Chapter 18

Cryptomonads

Brec L. Clay
CH Diagnostic & Consulting Service, Inc., Berthoud, Colorado, USA

Chapter Contents

I INTRODUCTION

Cryptomonads, cryptoprotists, or cryptophytes, as these algae are commonly called, are a well circumscribed group of unicellular, biflagellate protists. They are variously classified within the division Cryptophyta, class Cryptophyceae, order Cryptomonadales, or phylum Cryptista *sensu* Cavalier-Smith (1986). Cryptomonads are important primary producers in freshwater and marine habitats (Gillot, 1990; Klaveness, 1988a,b). Many are cosmopolitan in their distribution, although they appear to be more common in cooler waters associated with high mountain and northern temperate lakes. Although routinely captured in phytoplankton samples, their cells are extremely delicate and easily rupture when fixatives are added or when temperatures are elevated. Consequently, their numbers are underestimated in preserved samples, reinforcing the perception that they are a rather obscure taxonomic group of protists. To the contrary, they often assume predominant phytoplankton status in temperate lakes and reservoirs where they often dominate the sub-ice, early spring, and late fall populations. Furthermore, the variations in cryptomonad cell structure discovered using specialized electron microscopic techniques (Hill, 1991a,b; Hill and Wetherbee, 1986, 1988, 1989; Kugrens and Lee, 1986, 1991; Kugrens et al., 1986, 1987; Lee and Kugrens, 1986; Clay and Kugrens, 1999a,b,c; Clay et al., 1999) strongly indicate that there are numerous freshwater genera and species that have yet to be formally recognized (Andersen, 1992).

While cryptomonads represent a well-circumscribed group of algae, there is another group of colorless flagellates that has been historically included within the Cryptophyta. Commonly referred to as kathablepharids, this little-known group of protists includes the genera *Kathablepharis, Leucocryptos*, and *Roombia*, which are common in both freshwater and marine habitats. Except for the presence of ejectisomes and placement of flagella, their cellular features do not support their

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inclusion within the cryptomonads (Lee and Kugrens, 1991; Lee et al., 1991; Vørs, 1992a,b; Clay and Kugrens, 1999a,b). Presently, phylogenetic analyses using molecular sequence data from several genes suggest that kathablepharids are a sister group to cryptomonads (Okamoto and Inouye, 2005; Okamoto et al., 2009). As a matter of convenience, and because a satisfactory treatment of this group rarely occurs elsewhere, kathablepharids are included along with the discussion of cryptomonads. In the interest of clarity and order, the discussion of cryptomonad characteristics is presented first followed by a separate description of kathablepharids.

II ULTRASTRUCTURE AND MORPHOLOGY (Figures 1 and 2)

A External Cell Architecture

Cells of cryptomonads have an asymmetrical shape that is strongly influenced by the vestibulum and furrow/gullet complex (Figures 1, 9A–C, 10A, 13A–C, 14A–C, 18A and C). Cell shapes may be oval, compressed, lunulate, caudate, acute, elongate, sigmoid, or otherwise contorted. All cells possess an anterior, outwardly facing depression called a vestibulum, which defines the ventral side of the cell. Two subapical flagella issue from the right margin (the back margin in *Goniomonas*) of the vestibulum, and the resulting asymmetry produces a distinctive gyrating motion about the long axis during swimming. In freshwater cryptomonads a contractile vacuole is located in the anterior end of the cell and usually discharges through a predetermined region in the dorsal portion of the vestibulum (Kugrens et al., 1986). In two genera, namely, the *Cryptomonas* campylomorph and *Chilomonas*, a small, flat appendage known as the vestibular ligule emanates from the dorsal rim of the vestibulum where it covers the discharge site of the contractile vacuole (Hill, 1991b; Kugrens and Lee, 1991; Kugrens et al., 1986).

A gullet, some type of furrow, or a combination of a furrow-gullet (Figure 9A–C) is one of the primary diagnostic traits for delineating genera. A furrow is a ventral groove, of variable length, that begins in the vestibular region of the cell and extends posteriorly, terminating somewhere in the anterior half of the cell depending on the species (Hill and Wetherbee, 1986, 1988, 1989; Klaveness, 1985; Kugrens et al., 1986; Munawar and Bistricki, 1979). A tubular invagination, called the gullet, may extend posteriorly from the vestibulum or from the end of the furrow (Munawar and Bistricki, 1979; Hill and Wetherbee, 1986, 1988, 1989; Kugrens et al., 1986).

Several types of furrows have been described in cryptomonads (Munawar and Bistricki, 1979; Klaveness, 1985; Kugrens et al., 1986). These include simple furrows that remain permanently open and complex furrows that dynamically open and shut, and such variations have served as fundamental characters in cryptomonad systematics (Hill and Wetherbee, 1986, 1988, 1989; Hill, 1990, 1991b; Clay et al., 1999). In fact, no less than five permutations in the furrow/gullet complex occur (Kugrens et al., 1986), including a gullet only (Figures 13A–C and 14A–C), simple furrow only (Figures 8A and 16A), simple furrow and gullet (Figure 4B), or a complex furrow structure with or without a gullet (Figures 4A and 5A).

Two types of furrow plates, scalariform (Figure 1) and fibrillar, may be associated with each type of furrow, thereby providing an additional source of variation on the overall furrow theme. A scalariform furrow plate takes on the form of a ladder, having sides connected by lateral, crystalline "rungs." Fibrillar furrow plates are made up of microfibrils that are oriented parallel to each other, occurring as a thin plate running along one side of the furrow.

Certain considerations must be borne in mind when ascertaining actual cell architecture in cryptomonads. Before Hill's studies, descriptions of such relied on light microscopic observations (Skuja, 1948; Huber-Pestalozzi, 1950; Butcher, 1967; Bourelly, 1970) because most electron microscopic preparatory procedures tended to grossly distort cell shapes. Parducz's (1967) fixation satisfactorily preserves cell shape; however, it has become established that freeze drying is the best method for examining the external features and determining whether a gullet and/or furrow is/are present. In fact, our understanding of the morphology of cryptomonad cells has been dramatically enhanced with the advent of cryofixation techniques. Characters such as tubular gullets, furrows, and furrow/gullet combinations have proved to be a significant delineator of genera and should serve to enhance light microscopic identifications. Unfortunately, few facilities use electron microscopic cryotechniques, and, in fact, finding a facility that does is becoming increasingly difficult.

B Periplast Structure (Figure 2)

Periplasts are unique coverings found in cryptomonads, consisting of a plasma membrane sandwiched between inner and surface components (Hibberd et al., 1971; Hill and Wetherbee, 1986, 1988, 1989; Kugrens et al., 1987; Kugrens and Lee, 1991; Wetherbee et al., 1986, 1987; Hill, 1990, 1991b; Clay and Kugrens, 1999a,b,c).

The inner periplast component (IPC) is comprised of proteinaceous plates of various shapes and sizes (Hibberd et al., 1971; Hill and Wetherbee, 1986, 1988, 1989; Kugrens and Lee, 1986; Hill, 1990, 1991b), or a solitary proteinaceous sheet

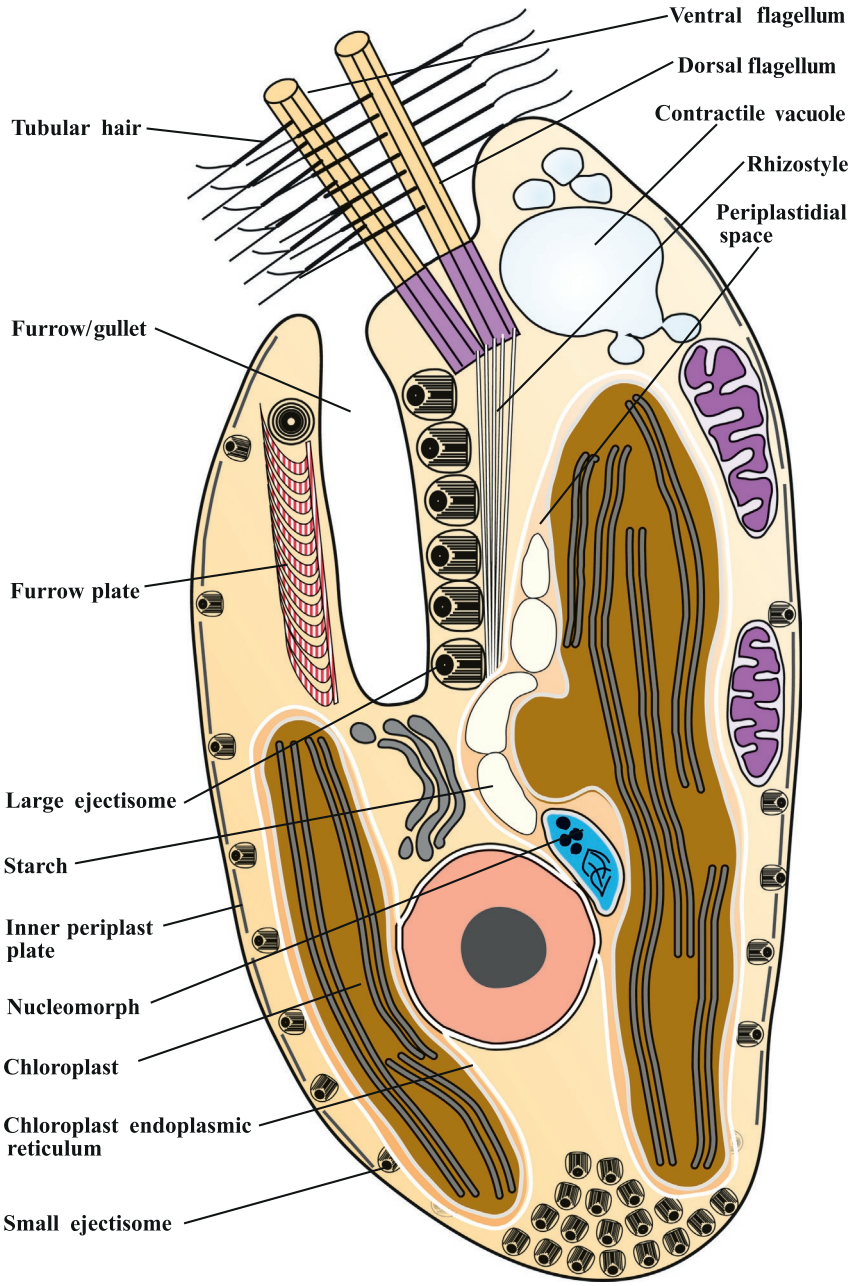

FIGURE 1 Diagram of a generalized cryptomonad cell showing the cellular details described in the text.

interrupted by pores where small ejectisomes may dock (Grim and Staehelin, 1984; Kugrens and Lee, 1986; Hill, 1991b). Plates of the IPC are attached to the cell membrane by intramembrane particles (IMPs) or proteins (Brett and Wetherbee, 1986; Hill and Wetherbee, 1986, 1988, 1989; Kugrens and Lee, 1986, 1991; Wetherbee et al., 1986, 1987; Clay et al., 1999), whereas proteinaceous sheets lack IMPs. The arrangement of these IMP domains conforms to the plate shapes (Brett and Wetherbee, 1986; Kugrens and Lee, 1986, 1991; Wetherbee et al., 1987; Clay et al., 1999). Variations in the shapes of these plates—they may be hexagonal, square, oval/round, rectangular, or irregular—are systematically informative and have been used to establish genera.

A sheet-like variant is lacking among the ensemble of surface periplast components (SPCs) and, instead, presents as plates, heptagonal scales, mucilage, or a combination of any of these. Apparently both types of IPCs as well as some of the surface plates are composed of protein (Gantt, 1971; Faust, 1974).

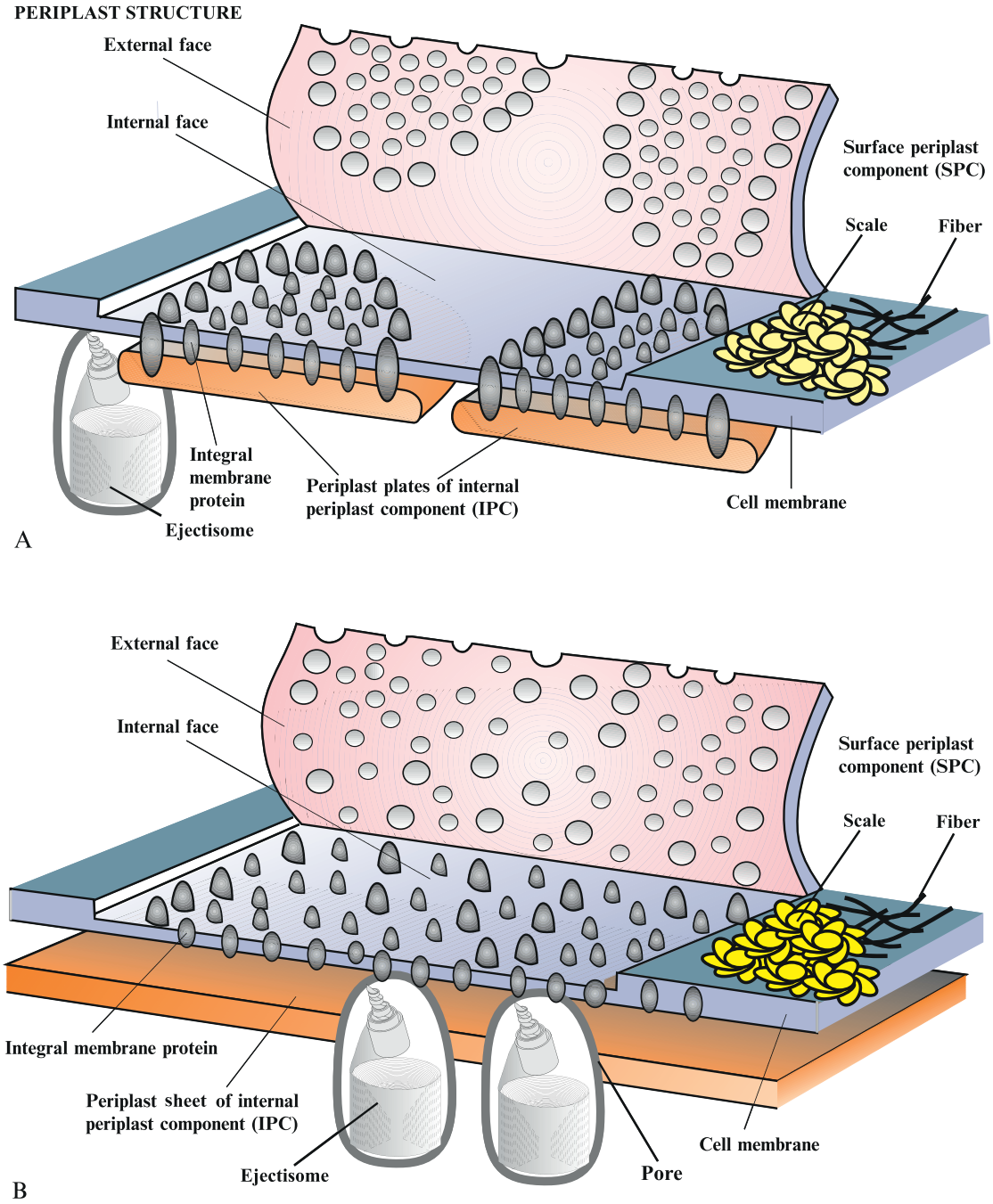

FIGURE 2 Diagrams of two major periplast types depicting their relationship with the plasma membrane and ejectisomes. (A) Periplast structure in cells where the inner periplast component is composed of plates. The plates are attached to the plasma membrane by transmembrane particles. (B) Periplast structure in cells having an inner periplast component consisting of a single sheet. The sheet is not intimately associated with the plasma membrane.

Because plate shapes are used for designating genera, it is critical that plate shapes are accurately determined. Oakley and Santore (1982), Gantt (1971), and Faust (1974) have suggested that periplast shapes may undergo natural conformational changes. However, it is now established that plates do not undergo shape changes unless they are subjected to drastic treatments such as fixation, desiccation, or excessive centrifugation. Therefore, any technique other than quick-freezing may introduce artifacts, particularly in those periplasts having circular or oval plates. The shapes of these plates are easily susceptible to altered geometries resulting from chemically and physically induced pressures among contiguous plates. For example, in periplasts where the plates are approximately the same size and each plate is surrounded by six others (1 by 6

arrangement), introduced pressures that force plates against each other transform round/oval plates into a hexagonal pattern (Kugrens et al., 1987).

Some studies have attempted to use either light (Novarino, 1993a,b) or scanning electron microscopy (Santore, 1977; Novarino, 1991a,b; Novarino and Lucas, 1993; Novarino et al., 1994) to determine plate shapes, but with a few exceptions (Munawar and Bistricki, 1979; Klaveness, 1985; Kugrens et al., 1986; Hill, 1990), scanning electron microscopy is inadequate for studying subsurface components. Where possible, quick-freezing freeze-fracture protocols should be used to study inner periplast plate shapes. By employing this technique, cells are quick-frozen (slammed) without any need for pretreatment or chemical fixation (Boyne, 1979; Chandler, 1984; Phillips and Boyne, 1984), thereby accurately preserving the delicate periplast shapes and the intimate associations that exist between the plates and the plasma membrane.

C Flagella and Flagellar Apparatus (Figure 1)

With the exception of *Goniomonas*, where the flagella are inserted on the dorsal side of the vestibulum (Figure 3), the flagella of cryptomonad cells are inserted subapically on the right side of the vestibulum. The two flagella are subequal in length and bipartite tubular hairs adorn at least one of the flagella (Kugrens et al., 1987; Clay et al., 1999). There appear to be at least five variations in the arrangement of tubular and nontubular hairs on the flagella (Kugrens et al., 1987), which only can be visualized using electron microscopy (EM).

The most common arrangement of flagellar hairs is one in which the longer or dorsal flagellum bears two laterally opposed rows of tubular hairs and the shorter or ventral flagellum bears a single row of hairs. Tubular hairs on the dorsal flagellum have one solid extension called a terminal filament, whereas the tubular hairs of the ventral flagellum have two

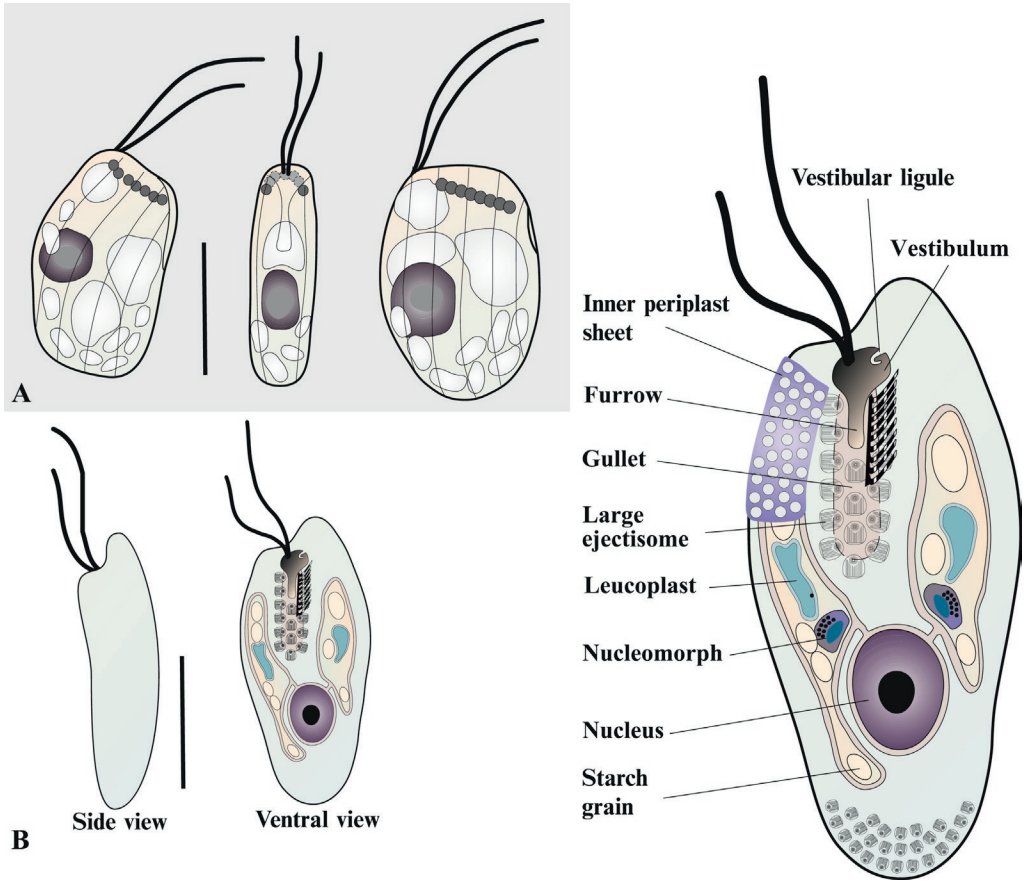

FIGURE 3 Light micrographic illustrations of the colorless *Goniomonas truncata* and the secondarily colorless *Cryptomonas* (*Chilomonas*) *paramecium* (A) *Goniomonas truncata* showing the laterally flattened nature of cells, the dorsal nucleus, and the dorsally inserted flagella. Vertical striations shown in the diagrams are visible with a light microscope. Scale bar = 5 µm. (B) *Cryptomonas* (*Chilomonas*) *paramecium* variously observed. Cells lack pigment but possess a reduced plastid known as a leucoplast. Scale bar = 10 µm.

unequal terminal filaments. In addition, flagella may bear small heptagonal scales (Pennick, 1981; Lee and Kugrens, 1986). Instead of tubular hairs, *Goniomonas* has a unilateral row of curved spikes decorating one of its flagella and fine, nontubular hairs on both flagella (Kugrens et al., 1987; Kugrens and Lee, 1991).

The flagellar transition region is unique and consists of a doublet system of septa in all cryptomonads (Grain et al., 1988; Kugrens and Lee, 1991). A rhizostyle is an integral component of the flagellar apparatus in most cryptomonads, consisting in part of a band of microtubules originating near the basal bodies and then extending posteriorly into the cell. One type of rhizostyle, found in *Chilomonas* (Roberts et al., 1981; Kugrens and Lee, 1991), *Hanusia phi* (Gillott and Gibbs, 1983; Gillot, 1990; Deane et al., 1998) *Teleaulax* (Hill, 1991b), *Storeatula* (Hill, 1991c), *Geminigera* (Hill, 1991c) and *Proteomonas* (Hill and Wetherbee, 1986), passes close to the nucleus and terminates near the posterior end of the cell. From each microtubule issues a wing-like extension (keel) and the length of each keel may vary with its respective microtubule (Gillott and Gibbs, 1983). A second type of rhizostyle, reported for *Cryptomonas ovata* (Roberts, 1984; Hill, 1990) and *Cryptomonas theta* (=*Guillardia theta*) (Gillott and Gibbs, 1983), lacks wings/keels coming off the microtubules. This type of rhizostyle usually terminates anterior to the nucleus (Roberts, 1984).

D Ejectisomes (Figures 2 and 6)

Cryptomonad ejectisomes (sometimes called trichocysts) were the first to be described (Anderson, 1962), but different types have been discovered in other organisms (noted below). Ejectisomes are extrusive organelles fabricated as tightly coiled ribbons within Golgi vesicles, and they appear to be structurally identical in all genera so far investigated (Kugrens et al., 1994). Two sizes of ejectisomes occur within all cryptomonad cells (Schuster, 1970; Kugrens et al., 1994; Clay et al., 1999). Large ejectisomes are located near the gullet/furrow complex, whereas small ejectisomes occur throughout the peripheral cytoplasm. Both types consist of two unequal sized wound, tapered ribbons that are joined together and enclosed by a membrane (Kugrens et al., 1994). The ribbons have a crystalline substructure (Morrall and Greenwood, 1980; Grim and Staehelin, 1984; Kugrens et al., 1994). Discharged ejectisomes from *Chroomonas* and *Cryptomonas* have been isolated and further characterized by Rhiel and Westerman (2012). The ejectisomes are forcibly discharged when the cell is disturbed, resulting in the formation of a long tube as the ribbons unfurl in spiral fashion. The tapered tube from the unfurling of the small ribbon projects off the large tube at a slight angle (Kugrens et al., 1994). In effect, the "detonated" ejectisome thrusts the cell in the direction opposite of its extension, thus appearing to function as a means of escape.

A few *Pyramimonas* species (chlorophytes) and the colorless protists *Kathablepharis* (Lee and Kugrens, 1991; Kugrens et al., 1994; Clay and Kugrens, 1999a,b) and *Leucocryptos* (Vørs, 1992a,b) also possess ejectisomes; however, these differ in structure from cryptomonad ejectisomes. In all three of these genera, the ejectisomes are constituted of large ribbons only. Upon discharge, and similar to cryptomonad ejectisomes, the ribbon unfurls to form a hollow tube. Unlike cryptomonad ejectisomes, however, the tube results from involution, whereby the edges of the unwound ribbon curl inwards toward each other until the tube is formed. Ejectisome discharge is rapid and forceful, propelling the organism in the direction opposite the discharge. Like cryptomonad ejectisomes, these likely function as an escape mechanism or as antiherbivory defenses (Kugrens et al., 1994).

E Ejectisome Digestion Vesicles

Ultrastructural evidence indicates that some vesicles in cryptomonads are specialized for ejectisome autolysis (Kugrens et al., 1994). These vesicles form by the fusion of several ejectisome chambers, and they continue to enlarge by accreting additional ejectisome membranes. Recently formed digestion vesicles contain disaggregated solitary ejectisomes, whereas components of expanded ejectisomes comprise the contents of older vesicles. In the final autolytic stages, most of the tubular, expanded components of ejectisomes are no longer recognizable, and the contents appear fibrillar or granular. The vesicle sizes are larger in cells from older cultures, and there may be several vesicles present per cell. Golgi vesicles have been observed to fuse with existing vesicles, presumably adding lytic enzymes to the mix. The vesicles apparently represent specific repositories for defective ejectisomes or for recycling older surplus ejectisomes. Under the light microscope, these structures may represent the refractive vesicles that are frequently reported and formerly may have been referred to as the Corps de Maupas (Lucas, 1970b).

F Mitochondrion and Chloroplasts (Figure 1)

A single reticulate mitochondrion with flattened cristae (Santore and Greenwood, 1977; Roberts et al., 1981; Kugrens and Lee, 1991) apparently occurs in cells of all cryptomonads.

The apparent color of chloroplasts may be brown to olive green, blue-green, or red, depending on the ensemble of pigments present. Pigments consist of chlorophylls a and c2, alpha and beta carotene, alloxanthin, diadinoxanthin, and several forms of blue and red phycobiliproteins called Cr-phycocyanin and Cr-phycoerythrin (Glazer and Appell, 1977; Hill and Rowan, 1989). With the exception of a marine endosymbiont (Hibberd, 1977), only one or two chloroplasts occur in the cells of pigmented genera (Santore, 1984, 1987; Hill, 1991a, b, c; Hill, 1991c). In addition, all pigmented forms possess either Cr-phycocyanin or Cr-phycoerythrin (Hill and Rowan, 1989), both of which are located in the intrathylakoidal lumens of the photosynthetic lamellae (Gantt et al., 1971; Faust and Gantt, 1973; Gantt, 1979, 1980; Ludwig and Gibbs, 1989).

Chloroplasts are surrounded by a double membrane variously referred to as the periplastidial envelope, periplastidial compartment, periplastidial complex, or chloroplast endoplasmic reticulum (CER) (Gillott and Gibbs, 1980). The CER is continuous with the outer membrane of the nuclear envelope and encompasses the chloroplast, starch granules, and a reduced red-algal nucleus known as the nucleomorph (Gillott and Gibbs, 1980; Santore, 1982c; Ludwig and Gibbs, 1985a). Starch granules are formed not within the chloroplast but, rather, within the periplastidial compartment where they generally associate with a pyrenoid if present. The number of thylakoids penetrating the matrix of the pyrenoid has been suggested as a possible systematic character (Santore, 1984). Thylakoids usually are arranged in pairs (Gantt et al., 1971; Dwarte and Vesk, 1982, 1983; Santore, 1984), sometimes in groups of three (Klaveness, 1981; Hill, 1991b), or in stacks of variable number (Hill, 1991b). Secondarily, colorless genus *Cryptomonas* species possesses a reduced chloroplast called a leucoplast (Figure 3), which lacks pigments (Sespenwol, 1973; Heywood, 1988; Kugrens and Lee, 1991). *Goniomonas* primitively lacks plastids and a nucleomorph, and consequently, it also lacks a periplastidial compartment.

G Nucleomorphs (Figure 1)

With the exception of *Goniomonas*, all cryptomonads harbor a peculiar compact nucleus termed a nucleomorph located within the periplastidial compartment (Gillott and Gibbs, 1980; McKerracher and Gibbs, 1982; Morrall and Greenwood, 1982; Santore, 1982c, 1984, 1987; Ludwig and Gibbs, 1985a; Kugrens and Lee, 1991). Nucleomorphs represent a vestigial nucleus derived from an ancestral red-algal endosymbiont (Douglas et al., 1991; McFadden et al., 1997; Archibald, 2007). This relic nucleus is limited by a double membrane, and its diminutive genome is organized into three linear chromosomes bearing genes that code for ribosomal RNAs, heat shock proteins, and several other plastid-specific proteins (Archibald, 2007; Lane et al., 2006). Several nucleomorph genomes have been karyotyped and comparisons among these are yielding new insights into the phylogenetic relationships of cryptomonads (Lane et al., 2006). In addition, the location of the pyrenoid within the periplastidial compartment is proving to be systematically informative (Santore, 1984; Hill and Wetherbee, 1989), specifically in *Rhodomonas* and *Storeatula* where the nucleomorph is located in the pyrenoidal bridge (Hill and Wetherbee, 1989; Novarino, 1991a,b).

H Reproduction and Life Cycles

The usual mode of reproduction is asexual and is occurs by mitotic divisions followed by cytokinesis. Complex sexual cycles have been documented for *Proteomonas* (Hill and Wetherbee, 1986), *Chroomonas* (Kugrens and Lee, 1988), and *Cryptomonas* (Hoef-Emden and Melkonian, 2003). That the life cycle of some cryptomonads can be quite complex was first demonstrated for *Proteomonas sulcata* (Hill and Wetherbee, 1986). This species manifests as two morphotypes, termed the diplomorph and the haplomorph, which are considerably distinct with respect to size, IPC component, flagellar apparatus configuration, and ploidy level. The life cycle of certain strains of *Cryptomonas* also have been shown to be equally complex (Hoef-Emden and Melkonian, 2003). Cultures of *Cryptomonas* derived from single cell isolates surprisingly gave rise to populations of cells that were identified as *Cryptomonas* and *Campylomonas*, implying that *Cryptomonas* is also a dimorphic genus and that the previously established *Campylomonas* genus may simply be an alternate form in the life cycle of *Cryptomonas*. As with *Proteomonas*, the two *Cryptomonas* morphotypes, the cryptomorph and campylomorph (Figure 4), exhibit profound differences with regard to cell size and periplast structure (Hoef-Emden and Melkonian, 2003). Others examples of dimorphic life cycles are suspected, emphasizing the need for caution when attempting to identify species solely on the basis of morphological characters.

Resistant spore production is rare but some species are able to generate thick-walled cysts or to transform into palmelloid stages to withstand adverse conditions (Santore, 1978). It has been suggested that palmelloid cell aggregates, enveloped by extensive mucilage, may be an adaptation to deter grazing (Klaveness, 1988a).

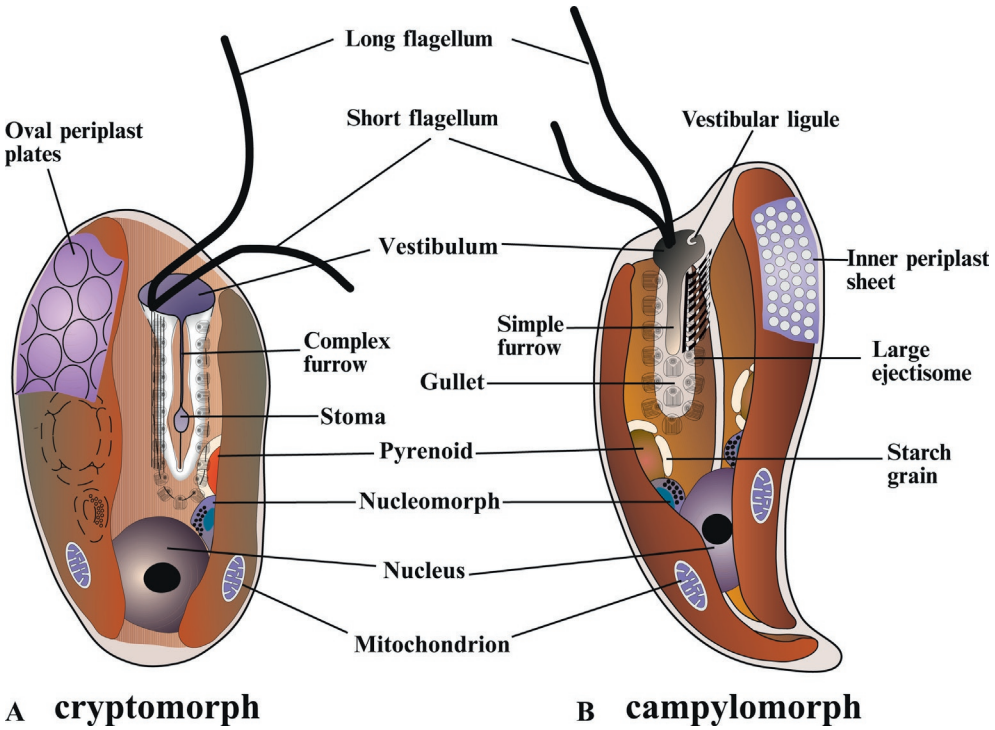

FIGURE 4 Diagrams of the dimorphic genus *Cryptomonas* spp. depicting the two alternate forms, termed the cryptomorph and the campylomorph, occurring as part of the life cycle. The cryptomorph and campylomorph express pronounced differences in cell size, morphology, periplast structure, and furrow/gullet structure.

I Nucleus and Mitosis (Figure 1)

The nucleus is typical of eukaryotes and is located in the cell posterior. During interphase it contains dispersed chromatin and a prominent and persistent nucleolus. The outer membrane of the nuclear envelope expands to form the CER around the chloroplast, nucleomorph, and starch. The Golgi apparatus, the gullet/furrow, and contractile vacuole in freshwater species are situated anterior to the nucleus.

Mitosis has been studied in *Chroomonas salina* (Oakley and Dodge, 1973, 1976; Meyer and Pienaar, 1981, 1984b), *Cryptomonas* sp. (Oakley and Bisalputra, 1977; Oakley and Heath, 1978), *Cryptomonas theta* (McKerracher and Gibbs, 1982), and *Chroomonas africana* (Meyer and Pienaar, 1981, 1984b). Generally, microtubules proliferate near the flagellar bases at the onset of mitosis. The nuclear envelope disaggregates as the microtubules migrate away from the basal bodies to form a mitotic spindle. At metaphase the chromosomes appear as a solid mass at the equator of the spindle. Chromosomal microtubules articulate to chromosomes within the mass (Oakley and Dodge, 1973, 1976; McKerracher and Gibbs, 1982), but specialized structures for microtubular attachment known as kinetochores have not been observed. At anaphase the solid chromosomal mass splits and the two masses move toward their respective poles. A cytokinetic ring is formed at metaphase, and the ring constricts following anaphase to cleave the cells into two daughter cells. Cytokinesis and daughter-cell separation follow a characteristic pole reversal as part of daughter-cell formation (Perasso et al., 1993).

J Contractile Vacuoles (Figures 1 and 14A)

Most freshwater cryptomonads possess a pulsating vacuole, known as a contractile vacuole, located in the anterior end and that functions in osmoregulation (Figure 1). The contractile vacuole receives and expels excess water and waste metabolites from the cell (Patterson, 1981). Because freshwater cryptomonads exist in a hypoosmotic medium, there is a net influx of water into the cell, and the contractile vacuole prevents osmotic rupture by actively expelling water.

K Starch (Figure 1)

The principal storage product is starch, and it is cached as granules in the periplastidial space outside of the chloroplast. If a pyrenoid is present, some starch accumulates as large plates around the pyrenoid (Figures 4B and 13A). Cryptomonad

TABLE 1 Characters Useful in Defining Freshwater Cryptomonads

GENUS	Furrow/Gullet[a]	IPC	SPC	Furrow Plate	VL	Rhizostyle	Nm.Loc	AP[b]
Goniomonas	Furrow only	RP	RP	??[c]	No	Nonkeeled	None	None
Cryptomonas (colorless)	1/4 furrow	IS	Fibrils	Scalariform	Yes	Keeled	Between L&N	None
Cryptomonas (cryptomorph)	2/3 furrow	OP	Fibrils	Fibrous	no	Nonkeeled	Between P&N	P.E. 566
Cryptomonas (campylomorph)	1/3 furrow	IS	Fibrils	Scalariform	Yes	Keeled	Between P&N	P.E. 566
Plagioselmis	Furrow only	HP	Scales	Fibrous	No	Nonkeeled	Between P&N	P.E. 545
Hemiselmis (red species)	Gullet only	IS?	HP	Fibrous	No	None	Between P&N	P.E. 555
Hemiselmis (blue-green species)	Gullet only	IS?	HP	Fibrous	No	None	Between P&N	P.C. 615
Pyrenomonas	1/8-1/2 furrow	SP	Fibrils	Fibrous	No	Keeled	In pyrenoid	P.E. 545
Storeatula	Gullet only	IS	Fibrils	Scalariform	No	Keeled	In pyrenoid	P.E. 545
Chroomonas	Gullet only	RP	RP/scales	None	No	None	A to P	P.C. 630
Komma	Gullet only	HP	HP/scales	Fibrous	No	None	Between P&N	P.C. 645

IPC, inner periplast component; SPC, superficial periplast component; VL, vestibular ligule; Nm.Loc, nucleomorph location; AP, accessory pigment; RP, rectangular plates; OP, oval plates; HP, hexagonal plates; SP, square plates; IS, inner sheet; L&N, leucoplast and nucleus; P&N, pyrenoid and nucleus; A to P, anterior to pyrenoid; PE, phycoerythrin; PC, phycocyanin.

[a]*Values given as fractions in this column denote the length of the furrow relative to the entire length of the furrow/gullet complex.*

[b]*Values indicate light absorption maxima, expressed in nanometers, for cryptomonad phycobilin pigments.*

[c]*No available data for this character.*

starch is an α-1-4-glucan composed of 30% amylose and amylopectin. This is similar to starch found in green algae and dinoflagellates (Antia et al., 1979), and it stains purple with iodine.

III ORIGIN OF CRYPTOMONADS

With the exception of the primitively aplastdial genus *Goniomonas*, cryptomonads are one of the most complex cells known, consisting of an amalgam of four genomes: the host nuclear genome, the mitochondrial genome, the chloroplast genome, and the nucleomorph genome. The chimaeric nature of these protists apparently resulted from three distinct symbiotic events: two prokaryote-eukaryote endosymbiotic associations and one eukaryote-eukaryote endosymbiotic association (McKerracher and Gibbs, 1982; Ludwig and Gibbs, 1985a,b, 1989; Gillot, 1990; Douglas et al., 1991; McFadden et al., 1994, 1997). Therefore, except for *Goniomonas* (McFadden et al., 1994), cryptomonads consist of a eukaryotic host cell, two prokaryotic endosymbionts (the mitochondrion and chloroplast), and a relic eukaryotic endosymbiont.

The evolutionary events leading to the establishment of the cryptomonad chloroplast result from a secondary endosymbiosis, which began when a colorless phagocytic protozoan ingested a red algal cell and failed to digest it (McFadden, 1993; McFadden et al., 1997). Once inside the food vacuole, and having averted digestion, the red-algal cell established itself as an endosymbiont. Over time, the endosymbiont was transformed into a plastid and became enveloped by two concentric membranes known as the CER. The inner membrane of CER is derived from the plasma membrane of the red-algal endosymbiont, whereas the outer membrane of the CER apparently resulted from the hybridization of the food vacuole membrane with the outer membrane of the nuclear envelope. Although initially "naked," the food vacuole membrane gradually became studded with 80S ribosomes that were present on the outer nuclear membrane. Presumably, this occurred by passive diffusion through the lipid bilayer of preexisting ribosome receptors (i.e., ribophorins) found within the outer nuclear membrane. The 80S ribosomes occurring on the outer membrane of the CER are distinct from the 80S ribosomes found in the cytoplasm of the red algal endosymbiont.

As the transformation from an endosymbiont to a plastid proceeded, many of the genes present within the genome of the endosymbiont were translocated to the host nucleus. Such bulk genetic migration necessitated the evolution of a complex protein import mechanism, allowing algal gene products transcribed on host cytosolic ribosomes to be targeted across the two topogenically unique membranes of the CER, through the periplastidial space, and back into the chloroplast. Having surrendered much of its control to the host cell, and having become absolutely dependent upon the host for its metabolism and survival, the red-algal endosymbiont assumed a new identity as a photosynthetic organelle.

As suggested above, the identity of the cryptomonad nucleomorph has been well established and is substantiated by several lines of evidence. Molecular data indicates that the nucleomorph is of red-algal origin, and the presence of starch in the periplastidial cytoplasm is a visual testament to this inference. Moreover, cryptomonad chloroplasts contain phycobiliproteins similar to those found in red algae (Glazer and Appell, 1977), and they possess the type I purple form of Rubisco, which among eukaryotes occurs only in red algae and cryptomonads (Martin et al., 1992).

Although the phylogenetic affinity of the nucleomorph to that of red algae is robustly supported, that surrounding the cryptomonad host is less certain. Based upon comparative ultrastructure, the host cell appears most related to the Heterokonta by the shared presence of chloroplasts surrounded by CER and of having at least one flagellum ornamented with tubular hairs (Cavalier-Smith and Chao, 2006). However, heterokonts lack the flattened cristae characteristic of cryptomonad mitochondria and, instead, have mitochondria with tubular cristae. Also, cryptomonad tubular hairs have a bipartite structure instead of the tripartite version present on heterokont flagella. Although ongoing phylogenetic analyses have narrowed the possibility of cryptomonad host affinities, none has yet to produce an unequivocal picture of the relationship of the host to that of other protists. Several sequence studies seem to infer that cryptomonads belong to a larger ensemble of protists known as chromalveolates that include heterokonts, haptophytes, dinoflagellates, and apicomplexans (Fast et al., 2001). Unfortunately, phylogenetic efforts have yet to consistently authenticate the Chromalveolata as a natural grouping. Multigene phylogenetic analyses do, however, robustly support the relationship between the cryptomonad host and haptophytes (Keeling, 2009). Further support for this inference comes from the observation that both groups share a signature horizontal gene transfer event resulting in the substitution of a certain ribosomal protein gene (Keeling, 2009). Also, the kathablepharids (see Section IX) represent another group of protists with flattened mitochondrial cristae that appear to be closely related to the cryptomonad host based upon SSU rDNA and beta-tubulin analyses (Okamoto and Inouye, 2005; Okamoto et al., 2009).

Given the convergence of structural, molecular, and biochemical data, it is now widely accepted that extant photosynthetic cryptomonads evolved monophyletically from an ancestral chimaeric protozoan that transformed a red-algal unicell, possessing both phycoerythrin and phycocyanin, into a permanent plastid (Cavalier-Smith et al., 1996). Apparently during evolution into an organelle the phycobilisomes within the chloroplast underwent biochemical erosion by loss of

allophycocyanin and phycocyanin, leaving only a truncated version of phycoerythrin. Initially existing as a hexameric structure, the remaining phycoerythrin biliprotein was reduced to a tetrameric form that eventually gave rise to all seven known varieties of cryptomonad phycobilins (Hoef-Emden, 2008). For that reason the known spectral properties of the various phycobilins are not due to differential loss of either phycoerythrin or phycocyanin as long thought but, instead, are due to various substitutions of linear chromophores that covalently bond to the biliprotein tetramer (Apt et al., 1995; Glazer and Wedemeyer, 1995; Marin et al., 1998). If phycoerythrin was the ancestral accessory pigment of all extant cryptomonads, then blue-green cryptomonads were derived from phycoerythrin-containing forebears. In fact, biochemical evidence reveals that blue-green cryptomonads do possess true phycoerythrins, but they appear blue-green because linear phycoerythrobilin chromophores (red tetrapyrroles) have been replaced with phycocyanobilin chromophores (blue-green tetrapyrroles). Molecular analyses combining sequences from nuclear small-subunit rDNA, partial nuclear large-subunit rDNA, and nucleomorph small-subunit rDNA strongly support the absence of phycocyanin in the ancestral cryptomonad and the monophyletic origin of all blue-green cryptomonads (Marin et al., 1998; Deane et al., 2002). As discussed, blue-green cryptomonads possess true phycoerythrins but are able to simulate the blue-green color of phycocyanin due to chromophore replacement. Interestingly, the genus *Hemiselmis* possesses both red and blue-green members but consistently groups within the blue-green clade despite being identical in their structure and morphology (Clay and Kugrens, 1999b). This observation has led some researchers to conclude that only slight biochemical modifications are necessary for cryptomonads to reverse color (Lane and Archibald, 2008). Such a reversal apparently happened at least once in *Hemiselmis*, yielding two recognized red species in a genus otherwise comprised of blue-green forms.

IV ECOLOGY AND DISTRIBUTION

Cryptomonads have been reported from nearly all types of water throughout the world including arctic, temperate, and tropical oceans; streams, lakes, and reservoirs; and environments of variable salinity (Klaveness, 1988a,b). Although cosmopolitan in distribution, it is within temperate lakes that cryptomonads reach their highest diversity and populations (Taylor et al., 1979), particularly in the deep layers of clear oligotrophic lakes (Nauwerk, 1968). Several forms have even exploited intracellular niches, serving as functional endosymbiotic chloroplasts for some ciliates (Hibberd, 1977; Klaveness, 1988a) and dinoflagellates (Lewitus et al., 1999). Unfortunately, the number of cryptomonad species and their population sizes generally are underestimated in phytoplankton inventories because collected samples are often preserved with destructive fixatives such as formaldehyde (Klaveness, 1988a).

A Abiotic Considerations

In lentic, estuarine, and marine habitats, cryptomonads are perennial residents of the phytoplankton community. In fact, when other algal populations are diminishing, cryptomonads increase in numbers (Rott, 1983; Klaveness, 1988a,b). In many lakes there appears to be a population peak during autumn destratification when the warmer waters of the epilimnion mix with the cooler waters of the hypolimnion (Pollingher, 1981). In an extensive survey conducted by Taylor et al. (1979) of lakes in the eastern and southeastern United States, cryptomonads seemed to prefer colder waters, an observation that matches those made in the Rocky Mountain region. In small temperate lakes they form stable stratified populations, although their place in the water column varies due to diel vertical migration (Salonen et al., 1984). It has been noted that populations are able to migrate vertically within the water column within a range that does not exceed 5 meters. Such water column dynamics serve at least two purposes including avoiding high irradiance during the day while exploiting the phosphorus-rich hypolimnion (Knapp et al., 2003) and minimizing grazing pressure by zooplankton (Loret et al., 2000). Formation of resting stages during some parts of the year is another seasonal strategy invoked by cryptomonads to withstand adverse conditions. In most lakes, cryptomonads exhibit maximal population densities far below the surface (Reynolds, 1980, 1984; Rott, 1983). Optimal depths have been reported from 15 to 25 m, with the deepest depths occurring in late spring and early summer, and the shallowest in late autumn and early winter. This pattern primarily is seen in more productive, buffered lakes with low turbulence and, therefore, reduced irradiance levels. In low buffered, eutrophic lakes, the decrease in pH due to photosynthesis favors productivity of cyanobacteria and a decrease in cryptomonads.

Most cryptomonad species appear well suited to low light conditions made possible by the phenomenon of chromatic adaptation. Moreover, some species are able to survive after prolonged periods of darkness. One particular strain was shown to viable after being subjected to a dark period lasting more than 24 weeks, whereas two other species, *Hemiselmis virescens* and *Rhodomonas lens*, survived for only a 4-week dark-period maximum. Survival during winter under lake ice in near-dark conditions has been shown to be due to efficient photosynthetic machinery, low rates of cellular respiration in colder waters, a change in lipid composition (Henderson and Mackinlay, 1989), and low grazing pressures (Morgan and Kalff, 1975).

Day length and its relation to water depth have been shown to be important factors shaping phytoplankton communities (Arvola et al., 1991). Because of the light-attenuating effects of water, daybreak occurs later and nighttime occurs earlier with increasing depth. At its extremes, light intensity that is too low translates into low productivity, and light intensity that is too high proves detrimental to some phytoplanktonic algae. Consequently, day length acts as a selective force that favors those planktonic algae most capable of responding to the day length-light intensity variable. As noted, cryptomonads are one group of algae highly attuned to light intensity, and thus are able to avoid the extremes through diel vertical migration behavior (Watanabe et al., 1976; Watanabe and Furuya, 1982a,b; Arvola et al., 1991).

Variation in pH among lakes differentially shapes cryptomonad populations, sometimes even among strains of the same species isolated from different sources (Pringsheim, 1968; Klaveness, 1988a). However, it is often difficult to determine what is ultimately responsible for these differences because other environmental factors are at play along with pH under natural conditions. Nevertheless, laboratory studies indicate that strains of a given species, when isolated from different geographic regions, exhibit their optimal growth at pH ranges correlated to those from the locality where they were isolated (Pringsheim, 1968). Another laboratory study conducted on a strain of *Rhodomonas lacustris* showed good growth between pH 6 and pH 8.5, although pH 10 was tolerated at the given light cycle in unbuffered media (Klaveness, 1977). As with other environmental parameters, cryptomonads as a whole are able to tolerate a wide range of pH, but the vast majority of strains generally tolerate a much narrower pH range. Personal observations in Colorado and Wyoming reveal that most cryptomonads favor alkaline conditions and are most prominent in lakes with pH values above 7.5.

It has been proposed that algae with a CER, such as the cryptomonads, might have an ecological advantage in high pH environments (Lee and Kugrens, 1998, 2000). Dissolved inorganic carbon (DIC) occurs mainly in bicarbonate form in waters that have high pH. The carbon-fixing enzyme Rubisco can only utilize DIC in the form of CO_2. Therefore, if the space within the CER is acidic, then it could serve as a reservoir of DIC in the form of CO_2, which is something that algae lacking CER would be incapable of. The ongoing availability of CO_2 within this space would impart a competitive advantage to these algae in waters high in pH and low in CO_2.

Cryptomonads are not thought of as a group of toxigenic algae. The only intimation of such traces to a report of massive catfish kills in some Texas ponds. The putative agent of these deaths was identified as a cryptomonad of the genus *Cyanomonas* (Pfiester and Holt, 1978). Unfortunately, neither the organism nor the toxin was ever isolated, and, therefore, the identity of *Cyanomonas* remains suspect. Furthermore, the light micrographs (LMs) presented in the cited publication lacked the image quality needed to determine whether it was indeed *Cyanomonas* or even a member of the cryptomonads. *Rhodomonas* sp. also has been implicated in exotoxin production (Stemberger and Gilbert, 1985), although it was not demonstrated that the observed inhibition of rotifer growth was specifically due to *Rhodomonas*. Substantial bacteria in the cultures may have been the causative agents.

B Biological Considerations

Cryptomonads are favored food organisms for a variety of zooplankton (Guillard, 1975; Klaveness, 1984; Stemberger and Gilbert, 1985; Sarnelle, 1993; Li et al., 1996). In addition, they are routinely ingested by various colorless dinoflagellates, ciliates, and *Kathablepharis* spp. (Stemberger and Gilbert, 1985; Clay and Kugrens, 1999a,b; Lewitus et al., 1999). Observations of *Daphnia hyalina* and *Diaptomus gracilis* feeding behavior showed that *Rhodomonas* sp. was the preferred food item when it was present (Ferguson et al., 1982). Moreover, various studies suggest that when given a choice among flagellate food items, rotifers appear to preferentially select cryptomonads (Stemberger and Gilbert, 1985), and the presence of cryptomonads has been shown to enhance the reproduction of planktonic rotifers (Edmondson, 1965). Pejler (1977) observed that various rotifers prefer *Rhodomonas* over *Chrysochromulina*. Given that many zooplankton selectively choose cryptomonads as prey, grazing probably plays a significant role in regulating cryptomonad population dynamics (Sarnelle, 1993). Cryptomonad populations generally peak following periods of moderate turbulence, which facilitates their dispersion throughout the water column and their mixing with higher nutrient waters (Reynolds, 1984). During such periods zooplankton grazing pressure is reduced, but as turbulence decreases and nutrients are depleted, grazing once again reduces population numbers.

Perhaps one of the more interesting aspects of cryptomonad ecology is the phenomenon known as kleptoplastidy (stolen plastids), which occurs when a phagotroph ingests a photoautotroph and selectively harvests its chloroplasts while digesting the remainder of the cell. The captured chloroplasts remain functional for a period of time and serve the purposes of the phagotroph (Schnepf et al., 1989; Larsen, 1992; Lewitus et al., 1999). Kleptoplastids derived from cryptomonad chloroplasts have been identified in ciliates (Stoecker and Silver, 1990; Stoecker et al., 1987, 1988/1989) and dinoflagellates (Skovgaard, 1998; Schnepf et al., 1989; Putt, 1990; Fields and Rhodes, 1991). These stolen chloroplasts actively

photosynthesize and the starch produced is presumably made available to the host (Putt, 1990; Larsen, 1992; Schnepf and Elbrächter, 1992), or may fulfill certain metabolic requirements under limited food availability (Lewitus et al., 1999).

Kleptoplastidy is particularly important in the survival of the ichthyotoxic dinoflagellate *Pfiesteria piscicida* Steidinger *et* Burkholder, where cryptomonad chloroplasts are selectively harvested by nontoxic zoospores. These stolen chloroplasts are retained for approximately 9 days and, they remain functional during that time as determined by the uptake of C_{14}-bicarbonate (Lewitus et al., 1999). It has been noted that the retention of these chloroplasts promotes the survival of this intermediate stage in the life cycle of *Pfiesteria*.

C Cryptomonad Endosymbionts and Pathogens

Cryptomonad cells are susceptible to prokaryotic or eukaryotic infections; however, pathogenicity in cryptomonads has not been the subject of detailed studies. On the other hand, bacteria have been known to enter the cell and become established as endosymbionts (Schnepf and Melkonian, 1990). These bacteria apparently do not have any adverse effects on cells and, thus, might represent a mutualistic association. There are, however, other bacteria and viruses that may adversely affect cryptomonads (Pienaar, 1976; Klaveness, 1982). Klaveness (1982) has shown that *Caulobacter* can attach to cells externally, causing cells to become morphologically malformed.

Canter (1968) reported that certain cryptomonads are vulnerable to attack by certain fungal parasites known as chytrids. For instance, *Rhizophydium fugax* has been observed infecting individuals of *Cryptomonas* resting in palmelloid colonies. Presumably, the chytrid is chemotactically attracted to the polysaccharide mucilage that envelopes the cells of the palmelloid colony. Upon making contact with a cell, the chytrid situates itself in the furrow and sends out an infection peg that pierces the cell. From the site of invasion the infection peg develops into a rhizoidal system that infiltrates the cytoplasm and begins to digest the cryptomonad from the inside out. Chytrids have also been reported parasitizing *Chilomonas striata* (Caljon, 1983). In addition to chytrid ectoparasites, intracellular parasites of undetermined taxonomic status have been observed in *Cryptomonas* (*Campylomonas*) *rostratiformis* (Ettl and Moestrup, 1980).

D Types of Nutrition—Carbon Sources

Cryptomonads are photoautotrophic, heterotrophic, or mixotrophic. Photoautotrophic species synthesize organic molecules from CO_2 by photosynthesis. Heterotrophic forms must satisfy their carbon needs via osmotrophy or phagotrophy. Osmotrophs are able to uptake dissolved organic matter (DOM) from the ambient medium, whereas phagotrophs ingest particulate matter including other organisms. Both modes of heterotrophy are restricted to colorless cryptomonads and kathablepharids. For instance, by virtue of being colorless, *Chilomonas* is unable to manufacture its own basic organic molecules via photosynthesis. It is strictly osmotrophic and obtains its nutrition by assimilating dissolved organic molecules into its cell (unpublished observations). It does not ingest particulates, which is known as phagotrophy, the mode of nutrition that this alga was assumed to have. In fact, the cell covering is a major obstacle to phagotrophy. *Goniomonas*, on the other hand, is phagotrophic and routinely ingests bacteria through a specialized structure in the anterior portion of the cell known as the infundibulum (Mignot, 1965; Kugrens and Lee, 1991). Whether it is also osmotrophic is unknown.

The vast majority of cryptomonads, however, are obligate photoautotrophs, and several studies have indicated that making DOM available does nothing to enhance the growth of photosynthetic cryptomonads (Lewitus and Caron, 1991; Arvola and Tulonen, 1998). Previous reports showing increased growth with the addition of DOM are now attributed to bacterial respiration, whereby the bacteria oxidized the organic molecules and released CO_2 as a byproduct. The increased CO_2 levels, and not the utilization of DOM, facilitated photosynthesis resulting in increased cryptomonad growth (Arvola and Tulonen, 1998).

Mixotrophy is an ecologically important mode of nutrition in many flagellates (Boraas et al., 1988), whereby a photosynthetic organism is able to supplement its carbon needs by ingesting particulate matter, primarily other cells including both prokaryotes and eukaryotes. This type of nutrition is common in chrysophytes, but it also has been reported in a few cryptomonads (Tranvik et al., 1989; Kugrens and Lee, 1991). For example, certain species of *Cryptomonas* are thought to be able to phagocytotically ingest bacteria (Tranvik et al., 1989; Urabe et al., 2000), but electron microscopic examinations were not conducted to confirm the presence of bacteria in food vacuoles. One species of *Chroomonas* may be mixotrophic, as determined by ultrastructural studies (Kugrens and Lee, 1991). This study revealed a specialized bacterial incorporation vesicle and the presence of bacteria in various stages of digestion, making *Chroomonas* the only genus of cryptomonad in which mixotrophy has been documented with EM. It is believed that mixotrophic ingestion of bacteria provides a means of acquiring nitrogen and phosphorus in waters where they are limited (Urabe et al., 2000).

V COLLECTION, ISOLATION, AND CULTURING

Planktonic cryptomonads can be collected from lakes or other standing bodies of water using phytoplankton nets or by grab samples from shore or boat. Attached cryptomonads embedded in mucilage can be scraped off various substrata with a putty knife and placed in a collecting bottle with water, where they soon become motile. Samples must be kept cold during transport, because cells easily lyse at elevated temperatures. Fixation of cells is not recommended because cells either rupture or hypertrophy, thereby destroying or altering cell morphology. If chemical preservation is required then Lugol's fixative generates the least amount of distortion; however, because iodine is a component of Lugol's, cells often appear purple instead of their true color because of stained starch. The proper approach for identifying cryptomonads is to examine living cells with a microscope. Photomicrography is obviously difficult because cells are usually swimming, and for this reason a flash attachment for photomicrography is helpful. Phase contrast or differential interference contrast (DIC) is also helpful in identifying architectural features of cells.

Before isolations are attempted, field samples should be enriched with growth media to select and establish populations that are capable of growing in a given medium. When individual cells are then isolated and placed into a given medium, there is confidence that they will take to it. Isolation of cryptomonads is most successful when employing the serial dilution pipetting technique (Hoshaw and Rosowski, 1973), which makes use of either a dissecting microscope or inverted microscope, depending on the skill and dexterity of the individual. All freshwater cryptomonads cultured so far grow prolifically in a recipe of sterilized lake water and Bold's Basal Medium (Nichols, 1973) or sterilized lake water and Alga-Gro concentrate (Carolina Biological Supply Company) at 40 mL per liter of lake water. Cultures should be grown in media with a pH of 7.8 or higher and maintained at approximately 18 °C (the optimum temperature range is 16-20 °C) in 16:8 h light:dark regimes. In addition, a sterilized wheat seed is an ideal addition to the medium for colorless cultures such as certain *Cryptomonas* (*Chilomonas*) species and *Goniomonas*. *Kathablepharis* is more challenging to culture and can be maintained only in a mineral medium that also contains its preferred food organism. For instance, *K. ovalis* requires *Chrysochromulina parva*, and *K. phoenikoston* requires *Chroomonas*.

VI CLASSIFICATION AND KEY (FIGURES 3–17)

A Introduction

Reliable identification of cryptomonads using light microscopy can be problematic due to their unicellularity, small size, and the occurrence of dimorphic life cycles. Electron microscopic and molecular studies have greatly enhanced our knowledge of the diversity within this group, and, therefore, ultrastructural and molecular data must continue to inform all taxonomic proposals. The salient morphological and ultrastructural features were described in detail earlier in this chapter (Brett and Wetherbee, 1986; Dodge, 1969; Dwarte and Vesk, 1983; Faust, 1974; Grim and Staehelin, 1984; Hibberd et al., 1971; Gantt, 1971, 1980; Greenwood et al., 1977; Hill, 1991a, b; Hill and Wetherbee, 1986, 1988, 1989; Klaveness, 1985; Kugrens and Lee, 1986, 1991; Kugrens et al., 1986, 1987; Lucas, 1970a, b, 1982; Munawar and Bistricki, 1979; Santore, 1977, 1982a, b, 1983, 1984, 1987; Sespenwol, 1973; Wetherbee et al., 1986). In light of the data discussed above, it has been possible to delineate 16 genera, 9 of which occur in freshwater. Two of the latter, *Cryptomonas* (cryptomorph and campylomorph) and *Komma*, are strictly freshwater genera.

It is noteworthy that the number of genera has increased considerably since Santore's (1984 and 1987) review articles, in which he recognized only five genera. Following these reviews, an expansion and revision of genera occurred, as well as the elimination of the genus *Rhodomonas* Santore (Erata and Chihara, 1989; Novarino, 1991a,b; Novarino and Lucas, 1993), although there were compelling arguments to retain the genus (Hill and Wetherbee, 1989; Hill, 1991a).

More recent ultrastructural investigations also point out the need for reexamining the structural traits that were proposed by Santore (1984, 1987); Novarino (1991a,b) and Novarino and Lucas (1993) as generic characters. As previously emphasized, important systematic characters such as cell architecture and the periplast are extremely susceptible to the introduction of artifacts when chemicals are used. Therefore, specialized techniques for scanning electron microscopy and freeze-fracture are indispensable for the accurate interpretation of these features (Munawar and Bistricki, 1979; Grim and Staehelin, 1984; Klaveness, 1985; Brett and Wetherbee, 1986; Wetherbee et al., 1986, 1987; Hill and Wetherbee, 1986, 1988, 1989; Hill, 1990, 1991b; Kugrens et al., 1986, 1987; Wetherbee et al., 1986; Kugrens and Lee, 1986, 1991). For information on earlier classification schemes based on light microscopy, the publications by Bourelly (1970), Huber-Pestalozzi (1950), and Skuja (1939, 1948) should be consulted for freshwater forms, whereas the extensive treatise by Butcher (1967) is the major reference for marine cryptomonads.

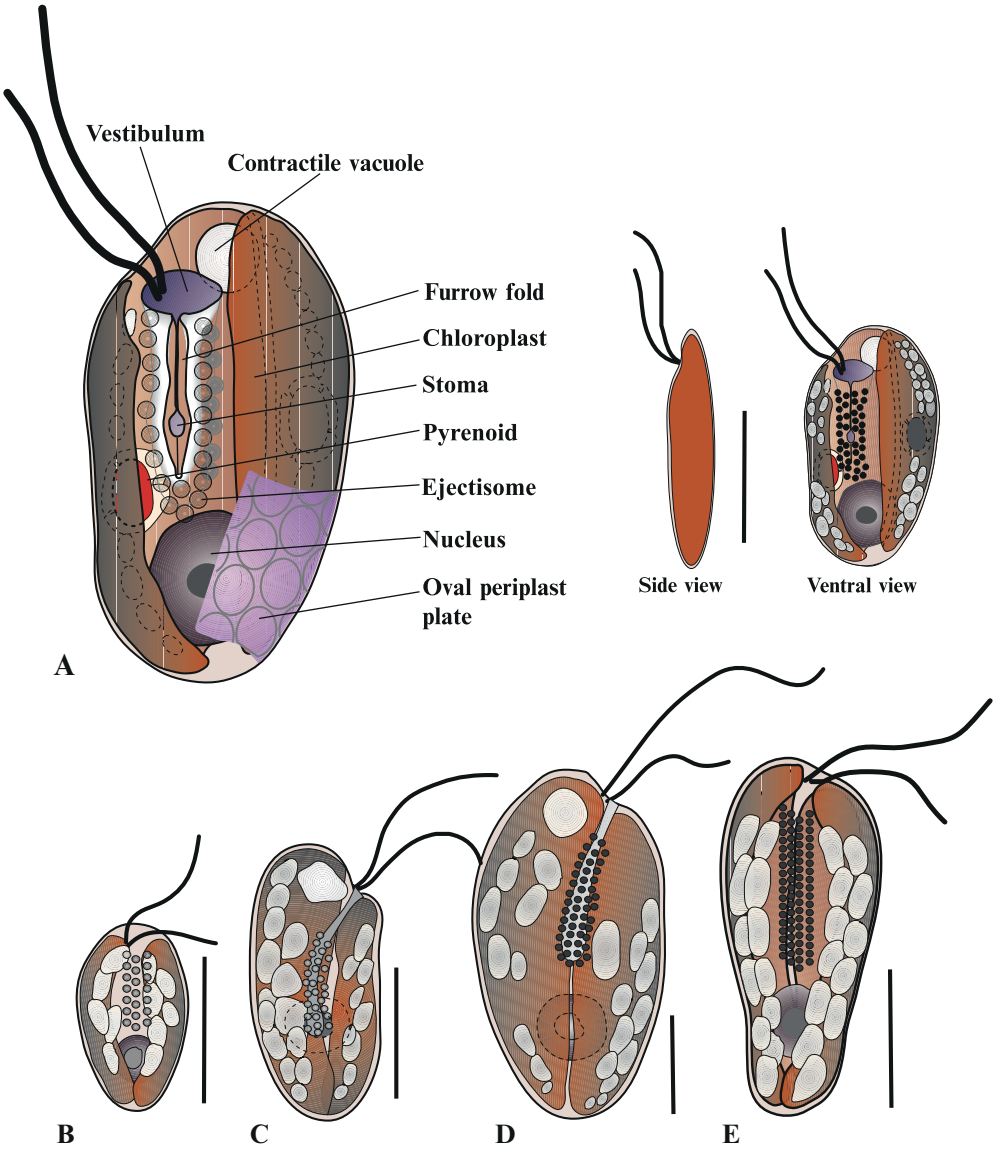

FIGURE 5 Light micrographic illustrations of *Cryptomonas* species showing representative cryptomorph species. (A) *Cryptomonas ovata* showing the laterally flattened nature of cells, the posterior nucleus, and the dorsally inserted flagella. The oval inner periplast plates are not apparent with a light microscope. (B) *Cryptomonas obovata.* (C) *Cryptomonas phaseolus*. (D) *Cryptomonas erosa*. (E) *Cryptomonas ozolinii*. Scale bars = 10 μm.

As indicated above, cryptomonad and kathablepharid presentations are combined in this chapter based upon such unifying characteristics as the presence of ejectisomes and the subapical insertion of flagella. The following section, however, addresses cryptomonads only, whereas kathablepharids are attended to separately at the end of the chapter.

B General Features Useful in Determining Genera and Species

As a starting point, strains can be separated artificially into two broad groups based upon the presence or absence of pigments. A second disjunction can be defined by the presence of either blue-green coloration (phycocyanobilin) or red and brown colorations (phycoerythrobilin) due to the various types of linear tetrapyrroles conjugated to the phycoerythrin tetramer (see Section II). A third disjunction could be based on life histories. Complex life histories involving two structurally distinct forms have been described for the marine genus *Proteomonas* and for the freshwater genus *Cryptomonas* (Figure 4). The remaining genera so far appear to reproduce asexually only or are haplobionts (Kugrens and Lee, 1988).

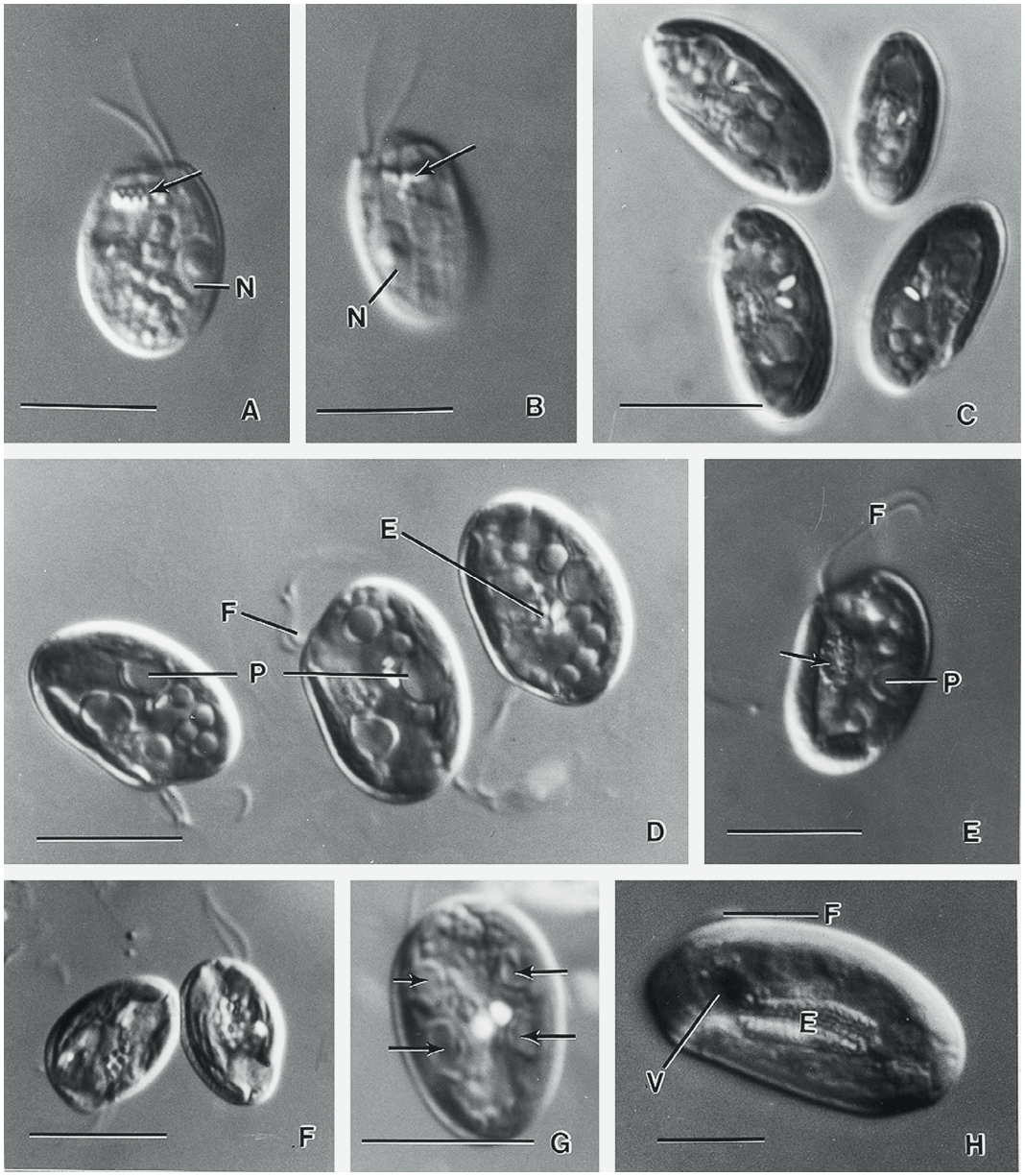

FIGURE 6 Light micrographs of *Goniomonas* and cryptomorph *Cryptomonas* species. (A) *Goniomonas truncata* cell showing the location of the nucleus (N) and ejectisomes (arrow). (B) *G. truncata* cell showing surface striations (arrows). (C) *Cryptomonas obovata* cells with a pyrenoid visible in some cells. (D) *Cryptomonas ovata* cells with two pyrenoids (arrows) in each cell. Flagella (F) and ejectisomes (E) are also evident. (E) *Cryptomonas ovata* cell showing ejectisomes (E) around furrow. Subapically inserted flagella and a nucleus (N) also visible. (F) *Cryptomonas phaseolus* cell. (G) *Cryptomonas tetrapyrenoidosa* showing four pyrenoids (arrows). (H) *Cryptomonas erosa* showing the furrow flanked by ejectisomes (E), a portion of a flagellum (F), and the location of the vestibulum. Scale bars = 10 µm.

The fourth and most accepted method for delineating genera is based upon a combination of characters involving the furrow/gullet complex (Figures 1, 9, 10, 12, 16, 17) and the type of periplast component, primarily the inner component (Figures 2 and 11). When considering the IPC, the first disjunction is based upon single-sheet forms versus multiple-plated forms. It is possible to further distinguish genera within these groups even when pigmentation is lacking. For instance, colorless *Cryptomonas* species bear a single inner periplast sheet (Figure 3B) (Grim and Staehelin, 1984; Kugrens and Lee, 1991), whereas the colorless *Goniomonas* bears rectangular plates (Kugrens and Lee, 1991). In multiple-plated forms, plate shapes, their sizes, and their arrangement serves to further delineate genera and is best served when used in conjunction with the furrow/gullet type and cell shape.

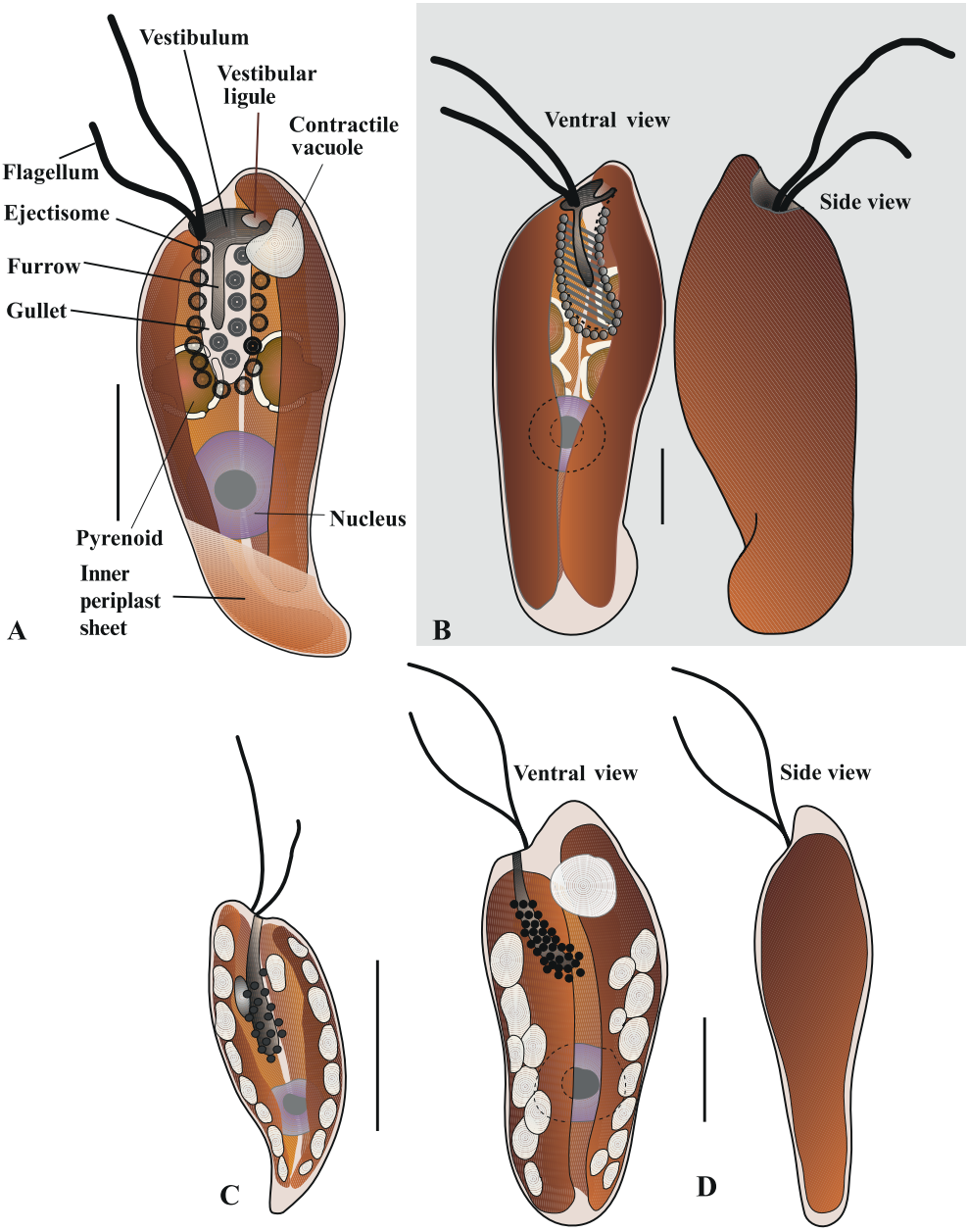

FIGURE 7 Light micrographic illustrations of campylomorph forms within the family Cryptomonadaceae. (A) *Campylomonas (Cryptomonas) reflexa*. (B) *Cryptomonas rostratiformis*. (C) *Cryptomonas marssonii*. (D) *Cryptomonas platyuris*. Scale bars = 10 µm.

The following characteristics are most useful in diagnosing species. Although several of these are discernible at the light microscopic level, many are based on features resolved with SEM.

1. *Flagellar Hair Arrangement and Scale Morphology* (Figure 1)

The arrangement of tubular and/or nontubular hairs, scales, and other structures on flagellar surfaces may assist in diagnosing species. These variations were reported by Kugrens et al. (1986) and Lee and Kugrens (1986).

2. *Variations in the Structure of the Flagellar Apparatus*

Reconstructing the entire flagellar apparatus for each strain, with particular attention to the complexity of the rhizostyle, is useful for comparative purposes, potentially providing another structural marker for defining species.

3. *The Presence and Structural Variations of the Furrow Plate* (Figure 1)

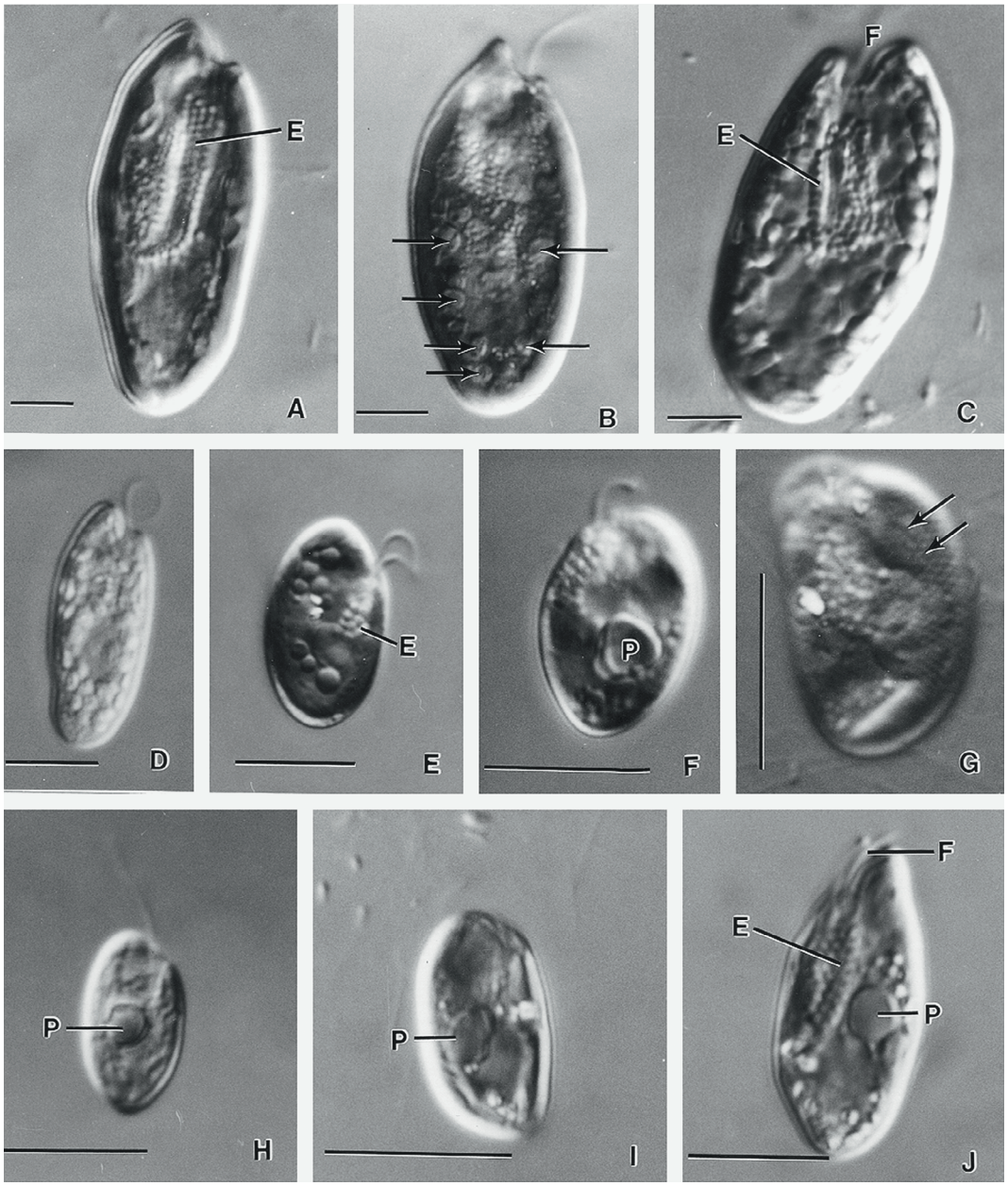

FIGURE 8 Light micrographs (differential interference contrast) of several species from the Cryptomonadaceae (campylomorph forms only) and Pyrenomonadaceae. (A) *Cryptomonas (Campylomonas) rostratiformis* cell with its characteristic rhinote anterior and numerous ejectisomes (E) lining the furrow. (B) Somewhat flattened cell of *Cryptomonas (Campylomonas) rostratiformis*, showing multiple pyrenoids (arrows). (C) *Cryptomonas (Campylomonas) platyuris* in ventral view showing the broad shape of the cell and the ejectisomes (E) lining the furrow. The cell is filled with considerable starch. (D) *Cryptomonas* (*Chilomonas*) *paramecium* cell with starch filling most of the cell. (E) *Pyrenomonas ovalis* cell with ejectisomes (E). (F) Pyr*enomonas ovalis* with the characteristic prominent pyrenoid (P). (G) Higher magnification of *a P. ovalis* cell showing periplast plates (arrows). (H) *Storeatula rhinosa* cell with a prominent pyrenoid (P). (I) *S. rhinosa* cell as viewed obliquely showing the asymmetrical cell shape and a prominent pyrenoid (P). (J) *Storeatula* sp. cell with an extensive ejectisome region (E) and a prominent pyrenoid (P). Portions of the flagella (F) are seen adhering to the cell. Scale bars = 10 µm.

There are currently two types of furrow plates associated with the furrow of cryptomonad cells. These appear to correlate with the type of periplast for a given species. Other variations are expected to be found and could serve as an additional character when reconstructing phylogenies based upon morphological characters.

4. *The Number and Location of the Nucleomorph(s) in the Periplastidial Compartment*

Variations on this theme have been summarized by Santore (1982c), and these variations were used in delineating *Pyrenomonas*/*Rhodomonas* and *Storeatula* (Hill and Wetherbee, 1989; Kugrens et al., 1999), and *Teleaulax*, *Geminigera*,

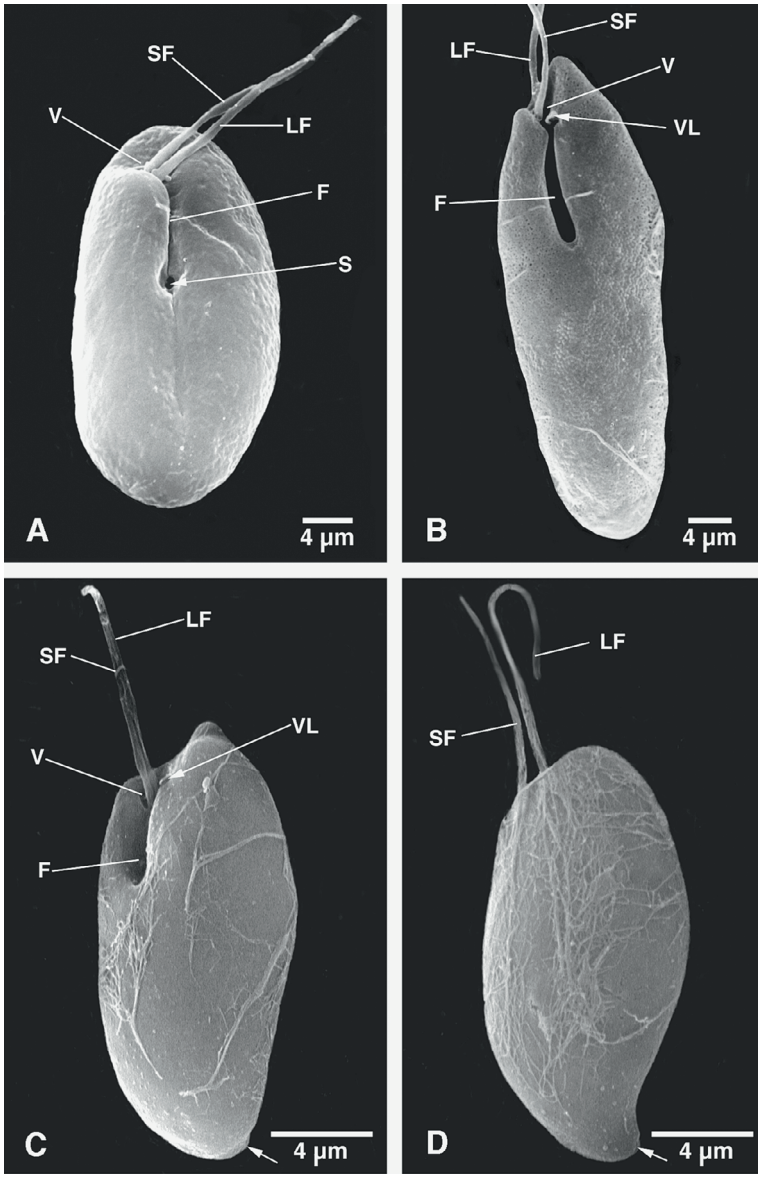

FIGURE 9 Scanning electron micrographs of *Cryptomonas* cryptomorph and *Cryptomonas* campylomorph species. (A) Cell of *Cryptomonas tetrapyrenoidosa* with long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). A long furrow (F) extends from the vestibulum and a stoma (S) is present. (B) Cell of *Cryptomonas (Campylomonas) rostratiformis* with long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). A vestibular ligule (VL) is seen attached to the dorsal wall of the vestibulum. A slightly curved, oblique furrow (F) runs for approximately one-third of the cell length. Note the rostrate anterior of the cell. (C) Oblique view of a cell of *Cryptomonas (Campylomonas) reflexa* showing the long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). A vestibular ligule (VL) attaches to the dorsal wall of the vestibulum. A furrow (F) extends posteriorly for a third of the cell length. Note the reflexed tail (arrow). (D) Lateral view *of C. reflexa* showing the reflexed shape of the cell. *(Parts (A) and (B) are from Kugrens et al. (1986) with permission).*

and *Campylomonas* (*Cryptomonas*) (Hill, 1991b). Those genera that have a nucleomorph embedded within the pyrenoid that bridges two lobes of a chloroplast appear to constitute a natural family described as the Pyrenomonadaceae (Clay et al., 1999). In fact, these genera form a robust clade based on ssurDNA data (Marin et al., 1998; Clay et al., 1999).

5. *Presence and Location of Eyespots*

Eyespots or stigmas have been confirmed ultrastructurally for *Chroomonas* spp. (Figure 14D and E) (Santore, 1987; Hill, 1991a) and *Hemiselmis amylosa* (Clay and Kugrens, 1999b). Many cryptomonads have highly retractile orange bodies seen under the light microscope but these do not represent true eyespots.

6. *Types of Thylakoid Arrangements within the Chloroplasts*

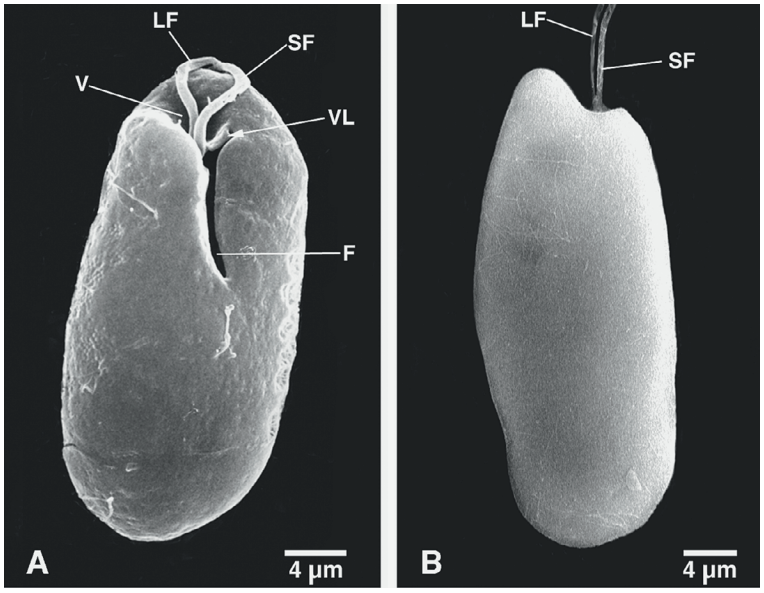

FIGURE 10 Scanning electron micrographs *Cryptomonas (Campylomonas) platyuris*. (A) Ventral view with long (LF) and short (SF) flagella inserted in the vestibulum (V). A vestibular ligule (VL) occurs on the dorsal side of the vestibulum. An oblique furrow (F) runs for almost one-half of the cell length from the vestibulum. (B) Dorsal view of *C. platyuris*. Note the slightly reflexed cell shape. *(Part (A) is from Kugrens et al. (1986) with permission).*

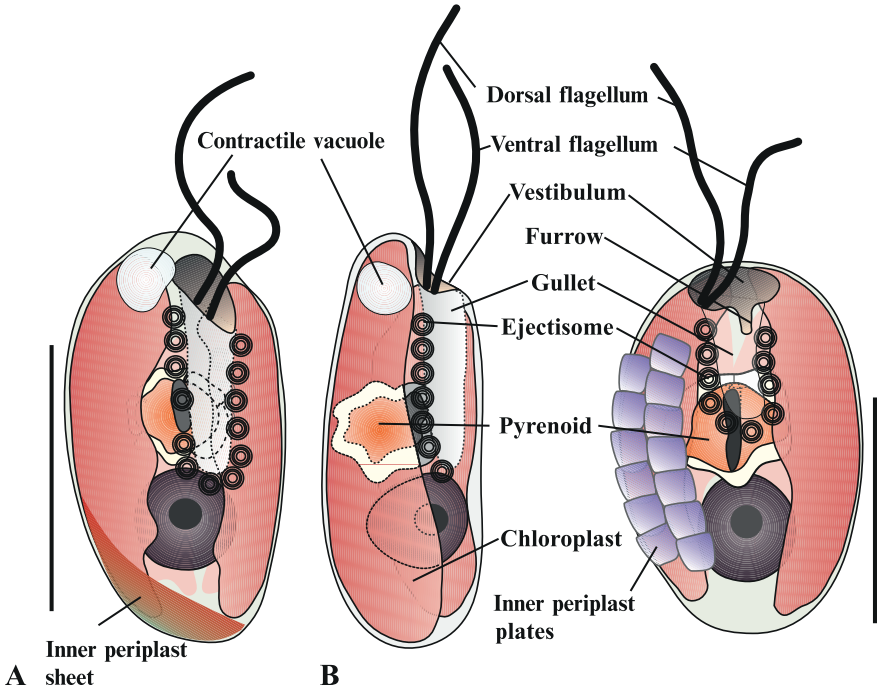

FIGURE 11 Light microscopic illustrations of red-colored cryptomonad species. (A) *Storeatula rhinosa*. (B) *Pyrenomonas ovalis*. Scale bars = 10 µm.

Several thylakoid arrangements have been reported, with paired thylakoids being the most common arrangement. However, caution must be exercised when using this trait for systematic purposes, because environmental conditions may influence this feature (Klaveness, 1981).

7. *Type of Scales Comprising the Outer Periplast Component*

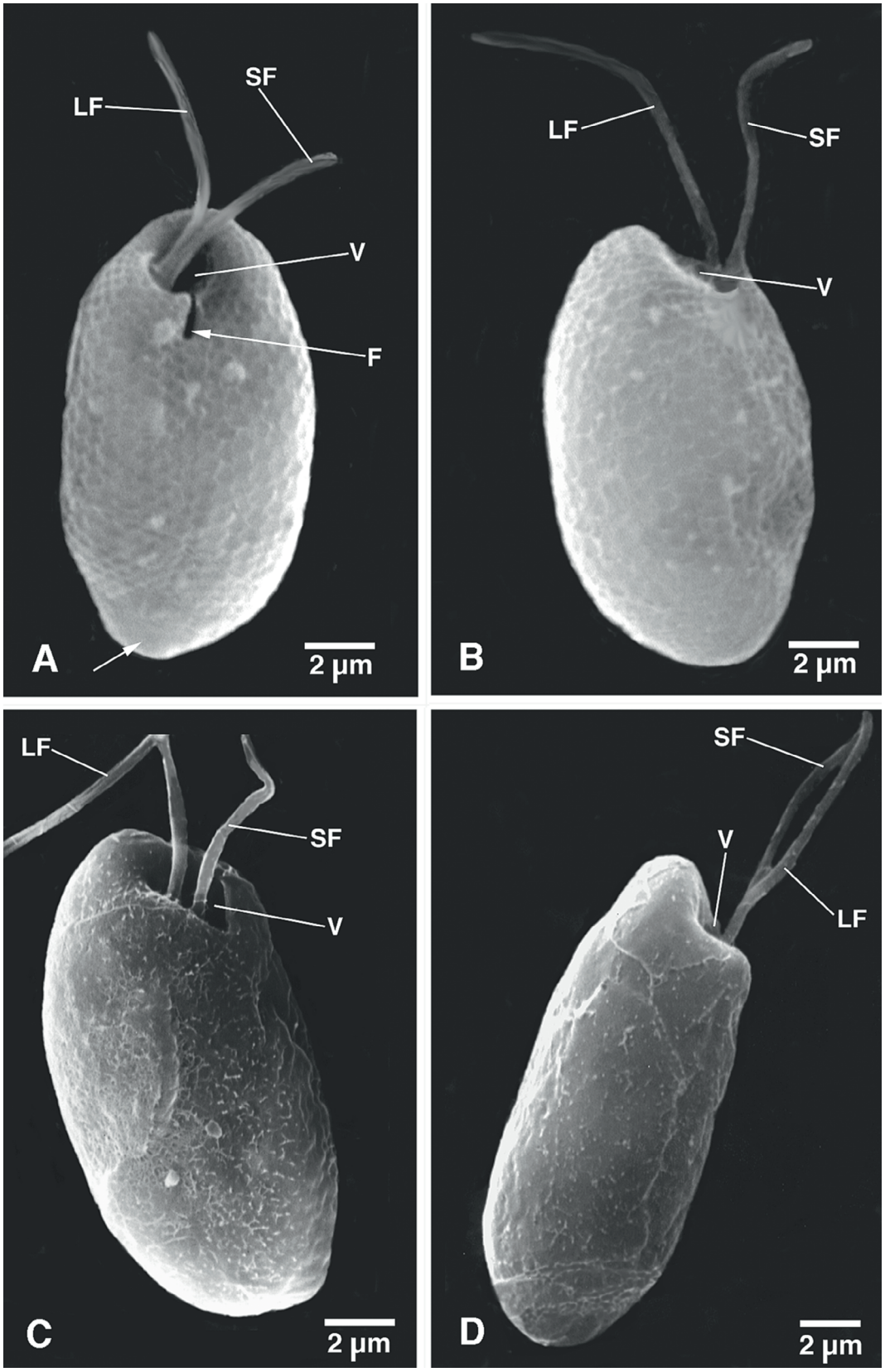

FIGURE 12 Scanning electron micrographs of red-colored cryptomonad species. (A) Ventral view of *Pyrenomonas ovalis* showing long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). A short furrow (F) and vestibulum are shown near the anterior, ventral surface. Note the absence of distinct plates at the cell posterior (arrow). (B) Lateral view of *P. ovalis*. (C) Oblique view of a cell of *Storeatula rhinosa* showing long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). (D) Lateral view of *S. rhinosa* showing the narrower elongate shape of the cell. *(Parts (C) and (D) from Kugrens et al. (1999) with permission).*

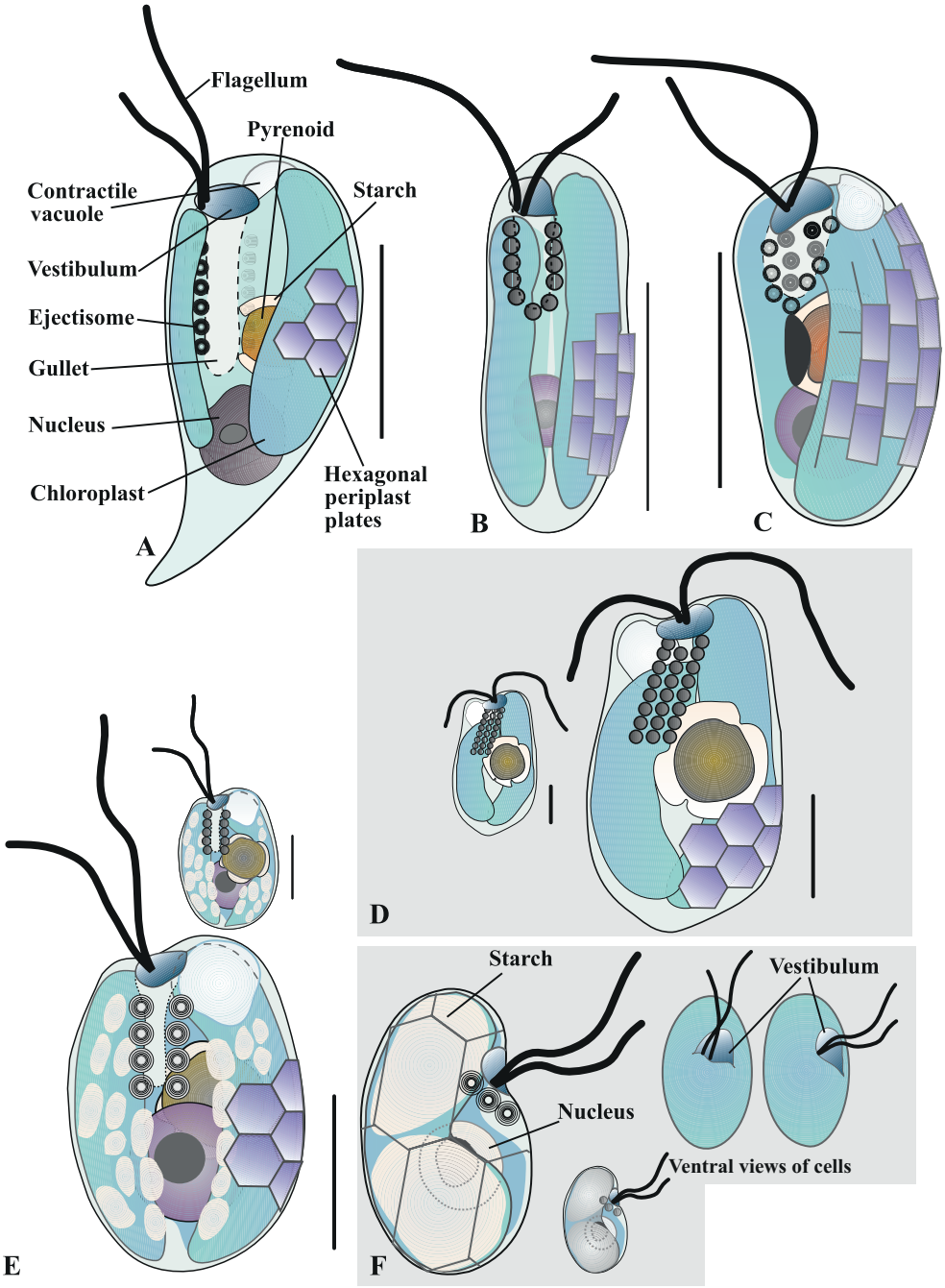

FIGURE 13 Light microscopic illustrations of blue-green cryptomonad species. (A) *Komma caudata*. (B) *Chroomonas oblonga*. (C) *Chroomonas coerulea*. (D) *Chroomonas nordstedtii*. (E) *Chroomonas pochmanni*. (F) *Hemiselmis amylosa*. Scale bars = 10 µm.

To date, only one type of scale has been identified, but the sample size has been limited and other types are expected to be discovered. The sole scale type is reported as heptagonal (Figure 2) (Pennick, 1981; Hill, 1990, 1991b; Lee and Kugrens, 1986).

8. *The Number, Location, and Types of Pyrenoids*

Pyrenoids may be absent, or there may be one, two, or several pyrenoids per chloroplast (Huber-Pestalozzi, 1950). In addition, thylakoids may or may not penetrate the pyrenoid, and this character may be a diagnostic feature for determining some species (Clay and Kugrens, 1999a,b,c; Clay et al., 1999).

9. *The Number of Chloroplasts per Cell*

Variation in chloroplast number may be species specific or may be useful in determining genera (Hill, 1991b). However, the systematic significance of this character has yet to be fully established.

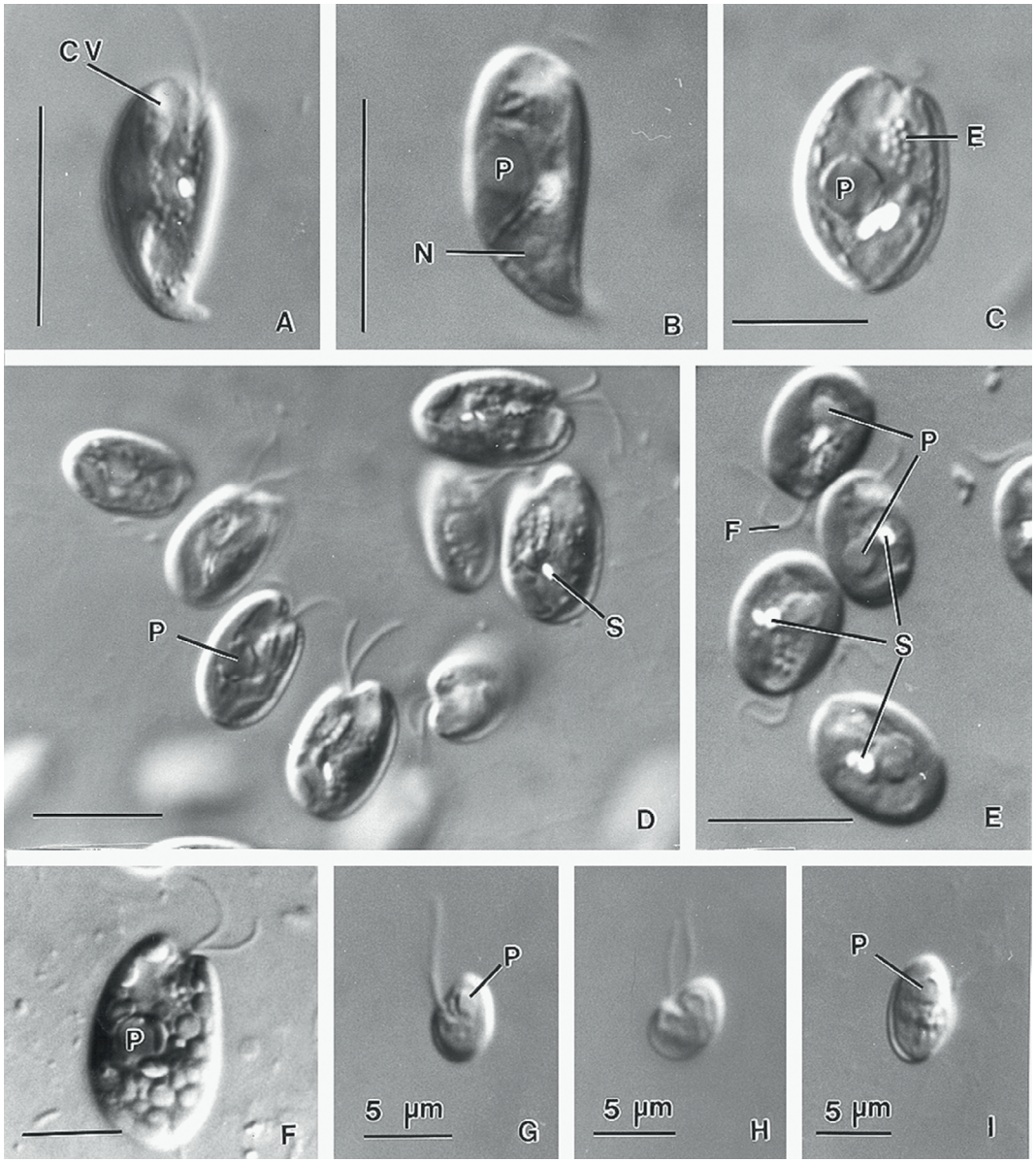

FIGURE 14 Light micrographs of blue-green cryptomonads species. (A) *Komma caudata* cell showing the typical shape. A contractile vacuole (CV) occurs in the anterior end of the cell. (B) *Komma caudata* cell with a dorsal pyrenoid (P) and a nucleolus located in the nucleus (N). (C) *Chroomonas pochmanni* cell displaying the typical shape, and a pyrenoid (P) and ejectisomes (E). (D) *Chroomonas coerulea* cells with some stigmas (S) and pyrenoids (P) visible. (E) Slightly flattened *C. coerulea* cells showing the stigma (S) associated with the pyrenoid (P). (F) *Chroomonas nordstedtii* cell displaying the typical cell shape. A pyrenoid (P) and starch grains are present in the cell. (G) *Hemiselmis amylosa* illustrating the typical bean-shaped cell in lateral view. A flagellum and pyrenoid (P) are evident. (H) *Hemiselmis amylosa* cell showing two flagella arising from a slight depression near the middle of the cell. (I) A slightly larger *Hemiselmis amylosa* cell with a pyrenoid (P). Scale bars = 10 µm unless otherwise indicated.

C Classification of the Phylum Cryptophyta

Our ability to identify and describe natural species within the Cryptophyceae continues to improve with the availability of additional sequence data, the recognition of complex dimorphic life cycles, and the use of more sophisticated techniques such as nucleomorph karyotyping. The following classification scheme conforms to the rules and regulations set forth by the International Code of Botanical Nomenclature (ICBN). Although this scheme is based upon the most reliable ultrastructural, biochemical, and phylogenetic information and borrows largely from the proposal by Clay et al. (1999a), the reader should bear in mind that taxonomy generally is an evolving endeavor and future amendments may be warranted as new data is collected. As will be evident, the type of accessory pigment strongly informs this classification scheme.

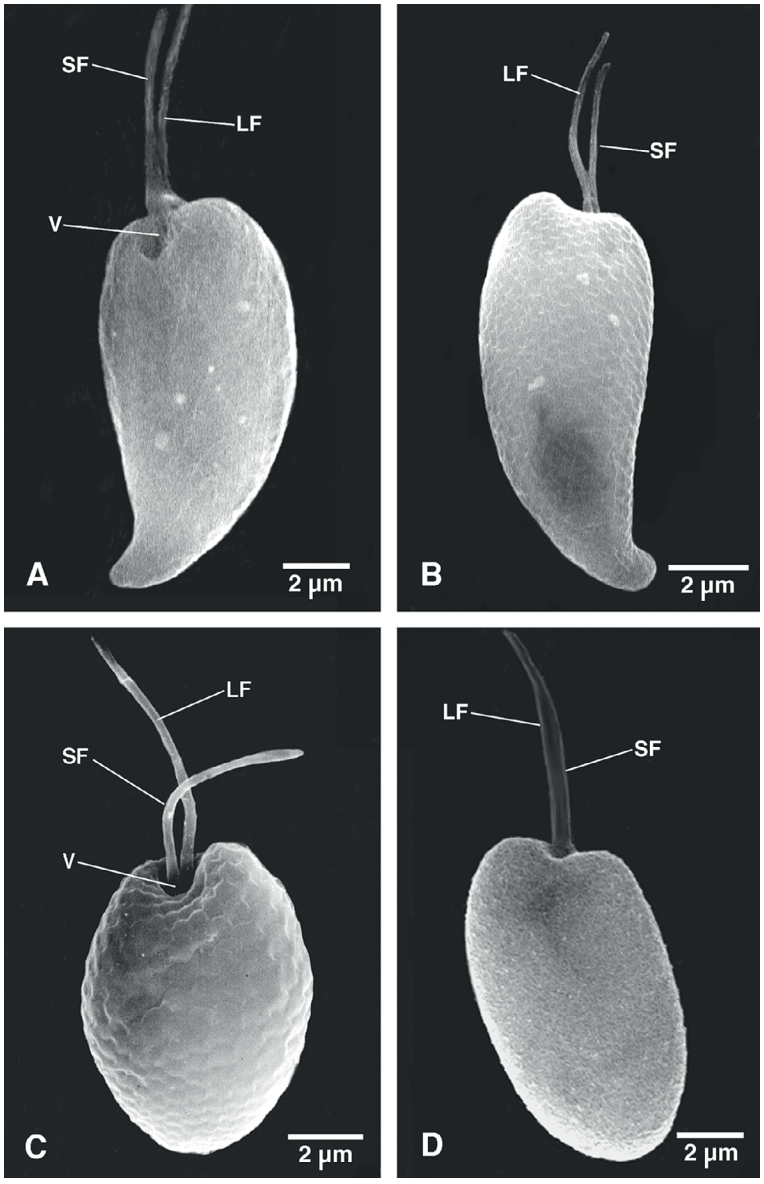

FIGURE 15 Scanning electron micrographs of two blue-green cryptomonad species. (A) Ventral view of a cell of *Komma caudata* showing a short flagellum (SF) and a long flagellum (LF) inserted subapically in the right side of the vestibulum (V). Note the acuminate tail. (B) Lateral view of *Komma caudata*. Plate elevations are visible. (C). Ventral view of a cell of *K. pochmanni* showing a short flagellum (SF) and a long flagellum (LF) inserted subapically in the right side of the vestibulum (V). (D) Lateral view of *K. pochmanni*. *(Part (C) is from Kugrens and Lee (1991) with permission).*

Division Cryptophyta (syn. Cryptista) Cavalier-Smith (1986)

Plastidial complex with nucleomorphs may be present or absent; chloroplasts (when present) contain chlorophylls a and c_2, and phycobiliproteins are located in the lumen of the thylakoids; bipartite tubular hairs on flagella occur in members possessing the plastidial complex; cell covering comprises inner and superficial periplast components (IPC and SPC); ejectisomes are present. Two classes:

Class Goniomonadophyceae (*syn. Goniomonadea*) Cavalier-Smith (1993)

Plastids and nucleomorphs are absent; bipartite tubular hairs on flagella are lacking; spikes occur on one flagellum; cells possess an infundibulum. One order:

Order Goniomonadales (Goniomonadida) Novarino and Lucas (1993)

Diagnosis identical to the class.

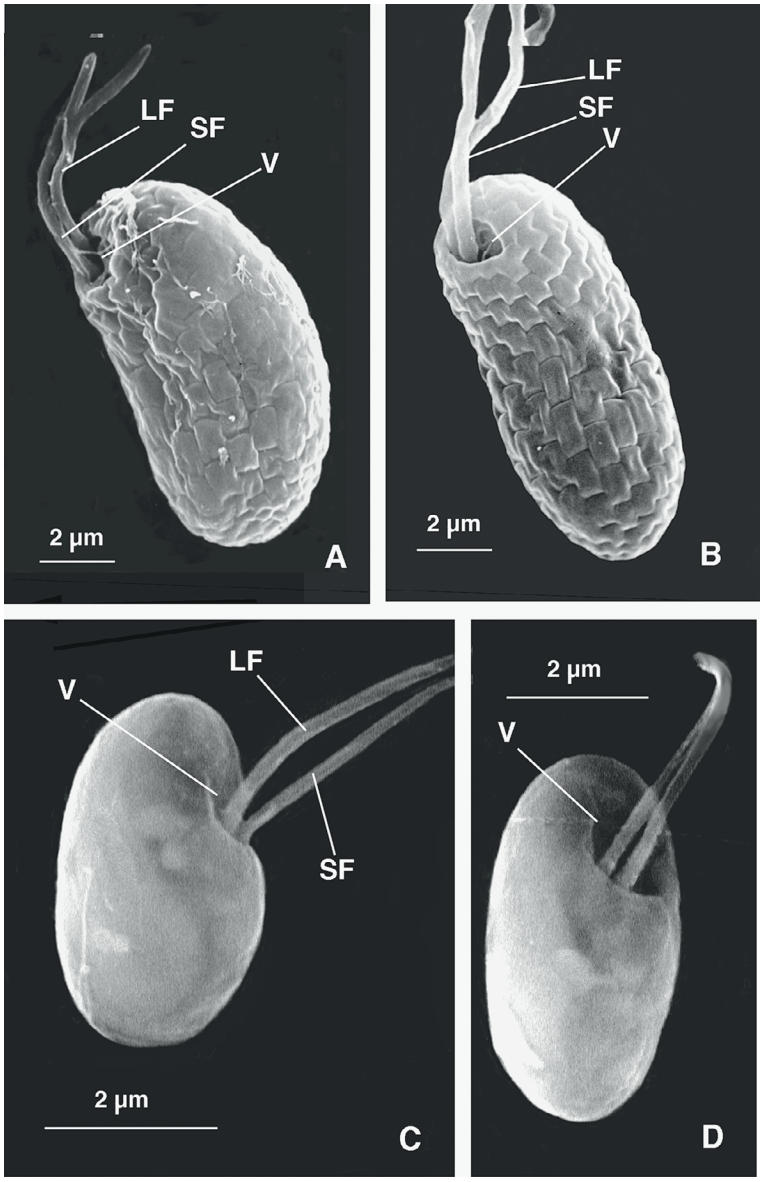

FIGURE 16 Scanning electron micrographs of two blue-green cryptomonad species. (A) Cell of *Chroomonas coerulea* showing a short flagellum (SF) and a long flagellum (LF) inserted subapically in the right side of the vestibulum (V). Rectangular surface plates are visible. (B) Cell of *Chroomonas oblonga* showing rectangular surface plates, and the short (SF) and long (LF) flagellar insertion. (C) Lateral view of *Hemiselmis amylosa* cell with long flagella (LF) and short flagella (SF) inserted in the vestibulum (V), approximately one-third of the distance down from the cell apex. (D) Ventral view of *H. amylosa* showing the location of the vestibulum (V). *(Parts (A) and (B) after Kugrens et al. (1986), and parts (C) and (D) are from Clay and Kugrens (1999b) with permission).*

Family Goniomonadaceae Hill (1991a) (*syn. Cyathomonadaceae*) Pringsheim (1944)
Characters as for order.
Goniomonas Stein.

Class Cryptophyceae

Plastidial complex with nucleomorphs present; chloroplasts possess either phycoerythrobilin (red and brown chromophores) or phycocyanobilin (blue-green chromophores); leucoplast present in some; bipartite tubular hairs present on at least one flagellum. Two orders:

Order Cryptomonadales Clay et al. (1999)

Not equivalent to Cryptomonadales *sensu* Novarino and Lucas (1993). Chloroplasts possess the phycobiliprotein Cr-phycoerythrin 566 (PE III); leucoplast present in some. One family:

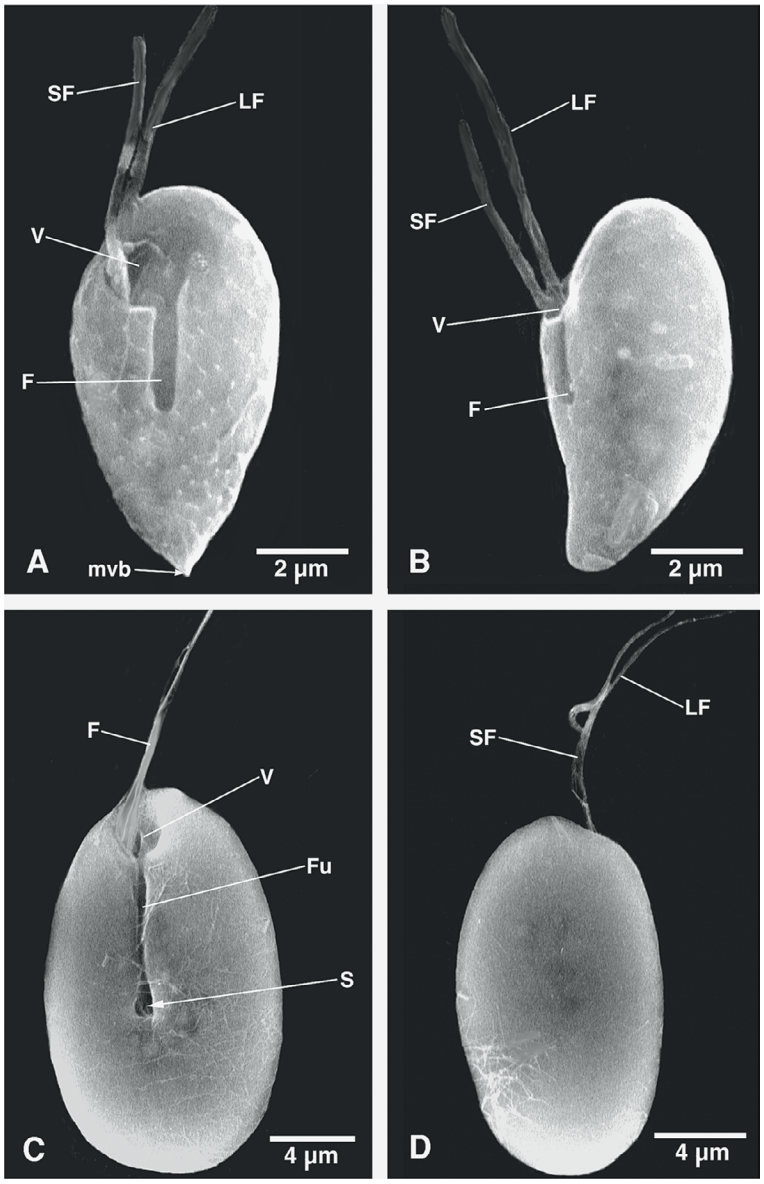

FIGURE 17 Scanning electron micrographs of *Plagioselmis* and *Cryptomonas ovata*. (A) Cell of *Plagioselmis nanoplanctica* showing the long (LF) and short (SF) flagella inserted on the right side of the vestibulum (V). A furrow (F) extends from the vestibulum for approximately half the length of the cell. Note the midventral band (mvb) on the cell posterior. (B) Lateral view of *Pn nanoplanctica* revealing additional cell shape. (C) Cell of *Cryptomonas ovata* with long and short flagella (F) inserted on the right side of the vestibulum (V). A long furrow (Fu) extends from the vestibulum. Note the stoma (S). (D) Lateral view of *Cryptomonas ovata*.

Family Cryptomonadaceae Clay et al. (1999)

Furrow and gullet complex present; nucleomorphs positioned between the pyrenoid and nucleus; complex life cycle (alternation of generations) with each phase presenting as a morphological distinct form (the cryptomorph and the campylomorph); IPC of the cryptomorph comprised multiple plates; IPC of the campylomorph composed of a single sheet; cryptomorphs possess a fibrous furrow plate and a short rhizostyle without wings (lamellae); campylomorphs possesses a scalariform plate and a long, keeled rhizostyle with wings (lamellae); campylomorphs and leucoplast forms have a vestibular ligule present. Two genera:

Cryptomonas Ehrenberg
Chilomonas Ehrenberg

Order Pyrenomonadales Clay et al. (1999)

Not equivalent to Pyrenomonadales *sensu* Novarino and Lucas (1993).

Chloroplasts possess red phycobiliproteins Cr-phycoerythrin 545 (PE I) or Cr-phycoerythrin 555 (PE II), never Cr-phycoerythrin 566 (PE 566); or chloroplast possess blue-green "Cr-phycocyanin." Four families:

Family Pyrenomonadaceae Novarino *et* Lucas (1993)

Chloroplasts possess Cr-phycoerythrin 545 (PE I); nucleomorphs positioned within pyrenoid. Three genera:

Pyrenomonas Santore (*syn. Rhodomonas*) Karsten
Storeatula Hill
Rhinomonas Hill *et* Wetherbee

Family Geminigeraceae Clay et al. (1999)

Chloroplasts possess the red pigment Cr-phycoerythrin 545 (PE I); IPC comprised of a sheet or a sheet and multiple plates if dimorphic; nucleomorphs never positioned in the pyrenoid; possessing a long, keeled rhizostyle with wings (lamellae); scalariform furrow plate present. Five genera and all are marine:

Geminigera Hill
Teleaulax Hill
Hanusia Deane, Hill, Brett, *et* McFadden
Guillardia Hill *et* Wetherbee *Proteomonas* Hill *et* Wetherbee
Proteomonas Hill *et* Wetherbee

Family Chroomonadaceae Clay et al. (1999)

Chloroplasts possess "Cr-phycocyanin" 630 (PC III), 645 (PC IV), or "Cr-phycocyanin" 569; rhizostyle absent. Three genera:

Chroomonas Hansgirg
Falcomonas Hill
Komma Hill

Family Hemiselmidaceae Butcher (1967)

Chloroplasts possess Cr-"phycocyanin" 615 (PC II) or Cr-phycoerythrin 555 (PE II), never possessing the other three types of "phycocyanins" or other two types of phycoerythrins; gullet only; nucleomorphs positioned anterior to pyrenoid; rhizostyle absent; thylakoids penetrate pyrenoid; flagella inserted laterally. One genus:

Hemiselmis Parke

D Key

1a. Cells colorless 2
1b. Cells pigmented 4
2a. Cells with leucoplast and copious starch granules ..... *Cryptomonas* (colorless)
2b. Cells without leucoplasts and abundant starch 3
3a. Cells with a furrow/gullet complex & anterior ring of ejectisomes ..... *Goniomonas*
3b. Cells lacking a furrow/gullet; two rows of ejectisomes on ventral side ..... *Kathablepharis*
4a. Cells blue-green in color due to presence of "phycocyanin" 5
4b. Cells olive, brown or red due to presence of phycoerythrin 7
5a. Cells reniform with flagella inserted one-third of cell length below anterior ..... *Hemiselmis*
5b. Subapically inserted flagella, near anterior end 6
6a. Cells comma-shaped with hexagonal inner periplast plates ..... *Komma*
6b. Cells oval-shaped with rectangular inner periplast plates ..... *Chroomonas*
7a. Cells red with plated or single sheet IPC 8
7b. Cells olive, brown with plated or single sheet-like IPC 9
8a. Cells red with square inner periplast plates ..... *Pyrenomonas*
8b. Cells red with single sheet IPC; gullet only ..... *Storeatula*
9a. Cells olive/brown w/hexagonal inner periplast plates; simple furrow ..... *Plagioselmis*
9b. Cells olive/brown w/sheet and oval inner periplast plates; dimorphic 10
10a. Cells flattened w/oval inner periplast plates; complex furrow/gullet ..... *Cryptomonas* (cryptomorph)
10b. Cells contorted w/single sheet inner periplast ..... *Cryptomonas* (campylomorph)

VII DESCRIPTIONS OF GENERA

A Freshwater Cryptomonad Genera and Species

The following diagnoses provide characteristics of genera and some species currently recognized. Scanning electron micrographs (SEMs), LMs, and diagrams are provided as a guide so that cellular features can be interpreted correctly. Given that SEM is becoming more common in phytoplankton identification, the SEMs provided should serve as an index to assist identification.

Goniomonas Stein (*syn. Cyathomonas*)

The aplastidial *Goniomonas* represents the only primitively colorless genus and most resembles the ancestral type of cryptomonad. Although lacking a periplastidial compartment, *Goniomonas* certainly is a cryptomonad based upon the presence of ejectisomes, the structure of the flagellar transition region, and the presence of a periplast. Only the large type of ejectisomes occur, which are arranged in a ring around the anterior rim of the cell (Mignot, 1965; Schuster, 1968; Kugrens and Lee, 1991; Kugrens, 1998). Cells are laterally compressed; the nucleus is situated in the dorsal portion of the cell; and food vacuoles containing ingested bacteria occur in the cell posterior. The flagella issue from the dorsal side of the vestibulum, with one flagellum bearing a unilateral row of recurved spines, whereas the other flagellum is decorated with fine fibrillar hairs (Kugrens and Lee, 1991). From the vestibulum a complex furrow with stoma extends posteriorly down the ventral side of the cell. A gullet is absent but another unusual type of tubular invagination called the infundibulum can be found on the left side of the cell, presumably functioning as an ingestion organelle. The periplast is constituted of inner and outer rectangular plates that are not offset. *Goniomonas* is represented in both freshwater and marine habitats. Refer to Mignot (1965), Schuster (1968), Hill (1991a), Kugrens and Lee (1991), and Kugrens (1999) for additional information.

Goniomonas truncata (Figures 3A, 6A and B, 20A)

Cells 5-12 µm long, 3-5 µm wide, and 4-10 µm deep. As the type species, *G. truncata* generally possesses the features described for the genus.

Cryptomonas Ehrenberg

The color of this exclusively freshwater genus manifests as different hues of olive and brown due to varying concentrations of a unique type of phycoerythrin (PE 566), or more rarely cells may be secondarily colorless. Cells are oval to obovoid and often are found embedded in an extensive mucilaginous biofilm known as a palmelloid colony. Motile cells possess two flagella that originate from the right side of the vestibulum, and flagella bear the most common arrangement of tubular hairs. Pigmented species are dimorphic, presenting as two dissimilar morphotypes known as the cryptomorph and the campylomorph (Hoef-Emden and Melkonian, 2003; Hoef-Emden, 2007). Cryptomorphs (Figure 4A) have a complex vestibular-furrow-gullet system consisting of furrow ridges, furrow folds, and a persistent oval opening called the stoma located near the posterior end of the furrow. The furrow is apparently dynamic, possessing the ability to open and close. The periplast of the cryptomorph consists of inner round to oval shaped plates, whereas the surface component is made up of a thin layer of fibrils. The periplastidial compartment contains two chloroplasts with two pyrenoids not traversed by thylakoids, and two nucleomorphs, each located between the nucleus and the pyrenoids.

The periplastidial compartment of the *Cryptomonas* campylomorph (formerly the genus *Campylomonas* and *Chilomonas*) holds the same contents as and is organized in a manner similar to that of the cryptomorph. However, the campylomorph differs from the cryptomorph in that it is sigmoid or contorted in shape, possesses an IPC made of a single sheet, has a scalariform furrow plate, and bears a vestibular ligule that covers the discharge site of the contractile vacuole (Figure 4B). The few colorless *Cryptomonas* species (formerly *Chilomonas*) reflect campylomorph morphology and ultrastructure but possess colorless leucoplasts instead of chloroplasts (Figure 3B).

This genus is ubiquitous in temperate lakes, reservoirs, and streams. Although frequently present in phytoplankton collections, the degree of intraspecific morphological plasticity and the occurrence of dimorphic life cycles make species identifications difficult with the light microscope, prompting some to include molecular sequence signatures as part of species diagnoses (Hoef-Emden, 2007). For further information on the structure of this genus refer to Santore (1977, 1984), Munawar and Bistricki (1979), Roberts (1984), Brett and Wetherbee (1986), Kugrens et al. (1986), Kugrens and Lee (1987), and Hill (1991b).

Representative Freshwater Cryptomorph Species

Cryptomonas ovata Ehrenberg (Figures 4A, 5A, 6D and E, 17C and D)

Cells are ellipsoid to oval and they may appear slightly curved. Cells measure 20-80 µm in length, 6-20 µm in width, and 5-18 µm in depth, imparting a somewhat flattened appearance. The furrow is complex and cells have a short gullet. There are two chloroplasts per cell, each with a pyrenoid, that are olive green to dark brown in color.

Cryptomonas obovata Skuja (Figures 5B and 6C)

Cells are 24-46 µm long and 13-24 µm in diameter and slightly curved. The vestibulum sits below the cell apex, imparting a lobed appearance to the cell apex. Cells have two olive or brown chloroplasts without pyrenoids. Numerous starch grains generally are present within the cells.

Cryp*tomonas phaseolus* Skuja (Figures 5C and 6F)

This is the smallest *Cryptomonas* species, measuring 8-13 µm in length and 5-8 µm in diameter. It appears ellipsoid in lateral view and oval in cross section, with rounded ends. The anterior end has a rounded protrusion just above the site of flagellar insertion. The posterior of the cell is slightly narrower than the anterior end. Each cell has two brownish chloroplasts without pyrenoids.

Cryptomonas tetrapyrenoidosa Skuja (Figures 6G and 9A)

Cells measure 20-60 µm in length, 10-27 µm in width, and 5-17 µm in depth. There are two chloroplasts per cell, each with two pyrenoids, making a total of four pyrenoids. The periplast type has not been investigated.

Cryptomonas erosa Ehrenberg (Figures 5D and 6H)

Cells are oval or slightly elliptical, flat and slightly contorted, ranging in size from 13 to 45 µm in length, and from 6 to 26 µm in width. There are two chloroplasts per cell that are olive, and pyrenoids are absent. The periplast type has not been investigated.

Cryptomonas ozolinii Skuja (Figure 5E)

Cells are slightly egg shaped and laterally compressed. The anterior end is the widest part of the cell. Cells measure 17-29 µm in length, 9-13 µm in width, and 6-9 µm in depth. Cells contain two olive-green chloroplasts, each with a pyrenoid. The ultrastructure of this species indicates that it should be a new genus (unpublished observations).

Representative Freshwater Campylomorph Species

Members of the *Cryptomonas* campylomorph have had a vacillating taxonomic history. Prior to electron microscopic studies, these members were originally identified and diagnosed as *Cryptomonas* species. However, ultrastructural evidence demonstrated that the campylomorph was considerably different, prompting Hill (1991b) to reclassify them into the newly erected genus *Campylomonas*. More recently, Hoef-Emden and Melkonian (2003) discovered that certain species of *Cryptomonas* display dimorphic life cycles, and that members thought of as *Campylomonas* are an alternate morphotype within the *Cryptomonas* life cycle. Given the unreliability of the morphological species concept, the genus *Cryptomonas* has undergone revision to include members that were formerly classified as *Campylomonas* and *Chilomonas* (Hoef-Emden and Melkonian, 2003; Hoef-Emden, 2007; Tanifuji et al., 2011). The fundamental differences between members of the cryptomorph and those of the campylomorph are that the latter manifest as slightly contorted, sigmoid-shaped cells in lateral view with a characteristic recurved posterior. At the electron microscopic level, the distinguishing features are the presence of an inner periplast sheet, a simple furrow with a gullet of variable length extending posteriorly from the furrow, and the presence of a vestibular ligule. A SPC may be lacking or it may consist of fibrillar material or heptagonal scales. The configuration of the campylomorph periplastidial compartment reflects that of the cryptomorph as described above. The campylomorph is strictly freshwater. For additional information refer to Munawar and Bistricki (1979), Klaveness (1985), Kugrens et al. (1986), Kugrens and Lee (1987), and Hill (1991b).

Cryptomonas reflexa Marsson (*syn. Campylomonas reflexa* Hill) (Figures 5A, 7C and D)

This species has the characteristics described above for the campylomorph. Two pyrenoids are present, and nucleomorphs are located posterior to the pyrenoids and anterior to the nucleus. Cells are highly variable in size and can range from 15 to 60 µm in length and from 10 to 30 µm in width.

Cryptomonas rostratiformis Skuja (Figures 7A, 9C and D)

This species is the largest cryptomonad, ranging in size from 45 to 80 µm in length, 16 to 40 µm in width, and from 14 to 24 µm in depth. Cells are slightly recurved at the posterior, and they have a pronounced rostrate anterior. The furrow is curved slightly toward the left, and the vestibular ligule is pointed and attached to the left side of the vestibulum. The cells have two chloroplasts, each with numerous pyrenoids. Starch is often present in large amounts throughout the chloroplast, sometimes obscuring the pyrenoids. Refer to Kugrens and Lee (1986) for additional structural information.

Cryptomonas platyuris Skuja (Figures 7D, 8C, 10)

Cells range in size from 30 to 55 µm in length, from 15 to 28 µm in width, and from 9 to 16 µm in depth, and have a distinctive flattened or paddle-like posterior tail when viewed from the side. There are two chloroplasts per cell but pyrenoids are lacking. Considerable starch usually is present within the cells. Refer to Kugrens and Lee (1986) and Kugrens et al. (1987) for additional structural details.

Cryptomonas marssonii Skuja (Figure 7C)

Cells range in size from 16 to 38 µm in length and from 8 to 14 µm in width. Cells are somewhat fusiform and slightly sigmoid in shape, with a pointed posterior end. Each cell contains two chloroplasts without pyrenoids, which differs from *C. reflexa*.

Representative Freshwater Colorless Species

Colorless members of *Cryptomonas* were formerly classified within the genus *Chilomonas* Ehrenberg. The furrow/gullet complex consists of a vestibulum, a short furrow, and a long tubular gullet (Kugrens and Lee, 1991). A vestibular ligule covers the area of contractile vacuole discharge. Each flagellum is ornamented by a unilateral row of tubular hairs (Kugrens and Lee, 1991). The IPC consists of a single sheet perforated by numerous ejectisome pores (Grim and Staehelin, 1984; Kugrens et al., 1986), whereas the SPC consists primarily of fibrils. Ejectisomes are docked to the pore edges within the periplast sheet. Two diminutive leucoplasts lacking thylakoids occur within the periplastidial compartment, each containing numerous starch grains and a nucleomorph. Refer to Anderson (1962), Roberts et al. (1981), Grim and Staehelin (1984), Kugrens et al. (1986), Kugrens and Lee (1987), Kugrens and Lee (1991), Heywood (1988), and Kugrens (1999) for additional structural information.

Cryptomonas (*Chilomonas*) *paramecium* Ehrenberg (Figures 3B, 8D, 20B)

Cells are 20-40 µm long and 10-20 µm in diameter, with a rhinote anterior and a blunt, reflexed posterior, imparting a sigmoid shape to the cells. Two leucoplasts and nucleomorphs are located within the periplastidial compartment. This species appears to be evolutionarily derived from a *Cryptomonas* (*Campylomonas*) campylomorph (Clay et al., 1999).

Cryptomonas (*Chilomonas*) *acuta* Schiller

This species has been described from freshwater, but it has not been cultured. It is possible that this genus might be *Leucocryptos acuta* in Bourelly (1970). Its ultrastructural features are unknown. This species was observed in an enrichment culture from a reservoir near Severance, Colorado, but attempts at isolation were unsuccessful.

Pyrenomonas Santore (=*Rhodomonas* Karsten)

Pyrenomonas is the consensus name but some authors continue to use *Rhodomonas*; the generic names are synonyms. Cells are oval and may be pink to red in coloration. Cells have a short furrow of variable length and a deep tubular gullet. The periplast consists of inner plates of approximate square shape with beveled corners. The plates taper slightly toward the posterior, and tapered ends slightly subduct under the broad end of the succeeding plate, imparting a serrated appearance to the cell when seen in section. The SPC consists of intertwining fibrils. The periplastidial compartment contains a single, bilobed chloroplast with a pyrenoid situated between the two lobes of the chloroplast. Thylakoids do not traverse the pyrenoid and a nucleomorph is located in an invagination of the pyrenoid. For additional information refer to Santore (1984), Erata and Chihara (1989), Hill and Wetherbee (1989), and Kugrens et al. (1999).

Pyrenomonas ovalis Kugrens, Clay *et* Lee (Figures 8E–G, 11B, 12A and B)

Cells are oval to ellipsoid, 14-15.5 µm long and 7-8 µm wide with a single red chloroplast with two lobes. The pyrenoid is attached to both lobes, forming a bridge between the two lobes, making the chloroplast appear H-shaped. The nucleomorph is embedded within the pyrenoid. Cells have a short furrow and an anterior tubular gullet. Currently this is the only species described from freshwater; it was collected from Great Western reservoir near Broomfield, Colorado (Kugrens et al., 1999).

Storeatula Hill

Cells are ellipsoid with a slightly rhinote anterior, and are red to pink in color. A furrow is lacking but a tubular gullet extends to approximately the middle of the cell and is lined with several rows of ejectisomes. The periplast consists of an inner sheet and an outer component made of coarsely deposited fibrils. The periplastidial compartment contains a single,

bilobed chloroplast with a pyrenoid that connects the two lobes of the chloroplast. A nucleomorph is located in an anterior groove or invagination of the pyrenoid. Only one freshwater species has been identified to date. For additional information refer to Hill (1991b) and Kugrens et al. (1999).

Storeatula rhinosa Kugrens, Clay, *et* Lee (Figures 8H and I, 11A, 20C and D)

Cells are 16-20 µm long, 7-8 µm wide, and 8-10 µm deep, and appear ellipsoid with a slightly pointed anterior. A furrow is lacking but a tubular gullet runs down through the anterior portion of the cell. A single H-shaped chloroplast with a dorsocentral pyrenoid occurs. This species has been collected from Hanratty's Ditch near Beulah, Colorado, and Sheldon Lake and North Shields Pond in Fort Collins, Colorado.

Komma Hill

Based upon a single isolate, this genus originally was diagnosed as being comma-shaped or acuminate with a rounded anterior end, tapering to a pointed or acutely rounded posterior. A furrow is lacking, but a tubular gullet extends posteriorly from the base of the vestibulum. The periplast consists of relatively small internal and surface hexagonal plates. The surface plates have a crystalline composition and occasionally rosulate, heptagonal scales overlay their surface. The periplastidial compartment contains a single, dorsal blue-green chloroplast with a central pyrenoid lacking traversing thylakoids that projects from the chloroplast. The nucleomorph is situated at the level of the pyrenoid. Refer to Hill (1990) for more specific descriptions. Any blue-green acuminate cryptomonads probably represent *Komma*. Other blue-green cryptomonads with hexagonal plates have been observed (Kugrens and Lee, 1991), but they are not comma shaped. The genus is strictly freshwater and only one species has been described.

Komma caudata (Geitl.) Hill (Figures 13A, 14A and B, 15A and B)

Cells are comma-shaped or acuminate with a sharply pointed posterior end and a rounded anterior end. Cells measure 8-12 µm in length and 4-6 µm in width. Cells contain single, dorsal, blue-green chloroplasts with a single pyrenoid projecting from the center of the chloroplast. Cells lack a furrow and possess a gullet only.

Chroomonas Hansgirg

Cells are subovate or barrel-shaped and often form pseudopalmelloid colonies in streams and rivers. A furrow is absent but a tubular gullet extends posteriorly from the vestibulum. Inner and outer components of the periplast consist of offset rectangular plates (Hill, 1991a), with the anterior plate edges raised because of rows of IMPs in the cell membrane attaching strongly to the plates at the posterior end of each plate. Scales or fibrils may be present on the external surfaces of the superficial plates in some species. The periplastidial compartment contains one or two chloroplasts that may have a pyrenoid. The nucleomorph usually is located near the pyrenoid if present. Chloroplasts contain phycocyanobilin chromophores, imparting a blue-green color to the cells. A reddish stigma may be present in chloroplasts of some species. Refer to Dodge (1969), Gantt (1971), Antia et al. (1973), Meyer and Pienaar (1984a,b), Kugrens et al. (1986), Kugrens and Lee (1987), and Hill (1990) for additional structural features.

Chroomonas oblonga Skvortzow (Figures 13B and 16B)

Cells are ellipsoid, measuring 15 µm in length and 6 µm in width. Two chloroplasts are present per cell, each with a pyrenoid. A stigma may be present. The periplast is comprised of small, inner and superficial rectangular plates. Isolated from Fossil Creek south of Fort Collins, Colorado.

Chroomonas coerulea (Geitler) Skuja (Figures 13C, 14D and E, 16A)

Cells are ellipsoid and sometimes slightly concave dorsoventrally, measuring 8-12 µm in length and 4-6 µm in width. The cell posterior is rounded. Inner and SPCs consist of small rectangular periplast plates. A single blue-green chloroplast is present with a single pyrenoid and a prominent stigma, which is associated laterally with the pyrenoid. Only a vestibulum and gullet are present. Flagella are shorter than the cell length. Cells often form extensive mucilaginous pseudocolonies, which enables secure attachment to a substrate. Ubiquitous in Rocky Mountain lakes, reservoirs, and streams.

Chroomonas pochmanni Huber-Pestalozzi (Figures 13E, 14C, 15C and D)

Cells are barrel shaped to ovoid, 10-18 µm long and 8-13 µm wide, and blue-green in color. A massive single chloroplast bearing a stalked pyrenoid is present in the cell. This species may be mixotrophic (Kugrens and Lee, 1990, 1991). Cells lack a furrow but have a gullet. A large, prominent contractile vacuole is located in the anterior of the cell. Uniquely, a bacterial

ingestion vacuole may also be visible with a light microscope. Numerous large starch grains usually are present in the cell. This species deviates from many of the diagnostic characters for *Chroomonas* and, therefore, the erection of a new genus may be warranted.

Chroomonas nordstedtii Hansgirg (Figures 13D and 14F)

Cells are elongated and slightly egg-shaped with the anterior end obliquely truncate. The posterior end is larger in diameter than the anterior end, and cells exhibit a slight dorsal curvature. *C. nordstedtii* ranges in size from 10 to 30 µm in length and 7 to 15 µm in diameter, which is larger than described by Huber-Pestalozzi (1950). A blue-green parietal chloroplast with a prominent pyrenoid and a small stigma fill the posterior three-fourths of the cell. Common in Wyoming lakes.

Hemiselmis Parke

Cells are rounded anteriorly and posteriorly, are slightly flattened dorsoventrally, and appear somewhat bean-shaped in lateral view. The vestibulum and gullet are uniquely located approximately one-third of the cell length from the anterior, and a furrow is absent. Inner and superficial periplast components are comprised of large hexagonal plates. The periplastidial compartment contains a single dorsal, boat-shaped chloroplast with a centrally situated, stalked pyrenoid that is traversed by a single thylakoid. The nucleomorph is located anterior to the pyrenoid. Chloroplasts generally appear blue-green due to the presence of phycocyanobilin chromophores, although there are several marine species that appear red by virtue of phycoerythrobilin chromophores. This genus is ubiquitous in Colorado and Wyoming lakes, specifically Lake John and Cowdrey Lake in Colorado and Diamond and Twin Buttes Lakes in southern Wyoming. Currently there is only one freshwater species described, and it is the smallest cryptomonad described from freshwater.

Hemiselmis amylosa Clay *et* Kugrens (Figures 13F, 14G-I, 16C and D)

Cells generally are suspended throughout the water column and usually do not swim unless disturbed. Cells range in size from 4 to 5.5 µm in length, from 2.5 to 3 µm in width, and 3 µm in depth. They are slightly compressed laterally, and they appear bean-shaped in the lateral view. The vestibulum and gullet are oval and shallow and are located one-third the distance from the anterior. Cells have few ejectisomes and a single parietal chloroplast with a prominent dorsal pyrenoid in the anterior portion of the cell.

Cryptomonads of Uncertain Taxonomic Status

Cyanomonas Oltmanns (Figures not available)

It should be noted that the existence of this genus is suspect. It has never been cultured and may represent a species of *Chroomonas*, in which large starch grains with a blue-green refraction have been mistaken for the presence of multiple chloroplasts (Hill, 1990). Cells are described as containing several blue-green chloroplasts, and the cell shape is similar to that of some *Chroomonas* spp. Again, since this genus has not been investigated with the electron microscope, and has not been cultured, its status as a legitimate genus remains in doubt.

Plagioselmis Butcher

This genus originally was described only from the marine environment by Butcher (1967) until Novarino et al. (1994) transferred the freshwater *Rhodomonas minuta* into the genus *Plagioselmis*, as *P. nanoplanctica*. Cells are comma-shaped with an acute tail that lacks plates, and appear pink, red, or brown in color. A gullet is absent, but there is a prominent ventral furrow (=sulcus). The periplast consists of an internal component of hexagonal plates, whereas rosette scales make up the superficial periplast component. The acute tail has a continuous periplast sheet rather than plates. Thylakoids usually occur in groups of three (Klaveness, 1981). Novarino et al. (1994) described several of these features from three isolates of *Plagioselmis*, and all bore features consistent with the above except for the nature of the furrow. In comparative studies, they noted that some strains of *P. prolonga* and *P. nanoplanctica* appeared to have a furrow, but they attributed such depressions to artifactual folds induced by cell shrinkage. The presence or absence of a furrow was then used by the investigators as a character for the diagnosis of species. If the absence of a furrow is indeed correct, then either the character set for *Plagioselmis* needs to be expanded or the nonfurrow strains might need to be described as a new genus. An investigation using proper techniques is needed to determine whether the folds are artifacts or whether the collapsed folds were some type of furrow.

Plagioselmis nanoplanctica (Skuja) Novarino. (Figures 15A and B, 18A, 19A and B)

Cells are comma-shaped with an acute posterior end, and chloroplasts are red or pink colored. Cells range in size from 12 to 18 µm in length and from 8 to 10 µm in diameter at the widest portion of the cell. This is the only recognized freshwater species at this time.

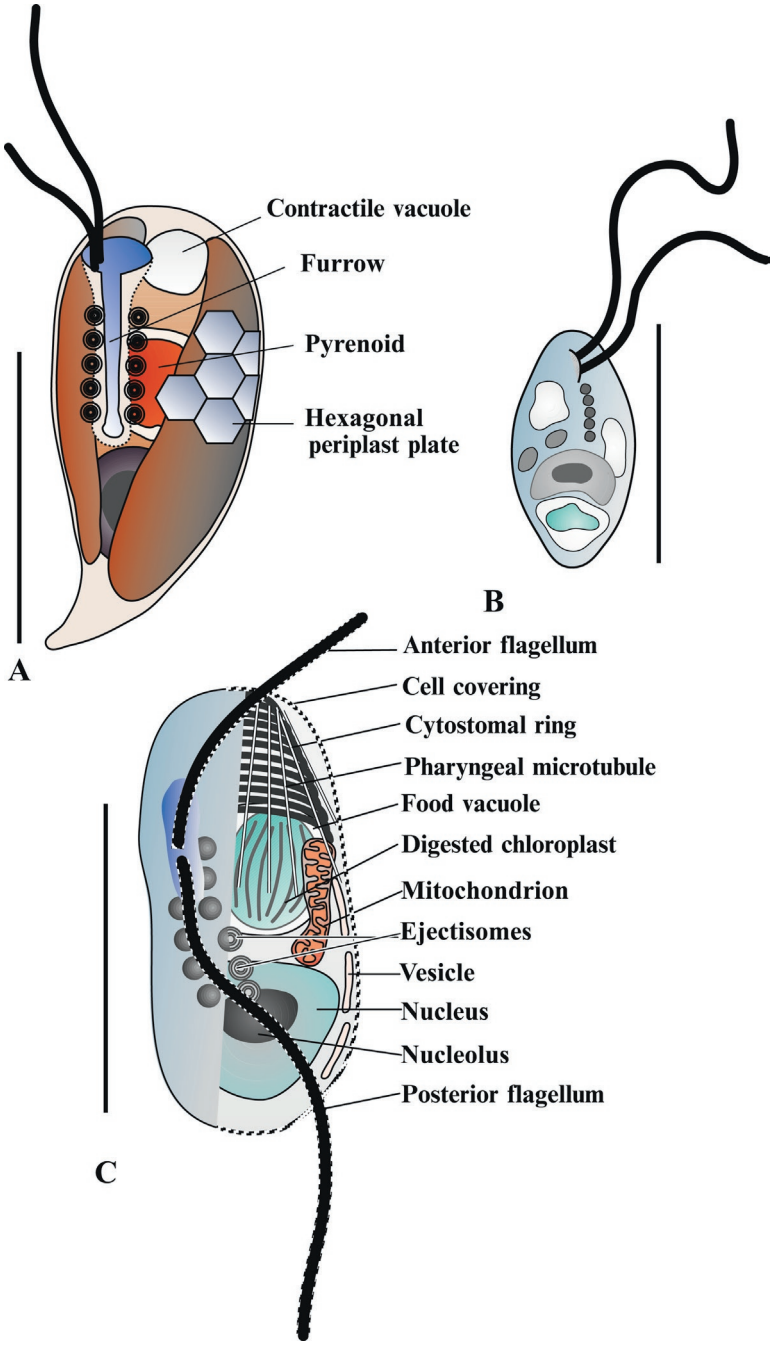

FIGURE 18 Light microscopic illustrations of *Plagioselmis* and *Kathablepharis* species. (A) Diagram of *Plagioselmis nanoplanctica*. (B) *Kathablepharis ovalis*, showing its general features. (C) Diagram of a *Kathablepharis phoenikoston* cell as interpreted from electron microscopic data.

B Guide to Literature for Species Identification

1. *Cryptomonas* (campylomorph)—Hill (1991c), Kugrens et al. (1986)
2. *Cryptomonas* (formerly *Chilomonas*)—Hill (1991a,b), Kugrens and Lee (1991)
3. *Chroomonas*—Hill (1991a)
4. *Cryptomonas* (cryptomorph)—Kugrens et al. (1986)
5. *Goniomonas*—Hill (1991a), Kugrens and Lee (1991)
6. *Hemiselmis*—Clay and Kugrens (1999b)
7. *Kathablepharis*—Lee and Kugrens (1991), Lee et al. (1991), Clay and Kugrens (1999a,c)

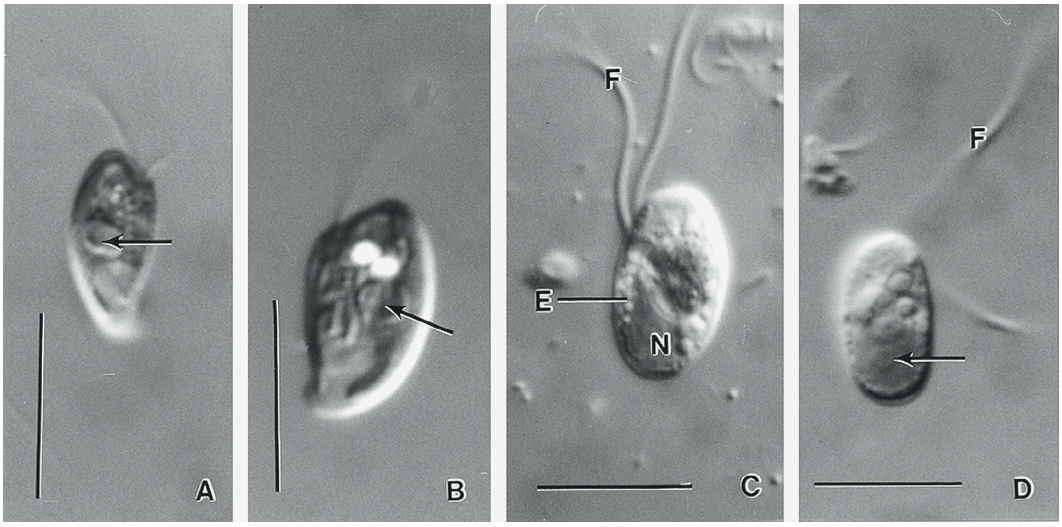

FIGURE 19 Light micrographs of *Plagioselmis* and *Kathablepharis*. (A) Lateral view of *Plagioselmis nanoplanctica* showing the typical comma shape of the cell. The pyrenoid is dorsal (arrow). (B) Ventral view of *Plagioselmis nanoplanctica* showing that this cell is broad in this view. The pyrenoid is on the dorsal side of the cell (arrow). (C) *Kathablepharis ovalis* with ingested food in an enlarged food vacuole. A row of ejectisomes (E) extends posteriorly from the site of flagellar insertion. The nucleus (N) is in the posterior of the cell. (D) *Kathablepharis phoenikoston* cell with the nucleus in the posterior (arrow). Globular contents likely represent ingested food. Scale bars = 10 µm.

8. *Komma*—Hill (1991a)
9. *Plagioselmis*—Novarino et al. (1994)
10. *Pyrenomonas/Rhodomonas*—Kugrens et al. (1999)
11. *Storeatula*—Kugrens et al. (1999)

VIII AVAILABILITY OF CRYPTOMONADS

Cryptomonad cultures are available from a variety of sources. The UTEX Culture Collection of Algae at the University of Texas at Austin houses a number of cryptomonad strains donated by Colorado State University. In addition, Dr. Michael Melkonian's laboratory at the University of Cologne and Dr. Dag Klaveness's laboratory at the University of Oslo, Norway, have numerous cryptomonads, each containing over 50 isolates. Other culture collections with a considerable number of cryptomonad cultures include the Japanese NIES Collection, the Culture Collection of Algae and Protozoa, and Provasoli-Guilliard Culture Collection of Marine Phytoplankton (CCMP) at the Bigelow Laboratories, Boothbay Harbor, Maine.

IX PHYLUM KATHABLEPHARIDA (Figures 18–20)

The colorless predatory flagellates known as the kathablepharids are a well-diagnosed group but their large-scale phylogenetic affinities have long proven elusive. The group was first described by Skuja and placed in the family Kathablepharidaceae within the class Cryptophyceae (Skuja, 1939). Subsequent ultrastructural investigations, however, revealed radically different cytological features than those of cryptomonads, prompting the notion that they be tentatively regarded as *incertae sedis* (Clay and Kugrens, 1999a,b,c). The first molecular data for kathablepharids were generated by Okamoto and Inouye (2005), and the phylogenies they reconstructed using SSU rDNA and beta-tubulin sequences strongly support that kathablepharids are a sister group to the cryptomonads. As a result, these researchers proposed and placed the kathablepharids into the new phylum Kathablepharida (=division Katablepharidiophyta) (Okamoto and Inouye, 2005). More recently, Okamoto et al. (2009) described a new genus and species of kathablepharid from marine waters called *Roombia truncata.* Conducting multigene analyses using Hsp90, SSU rDNA, and LSU rDNA gene sequences, *Roombia* was shown to be a sister group to all other known kathablepharids, and most of these multigene molecular phylogenies suggest also that kathablepharids are a sister group to the cryptomonads. Moreover, cryptomonads and kathablepharids together with haptophytes, telonemids, centrohelids and sometimes biliphytes form a larger, robust clade informally referred to as the Hacrobia (Okamoto et al., 2009).

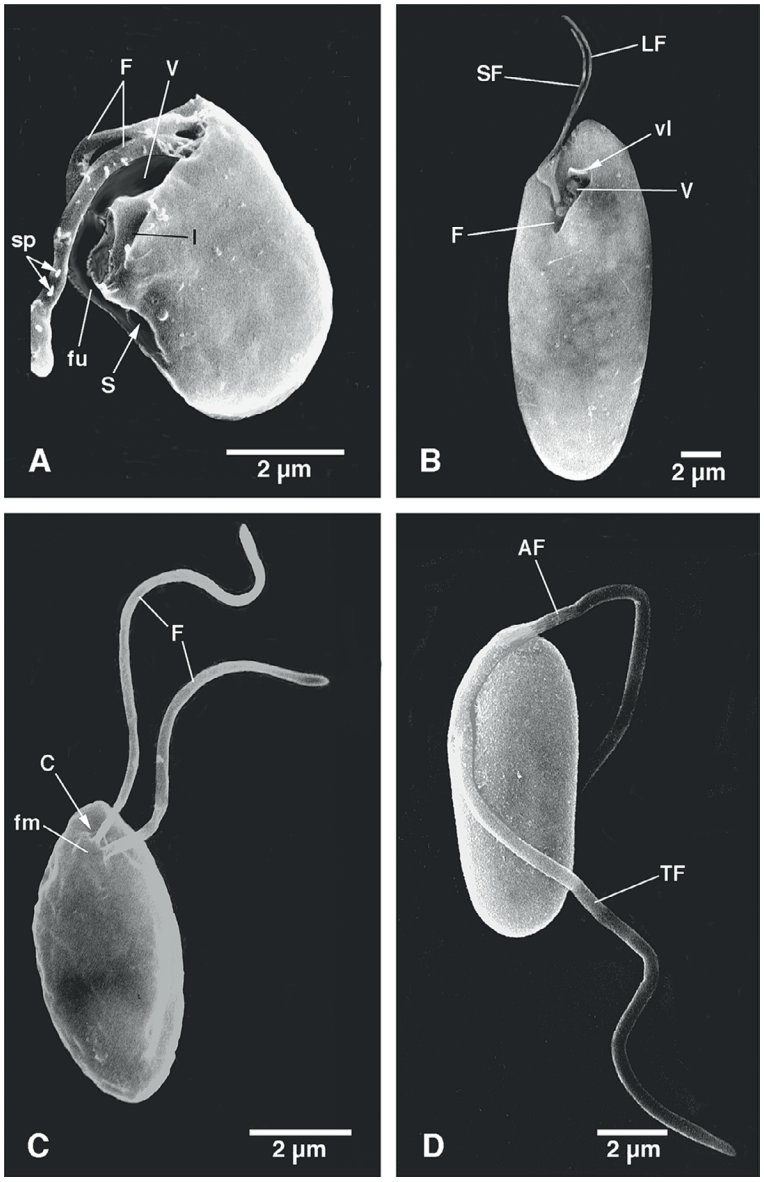

FIGURE 20 Scanning electron micrographs of colorless cryptomonad and kathablepharid species. (A) Cell of *Goniomonas truncata* with both flagella (F) inserted on the dorsal side of the vestibulum (V). Note some unilateral spikes (sp) remaining on the left flagellum. A ventral furrow (fu) continues from the vestibulum and features a persistent opening termed the stoma (S). A second tubular invagination termed the infundibulum (I) is present on the left side of the cell. (B) Cell of *Cryptomonas* (*Chilomonas*) *paramecium* showing two subapically inserted flagella (F) on the right side of the vestibulum (V). Note the vestibular ligule (vl) attached to the dorsal side of the vestibulum. A short furrow (F) extends from the vestibulum. (C) Cell of *Kathablepharis ovalis* showing two subapically inserted flagella (F) arising from a flagellar mound (fm). A cytostome (C) is located between the flagellar mound and the cell apex. (D) Cell of *Kathablepharis phoenikoston* showing an anteriorly directed flagellum (AF) and a trailing flagellum (TF), both inserted subapically. *(Parts (A) and (B) are from Kugrens and Lee (1991), part (C) is from Lee and Kugrens (1991), and part (D) is from Clay and Kugrens (1999a) with permission).*

A Ecology

Based upon field sampling data from the Rocky Mountain region, kathablepharids appear to be tolerant of a variety of temperatures, pH, salinities, and nutrient conditions, an observation made also by Vørs (1992b) in her autecological studies. The lack of more global information regarding these flagellates is likely a consequence of their being overlooked in plankton samples or misidentified, especially in fixed material.

Kathablepharids are voracious predators, attacking their prey individually but more often in groups that can range up to several hundred cells. They feed on both bacteria and various eukaryotes, but each freshwater species that has been studied in detail appears to prefer a specific food organism (Lee and Kugrens, 1991; Lee et al., 1991). For instance, *Kathablepharis ovalis* feeds on the chrysophyte *Chrysochromulina parva* and *K. phoenikoston* preferentially feeds on *Chroomonas*.

B Cell Structure

1. Cell Covering

A distinctive cell covering, termed a surface sheath, jackets the cell including the flagella (Figure 18C). It is composed of outer hexagonal subunits and an inner basement layer. When viewed in section, the outer component consists of equally spaced lamella extending outward at a 45° angle, giving a serrated appearance to the cell perimeter. The inner component or basement layer is appressed to the plasma membrane and is composed of randomly arranged fibrils that cover the whole cell. The outer compartment of the surface sheath is absent over the area of the cytostome, the area where the flagella are inserted into the cell, and the area posterior to the cytostome where the rows of ejectisomes occur under the plasma membrane (Lee and Kugrens, 1991).

2. Flagella

Flagella are inserted subapically, and their length and orientation are variable among species. The flagella appear thick when viewed with the light microscope because the surface sheath surrounding the cell body is continuous with the flagella (Figures 18C, 19C and D, 20C and D).

3. Feeding Apparatus

All kathablepharids have a distinctive, complex feeding apparatus that is similar to that of suctorian ciliates or apicomplexans (Kugrens et al., 1994; Lee et al., 1991). Depending on the species, the feeding apparatus consists of a stack of 2-12 cytopharyngeal rings in the form of a truncated cone located just below an anterior depression known as a cytostome or mouth (Figure 18C). Small vesicles, presumably holding digestive enzymes, are concentrated inside the feeding apparatus. Microtubular bundles are attached to the external face of these rings, and the microtubules extend toward the posterior of the cell. The collection of microtubules occurs as inner and outer circular arrays and together they constitute the cytopharyngeal skeleton (Vørs, 1992a,b). The inner array is associated with the cytopharyngeal rings, and the outer array occurs just beneath the cell cover. This outer array of microtubules is known also as the pellicular skeleton (Vørs, 1992a,b).

4. Nucleus and Mitosis

The interphase nucleus is of the typical eukaryotic type with chromatin attached to the inner membrane of the nuclear envelope. A single nucleolus occurs inside the nucleus. As cells divide, the nucleolus disperses, the nuclear envelope detaches from the chromatin and converts into rough endoplasmic-reticulum, and the chromatin condenses into a single disc-shaped mass where individual chromosomes are no longer resolvable. Microtubules penetrate the chromosome mass but kinetochores have not been observed. Spindle microtubules end in a number of minipoles in the cytoplasm. The chromosome mass separates at anaphase, and each mass migrates to the poles. Then the nuclear envelope reforms and attaches to the chromatin, and the nucleolus reappears. Cytokinesis is longitudinal, forming two daughter cell products (Lee et al., 1993).

5. Mitochondria

Mitochondria have flattened cristae and usually are found between the outer and inner microtubular arrays of the cytopharyngeal skeleton. Serial sections of cells have not been made, and, thus, there is the possibility that only one large reticulate mitochondrion occurs per cell.

6. Ejectisomes

Kathablepharid ejectisomes generally come in two sizes and are enclosed in membranous vesicles. Small ejectisomes occur in the cell posterior in particular and around the cell periphery in general. The large ejectisomes occur in one or two rows underneath the plasma membrane on the ventral side and are oriented parallel to the long axis of the cell (Figures 18C and 19C). Both large and small ejectisomes are formed of single ribbons that are tightly wound into a spiral. In the discharged state, the ejectisomes appear as long, straight hollow tubes, resulting when the edges of the discharged ribbon roll inward and touch. Near the tip of the discharged ejectisome, the ribbon tapers rapidly to a spatula-like point.

7. Food Vacuoles

Food vacuoles are generally located in the posterior portion of the cell, and food particles ingested through the cytosome are transferred to these where they are digested and their products assimilated. Both bacteria and chloroplasts from the food organisms are commonly observed in these food vacuoles with a light microscope.

8. Alveoli-like Structures

Kathablepharid cells possess alveoli-like flattened vesicles of endoplasmic reticulum underneath the plasma membrane that are associated with outer array or subpellicular microtubules. They are similar in structure, although unlikely homologous, to the cortical alveoli present in ciliates, apicomplexans, and dinoflagellates. The primary structural difference between the alveoli-like sacs of kathablepharids and true cortical alveoli is that the flattened sacs of the former are studded, whereas the sacs of the latter are smooth.

C Classification of Kathablepharida

Currently, three genera of kathablepharids are recognized: *Kathablepharis*, *Leucocryptos*, and *Roombia*. The latter two genera are known only from marine environments, whereas *Kathablepharis* is represented in both marine and freshwater habitats.

Kathablepharis Skuja

Cells of *Kathablepharis* vary in size but tend to be oval to cylindrical in shape. All have two subapically inserted flagella, a continuous covering of fused scales (surface sheath) outside of the plasma membrane, and single-ribbon ejectisomes. Chloroplasts are absent, although when pigmented prey present in food vacuoles, they could be mistaken for organelles. Skuja (1939) erected the genus *Kathablepharis* and described eight freshwater species based upon light microscopic observations. Two of the most common freshwater species, *K. ovalis* and *K. phoenikoston*, are described here.

Kathablepharis ovalis Skuja (Figures 18B, 19C, 20C)

K. ovalis is a common flagellate in freshwater habitats in the Rocky Mountain region, occurring in a variety of lentic and lotic habitats. It is small and colorless and often contains one to several ingested cells of *Chrysochromulina parva*, making it appear as though it contains chloroplasts. Cells range in size from 8 to 15 µm in length, but the size is dependent on the number of ingested cells, which can distend the cell considerably. Two subapical flagella emerge laterally from a subapical mound and are encased in the surface sheath, imparting to them a somewhat thickened appearance. The anterior flagellum is approximately 15 µm long and the posterior flagellum is approximately 12 µm long. The cells have a conspicuous central nucleus. One to several large food vacuoles generally occupies the posterior portion of the cell. Two concentric arrays of microtubules begin at the anterior end of the cell and continue into the posterior region. A Golgi apparatus can be found just anterior to the nucleus and inside the inner array of microtubules. Six large ejectisomes occur in two rows posterior to and slightly to the right of the flagella, while smaller ejectisomes occur under the plasma membrane in the posterior and medial portion of the cell. At the light microscopic level, cells of *K. ovalis* appear ovate to subovate, with both flagella directed anteriorly during swimming. At the ultrastructural level, the feeding apparatus in *K. ovalis* has two distinctive cytopharyngeal rings.

K. ovalis has been collected from ponds in the Department of Energy's Rocky Flats Nuclear Weapons Plant, Jefferson County, in Horsetooth Reservoir and North Shields Pond, Larimer County, and in South Delaney Buttes Lake and Lake John, Jackson County, Colorado, USA. This protist, however, is easily overlooked in plankton samples because it is small and colorless and lacks any striking features when examined in the light microscope. In addition to solitary cells, swarms of *Kathablepharis* are common when attacking prey, consisting of aggregations of 20-100 cells.

Kathablepharis phoenikoston Skuja (Figures 18C, 19D, 20D)

Cells of *K. phoenikoston* are cylindrical and have one anteriorly directed flagellum and one trailing flagellum when swimming. *K. phoenikoston* possesses 9-10 conoid-like rings that are associated with the feeding apparatus. The cell covering and other features are similar to those described for *K. ovalis*.

D Isolation and Culturing Techniques for Kathablepharids

Isolation involves the same serial dilution technique as described for cryptomonads. Because they do not survive on bacteria alone, kathablepharids require the presence of their specific food organism and, therefore, they must be maintained as biprotist cultures. Generally, the food organism is isolated first and then established cultures can be inoculated with several kathablepharid cells. The mineral medium promotes growth of the photosynthetic prey organism, providing kathablepharids with a sustainable food source, which allows them to thrive.

LITERATURE CITED

Andersen, R.A., 1992. Diversity of eukaryotic algae. Biodivers. Conserv. 1, 267–292.

Anderson, E., 1962. A cytological study of *Chilomonas paramaecium* with particular reference to the so-called trichocysts. J. Protozool. 9, 380–395.

Antia, N.J., Kalley, J.P., McDonald, T., Bisalputra, T., 1973. Ultrastructure of the marine cryptomonad *Chroomonas salina* cultured under conditions of photoautotrophy and glyceroheterotrophy. J. Protozool. 20, 377–385.

Antia, N.J., Cheng, J.Y., Foyle, R.A., Percival, E., 1979. Marine cryptomonad starch from autolysis of glycerol-grown *Chroomonas salina*. J. Phycol. 15, 57–62.

Apt, K.E., Collier, J.L., Grossman, A.R., 1995. Evolution of the phycobiliproteins. J. Mol. Biol. 248, 79–96.

Archibald, J.M., 2007. Nucleomorph genomes: structure, function, origin and evolution. BioEssays 29, 377–385.

Arvola, L., Tulonen, T., 1998. Effects of allochthonous dissolved organic matter and inorganics on the growth of bacteria and algae from a highly humic lake. Environ. Int. 24, 509–520.

Arvola, L., Ojala, A., Barbosa, F., Heaney, S.I., 1991. Migration behaviour of three cryptophytes in relation to environmental gradients: an experimental approach. Phycologia 26, 361–373.

Boraas, M.E., Estep, K.W., Johnson, P.W., McN, Sieburth J., 1988. Phagotrophic phototrophs: the ecological significance of mixotrophy. J. Protozool. 35, 249–252.

Bourelly, P., 1970. Les Algues D'eau Douce. Tome III: Les Agues Bleues et Rouges. Les Eugleniens, Peridiniens et Cryptomonadines. Editions N. Boubee & Cie, Paris, 512 pp.

Boyne, A.F., 1979. A gentle, bounce free assembly for quick freezing tissues for electron microscopy: application to isolated *Torpedine* ray electrode stacks. J. Neurosci. Methods 1, 353–364.

Brett, S.J., Wetherbee, R., 1986. A comparative study of periplast structure in *Cryptomonas cryophila* and *C. ovata* (Cryptophyceae). Protoplasma 131, 23–31.

Butcher, R.W., 1967. An Introductory account of the smaller algae of British coastal waters IV. Cryptophyceae. Fishery Invest. Lond. Ser. 4, 1–54.

Caljon, A., 1983. Brackish-water phytoplankton of the Flemish lowland. Develop. Hydrobiol. 18, 1–272.

Canter, H.M., 1968. Studies on British chytrids: XXVII. *Rhizophydium fugax* sp. nov., a parasite of planktonic cryptomonads with additional notes and records of planktonic fungi. Trans. Br. Mycol. Soc. 51 (5), 699–705.

Cavalier-Smith, T., 1986. The Kingdom Chromista: origin and systematics. In: Round, F.E., Chapman, D.J. (Eds.), Progress in Phycological Research, Vol. 4. Biopress Ltd., Bristol, pp. 309–347.

Cavalier-Smith, T., 1993. Kingdom protozoa and its 18 phyla. Microbiol. Rev. 57, 953–994.

Cavalier-Smith, T., Chao, E.E., 2006. Phylogeny and megasystematics of phagotrophic heterokonts (kingdom Chromista). J. Mol. Evol. 62, 388–420.

Cavalier-Smith, T., Couch, J.A., Thorsteinsen, K.E., Gilson, P., Deane, J.A., Hill, D.R.A., McFadden, G.I., 1996. Cryptomonad nuclear and nucleomorph 18S rRNA phylogeny. Eur. J. Phycol. 31, 315–328.

Chandler, D.E., 1984. Comparison of quick-frozen and chemically fixed sea urchin eggs: structural evidence that cortical granule exocytosis is preceded by a *local* increase in membrane mobility. J. Cell Sci. 72, 23–36.

Clay, B.L., Kugrens, P., 1999a. Systematics of the enigmatic kathablepharids, including EM characterization of the type species, *Kathablepharis phoenikoston*, and new observations on K. *remigera* comb. nov. Protist 150, 43–59.

Clay, B.L., Kugrens, P., 1999b. Characterization of *Hemiselmis amylosa* sp. nov. and phylogenetic placement of the blue-green cryptomonads *H. amylosa* and *Falcomonas daucoides*. Protist 150, 297–310.

Clay, B.L., Kugrens, P., 1999c. Description and ultrastructure of *Kathablepharis tenuis* sp. nov. and *K. obesa* sp. nov.—two new freshwater kathablepharids (Kathablepharididae) from Colorado and Wyoming. Eur. J. Protistol. 35, 435–447.

Clay, B.L., Kugrens, P., Lee, R.E., 1999. A revised classification of Cryptophyta. Bot. J. Linn. Soc. 131, 131–151.

Deane, J.A., Hill, D.R.A., McFadden, G.I., 1998. *Hanusia phi* gen. *et* sp. nov. (Cryptophyceae): characterization of *Cryptomonas* sp. Eur. J. Phycol. 33, 149–154.

Deane, J.A., Strachan, I.M., Saunders, G.W., Hill, D.R.A., McFadden, G.I., 2002. Cryptomonad evolution: nuclear 18S rDNA phylogeny versus cell morphology and pigmentation. J. Phycol. 38, 1236–1244.

Dodge, J.D., 1969. The ultrastructure of *Chroomonas mesostigmatica* Butcher (Cryptophyceae). Arch. Mikrobiol. 69, 266–280.

Douglas, S.E., Murphy, C.A., Spencer, D.F., Gray, M.W., 1991. Cryptomonad algae are evolutionary chimeras of two phylogenetically distinct unicellular eukaryotes. Nature 350, 148–151.

Dwarte, D., Vesk, M., 1982. Freeze-fracture thylakoid ultrastructure of representative members of chlorophyll c algae. Micron 13, 325–326.

Dwarte, D., Vesk, M., 1983. A freeze-fracture study of cryptomonad thylakoids. Protoplasma 117, 130–141.

Edmondson, W.T., 1965. Reproductive rate of planktonic rotifers as related to food and temperature in nature. Ecol. Monogr. 35, 61–111.

Erata, M., Chihara, M., 1989. Re-examination of *Pyrenomonas* and *Rhodomonas* (Class Cryptophyceae) through ultrastructural survey of red pigmented cryptomonads. Botanical Magazine Tokyo 102, 429–442.

Ettl, H., Moestrup, O., 1980. Uber einen intrazellularen Parasiten be *Cryptomonas* (Cryptophyceae), I. Plant Syst. Evol. 135, 211–226.

Fast, N.M., Kissinger, J.C., Roos, D.S., Keeling, P.J., 2001. Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. Mol. Biol. Evol. 18, 418–426.

Faust, M.A., 1974. Structure of the periplast of *Cryptomonas ovata* var. *palustris*. J. Phycol. 10, 121–124.

Faust, M.A., Gantt, E., 1973. Effect of light intensity and glycerol on the growth, pigment composition and ultrastructure of *Chroomonas* sp. J. Phycol. 9, 489–495.

Ferguson, A.J.D., Thompson, J.M., Reynolds, C.S., 1982. Structure and dynamics of zooplankton communities maintained in closed systems, with special reference to the algal food supply. J. Plankton Res. 4, 523–543.

Fields, S.D., Rhodes, R.G., 1991. Ingestion and retention of Chroomonas spp. (Cryptophyceae) by Gymnodinium acidotum (Dinophyceae). J Phycol 27, 525–529.

Gantt, E., 1971. Micromorphology of the periplast of *Chroomonas* sp. (Cryptophyceae). J. Phycol. 7, 177–184.

Gantt, E., 1979. Phycobiliproteins of Cryptophyceae. In: Levandowsky, M., Hunter, S.H. (Eds.), Biochemistry and Physiology of Protozoa, Vol. 1. Academic Press, New York and London, pp. 121–138.

Gantt, E.R., 1980. Photosynthetic cryptophytes. In: Cox, E.R. (Ed.), In: Phytoflagellates, Developments in Marine Biology, Vol. 2. Elsevier, North Holland Amsterdam, pp. 381–405.

Gantt, E., Edwards, M.R., Provasoli, L., 1971. Chloroplast structure of the Cryptophyceae, evidence for Phycobiliproteins within intrathylakoidal spaces. J. Cell Biol. 48, 280–290.

Gillot, M., 1990. Phylum cryptophyta (cryptomonads). In: Margulis, L., Corliss, J.O., Melkonian, M., Chapman, D.J. (Eds.), Handbook of Protoctista. Jones & Bartlett Publishers, Boston, pp. 139–151.

Gillott, M.A., Gibbs, S.P., 1980. The cryptomonad nucleomorph: its ultrastructure and evolutionary significance. J. Phycol. 16, 558–568.
Gillott, M.A., Gibbs, S.P., 1983. Comparison of the flagellar rootlets and periplast in two marine cryptomonads. Can. J. Bot. 61, 1964–1980.
Glazer, A.N., Appell, G.S., 1977. A common evolutionary origin for the biliproteins of cyanobacteria, rhodophyta, and cryptophyta. FEMS Microbiol. Lett. 1, 113–116.
Glazer, A.N., Wedemeyer, G.J., 1995. Cryptomonad biliproteins—an evolutionary perspective. Photosynth. Res. 46, 93–105.
Grain, J., Mignot, J.P., Puytorac, P., 1988. Ultrastructures and evolutionary modalities of flagellar and ciliary systems in protists. Biol. Cell. 63, 219–237.
Greenwood, A.D., Griffiths, H.B., Santore, U.S., 1977. Chloroplast and cell compartments in Cryptophyceae. Br. Phycol. J. 12, 112–119.
Grim, J.N., Staehelin, L.A., 1984. The ejectisomes of the flagellate *Chilomonas paramecium*: visualization by freeze-fracture and isolation techniques. J. Protozool. 3, 259–267.
Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of marine invertebrate animals. Plenum Press, New York, pp. 29–60.
Henderson, R.J., Mackinlay, E.E., 1989. Effect of temperature on lipid composition of the marine cryptomonad *Chroomonas salina*. Phytochemistry 28, 2943–2948.
Heywood, P., 1988. Ulttrastructure of *Chilomonas paramecium* and the phylogeny of cryptomonads. BioSystems 21, 293–298.
Hibberd, D.J., 1977. Observations on the ultrastructure of the cryptomonad endosymbiont of the red-water ciliate *Mesodinium rubrum*. J. Mar. Biol. Assoc. UK 57, 45–61.
Hibberd, D.J., Greenwood, A.D., Griffiths, H.B., 1971. Observations on the ultrastructure of the flagella and periplast in the Cryptophyceae. Br. Phycol. J. 6, 61–72.
Hill, D.R.A., 1990. *Chroomonas* and other blue-green cryptomonads. J. Phycol. 26, 133–145.
Hill, D.R.A., 1991a. Diversity of heterotrophic cryptomonads. In: Patterson, D.J., Larsen, J. (Eds.), The Biology of Free-Living Heterotrophic Flagellates. In: Systematics Association Special, Vol. 45. Clarendon Press, Oxford, pp. 235–240.
Hill, D.R.A., 1991b. A revised circumscription of *Cryptomonas* (Cryptophyceae) based on examination of Australian strains. Phycologia 30, 170–188.
Hill, D.R.A., Rowan, K.S., 1989. Biliproteins of the Cryptophyceae. Phycologia 28, 455–463.
Hill, D.R.A., Wetherbee, R., 1986. *Proteomonas sulcata* gen. *et* sp. nov. (Cryptophyceae) a cryptomonad with two morphologically distinct and alternating forms. Phycologia 27, 521–543.
Hill, D.R.A., Wetherbee, R., 1988. The structure and taxonomy of *Rhinomonas pauca* gen. et sp. nov. (Cryptophyceae). Phycologia 27, 355–365.
Hill, D.R.A., Wetherbee, R., 1989. A reappraisal of the genus *Rhodomonas* (Cryptophyceae). Phycologia 28, 143–158.
Hoef-Emden, K., 2007. Revision of the genus *Cryptomonas* (Cryptophyceae) II: incongruences between the classical morphospecies concept and molecular phylogeny in smaller pyrenoid-less cells. Phycologia 46, 402–428.
Hoef-Emden, K., 2008. Molecular phylogeny of phycocyanin-containing cryptophytes: evolution of biliproteins and geographical distribution. J. Phycol. 44, 985–993.
Hoef-Emden, K., Melkonian, M., 2003. Revision of the genus *Cryptomonas* (Cryptophyceae): a combination of molecular phylogeny and morphology provides insights into a long-hidden dimorphism. Protist 154, 371–409.
Hoshaw, R.W., Rosowski, J.R., 1973. Isolation and purification. 3: methods for microscopic algae. In: Stein, J.R. (Ed.), Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, New York, pp. 53–67.
Huber-Pestalozzi, G., 1950. Das Phytoplankton das Süsswassers, Teil 3. Cryptophyceen, Chloromonadinen, Peridineen. In: Thienemann, A. (Ed.), Die Binnengewasser, Stuttgart, pp. 2–78.
Keeling, P.J., 2009. Chromalveolates and the evolution of plastids by secondary endosymbiosis. J. Eukaryot. Microbiol. 56, 1–8.
Klaveness, D., 1977. Morphology, distribution and significance of the manganese-accumulating microorganism *Metallogenium in* lakes. Hydrobiologia 56, 25–33.
Klaveness, D., 1981. *Rhodomonas lacustris* (Pascher & Ruttner) Javornicky (Cryptomonadida): ultrastructure of the vegetative cell. J. Protozool. 28, 83–90.
Klaveness, D., 1982. The *Cryptomonas-Caulobacter* consortium: facultative ectocommensalism with possible taxonomic consequences? Nord. J. Bot. 2, 183–188.
Klaveness, D., 1984. Studies on the morphology, food selection and growth of two planktonic freshwater strains of *Coleps* sp.. Protistologica 20, 335–349.
Klaveness, D., 1985. Classical and modern criteria for determining species of Cryptophyceae. Bull. Plankt. Soc. Japan 32, 111–128.
Klaveness, D., 1988a. Ecology of the Cryptomonadida: a first review. In: Sandgren, C.D. (Ed.), Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, New York, pp. 105–133.
Klaveness, D., 1988b. Biology and ecology of the Cryptophyceae: status and challenges. Biol. Oceanogr. 6, 257–270.
Knapp, C.W., deNoyelles, F., Graham, D.W., Bergin, S., 2003. Physical and chemical conditions surrounding the diurnal vertical migration of *Cryptomonas* spp. (Cryptophyceae) in a seasonally stratified mid-western reservoir (USA). J. Phycol. 39, 855–861.
Kugrens, P., 1999. Cryptomonad systematics—an algal enigma? In: Seckbach, J. (Ed.), Enigmatic Algae. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 127–138.
Kugrens, P., Lee, R.E., 1986. An ultrastructural survey of cryptomonad periplasts using quick-freezing freeze-fracture techniques. J. Phycol. 23, 365–376.
Kugrens, P., Lee, R.E., 1988. Ultrastructure of fertilization in a cryptomonad. J. Phycol. 24, 510–518.
Kugrens, P., Lee, R.E., 1990. Ultrastructural evidence for bacterial incorporation and mixotrophy in the photosynthetic cryptomonad *Chroomonas pochmanni* Huber-Pestalozzi (Cryptomonadida). J. Protozool. 37, 263–267.
Kugrens, P., Lee, R.E., 1991. Organization of cryptomonads. In: Patterson, D.J., Larsen, J. (Eds.), The Biology of Free-Living Heterotrophic Flagellates. In: The Systematics Association Special, Vol. 45. pp. 219–233.

Kugrens, P., Lee, R.E., Andersen, R.E., 1986. Cell form and surface patterns in *Chroomonas* and *Cryptomonas* cells (cryptophyta) as revealed by scanning electron microscopy. J. Phycol. 22, 512–522.
Kugrens, P., Lee, R.E., Andersen, R.E., 1987. Ultrastructural variations in cryptomonad flagella. J. Phycol. 23, 511–518.
Kugrens, P., Lee, R.E., Corliss, J.O., 1994. Ultrastructure, function and biogenesis of extrusive organelles in selected non-ciliate protists. Protoplasma 181, 164–190.
Kugrens, P., Clay, B.L., Lee, R.E., 1999. Ultrastructure ans systematics of two new freshwater red cryptomonads, *Storeatula rhinosa*, sp. nov. and *Pyrenomonas ovalis*, sp. nov. J. Phycol. 35, 1079–1089.
Lane, C.E., Archibald, J.M., 2008. New marine members of the genus *Hemiselmis* (cryptomonadales, cryptopheceae). J. Phycol. 44, 439–450.
Lane, C.E., Khan, H., MacKinnon, M., Fong, A., Theophilou, S., Archibald, J.M., 2006. Insight into the diversity and evolution of the cryptomonad nucleomorph genome. Mol. Biol. Evol. 23, 856–865.
Larsen, J., 1992. Endocytobiotic consortia with dinoflagellate hosts. In: Reisser, W. (Ed.), Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored. Biopress, Ltd., Bristol, United Kingdom, pp. 427–442.
Lee, R.E., Kugrens, P., 1986. The occurrence and structure of flagellar scales in some freshwater cryptophytes. J. Phycol. 22, 549–552.
Lee, R.E., Kugrens, P., 1991. *Kathablepharis ovalis*, a colorless flagellate with interesting cytological characteristics. J. Phycol. 27, 505–513.
Lee, R.E., Kugrens, P., 1998. Hypothesis: the ecological advantage of chloroplast endoplasmic reticulum—the ability to outcompete at low dissolved CO2 concentrations. Protist 149, 341–345.
Lee, R.E., Kugrens, P., 2000. Ancient atmospheric CO2 and the timing of evolution of secondary endosymbioses. Phycologia 39, 167–172.
Lee, R.E., Kugrens, P., Mylnikov, A.P., 1991. Feeding apparatus of the colorless flagellate *Kathablepharis* (Cryptophyceae). J. Phycol. 27, 725–733.
Lee, R.E., Miller-Hughes, C., Kugrens, P., 1993. Ultrastructure of mitosis and cytokinesis in the colorless flagellate *Kathablepharis ovalis* Skuja. J. Eukaryot. Microbiol. 40, 377–383.
Lewitus, A.J., Caron, D.A., 1991. Physiological responses of phytoflagellates to dissolved organic substrate additions. 2. Dominant role of autotrophic nutrition in *Pyrenomonas salina* (Cryptophyceae). Plant Cell Physiol. 32, 791–801.
Lewitus, A.J., Glasgow, H.B., Burkholder, J.M., 1999. Kleptoplastidy in the toxic dinoflagellate *Pfiesteria piscicida* (dinophyceae). J. Phycol. 35, 303–312.
Li, A., Stoecker, D.K., Coats, D.W., Adam, E.J., 1996. Ingestion of fluorescently labeled and phycoerythrin-containing prey by mixotrophic dinoflagellates. Aquat. Microb. Ecol. 10, 139–147.
Loret, P., Pastoureaud, A., Bacher, C., Delsalle, B., 2000. Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaitifera* in the Takapoto lagoon (Tuamoto Archipelago, French Polynesia): is situ study using optical microscopy and HPLC pigment analysis. Mar. Ecol. Prog. Ser. 199, 55–67.
Lucas, I.A.N., 1970a. Observations on the ultrastructure of representatives of the genera *Hemiselmis* and *Chroomonas* (Cryptophyceae). Br. Phycol. J. 5, 29–37.
Lucas, I.A.N., 1970b. Observation on the fine structure of the Cryptophyceae. I. The genus cryptomonas. J. Phycol. 6, 30–38.
Lucas, I.A.N., 1982. Observation on the fine structure of the Cryptophyceae. II. The eyespot. Br. Phycol. J. 17, 113–119.
Ludwig, M., Gibbs, S.P., 1985a. DNA is present in the nucleomorph of cryptomonads: further evidence that the chloroplast evolved from a eukaryotic endosymbiont. Protoplasma 127, 9–20.
Ludwig, M., Gibbs, S.P., 1989. Localization of phycoerythrin at the lumenal surface of the thylakoid membrane in *Rhodomonas lens*. J. Cell Biol. 108, 875–884.
Marin, B., Klingberg, M., Melkonian, M., 1998. Phylogenetic relationships among the cryptophyta: analysis of nuclear-encoded SSU rRNA sequences support the monophyly of extant plastid-containing lineages. Protistology 149, 265–276.
Martin, W., Sommerville, C.C., Loiseaux-de Goer, S., 1992. Molecular phylogenies of plastid origins and algal evolution. J. Mol. Evol. 35, 385–404.
McFadden, G.I., 1993. Second-hand chloroplasts: evolution of cryptomonad algae. Adv. Bot. Res. 19, 189–230.
McFadden, G.I., Gilson, P.R., Hill, D.R.A., 1994. *Goniomonas*: rRNA sequences indicate that this phagotrophic flagellate is a close relative of the host component of cryptomonads. Eur. J. Phycol. 29, 29–32.
McFadden, G.I., Gilson, P.R., Douglas, S.E., Cavalier-Smith, T., Hofmann, C.J.B., Maier, U.-G., 1997. Bonsai genomics: sequencing the smallest eukaryotic genomes. Trends Genet. 13, 46–49.
McKerracher, L., Gibbs, S.P., 1982. Cell and nucleomorph division in the alga *Cryptomonas*. Can. J. Bot. 60, 2440–02452.
Meyer, S.R., Pienaar, R.N., 1981. The ultrastructure of mitosis and cytokinesis in a new species of *Chroomonas* (Cryptophyceae). Electron Microsc. Soc. South Africa 11, 163–164.
Meyer, S.R., Pienaar, R.N., 1984a. The microanatomy of Chroomonas africana sp. nov. (Cryptophyceae). S. Afr. J. Bot. 3, 306–319.
Meyer, S.R., Pienaar, R.N., 1984b. Mitosis and cytokinesis in *Chroomonas africana* Meyer & Pienaar (Cryptophyceae). S. Afr. J. Bot. 3, 320–330.
Mignot, J.P., 1965. Etude ultrastructurale de *Cyathomonas truncata* From. (Flagelle Cryptomonadine). J. Microsc. 4, 239–252.
Morgan, K., Kalff, J., 1975. The winter dark survival of an algal flagellate *Cryptomonas erosa* (Skuja). Verh. Internat. Verein. Limnol. 19, 2735–2740.
Morrall, S., Greenwood, A.D., 1980. A comparison of the periodic substructure of the trichocysts of the Cryptophyceae and Prasinophyceae. Biosystems 12, 71–83.
Morrall, S., Greenwood, A.D., 1982. Ultrastructure of nucleomorph division in species of Cryptophyceae and its evolutionary implications. J. Cell Sci. 54, 311–328.
Munawar, M., Bistricki, T., 1979. Scanning electron microscopy of some nanoplankton cryptomonads. Scan. Electron Microsc. 3, 247–252.
Nauwerk, A., 1968. Das Phytoplankton des Latnjajaure 1954–55. Schweiz. Z. Hydrol. 30, 188–216.

Nichols, H.W., 1973. Growth media—freshwater. In: Stein, J.R. (Ed.), Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, New York, pp. 7–24.

Novarino, G., 1991a. Observations on *Rhinomonas reticulata* comb. nov. and *R. reticulata* var. eleniana var. nov. (Cryptophyceae), with comments on the genera *Pyrenomonas* and *Rhodomonas*. Nord. J. Bot. 11, 243–252.

Novarino, G., 1991b. Observations on some new and interesting Cryptophyceae. Nord. J. Bot. 11, 599–611.

Novarino, G., 1993a. A comparison of some morphological characters in *Chroomonas ligulata* sp. nov. and *C. placoides* sp. nov. (Cryptophyceae). Nord. J. Bot. 13, 583–589.

Novarino, G., 1993b. Possible detection of the periplast areas and the nucleomorph of cryptomonads by light microscopy: some early observations by Künstler, Skuja and Hoolande. Quekett J. Microsc. 37, 45–51.

Novarino, G., Lucas, I.A.N., 1993. Some proposals for a new classification system of the Cryptophyceae. Bot. J. Linn. Soc., Lond. 111, 3–21.

Novarino, G., Lucas, I.A.N., Morrall, S., 1994. Observations on the genus *Plagioselmis* (Cryptophyceae). Cryptogam. Algol. 15, 87–96.

Oakley, B.R., Bisalputra, T., 1977. Mitosis and cell division in *Cryptomonas* (Cryptophyceae). Can. J. Bot. 55, 2789–2800.

Oakley, B.R., Dodge, J.D., 1973. Mitosis in the Cryptophyceae. Nature 244, 521–522.

Oakley, B.R., Dodge, J.D., 1976. The ultrastructure of mitosis in *Chroomonas salina* (Cryptophyceae). Protoplasma 88, 241–254.

Oakley, B.R., Heath, I.B., 1978. The arrangements of microtubules in serially sectioned spindles of the alga *Cryptomonas*. J. Cell Sci. 31, 53–70.

Oakley, B.R., Santore, U.J., 1982. Cryptophyceae: introduction and bibliography. In: Rosowski, J.R., Parker, B.C. (Eds.), Selected Papers of Phycology. Allen Press, Lawrence, KS, pp. 682–686.

Okamoto, N., Inouye, I., 2005. The katablepharids are a distant sister group of the Cryptophyta: a proposal fot Katablepharidiophyta divisio nova/ Kathablepharida phylum nova based on SSU rDNA and beta-tubulin phylogeny. Protist 156, 163–179.

Okamoto, N., Chantangsi, C., Hora´k, A., Leander, B.S., Keeling, P.J., 2009. Molecular phylogeny and description of the novel katablepharid *Roombia truncata* gen. et sp. nov., and establishment of the Hacrobia Taxon nov. PLoS One 4 (9), e7080. http://dx.doi.org/10.1371/journal.pone.0007080.

Parducz, B., 1967. Ciliary movement and coordination in ciliates. Int. Rev. Cytol. 21, 91–128.

Patterson, D.J., 1981. The behaviour of contractile vacuole complexes of cryptophycean flagellates. Br. Phycol. J. 16, 429–439.

Pejler, B., 1977. Experience with rotifer cultures based on Rhodomonas. Archiv für Hydrobiologie Beih. Ergebn. Limnologie 8, 264–266.

Pennick, D.L., 1981. Flagellar scales in *Hemiselmis brunnescens* Butcher and *H. virescens* Droop (Cryptophyceae). Arch. Protistenkd. 124, 267–270.

Perasso, L., Brett, S.J., Wetherbee, R., 1993. Pole reversal and the development of cell asymmetry during division in cryptomonad flagellates. Protoplasma 174 (1–2), 19–24.

Pfiester, L.A., Holt, J.R., 1978. A freshwater "red tide" in Texas. Southwest. Nat. 23, 103–110.

Phillips, D., Boyne, A.F., 1984. Liquid nitrogen-based quick freezing: experiences with bounce-free delivery of cholenergic nerve terminals to a metal surface. J. Electron Microsc. Tech. 1, 9–29.

Pienaar, R.N., 1976. Virus-like particles in three species of phytoplankton from San Juan Island, Washington. Phycologia 15, 185–190.

Pollingher, U., 1981. The structure and dynamics of the phytoplankton assemblages in lake Kinneret, Israel. J. Plankton Res. 3, 93–105.

Pringsheim, E.G., 1944. Some aspects of taxonomy in the Cryptophyceae. New Phytologist. 43, 143–150.

Pringsheim, E.G., 1968. Zur kenntinis der Cruptomonaden des Süsswassers. Nova Hedwigia 16, 367–401.

Putt, M., 1990. Metabolism of photosynthate in the chloroplast-retaining ciliate *Loboea strobila*. Mar. Ecol. Prog. Ser. 60, 271–282.

Reynolds, C.S., 1980. Phytoplankton assemblages and their periodicity in stratifying lake systems. Holarctic Ecology 3, 141–159.

Reynolds, C.S., 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, United Kingdom.

Rhiel, E., Westerman, M., 2012. Isolation, purification and some ultrastructural details of discharged ejectisomes of cryptophytes. Protoplasma 249, 107–115.

Roberts, K.R., 1984. Structure and significance of the cryptomonad flagellar apparatus. I. *Cryptomonas ovata* (Cryptophyta). J. Phycol. 20, 159–167.

Roberts, K.R., Stewart, K.D., Mattox, K.R., 1981. The flagellar apparatus of *Chilomonas paramecium* (Cryptophyceae) and its comparison with certain zooflagellates. J. Phycol. 17, 159–167.

Rott, E., 1983. Sind die Veränderung im Phytoplanktonbild dem Pilburger Sees Auswirkungen der Tiefenwasserableitung? Archiv für Hydrobiologie Supplementband 67, 29–80.

Salonen, K., Jones, R.I., Arvola, L., 1984. Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplankton. Freshw. Biol. 14, 431–438.

Santore, U.J., 1977. Scanning electron microscopy and comparative micromorphology of the periplast of *Hemiselmis rufescens*, *Chroomonas* sp., *Chroomonas salina* and members of the genus *Cryptomonas* (Cryptophyceae). Br. Phycol. J. 12, 255–270.

Santore, U.J., 1978. Light- and electron-microscopic observations of the palmelloid phase in members of the genus *Cryptomonas* (Cryptophyceae). Arch. Protistenkd. 120, 420–435.

Santore, U.J., 1982a. Comparative ultrastructure of two members of the Cryptophyceae asssigned to the genus *Chroomonas*—with comments on their taxonomy. Arch. Protistenkd. 125, 5–29.

Santore, U.J., 1982b. The ultrastructure of *Hemiselmis brunnescens* and *Hemiselmis virescens* with additional observations on *Hemiselmis rufescens* and comments about the Hemiselmidaceae as a natural group of the Cryptophyceae. Br. Phycol. J. 17, 81–89.

Santore, U.J., 1982c. The distribution of the nucleomorph in the Cryptophyceae. Cell Biol. Int. Rep. 6, 1055–1063.

Santore, U.J., 1983. Flagellar and body scales in the Cryptophyceae. Br. Phycol. J. 18, 239–248.

Santore, U.J., 1984. Some aspects of taxonomy in the Cryptophyceae. New Phytol. 98, 627–646.

Santore, U.J., 1987. A cytological survey of the genus *Chroomonas*—with comments on the taxonomy of this natural group of the Cryptophyceae. Arch. Protistenkd. 134, 83–114.

Santore, U.J., Greenwood, A.D., 1977. The mitochondrial complex in Cryptophyceae. Arch. Mikrobiol. 112, 207–218.
Sarnelle, O., 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. Ecol. Monogr. 63, 129–149.
Schnepf, E., Elbrächter, M., 1992. Nutritional strategies in dinoflagellates: a review with emphasis on cell biological aspects. Eur. J. Protistol. 28, 3–24.
Schnepf, E., Melkonian, M., 1990. Bacteriophage-like particles in endocytic bacteria of *Cryptomonas* (Cryptophyceae). Phycologia 29, 338–343.
Schnepf, E., Winter, S., Mollenhauer, D., 1989. *Gymnodinium aeruginosum* (Dinophyta): a blue-green dinoflagellate with a vestigial, anucleate, cryptophycean endosymbiont. Plant Syst. Evol. 164, 75–91.
Schuster, F.L., 1968. The gullet and trichocysts of *Cyathomonas truncata*. Exp. Cell Res. 49, 277–284.
Schuster, F.L., 1970. The trichocysts of *Chilomonas paramecium*. J. Protozool. 17, 521–526.
Sespenwol, S., 1973. Leucoplast of the cryptomonad *Chilomonas paramecium*; evidence for the presence of a true plastid in a colorless flagellate. Exp. Cell Res. 76, 395–409.
Skovgaard, A., 1998. Role of chloroplast retention in a marine dinoflagellate. Aquat. Microb. Ecol. 15, 293–301.
Skuja, H., 1939. Beitrag zur Algenflora Lettlands. II. Acta Horti. Bot. Univ. Latviensis 11/12, 41–168.
Skuja, J., 1948. Taxonomie des Phytoplanktons einiger Seen in Uppland. Schweden. Symb. Bot. Upsaliensis 9, 1–399.
Stemberger, R.S., Gilbert, J.J., 1985. Body size, food concentration, and population growth in planktonic rotifers. Ecology 66, 1151–1159.
Stoecker, D.K., Silver, M.W., 1990. Replacement and aging of chloroplasts in *Strombidium capitatum* (Ciliophora: Oligotrichida). Mar. Biol. (Berlin) 107, 491–502.
Stoecker, D.K., Michaels, A.E., Davis, L.H., 1987. Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature 3216, 790–792.
Stoecker, D.K., Silver, M.W., Michaels, A.E., Davis, L.H., 1988/1989. Enslavement of algal chloroplasts by four *Strombidium* spp. (Ciliophora, Oligotrichida). Mar. Microb. Food Webs 3, 79–100.
Tanifuji, G., Onodera, N.T., Wheeler, T.J., Dlutek, M., Donaher, N., Archibald, J.M., 2011. Complete nucleomorph genome sequence of the non-photosynthetic alga *Cryptomonas paramecium* reveals a core nucleomorph gene set. Genome Biol. Evol. 3, 44–54.
Taylor, W.D., Hern, S.C., William, L.R., Lambou, V.W., Morris, M.K., Morris, F.A., 1979. Phytoplankton Water Quality Relationships in U.S. Lakes. Part 6. The Common Phytoplankton Genera from Eastern and Southeastern Lakes. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Working Paper No. 710.
Tranvik, L.J., Porter, K.G., Sieburth, J.M., 1989. Occurrence of bacterivory in *Cryptomonas,* a common freshwater phytoplankter. Oecologia 78, 473–476.
Urabe, J., Gurung, T.B., Yoshida, T., et al., 2000. Diel changes in phagotrophy by *Cryptomonas* in Lake Biwa. Limnol. Oceanogr. 45, 1558–1563.
Vørs, N., 1992a. Heterotrophic amoebae, flagellates and Heliozoa from the Tvarminne area, Gulf of Finland, in 1988–1990. Ophelia 36, 1–109.
Vørs, N., 1992b. Ultrastructure and autoecology of the marine, heterotrophic flagellate *Leucocryptos marina* (Braarud) Butcher 1967 (Katablepharidaceae/Kathablepharidae), with a discussion of the genera *Leucocryptos* and *Kathablepharis/Kathablepharis*. Eur. J. Protistol. 28, 369–389.
Watanabe, M., Furuya, M., 1982a. Phototactic behaviour of cells of *Cryptomonas* sp. in response to continuous and intermittent light stimuli. Photochem. Photobiol. 35, 559–563.
Watanabe, M., Furuya, M., 1982b. Effects of viscosity on phototactic movement and period of cell rotation in *Cryptomonas* sp.. Physiol. Plant J. 56, 194–198.
Watanabe, M., Miyoshi, M., Furuya, M., 1976. Phototaxis in *Cryptomonas* sp. under conditions suppressing photosynthesis. Plant Cell Physiol. 17, 683–690.
Wetherbee, R., Hill, D.R.A., McFadden, I., 1986. Periplast structure of the cryptomonad flagellate *Hemiselmis brunnescens*. Protoplasma 131, 11–22.
Wetherbee, R., Hill, D.R.A., Brett, S.J., 1987. The structure of the periplast components and their association with the plasma membrane in a cryptomonad flagellate. Can. J. Bot. 65, 1019–1026.