughout steps a - b, the specimen containers were kept under constant agitation, provided by a revolving stage operating at 2 rpm.

Cured blocks were trimmed by hand under a stereomicroscope, and sectioned with glass or diamond (Diatome) knives using a Reichert OM U3 ultramicrotome. Sections were collected on uncoated copper grids (mesh sizes: 300, 400, or 1000).

Grids were double-stained with uranyl acetate (2 % acqueous solution, 1 - 2 hours) and Reynold's lead citrate (5 - 10 minutes according to the age of the staining solution), using a Hiraoka staining dish. Solutions were filtered through a 0.22 µm membrane filter immediately prior to use. In order to minimize the formation of precipitates on the sections, grids were rinsed briefly in a 0.05 M NaOH solution after staining with lead citrate.

Grids were viewed with a Philips EM 301 operating at 80 kV, or an AEI Corinth Kratos operating at 60 kV.

The osmium-thiocarbohydrazide-osmium method was used as alternative fixation schedule for strains of *Rhinomonas* (Chapter 4), and *Chroomonas* (Chapter 7). It is described in detail in Chapter 4.

6. PHOTOMICROGRAPHY

For light microscopy and SEM, Kodak Panatomic-X and Technical Pan roll films (6 x 9 cm frame size) were generally used, although a number of micrographs were taken on Kodak Plus-X Pan and Kodak T-Max 400. All films were developed in Kodak HC-110, dilution B. Several combinations of exposure settings and development times were tried, with a view to

obtaining the maximum contrast compatible with small grain size; the following ones gave acceptable results:

a) Panatomic-X exposed at 100 ASA, developed for 7 minutes at 20° C;

b) Technical Pan exposed at 200 ASA, developed for 11 minutes at 20° C;

c) Kodak Plus-X exposed at 200 ASA, developed for 7 minutes at 20° C; and

d) T-Max 400 exposed at 400 ASA, developed for 6 - 7 minutes at 20°C.

Combinations b) and a) yielded the smallest grain size, and d) the maximum contrast. All combinations showed some exposure latitude, except for b) which did not tolerate any degree of over- or underexposure.

The TEM negatives were developed by Mr D.A. Davies, School of Biological Sciences, Bangor, according to the standard schedules used in his laboratory.

Photographs were printed on Ilford Ilfospeed Multigrade III RC Deluxe paper, using a Durst Laborator enlarger fitted with a Schneider Componon 105 mm lens.

7. PIGMENTS

The spectral properties of crude phycobilin extracts from five strains were briefly investigated for taxonomic purposes. Extracts were obtained by repeatedly freezing and thawing cell pellets in 0.05 M phosphate buffer, pH 6.8, in light-proof polyethylene tubes. Care was taken to avoid exposure to light during subsequent handling of the extracts. Debris was eliminated by centrifuging for 30 minutes

at 4000 *g*. Absorbance of the extracts in the range 400 - 700 nm was measured with a Unicam SP500 spectrophotometer. Designation of spectral types of phycobilins follows Hill & Rowan (1989).

Table 2.1. Strains studied in this thesis. (*) = freshwater strain

Strain	Name given in collection catalogue (new names in brackets)
BUTCH 11/8	*Chroomonas salina* Butcher f. *adolescens* Butcher (*Pyrenomonas* sp.)
BUTCH 16/1	*Chroomonas atrorosea* Butcher (*Rhinomonas reticulata* var. *atrorosea* comb. et stat. nov.
CCAP 978/8	*Chroomonas placoidea* Butcher
CCAP 978/11	*Chroomonas collegionis* Butcher
CCAP 979/9	*Cryptomonas pseudobaltica* Butcher (*Proteomonas pseudobaltica* comb. nov.)
CCAP 979/18	*Cryptomonas* sp.
CCAP 995/2	*Rhodomonas* sp. (*Rhinomonas reticulata* comb. nov.)
CCMP 3C	*Chroomonas salina* (Wislouch) Butcher (*Rhinomonas reticulata* comb. nov.)
DK N 750301	*Rhodomonas lacustris* (Pascher & Ruttner) Javornicky (*)
GN A	*Proteomonas pseudobaltica* comb. nov.
GN BETA	*Proteomonas pseudobaltica* var. *leonardiana* comb. nov.
IANL 97825/C	unidentified (*Rhinomonas reticulata* var. *eleniana* var. nov.)
IANL E.O.	unidentified (*Pyrenomonas salina* var. *curvata* var. nov.)
IANL M	unidentified (*Rhinomonas* sp.)
IANL Rhodo	*Rhodomonas baltica* Karsten (*Rhinomonas reticulata* comb. nov.)
IANL TB.O	unidentified (*Rhinomonas reticulata* var. *compressa* var. nov.)
IANL Theta/C	unidentified (*Rhinomonas reticulata* comb. nov.)
PLY 157	*Hemiselmis virescens* Droop
PLY 175	*Cryptomonas maculata* Butcher (?*Pyrenomonas maculata* [Butch.] Santore)
PLY 355	unidentified (*Rhinomonas reticulata* comb. nov.)
PLY 358	*Cryptomonas reticulata* Lucas (*Rhinomonas reticulata* comb. nov.)
PLY 375	*Cryptomonas reticulata* Lucas (*Rhinomonas reticulata* comb. nov.)
PLY 403	*Cryptomonas* sp. (*Rhinomonas reticulata* comb. nov.)
PLY 409	*Chroomonas* sp. (*Rhinomonas reticulata* comb. nov.)
PLY D	*Hemiselmis rufescens* Parke
SAG 26.80	*Cryptomonas* sp., formerly *Rhodomonas* sp. (*Cryptomonas marssonii* Skuja) (*)
SAG 980-1	*Chroomonas* sp. (*)
UTEX LB 2422	*Chroomonas diplococca* Butcher

Chapter 3

Observations on *Hemiselmis rufescens* and *Hemiselmis virescens*, with particular reference to the periplast

Chapter outline: 1. Introduction. 2. Material and Methods. 3. Observations. 4. Discussion.

Chapter summary: In scanning electron microscopy, the periplast of *Hemiselmis rufescens* strain PLY D is usually indistinct. However, it may also show hexagonal periplast areas, whose shape and size compare favourably with those shown for *Hemiselmis brunnescens* using freeze-fracture/etch techniques. Rectangular areas, probably arising from preparation artifacts, can also be present. Hexagonal periplast areas are also present in *Hemiselmis virescens* strain PLY 157. *H. rufescens* being the type-species of its genus, the hexagonal areas should be considered as a distinctive feature of the genus *Hemiselmis* as a whole. This contrasts with the view that the genus is characterized by a periplast with rectangular areas. A specimen named *H. virescens*, of unknown origin, was examined using transmission electron microscopy; the internal periplast component and the fibrous layer on the outer surface of the cell are apparently missing. The observations underline the usefulness of scanning electron microscopy for providing taxonomic information on the Cryptophyceae.

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1. INTRODUCTION

The morphology of the periplast in the genus *Hemiselmis* Parke has been studied in scanning electron microscopy (SEM) by Santore (1977, 1982b). Periplasts showing a more or less regular array of rectangular 'plates' were described for *H. brunnescens* Butcher (1967), strain 984/2 from the Culture Collection of Algae and Protozoa (CCAP), and strain 14 from the Plymouth Collection (PLY); and also for *H. rufescens* Parke (1949), strain CCAP 984/1, which is descended from strain PLY D used during the original description of the genus. (Strain D was studied using transmission electron microscopy by Lucas 1970b). The plates were visible only in collapsed cells; in uncollapsed cells, smooth periplasts were seen. In *Hemiselmis virescens* Droop (1955), strains CCAP 984/5 and PLY 157, a member of the genus for which adequate fixation is apparently difficult to achieve, the periplast was tentatively described as having a smooth surface (Santore 1982b). Wetherbee *et al.* (1986) re-examined the CCAP strain of *H. brunnescens* used by Santore (1982b). The SEM yielded little information, whereas freeze-fracture/etch techniques showed that the surface component of the periplast was made up, among others, of more or less hexagonal plates having a crystalline substructure, and delineated by 'plate borders'.

The present chapter reports some interesting or previously undescribed features of the periplast of *Hemiselmis rufescens* and *Hemiselmis virescens*, as revealed by the SEM. It is believed that the observations:

1. contribute to a modification of the commonly

accepted concept of the genus *Hemiselmis*; and

2. support the view than SEM studies can yield useful information on the periplast of the Cryptophyceae, adequate for taxonomic purposes.

Unfortunately, owing to difficulties in achieving good fixation, it was not possible to examine strains PLY D and 157 with the transmission electron microscope. However, adequate sections were obtained of a specimen named *Hemiselmis virescens*, of unknown origin, whose periplast and vestibular complex show some interesting features.

2. MATERIAL AND METHODS

Hemiselmis rufescens, strain PLY D, and *Hemiselmis virescens*, strain PLY 157, were grown as described in Chapter 2. Cells of *H. rufescens* were prepared for SEM according to the standard procedure (Chapter 2). Cells of *Hemiselmis virescens* were filtered directly onto polycarbonate filters. Fixation was based on that used by Green & Manton (1970) for preserving the cell surface of *Pavlova gyrans*, and consisted in a brief application (20 mins at 0°C) of a 0.5% solution of osmium tetroxide in buffer; rinsing, dehydration, drying and coating were as for *H. rufescens*.

A resin block labelled '*Hemi. viresc.*' was obtained from Dr I.A.N. Lucas; strain details and fixation and embedding schedules are unknown. Sections were made and observed as described in Chapter 2.

3. OBSERVATIONS

As far as the general appearance of cells is

concerned, some changes in size were noted with respect to unfixed cells measured by light microscopy. The linear shrinkage of *Hemiselmis rufescens* was estimated as 17.5 - 22.2 %, and was statiscally significant (*t* test). *Hemiselmis virescens* showed approximately 2.6 - 3.6 % of linear swelling, but these figures were not significant (Tables 3.1 & 3.2).

The majority of observed cells of *H. rufescens* had a smooth periplast, similar to that illustrated in Pl. 2, Fig. 1. However, several cells possessed a periplast similar to that seen in Pl. 2, Fig. 2. This was composed of hexagonal or roughly circular periplast areas (PA's), delineated by a lattice showing a repeated hexagonal or, less frequently, heptagonal pattern. At higher resolution the PA's had a finely granular appearance, due to the presence of particles ca. 25-40 nm in diameter. The delineating lattice was also granular, although more coarsely so than the PA's. Cells similar to that shown in Pl. 2, Fig. 3 were also observed. Here, the PA's were irregularly polygonal to rectangular. They had a coarse texture, with particles several times larger than those seen in Pl. 2, Fig. 2. Owing to the fact that the particles of the delineating lattice were similar in size to those of the PA's, the lattice itself could only be made out at high resolution and by making extensive use of differential contrast correction.

The periplast of *Hemiselmis virescens* (Pl. 3) was somewhat more difficult to visualize than that of *H. rufescens*. Some cells possessed PA's and hexagonal delineating lattice (Pl. 3, Fig. 1). In other cells, the lattice was replaced by fissures (Pl. 3, Fig. 3). As in the case of *H. rufescens*, the

majority of cells had a periplast showing little detail (Pl. 3, Fig. 2).

Plate 4 shows some interesting features of the '*Hemi. viresc.*' specimens in TEM sections. The vestibular complex appeared to be composed of a long longitudinal gullet lined with large ejectosomes, and a shorter dorso-ventral branch (= vestibule *sensu* Santore 1982b), without ejectosomes (Pl. 4, Fig. 1); the spatial relationships between the vestibular complex and the flagellar bases were unclear. The periplast was composed of a dense layer on the external face of the plasma membrane (PM) (Pl.4, Figs 3, 4); this layer was approximately as thick as the PM, and showed several interruptions, usually in the proximity of peripheral ejectosomes (Pl.4, Fig 4). Grazing sections (Pl. 4, Fig. 2) often showed profiles of regularly arranged tubules of the endoplasmic reticulum.

4. DISCUSSION

The degree of shrinkage of *H. rufescens* is within the range of the figures obtained for a wide variety of strains examined during the course of this work; a more detailed account of shrinkage associated with the preparation of cells for SEM will be given in Chapter 6. On the other hand, the size of *Hemiselmis virescens*, fixed according to the cold osmium schedule, remains more or less unaltered. Hayat (1981) reported that fixation based on osmium alone may cause a small amount of cellular swelling. If this is the case, then any swelling of *H. virescens* must have been largely counteracted by shrinkage occurring during critical point-drying.

The indistinct appearance of the periplast in

the majority of observed cells may derive, perhaps, from the presence of an outermost layer of material on the periplast areas and the delineating lattice. Fibrous material was observed by Santore (1982b) on the cell surface of *H. brunnescens* and *H. virescens*; Santore also noted that the material was occasionally absent; therefore, he suggested that it could be removed during fixation. This could be the case of the '*Hemi. viresc.*' specimens sectioned here, where no such material was visible.

The hexagonal shape of the periplast areas of *H. rufescens* and *H. virescens*, as revealed by SEM, is similar to that shown for *H. brunnescens* using freeze-fracture/etch (Wetherbee *et al.* 1986). Thus, it is probably not artifactual. By contrast, the rectangular or irregularly polygonal shapes seen in *H. rufescens* could be artifactual. Apparently, these shapes are associated with a coarsely granular appearance of the PA's. This may derive, perhaps, from the coalescence of the 25 to 40 nm particles into larger aggregates, causing at the same time a change in shape of the PA's. It could also be asked whether a rearrangement of peripheral ejectosomes, known to bear a spatial relationship with the periplast in *H. brunnescens* (Wetherbee Hill & McFadden, 1986) may have any consequences on the shape of the PA's. In *H. rufescens*, however, peripheral ejectosomes are apparently absent (Santore 1977), or found only occasionally (Santore 1982b).

The '*Hemi. viresc.*' specimens are interesting in several respects. Apparently, there is no internal periplast layer, present in all examined members of *Hemiselmis* (Santore 1977, 1982b, Brett & Wetherbee 1986). To the author's knowledge, the only

other report of a cryptomonad visibly lacking one of the expected periplast layers is *Chroomonas africana*, where the external periplast component is missing (Meyer & Pienaar 1984). In addition, the morphology of the vestibular complex of the specimens is somewhat similar to that found in the genus *Chroomonas*, where there are also two gullet branches, one with ejectosomes and the other without (Meyer & Pienaar 1984, Santore 1987, this thesis, Chapter 7). However, there is a difference in that in *Chroomonas* the ejectosomes line the dorso-ventral branch, whereas in the '*Hemi. viresc.*' specimens they line the longitudinal branch.

From the viewpoint of systematics, it is interesting to note that hexagonal periplast areas are present in all of the species of *Hemiselmis* currently available in culture, including the type-species. This strongly suggests that this character should be considered as a distinctive feature of the genus *Hemiselmis* as a whole, in contrast with Santore's (1977, 1982b, 1984) view that the genus is characterized by a periplast with rectangular 'plates'. Butcher (1967), recognized two subgeneric divisions, based on the cell colour: *Hemiselmis* (red), and *Plagiomonas* (green).

Table 3.1. Comparison of cell sizes in *Hemiselmis rufescens* strain PLY D, measured with the light microscope (LM: unfixed material), and the scanning electron microscope (SEM; fixed, critical point-dried material). Measurements (in µm) are given as Mean ± Standard Deviation.

Parameter	Measured with the LM	Measured with the SEM	Difference (LM - SEM)	*t*, degrees of freedom, and *p*
Length	6,3 ± 1,1	5,2 ± 0,8	1,1 (= 17,5 %)	3,99 (56; $p < .001$)
Thickness	3,6 ± 0,5	2,8 ± 0,4	0,8 (= 22,2 %)	6,51 (52; $p < .001$)

Table 3.2. Comparison of cell sizes in *Hemiselmis virescens* strain PLY 157, measured with the light microscope (LM) on unfixed material, and with the scanning electron microscope (SEM) on fixed, critical point-dried material. Measurements (in µm) are given as Mean ± Standard Deviation. * = not significant.

Parameter	Measured with the LM	Measured with the SEM	Difference (LM - SEM)	t and degrees of freedom
Length	5,6 ± 1,7	5,8 ± 0,6	- 0,2 (= 3,6 %)	* - 0,33 (13)
Thickness	3,8 ± 0,4	3,9 ± 0,5	- 0,1 (= 2,6 %)	* - 0,34 (13)

Chapter 4

Observations on new varieties and combinations in the genus *Rhinomonas*, with comments on the genera *Pyrenomonas* and *Rhodomonas*

Information contained in the present chapter was included in the following papers:

Novarino G., 1991 - Observations on *Rhinomonas reticulata* comb. nov. and *R. reticulata* var. *eleniana* var. nov., with comments on the genera *Pyrenomonas* and *Rhodomonas*. *Nordic Journal of Botany*, 11(2) [in press]; and

Novarino G., in prep. - Observations on some new and interesting Cryptophyceae, with a diagnosis of the family *Pyrenomonadaceae* fam. nov. Submitted to *Nordic Journal of Botany*.

Chapter outline: 1. Introduction. 2. Material and methods. 3. Diagnoses. 4. Observations. 5. Discussion.

Chapter summary: Nine marine, red strains of Cryptophyceae were examined using scanning and transmission electron microscopy. Eight of these, possessing a nucleomorph housed within the pyrenoid, and a periplast showing distinctly hexagonal periplast areas, are assigned to *Rhinomonas reticulata* comb. nov. and its varieties. *Rhinomonas reticulata* var. *atrorosea* comb. et stat. nov. and *Rhinomonas reticulata* var. *compressa* var. nov. are described based upon the lateral compression of the

cells. *Rhinomonas reticulata* var. *elenïana* var. nov. is described on the basis of the smaller size of the periplast areas. In a strain assigned to the genus *Pyrenomonas*, which *Rhinomonas* otherwise resembles, the periplast areas are more or less rectangular. Since it is impossible to know with certainty which red, marine forms fit into the original concept of the genus *Rhodomonas*, the name *Pyrenomonas* should be preferred to a recent emendation of *Rhodomonas*.

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1. INTRODUCTION

In the previous chapter, it has been shown that scanning electron microscope studies (SEM) of the cryptophycean periplast may yield useful taxonomic information. In the present one, SEM observations are combined with information on internal cell fine-structure derived from transmission electron microscopy (TEM). These are used for addressing a specific taxonomic question, namely, the identity and position of several 'red' marine forms, containing one of three spectral types of phycoerythrin as an accessory photosynthetic pigment. (For a review of the phycobiliproteins of the Cryptophyceae, see Hill & Rowan 1989.)

As far as can be inferred from Butcher's (1967) monograph, in marine habitats the red forms seem to outnumber the blue-green ones, containing phycocyanin instead of phycoerythrin. Indeed, this is also the impression gathered here during the process of establishing cultures from various marine samples: red forms always appeared promptly in the

raw cultures, whereas blue-green ones were rarely seen. Despite the red forms being of common occurrence, their taxonomy and systematics are very uncertain; the difficulties which may be encountered while attempting to identify specimens with the light microscope, discouraging to say the least.

With the exception of two phycoerythrin-containing members of the genus *Hemiselmis* Parke (*H. rufescens* Parke and *H. brunnescens* Butcher), and omitting some poorly known genera (e.g., *Isoselmis* Butcher and *Plagioselmis* Butcher), the red forms are or have been accommodated at various times in the genera *Cryptomonas* Ehrenberg (1831), *Rhodomonas* Karsten (1898), *Chroomonas* Hansgirg *sensu* Butcher (1967) (subgenus *Cryptochrysis*), *Pyrenomonas* Santore (1984) (= *Chroomonas* Hansg. *sensu* Butch. subgenus *Cryptochrysis*), *Proteomonas* Hill & Wetherbee (1986), *Rhinomonas* Hill & Wetherbee (1988), and *Rhodomonas* Karsten *emend.* Hill & Wetherbee (1989), of which *Pyrenomonas* was considered a synonym. A comparison of the general fine-structural features of these genera shows that the position of the nucleomorph is a diacritical character. In *Rhinomonas* and *Pyrenomonas / Rhodomonas emend.* Hill & Wetherbee the nucleomorph, unlike *Cryptomonas* and *Proteomonas*, is positioned in a groove within the pyrenoid (Santore 1984, 1985; Hill & Wetherbee 1986, 1988, 1989). A discriminating feature between those genera with a nucleomorph housed within the pyrenoid is given by the morphology of the cell covering (periplast). In *Pyrenomonas / Rhodomonas emend.* Hill & Wetherbee, this is composed of small, more or less rectangular 'plates' on the internal face of the plasma membrane, and a fibrillar or finely granular layer

on the external face (Santore 1984, 1986; Hill & Wetherbee 1989). In *Rhinomonas* the periplast is composed of hexagonal plates, on the external face of the plasma membrane as well as on the internal one (Hill & Wetherbee 1988).

Eight marine, red strains of Cryptophyceae were observed, which showed a combination of nucleomorph and periplast features that do not ally them to the genera *Cryptomonas*, *Proteomonas*, *Pyrenomonas / Rhodomonas emend.* Hill & Wetherbee, nor to the genus *Rhinomonas* with its present definition. This has lead to a modification of the concept of the latter genus, and the description of a new combination and a new variety. Some comments are also made on the genera *Pyrenomonas* and *Rhodomonas emend.* Hill & Wetherbee.

2. MATERIAL AND METHODS

Details of the examined strains are given in Table 4.1. All strains were cultured and prepared for electron microscopy according to the standard schedules described in Chapter 2. The osmium-thiocarbohydrazide-osmium method (OTO) was used for some strains as an alternative TEM fixation schedule, with a view to enhancing the contrast of the plasma membrane. The method was based on that of Willingham & Rutherford (1984), and consisted in a 15 minute treatment in thiocarbohydrazide at room temperature (1 % aqueous solution, dissolved by sonicating for 10 minutes, and filtered through a 0.22 um membrane filter prior to use) after post-fixation in osmium tetroxide. Following 2 rinses in buffer, cells were re-osmicated (10 minutes in 1 % osmium tetroxide in buffer at room temperature),

after which they were rinsed, dehydrated, embedded, sectioned, and stained according to the standard preparation schedule. Measurements of cell size in SEM were taken using the point-to-point measuring facility of the microscope, whereas measurements of the periplast areas were made on enlarged photographic negatives (final magnification: x 50,000 - x 100,000).

Crude phycobilin extracts were studied as outlined in Chapter 2.

3. DIAGNOSES

Rhinomonas reticulata (Lucas) Novarino combinatio nova
Basionym: *Cryptomonas reticulata* Lucas 1968: 535 (fig. 1B holotype, figs 6, 7 paratypes). Synonyms: *Pyrenomonas reticulata* (Lucas) Santore 1986: 80; *Rhodomonas reticulata* (Lucas) Hill & Wetherbee 1989: 157.
Plates 5A (p.p.), 5B - 5D, 8 - 10 (p.p.), 25, 27 - 29, 31

Rhinomonas reticulata (Lucas) Novarino var. *atrorosea* (Butcher ex Hill & Wetherbee) Novarino comb. et stat. nov.
Basionym: *Rhodomonas atrorosea* Butcher ex Hill & Wetherbee 1989: 156. Synonym: *Chroomonas atrorosea* Butcher 1967: 33. Typ. non desig. Lectotype sec. Hill & Wetherbee 1989: Butcher 1967: pl. 2, fig. 11.
Plate 6

Rhinomonas reticulata (Lucas) Novarino var. *compressa* var. nov.
A typo cellulis lateraliter compressis differt.

Holotypus: Tabula 7 Figura 2, ex cultura mihi 'IANL TB-0'.

Differs from the type in the laterally compressed cells.

Holotype: Plate 7 Figure 2, from culture IANL TB-0. Plate 7

Rhinomonas reticulata var. *eleniana* Novarino var. nov.
A typo areis periplastis minoribus differt (latus hexagonorum circiter 0.22 µm longus).
Holotypus: Tabula 5A Figura 5, ex cultura mihi 'IANL 97825/C'.

Differs from the type in the smaller periplast areas (side of the hexagons about 0.22 µm long).

Holotype: Plate 5A Figure 5, from culture IANL 97825/C, which originally grew as a contaminant of strain no. 978/25 (*Chroomonas virescens*) from the Culture Collection of Algae and Protozoa, Oban, U.K. Plates 5A (Fig. 5), 5E, 9 (p.p.), 18, 19

4. OBSERVATIONS

Rhinomonas reticulata et var. are red forms, containing type I phycoerythrin of Hill & Rowan (1989) (Pl. 31B, Fig. 5).

Cells of *Rhinomonas reticulata* et var. *eleniana* (strains Rhodo, Theta/C, 3C, 995/2, 358, and 97825/C) were elliptic in shape, uncompressed (Pl. 5A). Cell sizes, measured with the SEM, are given in Table 4.2. When comparing these values with measurements of unfixed cells made with the light microscope, up to about 23% linear shrinkage should

be taken into account (this thesis, Chapter 6). The cell covering (Pl. 5A, Pl. 8 Figs 1-3, 5) was composed of hexagonal, at times roughly circular periplast areas (PA's), having a granular appearance and delineated by a lattice. This itself showed a repeated hexagonal pattern of variable size (Table 4.3). The lattice appeared as a network slightly raised above the cell surface and composed of particles about 1.5 times larger than those making up the PA's (strain Theta/C). In *Rhinomonas reticulata* var. *reticulata*, var. *atrorosea* and var. *compressa* (strains Rhodo, Theta/C, 3C, 995/2, 358, 16/1 and TB-0), the periplast areas were larger than var. *eleniana* (strain 97825/C): the mean length of the hexagon side was between 0.28 - 0.43 µm, as opposed to 0.22 µm in the new variety. Variations in the periplast pattern, consisting of one or two unornamented regions on the ventral side of the cells (Pl. 8, Figs 1, 5), were observed in strains 358 and 3C. In sections, these regions corresponded to internal cell components, presumably reserve materials, bulging towards the cell surface (Pl. 8, Fig. 4).

By contrast, in strain 11/8 the periplast pattern was rectangular, or composed of elongated six-sided polygons, with PA's between 0.4 - 0.7 µm in length and 0.2 - 0.3 µm in width (Pl. 8, Figs 6, 7). TEM sections showed that the nucleomorph is housed within the pyrenoid. The combination of periplast and nucleomorph features assigns strain 11/8 to the genus *Pyrenomonas*.

Sections showed that the periplast of *Rhinomonas reticulata* et var. was composed of an electron-dense layer (IPL) on the internal face of the plasma membrane (PM), and a layer of different

appearance on the external face (Pl. 9, Figs 3-7). The IPL was not continuous, in that it was always interrupted in the vicinity of the peripheral ejectosomes; the size of the areas resulting therefrom was comparable to that of the PA's seen with the SEM. The thickness of the IPL varied between a minimum of about 2.5 nm (strain 3C), to a maximum of about 8 nm (strain 995/2), with an average of about 5.5 nm. The distance separating the IPL from the PM was also variable. In strain Theta/C, for instance, it was only possible to discern the IPL in regions where the PM was swollen (Pl. 9, Fig. 3). In strains Rhodo, 97825/C, 3C, and 995/2, on the other hand, a gap was always visible (Pl. 9, Figs 4-7). The additional layer on the external face of the PM was composed either of fine fibrils projecting more than 100 nm away from the cell surface (strain Rhodo, Pl. 9 Fig. 4), or else appeared granular and was only a few nanometres thick (e.g., strains 97825/C, Pl. 9 Fig. 5, and 995/2, Pl. 9 Fig. 7). It was more easily observed when cells were prepared according to the OTO schedule (Pl. 9, Figs 4, 5, 7).

The features of the nucleomorph of *Rhinomonas reticulata* et var., and also of *Pyrenomonas* sp. (strain 11/8), are conveniently illustrated by those observed in strain Theta/C (Pl. 9, Figs 1, 2). It was always single, and located in a groove in the pyrenoid, from which it was separated by the double membrane of the chloroplast envelope. It was not possible to examine strain 358 with the TEM when the present work was carried out. However, as in the case of the other strains of *Rhinomonas reticulata* et var., this has a nucleomorph closely associated with the pyrenoid (the 'cytoplasmic

tongue' of Lucas 1970a), and a periplast composed of an internal electron-dense layer and an external fibrillar one (Lucas, pers. comm.)

5. DISCUSSION

Some insight into the taxonomic challenge posed by the red marine Cryptophyceae is provided by the fact that the strains of *Rhinomonas reticulata* et var. examined here were originally identified as belonging to distinct genera, or remained unidentified (Table 4.1). Further confusion arose from subsequent attempts to rename strains. Strain 3C, originally identified as *Chroomonas salina* (Wislouch) Butcher, was considered as a member of the genus *Pyrenomonas* by Santore (1986), and was referred to as *Rhodomonas salina* (Wislouch) Hill & Wetherbee by Hill & Rowan (1989); strain 358 was successively recombined as *Pyrenomonas reticulata* (Lucas) Santore (1986), and *Rhodomonas reticulata* (Lucas) Hill & Wetherbee (1989), with *Pyrenomonas reticulata* as a synonym.

It appears that no generic name, other than *Rhinomonas* itself, can be applied to the strains assigned here to *R. reticulata* et var. In fact:

1. *Cryptomonas* and *Proteomonas* are inappropriate owing to the different position of the nucleomorph (outside the pyrenoid);

2. the possibility of resurrecting Butcher's (1967) *Chroomonas* subgenus *Cryptochrysis*, which included supposed members of that genus lacking a blue-green colour, and was transferred to *Pyrenomonas* by Santore (1984), should be excluded. It has been pointed out that the name *Chroomonas* should be restricted to blue-green forms, containing

phycocyanin instead of phycoerythrin (Hill & Rowan 1989). Despite recent cautionary comments by Santore (1987), this view is strongly supported by fine-structural data (Santore 1984, 1987), and seems to better reflect Hansgirg's (1885) original concept of that genus ('*chromatophoris* ... *aerugineo-coeruleis*': Hansgirg 1885, p. 230);

3. the use of the name *Rhodomonas* Karsten should be avoided for the following reason. When examined with the light microscope, all of the strains of *Rhinomonas reticulata* et var., as well as several other red, marine forms, fit the original diagnosis or subsequent descriptions of *Rhodomonas baltica* Karsten, the type-species of its genus (Karsten 1898, Zimmermann 1923, 1925, Kylin 1935). Compare for instance the light micrographs of strain Theta/C and strain A from the author's collection (Pl. 10), with the illustrations of *Rhodomonas baltica* reproduced in Pl. 11. When examined by electron microscopy, however, differences are apparent. Strain A has a papillate periplast lacking any visible hexagonal pattern, and possessing a continuous internal layer; furthermore, the nucleomorph is not housed within the pyrenoid. These features assign strain A to the genus *Proteomonas* (this thesis, Chapter 5). As there is no way of knowing with certainty which of these forms truly represent the genus *Rhodomonas*, this name should not be applied to any one of them. Similar considerations should also apply to the strains studied by Hill & Wetherbee (1989), suggesting that the name *Rhodomonas* Karsten *emend.* Hill & Wetherbee is of uncertain application. There is little doubt that the names *Pyrenomonas* Santore and *Rhodomonas* Karsten *emend.* Hill & Wetherbee both designate the

same genus; however, in contrast with Hill & Wetherbee's (1989) view, it is the former which should be conserved in place of the second. (This usage will be followed from here onwards.)

There are two major similarities between the *Rhinomonas* strains and the genus *Pyrenomonas*, namely, the position of the nucleomorph and, as far as can be inferred from a comparison of the sectioned material with published TEM micrographs, the structural features of the periplast as a whole. A difference is given by the shape of the periplast areas. In *Pyrenomonas* the periplast areas are more or less rectangular (Santore 1984, 1986, Hill & Wetherbee 1989), as opposed to hexagonal in *Rhinomonas*. However, the precise meaning of some of the observations reported in the literature is unclear. During the course of observations on *Chroomonas salina* prior to its recombination under *Pyrenomonas*, Santore (1977, legend to Fig. 11, SEM) commented on the 'hexagonal appearance of the periplast', and emphasised this by marking the location of the ejectosomes on the micrograph; however, elsewhere in the paper the periplast 'plates' were described as 'rectangular'. Hill & Wetherbee (1989) exercised some caution when describing the periplast 'plates' of their strains as 'rectangular'; none of their freeze-fracture micrographs, however, illustrate periplasts with distinctly hexagonal patterns comparable to those observed here in the *Rhinomonas* strains. There are also some reports on the variation of the periplast pattern in other members of the Cryptophyceae. Gantt (1971) noted that the normally rectangular 'plate areas' of the periplast of a strain named *Rhodomonas lens* became hexagonal when placed in

hypotonic conditions. Kugrens & Lee (1987), who studied the periplast structure in several strains using freeze-fracture techniques, reported that centrifugation of cells of an unidentified, freshwater *Cryptomonas* strain frequently caused a 'somewhat hexagonal' appearance of the otherwise rectangular 'plates' (Kugrens & Lee 1987, p. 371, Figs 12 & 26). Kugrens & Lee (1987) also studied a strain of *Cryptomonas ovata* Ehrenb. var. *palustris* Pringsheim, descended from that studied by Brett & Wetherbee (1986); this showed somewhat hexagonal plates (as opposed to circular-oval) following treatment with glycerol. However, as pointed out by the authors themselves, in the same strain Brett & Wetherbee (1986) had previously noted hexagonal patterns, under conditions similar to those in which Kugrens & Lee (1987) observed circular-oval plates. In *Hemiselmis rufescens* viewed with the SEM, both rectangular and hexagonal patterns have been observed (Santore 1977; this thesis, Chapter 3). A comparison with freeze-fracture/etch micrographs of *Hemiselmis brunnescens* Butcher, clearly showing hexagonal patterns (Wetherbee, Hill & McFadden, 1986), suggests that the rectangular pattern of *H. rufescens* is artifactual. By contrast, other rectangular patterns, such as those of *Chroomonas* (Santore 1977, 1987, Meyer & Pienaar 1984, Klaveness 1985, Kugrens *et al.* 1986), and *Pyrenomonas* (Santore 1984, 1986, Hill & Wetherbee 1989, this chapter), are probably not artifactual. If it is accepted that freeze-fracture/etch gives a true-to-life picture of the cell surface, then judging whether a given appearance of the periplast in the SEM is artifactual or not becomes a simple matter of comparing it with freeze-fracture/etch micrographs.

In the *Rhinomonas* strains examined here, no deviations were observed from hexagonal periplast patterns, except for the presence of unornamented regions where internal cell components bulge towards the cell surface. Even when the PA's appeared roughly circular, the overall hexagonal appearance of the periplast was maintained thanks to the delineating lattice (Pl. 12). The assignment of those strains to *Rhinomonas* requires a modification of the concept of that genus. This was originally described as having a periplast with internal and external 'plates' (Hill & Wetherbee 1988), whereas the TEM sections made here lead to believe that only internal ones are present in *R. reticulata* et var.. Can a single genus conveniently accommodate forms with periplasts having a different construction? In this respect, it may be noted that in the type-species of *Proteomonas* Hill & Wetherbee, described as having two distinct morphological phases, the structure of the periplast varies according to the phase considered (Hill & Wetherbee 1986). (In *Proteomonas pseudobaltica* et var., only one morphological phase was found: this thesis, Chapter 5).

There are a few other differences between the *Rhinomonas* strains examined here and the original description of that genus. This mentions cells with a distinctly rhinote anterior, and a vestibular depression located at about one-third the cell length from the cell apex; also, in the SEM micrographs cells are mostly rugulose and show little or no evidence of hexagonal periplast patterns (Hill & Wetherbee 1988, diagnosis on p. 356, and Figs 5, 6). Within numerous strains of Cryptophyceae, however, the vestibular depression

may arise at variable distances from the cell apex; when the distance is equal or greater than the diameter of the depression, the cells may have a distinctly rhinote shape. This is particularly apparent, for instance, in strains of *Proteomonas* (this thesis, Chapter 5). As for the appearance of the cell surface in the SEM, the differences could be attributed, perhaps, to differences in fixation methods, Lugol's being that adopted by Hill & Wetherbee (1988). The method used here seems to give an adequate preservation of the cell micromorphology. For instance, the values of cell shrinkage seem acceptable (Chapter 6); in addition, the shape of the periplast areas of *Rhinomonas reticulata* et var., as revealed by SEM, compares favourably with that shown for the type-species *R. pauca* using freeze-fracture/etch techniques (Hill & Wetherbee 1988, figs 14-18).

As far as can be inferred from Hill & Wetherbee's (1988) freeze-fracture/etch micrographs, *R. pauca*, the type-species, seems to have larger periplast areas than *R. reticulata* var. *reticulata*, var. *atrorosea*, and var. *compressa*; these, in turn, have larger areas than *R. reticulata* var. *elenlana*. This suggests that, in general, differences in the size of the periplast areas can be used for distinguishing between taxa at the species level or below, once the variability existing within taxa has been taken into account. The exact significance of the variability within taxa is unclear. In *R. reticulata* var. *reticulata*, larger periplast areas tend to occur in those strains where, on average, the cell length is greater. By contrast, in ten strains of *Cryptomonas* there seems to be no significant correlation (Santore 1977). In addition,

periplast areas differing in size can also occur within single cells. In members of the genus *Chroomonas*, for instance, the periplast areas may be smaller towards the cell antapex (see for instance Meyer & Pienaar 1984).

Rhinomonas reticulata var. *atrorosea* comb. et stat. nov. is based on strain 16/1 from the Butcher collection prior to its relocation at Westfield College, University of London. This is one of the original isolates of the 'red' *Chroomonas atrorosea* Butcher, described from salt-marsh pools in several regions of the British Isles. Butcher's species was recently transferred to the genus *Rhodomonas* Karsten *emend.* Hill & Wetherbee (1989). Based upon the nucleomorph position, as revealed by TEM observations carried out during the course of this work, and the shape and size of the periplast areas, strain 16/1 can be considered as a variety of *Rhinomonas reticulata*, distinguished by the slight lateral compression of the cells (mean thickness to mean width ratio: ca. 1.1). It may be noted that Butcher (1967, p. 33), described *Chroomonas atrorosea* as 'not compressed'; however, some lateral compression is apparent in the illustrations (Butcher 1967, pl. 2 fig. 11). According to the International Code of Botanical Nomenclature (Art. 7.12), the type of a new combination is that of the basionym. In the present case, this is a lectotype, proposed by Hill & Wetherbee (1989).

Rhinomonas reticulata var. *compressa* var. nov. is based on strain TB-0 from the IANL collection. This was isolated by Dr I.A.N. Lucas in 1972, from a marine sand and water sample collected from the shore at Traeth Bychan, I'sle of Anglesey, U.K. The greater degree of lateral compression, as well as

the slightly shorter cells, enable differentiation from var. *atrorosea* described above, which it otherwise resembles.

Table 4.4 summarizes the differences between the known taxa of *Rhinomonas*. It may be seen that taxa are defined, and distinguished from one another, by a particular combination of two features (lateral compression of the cells, and size of the periplast areas), each one considered as a character with three mutually exclusive states.

Legends to tables overleaf

Table 4.1. Details of the examined strains. IANL = Dr I.A.N. Lucas; CCMP = Center for Culture of Marine Phytoplankton, Bigelow Laboratories, U.S.A.; CCAP = Culture Collection of Algae and Protozoa, Oban, U.K.; PLY = Plymouth Collection, U.K.

Table 4.2. Cell size in *Rhinomonas reticulata* var. *eleniana* (strain 97825/C), var. *reticulata* (strains Rhodo, 3C, Theta/C, 995/2, and 358), var. *atrorosea* (strain 16/1), and var. *compressa* (strain TB-0), measured using the point-to-point measuring facility of the SEM. L = length, T = thickness (dorso-ventral), W = width (perlateral). Values are given as mean ± standard deviation, with the number of observations in brackets.

Table 4.3. Size of the periplast areas in *Rhinomonas reticulata* var. *eleniana* (strain 97825/C), var. *reticulata* (strains Rhodo, 3C, Theta/C, 995/2, and 358), var. *atrorosea* (strain 16/1) and var. *compressa* (strain TB-0). Number of observations = 50 except where noted; SD = standard deviation.

Table 4.4. Cell and periplast features in *Rhinomonas* taxa, based on Hill & Wetherbee (1988), and this work. CC = cell compression (lateral), SPA = mean length of side of the periplast areas. CC (0) = cells not compressed, CC (1) = thickness ca. 10 % greater than width, CC (2) = thickness more than 10 % greater than width; SPA (0) = less than 0.28 um, SPA (1) = 0.28 - 0.43 µm, SPA (2) = greater than 0.43 µm.

TABLE 4.1

Strain	Collection or origin	Source	Specific name (as in collection)	Previous studies
97825/C	contaminant of CCAP 978/25	IANL	unidentified	-
Rhodo	IANL	IANL	*Rhodomonas baltica* Karsten	-
Theta/C	reisolated from CCMP Theta	IANL	*Chroomonas* sp.	CCMP Theta; Gillot & Gibbs 1980, McKerracher & Gibbs 1982, Gillot & Gibbs 1983, Vetherbee *et al.* 1987
3C	CCMP	IANL	*Chroomonas salina* (Wislouch) Butcher	Antia *et al.* 1973; Gillot & Gibbs 1980
995/2	CCAP	M.D. Budd	*Rhodomonas* sp.	-
358	PLY	IANL	*Cryptomonas reticulata* Lucas	Lucas 1968, 1970a
11/8	R.W.Butcher	IANL	*Chroomonas salina* f. *adolescens* Butcher	-
16/1	R.W.Butcher	IANL	*Chroomonas atrorosea* Butcher	-
TB-0	IANL	IANL	unidentified	-

TABLE 4.2

Strain	L (µm)	T (µm)	W (µm)
97825/C	8,4 ± 0,98 (30)	4,4 ± 0,47 (22)	4,2 ± 0,55 (8)
Rhodo	8,9 ± 0,85 (30)	5,0 ± 0,53 (19)	4,8 ± 0,33 (11)
3C	9,9 ± 0,82 (30)	5,4 ± 0,44 (17)	4,9 ± 0,57 (13)
Theta/C	9,4 ± 1,16 (30)	4,6 ± 0,61 (20)	4,6 ± 0,51 (10)
995/2	9,4 ± 0,88 (30)	5,3 ± 0,61 (19)	5,1 ± 0,45 (11)
358	11,6 ± 1,16 (30)	6,8 ± 0,71 (18)	6,4 ± 0,69 (12)
16/1	9,2 ± 0,87 (30)	4,8 ± 0,30 (17)	4,4 ± 0,50 (13)
TB-0	8,1 ± 0,82 (30)	4,4 ± 0,50 (28)	not measured (*)

(*) Owing to the lateral compression, most cells were observed lying on one side.

TABLE 4.3

Strain	Length of hexagon side (μm)		
	Minimum	Maximum	Mean ± SD
97825/C	0,16	0,37	0,22 ± 0,056
Rhodo	0,21	0,41	0,28 ± 0,041
3C	0,25	0,42	0,32 ± 0,040
Theta/C	0,26	0,45	0,32 ± 0,044
995/2	0,28	0,40	0,32 ± 0,030
358	0,38	0,59	0,43 ± 0,054
16/1	0,28	0,45	0,37 ± 0,041 (*)
TB-0	0,25	0,38	0,31 ± 0,028 (*)

(*): number of observations = 30.

TABLE 4.4

Taxon	CC	SPA
R. pauca	1	2
R. reticulata var. *reticulata*	0	1
R. reticulata var. *atrorosea*	1	1
R. reticulata var. *compressa*	2	1
R. reticulata var. *eleniana*	0	0

Chapter 5

Observations on *Proteomonas pseudobaltica* comb. nov. and *Proteomonas pseudobaltica* var. *leonardiana* var. nov., with comments on a freshwater *Cryptomonas*

Information contained in this chapter was included in the following paper:

Novarino G., in prep. - Observations on some new and interesting Cryptophyceae, with a diagnosis of the family *Pyrenomonadaceae* fam. nov. Submitted to *Nordic Journal of Botany*.

Chapter 6

A brief scanning electron microscope survey of marine Cryptophyceae, with comments on a freshwater form, cell shrinkage, and some effects of fixation schedules

Information contained in the present chapter was included in the following paper:

Novarino G. (in prep.) - Observations on some new and interesting Cryptophyceae, with a diagnosis of the family *Pyrenomonadaceae* fam. nov. Submitted to *Nordic Journal of Botany*.

Chapter 7

Observations on some species of *Chroomonas*

A species of *Chroomonas* not included in the present chapter formed the object of the following demonstration:

Novarino G., 1980 - Observations on non-motile cryptophycean cells. *In*: The Chromophyte Algae. An International Symposium held at Plymouth Polytechnic, England, 5-8 April 1988 (An abstract is enclosed at the end of the present volume).

Chapter outline: 1. Introduction. 2. Material and methods. 3. Observations. 4. Discussion.

Chapter summary: Some aspects of the fine-structure of three true, blue-green species of *Chroomonas* are examined using scanning and transmission electron microscopy. The size and shape of the periplast areas, as revealed by the SEM, appear to be useful for discriminating between species. Two species can also be distinguished on the basis of the presence or absence of two large refringent bodies. The non-motile, palmelloid cells are surrounded by an extracellular sheath. This is more easily observed in cells fixed according to the osmium - thiocarbohydrazide - osmium method; it is composed of two reticular layers of fibrils, a compact one close to the cell surface, and a loose one further away from it. New fine-structural information includes the presence of a vestibular ligule in *C. collegionis*. A suggestion is made that a structural relation may exist between the eyespot and the flagellar bases in *C. collegionis*.

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1. INTRODUCTION

With the exception of *Hemiselmis virescens* (Chapter 3), the previous chapters have dealt exclusively with red cryptomonads. The present one deals with *Chroomonas*, a genus described by Hansgirg (1885) as being blue-green in colour and, thus, possessing phycocyanin as an accessory photosynthetic pigment (Hill & Rowan 1989). In the past, some confusion has arisen from the fact that the generic name *Chroomonas* was also applied to red (phycoerythrin-containing) forms. These were considered by Butcher (1967), as members of his subgeneric grouping *Cryptochrysis* of the genus *Chroomonas*, later assigned to a distinct genus (*Pyrenomonas* Santore 1984: see Chapter 4).

Fine-structural information on some members of the genus can be found in Dodge (1969, 1973), Faust & Gantt (1973), Gantt (1971), Klaveness (1985), Kugrens *et al.* (1986), Lucas (1982), Meyer & Pienaar (1984), Meyer (1987), and Santore (1977, 1982a, 1987). The salient features of the genus include the following:

- The periplast is composed of internal and external, usually rectangular 'plates';
- There is a single chloroplast, and a single nucleomorph not embedded in the pyrenoid;
- The pyrenoid often bears an extension carrying an eyespot;
- The vestibular region of the cell is composed of two branches, a longitudinal one through which the flagella emerge, and lacking ejectosomes, and a transverse one bearing ejectosomes; and .
- Non-motile palmelloid cells, surrounded by a

mucilage sheath, are frequently seen in culture.

The taxonomic characters significant at the species level are poorly understood. Butcher (1967), recognized twelve blue-green members of the genus on the basis of characters visible with the light microscope: for instance, the cell shape and size, the presence of a pyrenoid, and the presence of 'refractive granules' or 'refractive bodies', whose nature was unknown.

In the present chapter, some aspects of the fine-structure of two marine and one freshwater strains are examined, with particular reference to the features of the periplast and the mucilage sheaths of palmelloid cells. The occurrence of a vestibular ligule, hitherto unknown in the genus *Chroomonas*, is also documented, and a suggestion is made that there may exist a structural relation between the eyespot and the flagellar bases. Comments are made on some characters useful for delimiting species.

2. MATERIAL AND METHODS

The marine *C. collegionis* and *C. placoidea*, strains CCAP 978/11 and 978/8, and the freshwater *Chroomonas* sp., strain SAG 980.1, were cultured, prepared, and observed using LM, SEM, or TEM, as described in Chapter 2. The marine strains were also observed using polarized light-microscopy, and *C. collegionis* was also prepared for TEM according to the osmium - thiocarbohydrazide - osmium method (OTO), described in detail in Chapter 4. *Chroomonas virescens*, strain CCAP 978/10, was studied using LM and SEM by Novarino (1988); unfortunately, the cultures perished before TEM observations could be under-

taken.

3. OBSERVATIONS

In addition to the motile flagellate unicells, non-motile palmelloid cells (single, paired, or occurring in large clusters), were also observed (Pls 32, 33, Pl. 35 Fig. 4). They were usually dominant in cultures of *C. collegionis*, constantly present in low numbers in *C. placoidea*, and found only in very old cultures in *Chroomonas* sp.

Plates 34 - 36 illustrate the features of the motile unicells, as revealed by SEM. Morphometric features of cells prepared for SEM are given in Table 8.1. The periplast was composed of rectangular periplast areas in *C. collegionis* and *C. placoidea*, and irregularly polygonal areas in *Chroomonas* sp. The size of the periplast areas is a discriminating feature between *C. collegionis* and *C. placoidea*: on average, 0.91 x 0.62 µm and 0.69 x 0.43 µm, respectively (Table 8.1). In all of the strains examined, the cell antapex lacked the rod-shaped structure observed in a strain of *Chroomonas* sp. by Gantt (1971).

Chroomonas collegionis and *C. placoidea* could also be distiguinhed from one another using polarized light microscopy, thanks to the presence or absence of two highly refringent bodies (Pl. 33). These were constantly present in *C. placoidea*, both in the motile and non-motile states, and always absent in *C. collegionis*. Unfortunately, the fine-structure of these bodies could not be ascertained.

Several TEM observations were made on *C. collegionis*, especially on the non-motile, palmelloid cells (Pls 37 - 45, Pl. 47 Fig. 1). The

main difference between motile and non-motile cells was the presence of a conspicuous extracellular sheath in the latter (Pls 38 - 40). This was usually more obvious in OTO - fixed cells (Pls 39, 40). However, even in the case of OTO - fixed material, care had to be taken to print the micrographs correctly for revealing the full extent of the sheath (Pl. 40). The sheath was composed of fine fibrils, forming a compact reticular layer close to the cell surface, and a loose layer further away from the cell (Pl. 39 Figs 3, 4, Pl. 40 Fig. 4). Fibrillar material was sometimes visible on the surface of motile cells; however, it never appeared to form a conspicuous, well-defined sheath (Pl. 37 Fig. 1). Elongated structures of unknown nature were sometimes seen on the external cell face, deeply embedded in the extracellular sheath (Pl. 38 Figs 1, 4).

Another difference between motile and non-motile cells of *C. collegionis* was given by the features of the periplast. In the non-motile cells, this often appeared to lack a subdivision into discrete periplast areas (see for instance Pl. 40). In addition, only few peripheral ejectosomes were present (see for instance Pl. 38). As a result, the serrated appearance of the periplast (compare Pl. 41 Fig. 4), also seen in other genera of cryptomonads (e.g., *Rhinomonas*: Pl. 46), was observed only rarely (Pl. 39 Fig. 3). A vestibular ligule was seen in numerous sections of *C. collegionis*. This occurred close to the external opening of the gullet, between it and the contractile vacuole (Pl. 37 Figs 1, 2, Pl. 41 Figs 1, 4).

Plates ·42 and 43 show some features of the chloroplast and associated structures of *C. colle-*

gionis. The thylakoids were frequently paired, with the pairs regularly spaced (Pl. 42 Fig. 1), or showing stack-like regions here and there (Pl. 42 Fig. 2). However, they also occurred in true stacks, with no evidence of being arranged in pairs (Pl. 42 Fig. 3). An eyespot, consisting in an extension of the pyrenoid bearing a few dark globules, was observed in several sections (e.g., Pl. 38 Fig. 2). Plate 43 shows that it occurs in a position close to the flagellar bases. Profiles of transverse and longitudinal tubules were present between it and the flagellar bases themselves (Pl. 43 Figs 2, 4).

Plate 44 shows some sections of the nucleomorph of *C. cdllegionis*. It was always single, and not embedded in the pyrenoid. It showed the typical inclusions: a dense or moderately dense fibrillo-granular body, and a cluster of dense globules. Apparently, these inclusions were not orientated in any particular direction with respect to the chloroplast.

Plate 45 shows some miscellaneous inclusions occasionally seen in *C. collegionis*; Fig. 1 is a section of a membrane-bound vesicle, occurring outside the chloroplast and the periplastidial compartment, and containing several virus-like particles (VLPs). The VLPs were roughly between 60 - 90 nm in size. They included a darker central region and a lighter peripheral one. Fig. 2 is a section of a chloroplast, showing two thylakoids forming what appears to be a closed loop around an amorphous inclusion.

The Golgi apparatus of *C. collegionis* was composed of a single stack of cisternae (dictyosomes) in motile cells, and up to three stacks in non-motile cells (Pl. 41 Fig. 3, Pl. 47

Fig. 1). Unlike several red cryptomonads (e.g., *Rhinomonas reticulata*: Pl. 47 Figs 2, 3), the dictyosomes were made up of only few cisternae, moderately appressed in the median region, and distinctly swollen at the periphery.

Plate 48 shows some features of the flagella and associated structures. The axoneme shows the typical 9 + 2 arrangement of microtubules (Fig. 1), whereas the flagellar base has the usual 9 + 0 configuration (Fig. 3). In the transition region between the flagellum and the flagellar base, the axoneme lacks the central doublet of microtubules (Fig. 2). The portion of the flagellum lying above the transition region can be distinctly swollen, or carry a 'platform' bearing a tuft of mastigonemes, much in the same way as other genera of cryptomonads (e.g., *Rhinomonas*: Pl. 48 Fig. 4, and Pl. 49). Transverse sections of the mastigonemes clearly show their tubular nature (Pl. 48 Fig. 1)

Plate 50 shows some features of the ejectosomes. The peripheral ones are much smaller than the ones lining the gullet, as in other genera of cryptomonads (e.g., *Rhinomonas*: Pl. 50 Fig. 1). As expected, the ejectosomes are composed of two coiled subunits of different size (Pl. 50 Figs 2, 3); Plate 50 Fig. 4 shows the typical lamellar substructure of a large ejectosome.

4. DISCUSSION

The species of *Chroomonas* studied here can be distinguished from one another on the basis of the features of the periplast of the motile cells. *Chroomonas collegionis* and *C. placoidea* have rectangular periplast areas (PA's), whereas in

Chroomonas sp. the PA's are irregularly polygonal. In addition, the size of the PA's appears to be a discriminating character between *C. collegionis* and *C. placoidea*.

Chroomonas collegionis and *C. placoidea* can also be recognized using polarized light microscopy. This reveals the presence of two large refringent bodies in *C. placoidea*, but not in *C. collegionis*. The nature of these structures remains unknown. Perhaps, they could be tentatively referred to as the 'Corps de Maupas', a term used in the past for paired refringent bodies seen in the cryptomonads and the ciliates (see for instance Deflandre 1938). Santore (1985), suggested that the Maupas bodies are neither specialized inclusions nor organelles and, therefore, the term 'Corps de Maupas' should be abandoned. He quoted information derived from Pringsheim, who stated that the Corps de Maupas disappear after subculturing. In *C. placoidea*, on the other hand, the paired refringent inclusions are constantly present, and in *C. collegionis* they are always absent. Lucas (1970a, b), illustrated structures termed 'Corps de Maupas' using the TEM, and suggested a lysosomal function (Lucas 1970a).

The nature of the extracellular sheath surrounding the non-motile palmelloid cells remains unknown. The fact that the sheath can be visualized more clearly using the OTO method could be due, perhaps, to an unspecific osmium staining, enhanced by the use of thiocarbohydrazide as a 'bridge' between osmium molecules. Preliminary attempts to stain the sheath with ruthenium red, which reacts specifically with acid mucopolysaccharides, were unsuccessful.

The fact that the thylakoids may or may not be

paired agrees with previous observations on a true member of the genus *Chroomonas* (Faust & Gantt 1973), and a phycoerythrin-containing '*Chroomonas*' sp. (Vesk & Jeffrey 1977). In the former, the thylakoids were more closely packed when cells were grown under low light intensities; in the latter, the thylakoids showed an increased amount of stacking when cells were grown under blue-green light. In *Chroomonas collegionis*, the causes of the variable degree of stacking and pairing are unknown. However, this variability prompts for much caution in using thylakoid features for delimiting species, as in the case of the cryptomonad studied by Andreoli *et al.* (1986).

As far as the nucleomorph features are concerned, in some of the cryptomonads examined by Gillot & Gibbs (1980), it was noted that the nucleomorph inclusions were often orientated in a particular way with respect to the chloroplast. The fibrillogranular body, for instance, was usually closest to the chloroplast. This contrasts with *Chroomonas collegionis* where, apparently, the inclusions are orientated more or less at random.

A structure resembling the vestibular ligule seen here was observed in some species of *Cryptomonas* using SEM (Kugrens *et al.* 1986). There is a good agreement between the present observations on the position of this structure, and the observation by Kugrens *et al.* (1986, pp. 516-518), that the vestibular ligule 'extends over the discharge site of the contractile vacuole'. Nothing is known about the possible function of the ligule. It might be speculated that it is somehow involved in the extrusion of mucilage from the gullet when the cells assume the palmelloid state.

The presence of tubules between the eyespot and the flagellar bases of *C. collegionis*, suggests that some structural relation may exist between these organelles. Neither Dodge (1969, 1973), nor Lucas (1982), allowed for the existence of such a relation, already known in other groups of algae (Dodge 1973).

In the cryptomonads, virus-like inclusions similar to those seen here have already been observed in the nucleus and cytoplasmic vesicles of a species of *Cryptomonas* (Pienaar 1976), and in intracellular bacteria infecting *Cryptomonas* sp. strain SAG 25.80 (Schnepf & Melkonian 1990). Chloroplast inclusions have been observed in a species of *Chlamydomonas* (Stein & Bisalputra 1968); however, unlike *Chroomonas collegionis*, these had a crystalline rather than amorphous appearance.

Table 8.1. Morphometric features of motile cells of *Chroomonas* spp. prepared for SEM according to the glutaraldehyde / osmium / critical point-drying schedule. Cells were measured using the point-to-point measuring facility of the microscope. The periplast areas (PA's) of *Chroomonas collegionis* and *C. placoidea* were measured on enlarged photographic negatives (final magnification: between x 50,000 - x 100,000). The surface area of the PA's of *Chroomonas* sp. SAG 980.1 was measured using a simple gravimetric method: the PA's were cut out from photographic prints of known weight per unit area, and accurately weighed. No corrections were applied to compensate for tilt angle (all samples were observed untilted). L = length, T = thickness (dorso-ventral), W = width, SPA = size of periplast areas. Values are given as mean ± standard deviation, with the number of observations in brackets.

Taxon	L (µm)	T (µm)	W (µm)	SPA (µm or µm²)
C. collegionis	6,1 ± 0,47 (30)	3,5 ± 0,31 (13)	3,5 ± 0,37 (17)	L: 0,91 ± 0,068 (28) W: 0,62 ± 0,054 (28)
C. placoidea	6,7 ± 0,97 (30)	4,0 ± 0,55 (19)	4,1 ± 0,38 (11)	L: 0,69 ± 0,086 (30) W: 0,43 ± 0,043 (30)
Chroomonas sp.	8,1 ± 0,82 (30)	4,9 ± 0,58 (18)	4,2 ± 0,52 (12)	surface area (µm²): 0,53 ± 0,097 (70)

Chapter 8

Some proposals for a new classification system of the Cryptophyceae

Chapter outline: 1. Introduction. 2. Some remarks on the cryptomonad species concept. 3. How many genera? 4. A brief taxonomic key to the red genera. 5. How many families and orders? 6. A new classification system of the Cryptophyceae.

Chapter summary: Comments are made on some existing classification systems of the Cryptophyceae. In addition, a new system is proposed. This allows for the existence of three orders, four families, seven pigmented genera and two colourless ones. Most diagnosis or descriptions of taxa above the generic level are based on only one character. This should make the system capable of accomodating new taxa with little difficulty. A brief taxonomic key to the red genera is given.

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1. INTRODUCTION

Our concepts of the systematics of the Cryptophyceae originate from the works of Pascher (1913, 1914), and Pringsheim (1944), on which most of the later systematic treatments have been based. These systems are no longer adequate for a number of reasons:

- they are necessarily based on light-microscopical observations, which yield limited taxonomic information, and cannot provide a framework where the genera recently described by electron microscopy can be easily accommodated; and
- they allow for the existence of families based on, or including, genera no longer recognized as belonging to the class, or of doubtful status.

Unfortunately, the numerous contributions to the knowledge of cryptomonad fine-structure by Santore (1975 *et seq.*), have not led to a well defined classification scheme. Their main systematic preoccupation has been the delineation of genera; the question of higher rank classification has been addressed in the case of the family Hemiselmidaceae Butcher (Santore 1982b), which he considered to be a justified assemblage. In a later review of the fine-structure of the genus *Cryptomonas*, Santore (1985, p. 47), noted that 'there should be just one family which incorporates all pigmented genera'; as far as can be judged by the earlier statement on the Hemiselmidaceae, it was probably that family that Santore was referring to. To my knowledge, there is only one classification system based on fine-structural features, namely, that proposed by Lee (1989).

2. SOME REMARKS ON THE CRYPTOMONAD SPECIES CONCEPT

When dealing with the systematics of any one group of organisms, it is useful to make a rough estimate of the number of species whose existence should be allowed for. Thus, it becomes necessary to have some idea of what a species is. Unfortunately, this is by no means clear. It appears that different species concepts apply to different groups of organisms. In a previous thesis (Novarino 1985), I commented briefly on the species concept in the diatoms. This should be largely understood in a typological (morphological) sense. Further investigations on the genetic make-up of natural populations are needed to establish whether or not there is a correspondence between morphospecies and biological species. A 'biological species' is understood here in its usual meaning, namely, a group of actually or potentially interbreeding populations, reproductively isolated from other such groups.

In the Cryptophyceae, it is by no means clear whether biological species may actually exist, the reason being that the occurrence of sexual reproduction is not extensively documented. Reports by Wawrik (1969, 1971), Hill & Wetherbee (1986), and Kugrens & Lee (1988), are still in need of further evaluation; in any case, all of the sexual processes investigated would seem to occurr only rarely. Therefore, until the importance of sexuality in the Cryptophyceae is better appreciated, it seems preferable to regard the class as a group where reproduction occurs almost entirely by asexual means.

Although with some cautiron, Klaveness (1985), quoted the 'ecospecies' concept as being applicable

to asexually reproducing organisms such as the Cryptophyceae. This term is applied here to entities deriving from a preexisting continuum, by means of ecological factors acting differently on different parts of the continuum itself.

How are the cryptomonad ecospecies to be recognized from one another? If genetic change takes place during their origin (a given selective pressure might be favourable to certain genotypes, and unfavourable to others), by measuring the amount of change it should be possible to assess the differences between them. This could be done, for instance, by studying the patterns of isoenzyme occurrence and distribution within and between populations, using one of several well-established electrophoretic methods (e.g., starch gel electrophoresis, recently used by Huber & Lewin [1986], in a study of flagellates of the genus *Tetraselmis* [Prasinophyceae]). However, we do not know if the Cryptophyceae are mostly diploid, or haploid, or may alternate between these two phases. *A priori*, it is likely that this would make it difficult to give correct interpretations of isoenzyme patterns.

The alternative might be to adopt a strictly typological (mostly morphological) approach to the question of (eco)specific differences. This has nothing particularly new to it, in that it is basically the traditional method used by the early, light-microscopical taxonomists. It might be asked whether it can be upheld, since it was implicitly considered inadequate by such an authoritative investigator as Pringsheim (1968). If a typological concept is to be adopted, it becomes necessary to decide what characters should be used for defining

types. In other words: which characters are taxonomically significant? Pringsheim's admitted failure to find such characters might have an historical explanation, namely, that he was mainly active before electron microscopy became a widespread tool in microalgal taxonomy. However, even in more recent years his warnings on the naming of species have been more or less explicitly accepted by an experienced electron microscopist such as Santore. As he did not succeed in finding fine-structural features taxonomically significant at the species level, this author put forward the idea that there may be only few, widely distributed species (Santore 1984, 1985).

The search for morphological characters significant at the specific level requires a preliminary assessment of their inter- and infraspecific variability. A character showing more 'inter' than 'infra' variability is more significant than one for which the opposite is true (Pankhurst 1978). Unfortunately, there is still very little information on variability as it occurs in the Cryptophyceae (Lund 1962, Javornicky 1976, Willen *et al.* 1980, Anton & Duthie 1981). Again, this could depend on the difficulties of observing specimens by light microscopy. In this thesis, attempts have been made to describe variability using scanning electron microscopy. The techniques involved are more complex than light microscopy. However, the SEM makes it possible:

1. to measure the specimens accurately;
2. to observe the shape of the cells and the periplast areas, if present; and
3. to observe the morphology of the vestibular region of the cell, in order to establish whether a

true furrow is present or not.

Providing that the possible occurrence of cell shrinkage, and its approximate amount, are taken into account, the features revealed by the SEM may be useful for delineating species. The SEM could also become a useful tool for the routine identification of 'wild' (uncultured) planktonic specimens. Samples fixed in the usual manner (Lugol's iodine), can be studied with the SEM with relatively little difficulty.

If my viewpoint can be upheld, then deciding on how many cryptomonads species can be recognized will require SEM investigations on a wider range of strains. In the meantime, the following figures for two genera can act as a rough guideline:

- *Rhinomonas*: 2 species (*R. pauca* and *R. reticulata*, the latter with 3 varieties in addition to the type-variety);
- *Proteomonas*: 2 species (*P. sulcata* and *P. pseudobaltica*, the latter with 1 variety in addition to the type-variety).

3. HOW MANY GENERA?

A variable number of genera have been recognized by different authors. For instance, Santore (1984), allowed for the existence of only four pigmented genera (*Hemiselmis* Parke, *Chroomonas* Hansgirg, *Cryptomonas* Ehrenberg, and *Pyrenomonas* Santore), and the colourless genus *Chilomonas* Ehrenberg. On the other hand, Bourrelly (1970), recognized as many as twenty-one genera including a total of about 100 species.

In my own view, features which may be taxonomically significant at the generic level

include:

1. the phycobilin type;
2. the point of flagellar insertion (distinctly lateral versus more or less apical);
3. the number of chloroplasts and nucleomorphs;
4. the features of the cell covering (periplast);
5. the presence of a posterior 'tail'; and
6. the habitat, i.e. freshwater versus marine or brackish.

Based upon these features, the following definitions can be given of seven pigmented genera. They take into account information available in the literature, as well as observations made in the present thesis.

Genus *Chroomonas* Hansgirg

Hansgirg, 1885: 230.

Phycocyanin (mostly PC 645). Flagella inserted apically. One chloroplast and one nucleomorph, located outside and close to the pyrenoid (and eyespot, if present). Periplast usually with internal and external components, made up of discrete, large, rectangular or polygonal periplast areas. Tail sometimes present. Marine/brackish and freshwater.

Genus *Cryptomonas* Ehrenberg

Ehrenberg, 1831: 56.

Phycoerythrin III. Flagella inserted apically. Two chloroplasts and two nucleomorphs, located outside the pyrenoid(s) (if pyrenoids are present).

Periplast with internal component, continuous (i.e., sheet-like), or made up of discrete, hexagonal periplast areas, forming numerous transverse rows. Apparently, a tail may sometimes be present. Mostly freshwater.

Genus *Hemiselmis* Parke

Parke, 1949: 279.

Subgenus *Hemiselmis* Butcher

Butcher, 1967: 13.
Phycoerythrin II. Flagella inserted laterally. One chloroplast and one nucleomorph, located outside the pyrenoid. Periplast usually with internal and external components, made up of hexagonal periplast areas, forming few transverse rows. Tail absent. Marine/brackish.

Subgenus *Plagiomonas* Butcher

Butcher, 1967: 16.
Phycocyanin. The rest as in the subgenus *Hemiselmis*.

Genus *Plagioselmis* Butcher

Butcher, 1967: 18.
Phycoerythrin (probably PE I.) Flagella inserted apically. One nucleomorph, placed outside the pyrenoid and closely appressed to the nucleus; thus, probably one chloroplast only. Periplast with hexagonal periplast areas. Tail present, and bearing

a periplast not composed of discrete areas. Marine.

Genus *Proteomonas* Hill & Wetherbee

Hill & Wetherbee, 1986: 522.
Phycoerythrin I. Flagella inserted apically. One chloroplast and one nucleomorph, placed outside the pyrenoid. Periplast with single sheet-like layer in the 'diplomorph' phase; with internal and external layers made up of distinct periplast areas in the 'haplomorph' phase. Tail absent. Marine.

Genus *Pyrenomonas* Santore

Santore, 1984: 637.
Phycoerythrin I. Flagella inserted apically. One chloroplast and one nucleomorph, positioned in a groove within the pyrenoid. Periplast with internal layer made up of discrete, more or less rectangular periplast areas, and external fibrillar-granular layer. Tail absent. Marine.

Genus *Rhinomonas* Hill & Wetherbee

Hill & Wetherbee, 1989: 356.
Phycoerythrin I. Flagella inserted apically. One chloroplast and one nucleomorph, positioned in a groove within the pyrenoid. Periplast with internal layer made up of discrete, distinctly hexagonal periplast areas; external layer similar to the internal one, or fibrillar-granular. Tail absent. Marine.

The following list is a selection of genera which have been synonymized, assigned to a different class, or whose affinities with the Cryptophyceae are uncertain. It also reports genera which should be considered doubtful, either because the original descriptions were not detailed enough, no fine-structural information is available, or else their publication was not in accordance with the rules of the International Code of Botanical Nomenclature.

Bjornbergiella Bicudo
Bicudo, 1966: 217.

This is a monospecific genus from Hawaian soil, known only from the original light-microscopical observations. Cell division occurs mostly in a non-motile state. Its affinities with the Cryptophyceae are very doubtful, as underlined by Javornicky & Hindak (1970).

Cryptochrysis Pascher
Pascher, 1911: 190.

= *Chroomonas* Hansg. *sensu* Butcher, subgenus *Cryptochrysis*; = *Pyrenomonas* Santore.

Cyanomonas Oltmanns
Oltmanns, 1904 (*Morphologie und Biologie der Algen*) (*non vidi*).

The affinities of this genus are very unclear. Bourrelly (1970), mentioned that there are 6 - 10 blue-green chloroplasts per cell. This feature has never been observed in any unequivocal member of the Cryptophyceae.

Cyanomonas Buttner *nom. illegit.*
Buttner, 1911: 129.

This is a later homonym of *Cyanomonas* Oltmanns, created by Buttner for a marine, blue-green flagellate between 10 - 12 µm in length, with a single chloroplast, a pyrenoid, and starch as a reserve product. Although the original figures are poor, they point towards a resemblance with a motile cell of *Chroomonas*.

Isoselmis Butcher
Butcher, 1967: 19.

Butcher created this genus 'with some hesitation' for a single *Plagioselmis*-like form. This had 'homodynamic instead of heterodynamic flagella' and 'a short double row of hardly perceptible trichocysts'.

Monomastix Scherffel
Scherffel, 1912 (*Archiv für Protistenkunde*, 27: 94 - 128) (*non vidi*).

Norris (1980), ranked this genus among the Prasinophyceae.

Pleuromastix Scherffel
Scherffel, 1912 (*Archiv für Protistenkunde*, 27: 94 - 128) (*non vidi*).

Bourrelly (1970), reported that *Pleuromastix* has a single flagellum, a feature never observed in any unequivocal member of the Cryptophyceae.

Protochrysis Pascher
Pascher, 1911: 191.

This genus is known only from Pascher's original observations. It shows a strong overall

resemblance with *Hemiselmis* Parke. No fine-structural information is available. Strain UW328 from the CCMP collection, Bigelow Laboratories, U.S.A., is listed in the collection catalogue as *Protochrysis* sp.

Rhodomonas Karsten
Karsten, 1898: 15.
Rhodomonas Karsten *emend.* Hill & Wetherbee
Hill & Wetherbee, 1989: 155.

In Chapter 4 of this thesis, some reasons were given for avoiding the use of the name *Rhodomonas* altogether. *Rhodomonas emend.* Hill & Wetherbee is a later synonym of *Pyrenomonas* Santore (1984).

Tetragonidium Pascher
Pascher, 1914: 160.

This genus is known only from Pascher's written diagnosis and the figures given in Fritsch (1935). Its main feature is the prevalence of cell division in the non-motile state. As far as can be judged from the available information, its affinities with the Cryptophyceae are uncertain.

Thecomonas Lee *nom. nud.*
Lee, 1989.

This name was used by Lee (1989), who quoted information supposedly given in Kugrens *et al.* (1986). However, to my knowledge no formal diagnosis of the genus has been published. In addition, the name is not found in Kugrens *et al.*. Lee's (1989) figure 10, p. 408 (*Thecomonas* sp.), was reproduced from figure 11 of Kugrens *et al.*, which they named *Cryptomonas platyuris*. It is suggested that *Thecomonas* be regarded as a name published without

associated descriptive material, or *nomen nudum* (International Code of Botanical Nomenclature, art. 32.1).

4. TAXONOMIC KEY TO THE RED GENERA OF CRYPTOPHYCEAE

As underlined elsewhere in this thesis, the taxonomic difficulties within the Cryptophyceae apply in particular to the red forms. The key which follows, constructed by combining the observations made in this thesis with information available in the literature, might be useful for identifying the red forms to the genus level. The average path length (i.e., the number of questions which need to be answered for identifying a given taxon), is equal to 3.0. This is only slightly greater than the ideal value, given by the logarithm in base 2 of the number of identifiable taxa ($\log_2 7 = 2.8$). Therefore, the key is considered to be sufficiently parsimonious (see Pankhurst 1978 for a brief discussion of parsimony within the context of the theory of keys).

1. Nucleomorph inside pyrenoid 2
1. Nucleomorph outside pyrenoid 3
2. Periplast areas more or less rectangular ..... *Pyrenomonas*
2. Periplast areas hexagonal ..... *Rhinomonas*
3. Periplast smooth or papillate, lacking periplast areas 6
3. Periplast with distinct periplast areas 4
4. Small cells (L < 8 µm) with few transverse rows of hexagonal periplast areas; flagella inserted laterally ..... *Hemiselmis* subgenus *Hemiselmis*

4. Larger cells, with numerous transverse rows of hexagonal periplast areas; flagella inserted apically 5
5. Chloroplast single .. *Proteomonas* (haplomorph)
5. Two chloroplasts ..... *Cryptomonas* p.p.
6. Chloroplast single .. *Proteomonas* (diplomorph)
6. Two chloroplasts ..... *Cryptomonas* p.p.

5. HOW MANY FAMILIES AND ORDERS?

Pascher (1913), divided the cryptomonads into two groups based upon the relative importance of the motile and non-motile stages throughout the life-cycle:
- the *Eucryptomonadinae*, where the flagellated stage is dominant; and
- the *Phaeocapsinae*, where the palmelloid stage is dominant.

In the Eucryptomonadinae, the characters used at lower taxonomic levels dealt mainly with the morphology of the vestibular region of the cell. For instance, the family Cryptomonadaceae was further subdivided in the *Cryptochrysidae*, possessing a furrow but lacking a gullet, and the *Cryptomonadeae*, with furrow and gullet.

The essence of this system was maintained in a later one by the same author (Pascher 1914). Here, four orders were recognized, based upon the general organization of the thallus: the *Cryptomonadales* (flagellated), *Phaeocapsales* (tetrasporalean), *Cryptococcales* (protococcalean), and *Cryptotrichales* (filamentous). Fritsch (1935), basically followed Pascher, although he did not include the filamentous forms.

Pascher's choice of diagnostic characters can no longer be upheld. As pointed out by Pringsheim (1944), in culture many Cryptophyceae tend to assume a palmelloid state, especially if the cultures have been allowed to age. Furthermore, several genera included in Pascher's system are doubtful (e.g., *Tetragonidium* Pascher), or do not belong to the Cryptophyceae at all: for instance, *Naegeliella* Correns and *Phaeothamnion* Lagerheim are members of the Chrysophyceae (Vignoli 1964, Bold & Wynne 1985). As far as the vestibular region of the cell is concerned, non-artifactual furrows appear to be taxonomically significant at most at the specific level (this thesis, Chapter 6).

Pringsheim (1944), did not address the question of classification at the order level. He recognized four previously described families:

- *Cryptomonadaceae sensu* Pascher, *sensu* Fritsch (forms with furrow and gullet);
- *Cryptochrysidaceae* Pascher (forms with furrow but lacking a gullet);
- *Nephroselmidaceae* Pascher (forms with laterally inserted flagella); and
- *Katablepharidaceae* Skuja, based on the rare colourless genus *Katablepharis* Skuja (1939: 97).

He also described the new family *Cyathomonadaceae* to accomodate the colourless genus *Cyathomonas* Fromentel (1874, *Etudes sur les Microzoaires*) (*non vidi*).

Huber-Pestalozzi (1950), recognized two subclasses, the *Monomastiginae*, with a single flagellum, and the *Cryptomonadinae*, with two flagella. As already mentioned, the genera included in the former (*Monomastix* Scherffel and *Pleuromastix* Scherffel), are not members of the Cryptophyceae. In

the Cryptomonadinae, he recognized Pascher's Cryptococcales and Cryptomonadales. For the latter, he adopted Pringsheim's (1944) subdivision into families, with a minor modification.

Much like Pringsheim, Butcher (1967) did not deal with orders. He considered the class to be composed of three families:

- the *Cryptomonadaceae*, with the 'depression - furrow - gullet along the long axis';
- the new family *Hemiselmidaceae*, 'with the gullet along or across the short axis'; and
- the new family *Hilleaceae*, 'with a vertical depression or ... a simple furrow not lined with trichocysts'.

Bourrelly (1970), maintained Pascher's concept that the basic subdivision of the class is that between motile and non-motile forms. The former were further subdivided in seven families. He adopted all of the families recognized by Pringsheim (1944), with the exception of the Cryptochrysidaceae, whose type-genus he considered to be a subgenus of *Chroomonas*, in accordance with Butcher's (1967) view. He used the name *Planonephracées* for the forms with laterally attached flagella (= the family *Nephroselmidaceae* Pascher). He also recognized Butcher's *Hilleaceae*, and two families whose affinities with the Cryptophyceae are very doubtful (*Pleuromastigacees* and *Butschliellacees*).

Christensen (1980), followed Bourrelly's system with few modifications: he used Butcher's name *Hemiselmidaceae* for Bourrelly's *Planonephracées*, and did not recognize the *Butschliellacées*.

As already mentioned, the system of Lee (1989), appears to be the only one based on fine-structural features. He recognized three orders:

- the *Cyathomonadales*, lacking plastids;
- the *Cryptomonadales*, with plastids, and a periplast made up of multiple 'plates'; and
- the *Thecomonadales*, with plastids, and a periplast composed of a single 'plate' on the internal face of the plasma membrane.

6. A NEW CLASSIFICATION SYSTEM OF THE CRYPTOPHYCEAE

In the system which follows, each diagnosis or description at the various levels is based on a small number of features (usually one), namely:
- the absence of plastids and nucleomorphs at the level of order;
- the nucleomorph position at the level of order;
- the point of flagellar insertion at the familial level.

In my view, using only few diagnostic characters could make the system easy to use, and capable of accomodating new taxa with little difficulty.

Ideally, morphological characters used at the order or familial level should be highly conservative, that is, present in most members of the class. They should also show an appropriate amount of inter-taxa variability, so that they can be used for defining the taxa themselves. This is the case, for instance, of the nucleomorph, present in most cryptomonads examined by TEM, and found in two different positions (i.e., inside or outside the pyrenoid). *Cyathomonas* is a genus where no nucleomorphs have been detected. As such, it does not fit into Cavalier-Smith's (1986) definition of the subkingdom and phylum Cryptophyta of the kingdom Chromista Cavalier-Smith. However, it is retained in the cryptomonads, in agreement with Lee's (1989)

view that an organism similar to it might have been the original host in the endosymbiosis which led to the evolution of the group.

CLASS *CRYPTOPHYCEAE sensu* Bold & Wynne; *sensu* Lee

Bold & Wynne, 1985: 634

Lee, 1989: 399 *et seq.*

= *Cryptophyceae sensu* Pascher (1914) p.p.

= *Cryptophyceae sensu* Fritsch (1935) p.p.

= *Cryptophyceae sensu* Pringsheim (1944) p.p.

= *Cryptophyceae sensu* Huber-Pestalozzi (1950) p.p.

= *Cryptophyceae sensu* Butcher (1967) p.p.

= *Cryptophycees sensu* Bourrelly (1970) p.p.

A. Order *Cyathomonadales sensu* Lee

Lee, 1989: 406.

Plastids and nucleomorphs absent. One family:

Cyathomonadaceae Pringsheim

Pringsheim, 1944: 149.

With the characters of the order. One genus:

Cyathomonas Fromentel

B. Order *Pyrenomonadales* ord. nov.

Nucleomorphus in pyrenoide posito.

Nucleomorph positioned in the pyrenoid. One family:

Pyrenomonadaceae fam. nov.

Diagnosis ut in ordo Pyrenomonadales.

Diagnosis as in the order *Pyrenomonadales*. Two genera:

Pyrenomonas Santore

Rhinomonas Hill & Wetherbee

C. Order *Cryptomonadales* ord. nov.

Non *Cryptomonadales* Pascher (1914); non *Cryptomonadales* Lee (1989); nec *Cryptomonadales* auctorum.

Nucleomorphus ex pyrenoide posito.

Nucleomorph positioned outside the pyrenoid. Two families:

Cryptomonadaceae sensu Butcher

Butcher, 1967: 7.

Non *Cryptomonadaceae* auctorum

Flagella inserted apically or subapically. Five genera:

Chilomonas Ehrenberg

Chroomonas Hansgirg

Cryptomonas Ehrenberg

Plagioselmis Butcher

Proteomonas Hill & Wetherbee

Hemiselmidaceae Butcher

Butcher, 1967: 11.

Flagella inserted laterally. One genus:

Hemiselmis Parke

subgenus *Hemiselmis* Butcher

subgenus *Plagiomonas* Butcher

Chapter 9

Discussion to Part I

Chapter outline: 1. Fine-structure. 2. Taxonomy. 3. Systematics.

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1. FINE-STRUCTURE

The present thesis contributes some new fine-structural information on the genus *Chroomonas*. The mucilage sheath produced by palmelloid cells of *Chroomonas collegionis* is different from that which may be present in the genus *Cryptomonas* (Santore 1978). The latter is composed of three distinct layers rather than two; this sequence may be repeated several times, resulting in a multi-layered investment. This was never the case of *Chroomonas collegionis*. Apparently, multi-layered investments may occur in other groups of algae, for instance the blue-greens (cyanobacteria) (Bazzichelli *et al.* 1985, 1986), and the Chlorophyceae (e.g., *Pectodictyon cubicum*: Lang *et al.* 1987).

Santore (1987), reported that the Golgi apparatus of *Chroomonas* is composed of a single stack of cisternae (dictyosomes). This thesis shows that in palmelloid, mucilage-producing cells of *Chroomonas collegionis*, there may be up to three dictyosomes. This strongly supports the view that the Golgi apparatus is involved in the production of

mucilage (Santore 1978).

The significance of the vestibular ligule of *Chroomonas collegionis* is unclear. This has been observed using SEM in some other cryptomonads (members of the genus *Cryptomonas*: Kugrens *et al.* 1986), but Chapter 7 of this thesis appears to be the first report in *Chroomonas*. It might be speculated that, in *Chroomonas collegionis*, it is involved in the extrusion of mucilage from the gullet when cells assume the palmelloid state.

Based on the case of *Chroomonas collegionis*, the present observations are the first claim that some structural relation may exist between the flagellar bases and the eyespot in the cryptomonads. This is a tentative assumption in need of further study, preferably using serial sections and three-dimensional reconstruction.

2. TAXONOMY

At the inception of this study, by using the SEM it was possible to show that the periplast areas of *Hemiselmis* are hexagonal rather than rectangular, in contrast with the view expressed by Santore (1984) (Chapter 3). This observation prompted further SEM studies on a wider variety of strains (Chapter 4 - 7). As a result, all genera possessing discrete periplast areas appeared to be characterized by one particular shape of the areas themselves. This suggests that, in general, the quality of the data likely to be gathered using SEM is adequate for taxonomic purposes. SEM information is also easier to obtain than freeze-fracture data, and may enable more detailed identifications of 'wild' (uncultured) material, as shown by the case of *Cryptomonas acuta*

and *Plagioselmis* sp. from the North Sea phytoplankton (Chapter 6).

Unfortunately, SEM data alone are not sufficient for identifying specimens to genus level. Some TEM information is also desirable (Chapters 4, 5, 8). This includes:

- the structure of the periplast, particularly the internal component;
- the number of chloroplasts; and
- the nucleomorph position.

Owing especially to the fact that they require large quantities of specimens, usually in the form of cultured material, TEM investigations can be time-consuming and, as such, are probably unpractical for routine taxonomic purposes. In particular, ascertaining the number of chloroplasts and the nucleomorph position can be difficult, and may require the examination of a large number of sections. However, in theory this could also be possible by using less complex, light-microscopy based methods. The nucleomorph stains with DAPI (Ludwig & Gibbs 1985), and, therefore, it should be possible to observe its position using fluorescence microscopy. Unfortunately, DAPI staining was not tried here; however, by using the fluorescent microscope I have been able to count the chloroplasts in living, actively swimming cells, thanks to the natural fluorescence of the chlorophyll pigments.

The question of delimiting and identifying species, defined as 'one of the greatest problems in working with cryptomonads' (Hill, pers. comm.), has been one of the main concerns of this thesis. By contrast, little attention has been given to the subject in recently published work. For instances,

the genus *Proteomonas* Hill & Wetherbee (1986) was described based on the type-species alone; *Rhinomonas* Hill & Wetherbee (1988), on the type-species and three new combinations erected solely on the basis of Butcher's (1967) observations in light microscopy; and '*Rhodomonas*' Karsten *emend.* Hill & Wetherbee (1989) (= *Pyrenomonas* Santore), on six new combinations, not re-examined, and four examined species. Amongst the latter, some show a considerable overlap of characters. Therefore, the usefulness of the particular combination of features used for delimiting them (cell length, length of 'furrow', chloroplast number and morphology, and periplast structure), may be questioned; indeed, this was done by the authors themselves. '*Rhodomonas baltica*' and '*R. salina*', for instance, show a nearly complete overlap of characters, the exception being the fibrillar surface periplast layer, 'inconspicuous' versus 'coarse' (Hill & Wetherbee 1989, p. 156); differences in chloroplast morphology were also mentioned, but alternative interpretations of the TEM micrographs (Hill & Wetherbee 1989, figs 8-10 & 18, 19), are possible.

Klaveness (1985) gave a comprehensive review of thirty-eight characters, each one with up to nine possible states, likely to be useful for identifying the Cryptophyceae to the species level. In the genus *Rhinomonas*, two characters (lateral compression of the cells, and size of the periplast areas), suffice for distinguishing the known taxa if each character is assigned three mutually exclusive states (Chapter 4). In the genus *Proteomonas*, taxa can be distinguished on the basis of the presence or absence of a furrow, and the degree of lateral compression of the cells (Chapter

5).

3. SYSTEMATICS

As far as it has been possible to ascertain, the classification scheme proposed in this thesis (Chapter 8), is the second one based on fine-structural features, the first being that of Lee (1989). The nucleomorph position is considered a highly informative character, and used as a diagnostic feature at the level of order. (For a review of the cryptophycean nucleomorph, see Santore 1982c.) Evidence that the nucleomorph is the vestigial nucleus of a eukaryotic endosymbiont is now well established (see for instances Gillot & Gibbs 1980, Hansmann *et al.* 1986 and references, and McFadden 1990). Its presence has been demonstrated in most members of the class examined by TEM; therefore, it may represent a highly conservative character. In the author's view, this justifies the use of the nucleomorph position in the diagnosis of orders (*Pyrenomonadales* with the nucleomorph inside the pyrenoid, and *Cryptomonadales* with the nucleomorph outside the pyrenoid). The occurrence of an intrapyrenoidal nucleomorph in the Chlorarachniophyceae (Hibberd & Norris 1984, Ludwig & Gibbs 1989), is reminiscent of the Pyrenomonadales; it may be due to convergent or parallel evolution, in that the two classes differ in numerous other features.

Part II

Bacillariophyceae and General Conclusions

Chapter 10

Introduction to Part II (Bacillariophyceae)

Chapter outline: 1. The diatom genus *Mastogloia*. 2. Aims of Part II.

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1. THE DIATOM GENUS *MASTOGLOIA*

The naviculoid diatom genus *Mastogloia* Thwaites ex Wm. Smith (1856), is second only to very large genera such as *Navicula*, *Nitzschia*, and *Chaetoceros*, in terms of the number of taxa it includes. These are mostly marine, and usually occur attached to macroalgae. Although it has a wide geographic distribution, many taxa have been reported only from subtropical and tropical regions (e.g., the Indomalayan region and the Atlantic coast of Central America: Hustedt 1955). A great deal of taxa may be found in shallow, warm, hyperhaline waters (Novarino & Bazzichelli 1986, Muftah, pers. comm.).

The distinctive feature of the frustule is the presence of a highly specialized girdle band (valvocopula), contiguous to the valve, and bearing a ring of box-like structures (partecta) on its internal face (Novarino 1987). Although this feature makes a *Mastogloia* easy to recognize, identifying specimens to the species level (or below), is notoriously difficult.

In a previous thesis (Novarino 1985), I have attempted to address the question of the practical identification of specimens, using two different

approaches. The first one was statistical. It consisted in the use of a discriminating function (Fisher's), for distinguishing between the variety *linearis* and the type-variety of the species *M. pusilla*. Apparently, the proposed function served its purpose well. However, owing to the fact that its calculation required an *a priori* distinction between the two varieties, there is a possibility that an element of tautology was present.

The second approach was to collect as much taxonomic literature as possible, and to organize the information in a series of appropriately conceived computer databases. One database was an update (= a *bona fide* list of taxa) of the genus. As a result, the number of taxa whose existence was to be allowed for (ca. 280), was more than doubled with respect to that given in the main reference work for the taxonomy of the genus (Hustedt 1933). At that time, I was unable to consult VanLandingham's (1971) catalogue of diatom taxa. When this eventually became possible, some two years later, by combining his catalogue with mine, and incorporating in it a good deal of newly acquired information, I made an estimate of ca. 410 described taxa of *Mastogloia*. This was about 20% greater than VanLandingham's (1971) figure.

Another database was conceived as a 'resemblance list'. Since its original compilation, and submission in my previous thesis (Novarino 1985), much new information has been added to it, and its original structure has been greatly modified. This was done while at the School of Ocean Sciences in Menai Bridge, U.K.. In my opinion, this justifies its inclusion in the present thesis.

At a later stage, a different line of enquiry was pursued. Following an investigation on the internal structure of the partecta, which documented for the first time with the SEM a structure named *intrapartectal cylinder* (Novarino 1987), a detailed SEM study of one species (*M. smithii*), was carried out. This showed that the valvocopula is a complex structure, whose great variability in *Mastogloia* casts some doubts on the idea that the genus is a monophyletic assemblage. It also posed the question of a possible affinity between the genera *Mastogloia* and *Cocconeis*.

2. AIMS OF PART II

The aims of Part II are the following:

1. to present the latest versions of the update and 'resemblance list' of *Mastogloia*, which suggest that the simple collation and retrieval of information is a useful taxonomic tool; and
2. to present the results of the SEM study of *M. smithii*.

Chapter 11

An update of the taxa of the genus *Mastogloia*, with a 'resemblance list' for the more recently described ones

The substance of this chapter was published as a paper with the same title by G. Novarino (*Diatom Research*, 4(2): 319-343, 1990). A reprint is enclosed at the end of the present volume of this thesis.

Chapter 12

Observations on the frustule architecture of *Mastogloia smithii*

The substance of this chapter formed the object of the paper:

Novarino G., 1990 - Observations on the frustule architecture of *Mastogloia smithii*, with particular reference to the valvocopula and its integration with the valve. *Diatom Research*, 5(2) [in press].

Chapter 13

Discussion to Part II

Chapter outline: 1. Identification, taxonomy and systematics of *Mastogloia*. 2. Frustule architecture of *Mastogloia*. 3. Affinities of *Mastogloia*.

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1. IDENTIFICATION, TAXONOMY AND SYSTEMATICS OF *MASTOGLOIA*

Owing to the large number of taxa of *Mastogloia*, Hustedt's (1933) account of the genus is of limited usefulness for identifying specimens. The 'resemblance list' given here might be helpful when used in conjunction with Hustedt's taxonomic keys. A fully functional computer database version, enabling the list to be searched in a number of different ways, is in the process of being conceived by the author; when completed, it will be available for unrestricted distribution on disc. A preliminary text-only (i.e., wordprocessor file) version of the list was seen, prior to its publication, by T.B.B. Paddock, and its usefulness appreciated (Paddock & Kemp 1990).

Another approach to the question of identification might be an 'illustrated VanLandingham' (Paddock, pers. comm.), that is, a computer graphics catalogue of illustrations of as many taxa as possible. This is technically feasible, and the costs involved accessible. However, the amount of work required is probably beyond the possibilities

of a single worker.

Updates of described taxa and 'resemblance lists' might also be useful from a purely taxonomic viewpoint. By consulting them when describing new taxa, it could be possible to avoid the use of names which have already been published, and to ascertain that the taxa being described are not superfluous.

The 'resemblance list' does not pretend to have a systematic value. The most recent classification scheme available is that of Hustedt (1933), who recognized 11 subgeneric groups of *Mastogloia*. Some comments on this scheme can be found in Novarino (1985), where it was suggested that, on the basis of characters visible with the light microscope, it has a good general usefulness. However, Paddock & Kemp (1990), wrote that it would be interesting to make a comparison between Hustedt's groups and those likely to be conceived on the basis of micromorphological characters, as revealed by the SEM.

2. FRUSTULE ARCHITECTURE OF *MASTOGLOIA*

The structure named 'intrapartectal cylinder' by Novarino (1987), may occur, perhaps, in a greater number of species than previously supposed. In a sense, the IPC resembles an unpartitioned, miniature partectal ring, occurring within the partectal ring proper. Its significance is unclear. In their TEM study of *M. grevillei*, Stoermer *et al.* (1964), noted that the IPC contained numerous mitochondria. Therefore, it might serve the purpose of secluding an energy-producing compartment from the rest of the cell. This energy might be the one necessary for producing the strands of mucilage material which are known to be extruded from the partecta to the cell

exterior, *via* the partectal ducts, in numerous *Mastogloia* (Stoermer *et al.* 1964). However, this does not account for the fact that several species clearly lacking IPCs are capable of producing mucilage strands (Muftah & Novarino, unpublished observations).

The specialized, partecta-bearing valvocopula of *Mastogloia* appears to be tightly interlocked with the valve, at least as far as can be judged from the observations on *M. smithii*. It is not clear how the integration between valvocopula and parent valve is achieved in those species where the valve lacks pseudosepta, e.g. many 'elliptical' forms, for instance those studied by Stephens & Gibson (1979b), and Yohn & Gibson (1982a). When pseudosepta are lacking, the septum of the valvocopula is also much reduced. Presumably, in those cases different interlocking mechanisms are present.

The tight interlock between valvocopula and valve of *M. smithii* suggests that, during cell division, each daughter cell might inherit one parent valve with its valvocopula attached to it. If this is the case, then it should be possible to find cells with only one valvocopula (inherited from the mother cell), with the second one still not formed. This agrees well with light microscopical observations on *M. belaensis* (Voigt 1956), who wrote that 'the second set of loculi [*partecta*] must be formed after the separation of the two daughter frustules'.

3. AFFINITIES OF *MASTOGLOIA*

The phylogenetic affinities of *Mastogloia* are poorly understood. The various hypothesees on the subject

have been summarized in a study on the evolution of the monoraphid diatoms, to which *Mastogloia* was considered to be related (Kociolek & Stoermer 1986). The monoraphid genus *Cocconeis* Ehrenb. was viewed as the closest ally of *Mastogloia*. This was in agreement with early comments by P.T. Cleve (*non vidi*), who suggested that the 'loculiferous rims' seen in valvar views of many species of *Cocconeis* were homologous to the partecta of *Mastogloia*. A few years before Kociolek & Stoermer's study, Holmes *et al.* (1982), showed that the 'loculiferous rims' of *Cocconeis* were fimbriate valvocopulae; the resemblance between these and the partecta-bearing valvocopulae of *Mastogloia* were considered to be superficial. Thus, the valvocopulae of the two genera could not be interpreted as homologous.

What is the significance and origin of the morphology of the *partes interiores* of the valvocopulae? Mann (1982), in a study of *Rhoicosphenia curvata*, suggested that, during frustule morphogenesis, each part of the frustule acted as a mould for another one. In particular, the *pars interior* of the valvocopula of *R. curvata* appeared to be moulded around the valve pseudosepta. Similar 'mould effects' might also occur in other species, for instance in *Diploneis finnica*, where the crenellate *pars interior* appears to fit precisely over the ribs of the internal valve face (Idei & Kobayasi 1989). If 'mould effects' are a widespread phenomenon, then caution is needed on using *pars interior* morphology alone for inferring phylogenetic relations (Mann 1982).

A frustule of *Mastogloia* without its partecta-bearing valvocopula might easily be mistaken for a *Navicula* (*sensu lato*). Does this suggest a

relationship? The question cannot be answered at present, nor can that relative to a relation between *Mastogloia* and *Cocconeis*. Plates 57 and 58 show the valvocopulae of *Cocconeis scutellum* var. *staurophora*, which occurred as an epiphyte on intertidal *Fucus* and *Laminaria* spp. collected on the shore of the Menai Straits.

Chapter 14

More philosophical considerations: general conclusions to this thesis

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Cryptomonads have little in common with diatoms in general, and the genus *Mastogloia* in particular. Nevertheless, the general taxonomic questions are similar in the two groups. These are both in need of a practical method for identifying specimens to the species level, and reliable taxonomic characters for delimiting species. In addition, the concept of what a 'species' is, appears to be remarkably similar in both groups, at least as far as the practical notion is concerned.

Perhaps, some of the analogies outlined above could be due, at least in part, to similar historical causes. In the case of the cryptomonads, it could be inferred that 'Pringsheim's warning about the naming of genera and species in this class of algae' [the sentence was used by the anonymous reviewer of one of the papers included in this thesis], was widely seen as a sort of dogma. Pringsheim's view is certainly very authoritative; however, if we fail to name the members of the class, we are missing one of the main purposes of taxonomic investigation. A worthwhile approach to the taxonomic questions could be that of an electron-microscopy based (especially SEM), revival of the traditional, comparative method of the early,

light-microscopy based phycologists (Chapter 8). While I do not maintain that SEM may yield the final answer to the taxonomic questions, I do feel that it provides a means for:

- *addressing* the questions; and
- bringing cryptomonad taxonomy within the reach of a larger number of workers: medium-range SEM's, such as the one used in my study, are no more costly than a good, well-equipped research light microscope; in addition, the preparation techniques involved are not particularly difficult.

Are alternative approaches feasible? A possible reason why biomolecular techniques (especially isoenzyme electrophoresis), have not yet been tried, has been suggested in Chapter 8.

Our knowledge of the taxonomy and systematics of the genus *Mastogloia* seems to have progressed rather slowly. It could be hypothesized that the work of Hustedt (1933), may have exercised a kind of intellectual monopoly on the subject, much in the same way as Pringsheim's ideas seem to have acted as a dogma in the Cryptophyceae. The value of Arnold's (1948) views on the usefulness of keeping classification systems in a flexible state, cannot be overemphasized.

Appendix

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A database of selected culture strains of Cryptophyceae

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This database contains information on more than 150 culture strains. It has been compiled from data reported in the collection catalogues or published papers, or from information obtained directly from individual workers. Abbreviations are as follows:

CCAP = Culture Collection of Algae and Protozoa, U.K.;
CCMP = Center for Culture of Marine Phytoplankton, Bigelow Laboratories, West Boothbay Harbor, Maine, U.S.A.;
DK = Dr Dag Klaveness, Oslo, Norway;
GN = the author of this thesis;
IANL = Dr I.A.N. Lucas, Menai Bridge, U.K.;
MCC = Microbial Culture Collection, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan;
PLY = Plymouth Collection, U.K.;
SAG = *Sammlung von Algenkulturen*, Goettingen, F.R.G.; and
UTEX = Culture Collection of Algae, University of Texas, Austin, Texas, U.S.A.

The collection catalogues used were as follows:

- CCAP: 1982 ('previous') and 1988 ('latest') editions;
- CCMP: a print-out database supplied by Dr J.C. Green, Plymouth, U.K., in 1988 ('previous'), and the official collection catalogue supplied by Dr R.A. Andersen, Director, CCMP, in 1990;
- MCC: a copy of the official catalogue, supplied by Dr J.C. Green in 1988;
- PLY: a list supplied by Dr J.C. Green in 1988;
- SAG: the published 1982 catalogue (Schlosser

1982); and

- UTEX: the published 1978 and 1987 catalogues (Starr 1978, Starr & Zeikus 1987).

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