A REVIEW OF GROUP FILIATION OF STRAMENOPILES, ADDITIONAL APPROACHES TO THE QUESTION

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ABSTRACT: Increased support has accrued for recognition of the major grouping, stramenopiles. The "Stramenopila" include: chromophytous algal groups such as Chrysophyceae, Eustigmatophyceae, Bacillariophyceae, Xanthophyceae, Raphidophyceae, and Phaeophyceae; the pseudofungal groups, Hyphochytriomycetes and Oomycetes; and the protozoan-like Bicosoecids and Labyrinthulids. Understanding of the underlying unity of stramenopiles has not been paralleled by equal understanding of the order of evolution of member groups. Ultrastructural and molecular data bases have led to different filiation postulates. We compared postulates based on ultrastructure (morphology) with those from molecular sequence analyses. Attempting resolution of often contradictory outcomes, we sought information from additional sources, i.e., paleontology and ontogeny. Our conclusions as to the paleontological history of stramenopiles, though group-limited, are underpinned by fossil evidence. Our ontogenetic approach, though more theoretical, offers a measure of methodological innovation. Assessments of phylogeny of stramenopiles based on these two latter approaches are not in conflict, and are more consistent with inferences from morphological than molecular data. Though stramenopiles are probably monophyletic, a deep division into chromophyte-algal and pseudofungal lineages is supported by a synthesis of evidence. Primitive heterotrophs, e.g., the often phagotrophic, aloricate Bicosoecids, appear basal to both chromophyte and pseudofungal lineages.

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INTRODUCTION

Recognition of the stramenopiles (Patterson, 1989), initially an informal grouping partially equivalent to the kingdom Chromista (Cavalier-Smith, 1986, 1989), represented an important advance in our understanding of a significant segment of protistal organisms. Many stramenopiles were awkwardly assigned to other kingdoms (e.g., Plantae, Protista, Chromista) prior to general acceptance of this group. The stramenopiles are a diverse but apparently phylogenetic grouping (cf. Leadbeater, 1989; Leipe et al., 1994, 1996), clearly defined by a particular morphological marker (discussed below). This large clade (stramenopiles, or more formally, Stramenopila) has been suggested for formal kingdom status (cf. Blackwell and Powell, 1995, 1999; Campbell et al., 1999).

Stramenopiles are defined as "tubulocristate protists with tripartite flagellar hairs or those derived from such organisms" (Leipe et al., 1994). The composite flagellar hairs (composite mastigonemes, also called "retronemes" in reference to a reverse-thrusting effect on the forwardly directed "tinsel" flagellum) are considered to be lineage defining (cf. Cavalier-Smith, 1986, 1989; Leadbeater, 1989; Round, 1989; Corliss, 1998). In addition to these stiff, glycoproteinaceous, usually three-parted, tubular, flagellar hairs (cf. Andersen et al., 1991; Graham and Wilcox, 2000), other features often shared by (but not necessarily exclusive to) stramenopiles include: subapical or lateral, heterokont flagella (the more anterior of the two flagella, or in some cases the only flagellum present, usually bears the tubular mastigonemes); cell walls with cellulose (and sometimes silica); beta-1,3 glucan food reserves; and, among photosynthetic members, chloroplasts with a ring-shaped genophore (cf. Van den Hoek et al., 1995), chlorophyll c (of several forms, cf. Jeffrey, 1989), chlorophyll a but not b, characteristic carotenoid pigments (Bjornland and Liaaen-Jensen, 1989), three-thylakoid lamellae, girdle lamellae, and four surrounding or boundary membranes (complex plastids)--in addition to the two membranes of the chloroplast envelope, there are two additional (outer) membranes, the "chloroplast endoplasmic reticulum" (the outermost membrane usually bears ribosomes and is often connected to the outer nuclear membrane), cf. Gibbs (1981), Cavalier-Smith (1986), Blackwell and Powell (1995). The presence of two extra (outer) chloroplast-encircling membranes suggests that photosynthetic members (the chromophytous algal lineage) are resultant from a secondary, probably eukaryote/eukaryote, endosymbiosis (Sitte, 1993; Sitte and Eschbach, 1992).

The stramenopiles include a large assemblage of "chromophytous algae" (chromophytes); chromophytous algae are referred to variously as: "Ochrista" (Cavalier-Smith, 1986, 1989); autotrophic stramenopiles or

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"stramenochromes" (Leipe et al., 1994); "heterokontophytes" (Van den Hoek et al., 1995); "Ochrophyta" (Cavalier-Smith, 1997); or "ochrophytes" (Graham and Wilcox, 2000). Groups of chromophytes now recognized are: Chrysophyceae (golden algae), Synurophyceae (if separate from chrysophytes, cf. Andersen, 1987; Lavau et al., 1997)), Pedinellophyceae (if separate from chrysophytes, cf. Guillou, 1999), Eustigmatophyceae, Dictyochophyceae (silicoflagellates), Pelagophyceae, Bolidophyceae, Bacillariophyceae (diatoms), Xanthophyceae or Tribophyceae (yellow-green algae), Raphidophyceae (chloromonads), Phaeothamniophyceae, and Phaeophyceae (brown algae). Also included in stramenopiles are the "pseudofungi" (formerly placed in the true Fungi, cf. Alexopoulos, 1962), i.e., the Oomycetes (a diverse group in their own right, including the water molds, Saprolegniaceae) and the Hyphochytriomycetes (a superficially chytrid-like group; these are not, however, closely related to true chytrids, Chytridiomycetes, which belong to the Fungi). Additionally, the protozoan-like labyrinthulids (including thraustochytrids), previously placed with Fungi (cf. Alexopoulos, 1962), and bicosoecids (considered, debatably, as colorless chrysophytes by some authors, e.g., Moestrup, 1995) are both now accepted as well in the overall stramenopile lineage. Certain other protozoal groups, e.g., proteromonads and opalines (opalinids), though lacking composite/tubular flagellar hairs, are possibly related to the stramenopile lineage (cf. Delvinquier and Patterson, 1993; Hausmann and Hulsmann, 1996; Leipe et al., 1996; Silberman et al., 1996; Guillou et al., 1999); however, other interpretations concerning proteromonands and opalines are counter to this conclusion (cf. Lipscomb, 1991). Cryptophytes (cryptomonads)--with bipartite tubular mastigonemes, and in some cases a second (residual or vestigial) nucleus, the "nucleomorph" (cf. Bold and Wynne, 1985; Cavalier-Smith, 1986; McFadden, 1998)--are considered to be affiliated with the stramenopile lineage by some authors (e.g., Cavalier-Smith, 1989, 1997), but not others (e.g., Van de Peer and De Wachter, 1997). Haptophytes (prymnesiophytes) are no longer thought to be closely related to stramenopiles (Daugbjerg and Andersen, 1997b).

The Stramenopila correspond generally to the phylum Heterokonta of Cavalier-Smith's kingdom Chromista (cf. Cavalier-Smith, 1989). Regardless of absolute circumscription, recognition of the stramenopiles (and the Chromista, for that matter) was instrumental in dismantling the large, and largely unnatural, kingdom Protista (cf. Whittaker, 1969; Corliss, 1984), also known as Protoctista (cf. Margulis, 1981). The heterogeneous membership of Protista may now be more precisely apportioned among other kingdoms or candidate kingdoms, i.e., the Stramenopila, the Protozoa, the Fungi, the Plantae, the Animalia, or even the Biliphyta (Blackwell and Powell, 1995, 1999). It seems reasonable to suggest that formal use of the kingdom Protista is now becoming a matter of historical interest, although various general biology texts still employ this kingdom (e.g., Johnson, 2000). The term "protist," however, will doubtless continue as a useful general reference term for one-celled eukaryotes, whether these be related or not.

A pertinent question here is, to which other kingdom is the candidate kingdom Stramenopila related? The answer is, probably more than one. A plastid genome connection has been suggested between chromophytous and biliphytous algae (Delaney et al., 1995; Wee et al., 1996; Bhattacharya, 1998). However, the clearest relationships of stramenopiles are with the Protozoa (cf., Round, 1989; Patterson, 1989; Cavalier-Smith, 1993; Corliss, 1998); "Protozoa" as an entity is admittedly hard to define (Sleigh, 1991a; Blackwell and Powell, in press); yet, Protozoa are nonetheless often presently recognized as a kingdom (cf. Blackwell and Powell, in press). Historically, chrysophytes, particularly amoeboid and monadal members, were often classified as Protozoa (e.g., Hall, 1953); chrysamoebas, now considered to be paraphyletic or polyphyletic (O'Kelley and Wujek, 1995), nonetheless exhibit both chromophytous and rhizopodal features. Round (1986) alluded to the "protozoal" character of certain chrysophycean chromophytes; also, the pedinellids, traditionally placed with Protozoa, are established as having chromophytous (Patterson, 1986), possibly chrysophycean (Moestrup, 1995), affinities. Recently, in molecular genetic analyses, the alveolate protozoa (ciliates, dinoflagellates, and apicomplexans) were shown to be a probable sister group of the stramenopiles (Bhattacharya and Medlin, 1995; Van de Peer and De Wachter, 1997); Cavalier-Smith (1999) substantiated this relationship on ultrastructural grounds as well. Developayella (Tong, 1995) possibly shares characters of stramenopiles (such as Oomycetes) and alveolates. As discussed, opalines and proteromonads, usually treated as protozoan groups, have a possible connection to stramenopiles (Hausmann and Hulsmann, 1996). The choanoflagellates, with relationships to (other) protozoa and to animals (Zrzavy et al., 1998), and possibly to fungi as well (Vickerman, 1998), were traditionally treated as a group of chrysophytes (the Craspedomonadales), cf. Bold and Wynne, 1978; this latter relationship, however, has been called into serious question (Hibberd, 1986). Regardless, the most promising avenues for exploring relationships of members of the stramenopiles with other kingdoms are with members of the Protozoa.

We turn our attention now to relationships within the stramenopiles. We are asking here two related questions: Which major groups of stramenopiles are most closely related? And, what was the order of evolution (filiation) of stramenopile groups? To answer, we consider three sets of information:

(1) The first category of information (itself two-parted) comes from comparison of contemporary (a) phenotypes and (b) genotypes--these, separately or together, being the usual types of evidence cited in literature on the systematics of stramenopiles. Phenotypic comparison includes morphological, structural, ultrastructural, and certain biochemical information (e.g., pigment systems). Genotypic comparison is based primarily on ribosomal RNA data, although other types of molecular evidence have been utilized. In either phenotypic or genotypic comparisons, we are putatively considering a comparable stage in the life cycle of the organisms being compared, not the entire life cycle.

(2) The second category of information is provided by the fossil record. We are hindered in this endeavor by the differential preservation of groups of stramenopiles, and are restricted mainly to those groups (such as chrysophytes, diatoms, and silicoflagellates) which have left a substantial fossil record. Though with a more debatable fossil history, Oomycetes may also be included in this consideration. Although we are group-limited in a paleontological approach, the groups which are well-represented in the fossil record also happen to be pivotal in deciphering stramenopile filiation.

(3) Additionally, we wish to determine if ontogeny, specifically, the development and life cycles of particular stramenopilous organisms, can shed light on the filiation of stramenopile groups. Such an ontogenetic approach has been, at most, sparingly used in the attempt to gain insight into stramenopile evolution; yet, such an approach possibly holds clues critical to phylogenetic understanding.

In these three approaches to understanding stramenopile group evolution, we are broaching the three basic methods for determining character polarity (de Queiroz, 1985), viz. comparative (as in out-group comparison), paleontological, and ontogenetic. These approaches correspond as well to Agassiz's (1857) three-fold parallelism in classification (cf. Gould, 1977; Nelson, 1978). The last two approaches, paleontologic and ontogenetic, are often overlooked in systematic and evolutionary investigations on stramenopiles. Some (e.g., Bryant, 1997) have felt that paleontologic and ontogenetic approaches to polarity determination should not be mixed in an analysis with comparative approaches (e.g., outgroup comparison methodology). However, it is our intention simply to assess what information to date, from each approach, may tell us about stramenopile filiation, and then determine the common ground among, and differences between, these information bases. Recent support for combined approach input may be taken from Xiao et al. (1998), who examined developmental stages in certain microscopic fossils to enhance systematic understanding.

APPROACHES TO DETERMINING STRAMENOPILE FILIATION

APPROACH 1. Comparative (a. morphological, b. molecular): In this approach we are, ideally, considering data which characterize comparable stages ("semaphoronts," in terminology of the cladist) in the life cycle of different organisms. In examining (different) organisms at a particular stage, at a specific point in time, we are in a sense just viewing comparative "snapshots" of the organisms--what some (e.g., de Queiroz, 1985) have termed "instantaneous morphologies" (or "instantaneous physiologies," as the case may be). While theoretically sound, this concept (of comparability) can be difficult to apply; the adult stage of one organism, a chrysophyte monad for example, may not be equivalent to the adult stage of another, e.g., an oomycete mycelium; rather, the greater comparability in this particular example is between the monad and an oomycete zoospore; and, as we discuss later, the type of oomycete zoospore can make a difference in the results. In fact, differential application of the basic theoretical notion of "equivalence" is to be found in the literature, perhaps more commonly so in molecular than in morphological and ultrastructural investigations.

1a. Morphology/Ultrastructure. A number of workers (e.g., Andersen, 1991; Barr, 1983; Barr and Allan, 1985; Barr and Desaulniers, 1989; Beakes, 1989; Belcher and Swale, 1972a,b; Bortnick et al., 1985; Cavalier-Smith, 1986, 1989; Clarke and Pennick, 1975; Cooney et al., 1985; Gotelli, 1974; Henry and Cole, 1982; Hibberd, 1971, 1979, 1986; Hibberd and Leedale, 1970, 1971, 1972; Ho et al., 1968; Leadbeater, 1972, 1990; Lunney and Bland, 1976; O'Kelly, 1989; Owen et al., 1990; Preisig, 1989; Randolph and Powell, 1992) have investigated the morphology and fine structure of chromophytous algae and/or pseudofungi, particularly zoospore

(or motile cell) ultrastructure. Phytochemical markers, such as pigment systems, are occasionally addressed in such studies as well (more extensively so in studies devoted specifically to considerations of pigments and systematics, e.g. Bjornland and Liaaen-Jensen, 1989). The predominantly ultrastructural studies of the late 1960's to the early 1990's provided a mainstay of chromistan research. These investigations served not only to clarify zoospore morphology in groups of stramenopiles, and establish the fundamental unity of the stramenopiles, but also to reveal relationships among chromophytous algae, between chromophytous algae and pseudofungi, and between the groups pseudofungi (Oomycetes and Hyphochytriomycetes, combined in Cavalier-Smith's "Pythiistea," 1989). Noteworthy in these assessments are aspects of the flagella (the usual reference, ultrastructurally, being to the tinsel flagellum) and the flagellar apparatus of the zoospores. The flagellar transition region (from flagellum to basal body), cf. Hibberd (1979), Barr and Desaulniers (1989), Preisig (1989); and the flagellar roots, cf. Andersen (1991) and Owen et al. (1990), have provided insight into the systematics of stramenopiles. The flagellar transition zone has proved useful in deciphering possible relationships of particular stramenopile groups (cf. Preisig, 1989; Cavalier-Smith, 1989). Study of microtubular flagellar roots helped establish homology between Oomycetes (Saprolegnia) and types of chromophytous algae, e.g., Dinobryon (Owen et al., 1990). Variation in the R3 microtubular rootlet has been suggested as phylogenetically meaningful among ochrophytes (cf. Graham and Wilcox, 2000). Likewise, the fibrous root (rhizoplast) is potentially instructive of relationships among chromophytous algal groups, e.g., Chrysophyceae and Synurophyceae (Andersen, 1991). Fibrous and microtubular flagellar roots are absent in bacillariophytes (cf. Anderson, 1991; Manton and von Stosch, 1966); the two central tubules of the microtubular core (axoneme) of the flagellar shaft, present in most flagellated protists, are also missing in diatoms (Bold and Wynne, 1985), and the basal body has microtubular doublets rather than triplets (cf. Van den Hoek et al., 1995). The diminished flagellar morphology in bacillariophytes perhaps corresponds as well to a general flagellar reduction--the sperm of a few centric taxa of diatoms being the only motile cells occurring in the group, and these with but a single flagellum; diatom ancestors were presumably biflagellate (cf. Bold and Wynne, 1985; Guillou et al., 1999), but structural evidence for a second flagellum in diatoms is apparently lacking (Bold and Wynne, 1985; Van den Hoek, et al., 1995). Among other features, reduction (or lack) of flagellar rootlet structure in dictyochophytes (silicoflagellates) and pedinellophytes led Andersen (1991) to speculate on the possible alignment of these particular groups.

The precise connection between the generally autotrophic chromophytous algae and the osmotrophic pseudofungi is still uncertain. An alga once considered a possible pseudofungal progenitor, but now viewed as an interesting sidelight to the filiation question, is Vaucheria. A robust, coenocytic, unusual member of the Xanthophyceae, Vaucheria is similar in appearance to some Oomycetes, e.g., Achlya , Saprolegnia (Saprolegniaceae). Overall morphology, a closed mitosis, and the nuclear cycle in general (diplont life cycle), are among characteristics apparently "shared" by Vaucheria and saprolegniaceous Oomycetes (cf. Ott and Brown, 1972; Beakes, 1987, 1989; Round, 1989; Elliot, 1994; Van den Hoek et al., 1995). Hence, Vaucheria, formerly (e.g., Smith, 1955), and even more recently (e.g., Lee, 1989), has been considered a possible direct ancestor of Oomycetes. However, Vaucheria has more commonly become regarded as an anomalous xanthophyte, with genetically aberrant plastids (Von Berg and Kowallik, 1992)--not in a direct lineage to Oomycetes (Beakes, 1989), or perhaps to anything else. Plastid movement during cytoplasmic streaming in Vaucheria, thought to be somewhat independent of movements of nuclei and other organelles (Lee, 1989), raised suspicion that Vaucheria evolved (perhaps directly) from Oomycetes (by acquisition, somehow, of xanthophyte plastids), rather than being ancestral to Oomycetes. Ott and Brown (1974) had previously observed a similar "microfilament" (microtubular) system in Vaucheria and certain Oomycetes, e.g., Saprolegnia. The synzoosporous condition in Vaucheria might appear related to, perhaps derived from, a phenomenon such as the immediate clustering of primary zoospores (cysts) in a saprolegniaceous form such as Achlya (cf. Sparrow, 1960; Powell and Blackwell, 1998). However, a recent summary of biochemical and molecular genetic information (Bailey and Andersen, 1998) indicated that Vaucheria and the Oomycetes are not especially closely related; similarities are probably due, as suggested by Beakes (1989), to parallel evolution. Also, the overall complexity and specializations (oogamy, synzoospory, extensive siphonous thallus development) and genetic uniqueness of Vaucheria (Von Berg and Kowailik, 1992), suggest that it is not ancestral to the rest of the Xanthophyceae. Vaucheria, a derived and/or early divergent form, is apparently rather distantly connected to other xanthophyte lineages (Bailey and Andersen, 1998). Thus, while the exact systematic position of Vaucheria remains puzzling; it is no longer thought to have arisen from, or given rise to, Oomycetes; nor, is it considered to be more than generally related to other extant xanthophytes.

Opinion, based on morphological and ultrastructural studies, as to the general direction of evolution of stramenopiles, has been on the whole consistent; this being, that some chromophytous algal monad (i.e., unicellular, photosynthetic, heterokont flagellate) was the ancestor of either an oomycete and/or hyphochytrid motile cell (cf. Barr, 1983). Thus, primitive chromophytous algae have been considered basal to pseudofungi (cf. Cavalier-Smith, 1989, 1997). Such a scenario, however, mandates loss of the plastid in the filiation--a difficult proposition if, as is usually the case in chromophytous algae, the outer membrane of the chloroplast endoplasmic reticulum and the outer nuclear membrane have developed a structural and functional "connection" (cf. Cavalier-Smith, 1986; Lee, 1989); a chloroplast loss could potentially endanger the nucleus as well, even if the "connection" is only that of former gene transfer, and protein and lipid targeting (cf. Cavalier-Smith, 1999; McFadden, 1999). This is not to suggest that chloroplast reductions have not occurred among chromistans. A reduction from chloroplast to leucoplast seems clearly to have been involved in origins of colorless (apochlorotic) chrysophytes, such as Anthophysa (Belcher, 1972a). Also, Cavalier-Smith et al. (1995) offered evidence that non-photosynthetic pedinellids are derived, by reduction, from photosynthetic ancestors.

Suggested as the chromophyte algal ancestor of pseudofungi (and other chromophytous algae) have been: primitive chrysophytes (Barr, 1983); eustigmatophytes (Beakes, 1989); xanthophytes (Beakes, 1989; Cavalier-Smith, 1989); and phaeophytes (Andersen, 1991). The Phaeophyceae are a cytologically consistent, multicellular, marine, often reproductively complex, probably derived group (Henry and Cole, 1982; Bold and Wynne, 1985; Clayton, 1989; O'Kelly, 1989; Graham and Wilcox, 2000), doubtfully ancestrally linked to the pseudofungal lineage--though some parallelisms occur, as we later discuss. The Xanthophyceae (Tribophyceae, cf. Ott, 1982; Sze, 1998) are also relatively cytologically uniform (cf. Hibberd and Leedale, 1971); a number are unicellular, but there is a trend toward filamentous or multinucleate (siphonous) development (cf. Round, 1986; Lee, 1989; Bold and Wynne, 1978, 1985); cell-walls are often specialized, sometimes bipartite--the half-walls (involving two adjacent cells) overlapping (cf. Hibberd and Leedale, 1972; Lee, 1999); xanthophytes are more common in terrestrial or semi-aquatic habitats than other chromophytous algal groups (cf. Ott, 1982; Van den Hoek et al., 1995). Both phaeophyte and xanthophyte lineages, along with raphidophytes, show the advancement trend of multiple discoid plastids per cell (cf. Bold and Wynne, 1978; Van den Hoek et al., 1995). Thus, neither xanthophytes nor phaeophytes are the best candidate-groups to furnish primitive chromophyte examples. As mentioned, some focus (e.g., Beakes, 1989) has been given to members of the Eustigmatophyceae as possibly representative of primitive chromophytous algae. The eustigmatophyte plastid lacks girdle lamellae; the chloroplast endoplasmic reticulum (outer membrane) and (outer) nuclear membrane are not connected (Hibberd and Leedale, 1970, 1972), except in small azoosporic members (Santos and Leedale, 1995); the eyespot (stigma) is not within the plastid (cf. Hibberd, 1990); the eyespot-associated flagellar swelling is on the anterior (usually only) flagellum, not on the posterior flagellum as in a number of other chromophyte algae (cf. Lee, 1999); and the R3 flagellar rootlet is lacking (Graham and Wilcox, 2000). If eustigmatophytes are viewed as basal among chromophytous algae, difficult reversals to achieve these "primitive" traits would not be required in a filiation sequence (compare vs. Leipe et al., 1994). Also, in a hypothetical derivation of motile cells of pseudofungi, plastid loss from a eustigmatophyte motile cell would not be especially likely to cause nuclear impairment (if nuclear and plastid membranes are not connected). However, eustigmatophytes are but one of several potentially primitive chromophytous algal groups. Allegedly primitive chrysophytes, such as Ochromonas, have also been suggested as possibly representative of basal autotrophic stramenopiles (e.g., Barr, 1983; Hibberd, 1986; Van den Hoek et al., 1995); the potential protozoan connections of certain chrysophytes (Round, 1986) give credence to this suggestion. Structurally, chrysomonads have been loosely linked to the primitive protozoal stramenopilous group, the bicosoecids (Dyer, 1990). Some bicosoecids are loricate, as are some chrysophytes. Meyer (1986) placed rhizopodal, loricate chrysomads in one presumably related family of the Chrysophyceae. Whether loricate chrysophytes and loricate bicosoecids are particularly related remains to be demonstrated.

Within pseudofungi, Barr and Allan (1985) and Cooney et al. (1985) established the similarity of the (ring-like) flagellar transitional region between Hyphochytriomycetes and Oomycetes. Barr and Allan (1985) considered Hyphochytriomycetes to be related and apparently basal to Oomycetes. Beakes (1987) noted a similar relationship, and also observed that the reduction in flagellar apparatus (rootlet structure) in hyphochytrids, relative to Oomycetes, is commensurate with the almost complete reduction of the smooth flagellum. Fuller (1990), however, questioned the precise relationships of hyphochytrids. Barr and Allan (1985), based on a limited similarity of the flagellar apparatus, including the obconic ("bell-shaped") yet ring-like transition zone,

considered Thraustochytrium (a labyrinthulid, cf. Porter, 1990) at least distantly related to the Oomycetes/Hyphochytriomycetes. Using organelle markers (such as K-bodies), Randolph and Powell (1992) confirmed the zoospore ultrastructural relationship between some members of the orders Leptomitales and Saprolegniales of the Oomycetes. The Leptomitales are separated from the Saprolegniales by the perhaps superficial character of hyphal contrictions (cf. Alexopoulos, 1962). The order Lagenidiales (Oomycetes) is probably artificially held together by the feature of a substantially reduced thallus, related to endoparasitic habit (e.g., Lagenidium, Myzocytium, Olpidiopsis). Based on both traditional morphology (Sparrow, 1973) and ultrastructure (Powell and Blackwell, 1990), the Lagenidiales probably constitute a heterogeneous assemblage. Olpidiopsis, traditionally placed in the Lagenidiales, has a helical flagellar transition zone structure (Bortnick et al., 1985), resembling that of chromophytous algae rather than Oomycetes; Cavalier-Smith (1989) considered the flagellar transition zone of Olpidiopsis suggestive of a link between chromophytous algae and pseudofungi. Preisig (1989) discussed the flagellar transition region of chromophytous algae, i.e., usually possessing a transitional helix structure, as indicative of a general relationship among chromophytes; however, phaeophytes, raphidophytes, bacillariophytes, bolidophytes, dictyochophytes, and pedinellids lack a transitional helix (cf. Preisig 1989; Van den Hoek et al., 1995; Guillou et al., 1999; Graham and Wilcox, 2000); we note, though, that the latter four of this list have reduced flagellar apparatus (cf. also Saunders et al, 1995). Oomycetes usually possess a similar, and probably related, but more complex flagellar transition region, characterized by a "concertina," i.e., concentric rings with struts (Barr and Desaulniers, 1989; Cavalier-Smith, 1989; Preisig, 1989). The "concertina" of Oomycetes has more recently been referred to as a "double ciliary transition helix" by Cavalier-Smith (1997), relating it to the simpler (single) helix of most chromophytous algae. The transitional zone of hyphochytrids is similar (ring-like) to Oomycetes but less complex, and usually with fewer rings (Cooney et al., 1985; Cavalier-Smith, 1989; Preisig, 1989). Providing an evolutionary interpretation to information on the flagellar transition zone structure, Cavalier-Smith (1989, 1997) considered it more plausible that the complex concentric ring structure of Oomycetes was ultimately derived from the simple helix of chromophytous algae, than the reverse proposition; hence, a polarity of this character and a phylogenetic direction are suggested.

Ultrastructural evidence thus (cf. Barr, 1983; Barr and Desaulniers, 1989; Cavalier-Smith, 1986, 1989; Preisig, 1989), based mainly on parts of the flagellar apparatus (e.g., the flagellar transition zone), has been generally indicative of an evolutionary sequence leading from a simple chromophytous monad to a hyphochytrid motile cell or an oomycete zoospore (i.e., to pseudofungi, cf. Fig. 1, after Cavalier-Smith, 1989). Morphological/ultrastructural evidence has led to the inference that, among chromophytous algal groups, the golden algae, i.e., chrysophytes (particularly certain monadal types, e.g., Ochromonas), are basal to presumed derived groups, such as diatoms (bacillariophytes), brown algae (phaeophytes), and perhaps yellow-green algae (xanthophytes), cf. Fig. 1 and also Bold and Wynne (1985), Cavalier-Smith (1989), O'Kelly (1989), Moestrup (1995), Preisig (1995). Both monadal chrysophytes (e.g., Ochromonas) and the eustimatophytes are plausible as groups relatively primitive among chromophytous algae (cf. Barr, 1983; Hibberd, 1986; Beakes, 1989).

1b. Molecular Approach. This approach has ascended to dominance since approximately 1990. Most of these data have come from sequencing of ribosomal RNA genes (both large and small subunits). Small subunit ribosomal RNA (SSU rRNA), in particular, has been considered to have value in phylogenetic reconstructions, especially in establishing branching orders of taxa or groups (Sogin et al., 1993; Lavau et al., 1997). The potential value of such molecular data in determining filiation renders such information vital to the questions we ask in this paper. However, the infallibility of SSU rRNA data in deciphering phylogenies has been seriously challenged (Marshall, 1997; Maley and Marshall, 1998; Phillippe and Adoutte, 1998); to cite one type of problem, group alignment may be significantly affected by the particular sample species selected (Maley and Marshall, 1998). However, in spite of some possible problems, we do not wish to downplay the great potential importance of molecular investigations to the understanding of systematics, evolution, and phylogeny--of stramenopiles, or of any group for that matter. Molecular data are clearly as vital to phylogenetic considerations as any other type of systematic information.

The earlier of the molecular investigations on stramenopiles appeared confirmational of ultrastructural studies. Based on ribosomal RNA data, support was found for relationships among chromophytous algae, and of these with oomycetous "fungi" (cf. Gunderson et al., 1987; Perasso et al.; 1989; and Ariztia et al., 1991). Although evolutionary branching orders were not necessarily clarified, further insight was gained into probable

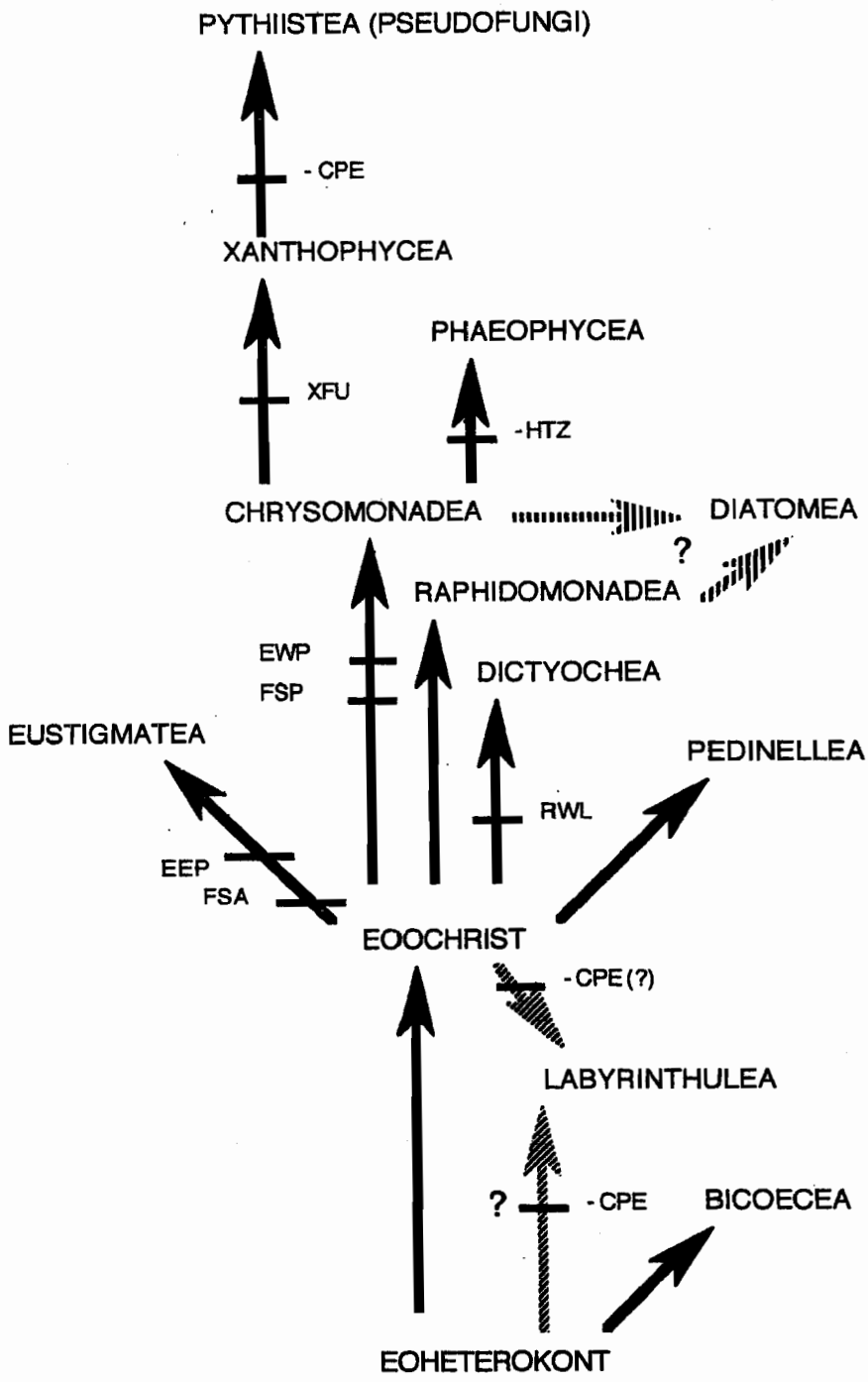

FIGURE 1. Adapted from portion of diagram in Cavalier-Smith (1989). This putative filiation of "phylum" Heterokonta, of kingdom Chromista, is based primarily on aspects of morphology and ultrastructure (some information on pigments also being included). Our abbreviations (see list for Fig. 6) for characters utilized in the diagram by Cavalier-Smith are: -CPE, loss of chloroplast (and periplastidal compartment); EEP, eyespot (stigma) external to plastid; EWP, eyespot within plastid; FSA, swelling on anterior flagellum (associated with eyespot); FSP, swelling on posterior flagellum (associated with eyespot); -HTZ, loss of transitional helix; RWL, reduction or loss of whiplash ("posterior") flagellum; XFU, loss of fucoxanthin.

relationships between particular groups of chromophytous algae, e.g., synurophytes with chrysophytes, xanthophytes with phaeophytes (Ariztia et al., 1991). Bhattacharya et al. (1992), using SSU rRNA data, recognized three lineages within chromophytous algae: the Bacillariophyceae (diatoms), the Phaeophyceae/Xanthophyceae, and the Chrysophyceae/Eustigmatophyceae/Synurophyceae. Andersen et al. (1999), also with SSU rRNA gene analysis, substantiated a close relationship of Chrysophyceae and Synurophyceae (synurophytes being, to an extent, taxonomically distributed among chrysophytes); the Eustigmatophyceae were selected as the outgroup in this analysis (a choice possibly related to findings of Daugbjerg and Andersen, 1997a). Additionally, Andersen et al. (1999) determined Ochromonas to be polyphyletic (within the chrysophyte/synurophyte grouping), O. tuberculatus (given as O. "tuberculata"), for example, being somewhat distant to other Ochromonas species; this finding has implications when Ochromonas, as a generic entity, is inferred as possibly representative of primitive chrysophytes (cf. Hibberd, 1986) or, more broadly, of primitive chromophyte or heterokont algae (cf. Barr, 1983). Daugbjerg and Andersen (1997a), using chloroplast-encoded rbcL sequences, determined the Eustigmatophyceae as the most basal group of chromophytes; however, Graham and Wilcox (2000) pointed out that certain other molecular studies are not in agreement with this assessment of eustigmatophytes (e.g., Cavalier-Smith and Chao, 1996, SSU rRNA gene analysis). Based on morphology and general biochemistry (Gayral and Billard, 1986; O'Kelly, 1989), a possible relationship to phaeophytes had been suggested for chrysomeridalean (multicellular, marine, sometimes laterally flagellate) chrysophytes; molecular data (Saunders et al., 1997) loosely supported this phaeophyte connection for the chrysomeridinean but not the sarcinochrysidinean group of the traditional Chrysomeridales (Sarcinochrysidales of some authors, cf. O'Kelly, 1989). Sarcinochrysidinean algae are related to and perhaps should be included in the pelagophytes (cf. Graham and Wilcox, 2000). Based on limited biochemical evidence (carotenoid distribution), Heywood (1989) speculated on a relationship of the seemingly taxonomically isolated Raphidophyceae with either the Chrysophyceae or Xanthophyceae. Analysis of combined data sets (nucleotide sequences, ultrastructure, pigments; Potter et al., 1997) indicated the Raphidophyceae possibly to be "the sister taxon to the Phaeophyceae-Xanthophyceae clade." Guillou et al. (1999) likewise grouped raphidophytes with phaeophytes and xanthophytes. On the other hand, Cavalier-Smith and Chao (1996) and Graham and Wilcox (2000) placed raphidophytes with the chrysophyte, synurophyte, and eustigmatophyte grouping. However, an unusual structure, the rhizostyle (perhaps differing only in degree from the rhizoplast of certain other ochrophytes), connecting the flagellar basal bodies with the nucleus, and, additionally, visible chromosomal kinetochores during a closed mitosis (cf. Bold and Wynne, 1985; South and Whittick, 1987), to an extent support the distinctiveness of raphidophytes. Honda et al. (1999), using 18S rRNA gene sequence data, indicated a relatively uncertain position for Raphidophyceae--variably related to Eustigmatophyceae, Chrysophyceae, Xanthophyceae, Phaeophyceae, in different trees--among chromophytous algae. The Phaeothamniales, treated traditionally as an order of the Chrysophyceae (see review in Preisig, 1995), was established, by rbcL sequences and other data (Bailey et al., 1998), as a "new" chromophyte class, the Phaeothamniophyceae--with relationships closer to the Xanthophyceae and Phaeophyceae than to the Chrysophyceae. Also utilizing both ultrastructural and molecular information, Guillou et al. (1999) proposed a new class, Bolidophyceae, a biflagellate yet apparent sister group of diatoms; however, the only motile cell found in diatoms, motile sperm (presently known only in certain marine, centric taxa), is uniflagellate; regardless, as we discuss later, the discovery of Bolidomonas is potentially significant to questions of stramenopiles phylogeny. Andersen et al. (1993), based on both ultrastructural and rRNA characteristics, had earlier described a new class of chromophytous algae, the Pelagophyceae. The Pelgaophyceae (uniflagellate, with 2-partite rather than 3-partite mastigonemes) were left in an unresolved position within the general chromophyte grouping by Andersen et al. (1993). Wee et al. (1996), citing SSU rRNA gene sequence evidence, affirmed the Pelagophyceae as a distinct lineage of chromophytes. However, Saunders et al. (1995), based on "phylogenetic analyses of 18S rRNA gene sequence data," determined a relationship of pelagophytes with other chromophyte groups which also exhibit a reduced flagellar apparatus, i.e., diatoms, silicoflagellates (dictyochophytes), and pedinellids (cf. Andersen, 1991). Graham and Wilcox (2000) noted that, in addition to molecular sequence similarities, pelagophytes, silicoflagellates, and pedinellids have in common particular structural features, such as a paraxonemal rod within the mastigonemate flagellum.

Williams (1991) developed a cladogram of "chromista" based on seventy-nine ultrastructural, cytological and biochemical characters; however, in his character breakdown, the scoring of a few characters might be questioned, such as that of character number six, flagellar transition region (indicated as "present" for

Phaeophyta). In William's cladogram, hyphochytrids and Oomycetes (i.e., pseudofungi) are in a basal position relative to other groups (mainly chromophytous algae). Leipe et al. (1994) indicated a branching order of groups of stramenopiles, in a cladogram (derived by parsimony and other cladistic methodology), based on sequence analysis of the 16S-like ribosomal rRNA gene (see Fig. 2, after Leipe et al., 1994). The earliest stramenopile group diverging, from a putative heterotrophic flagellated ancestor with tripartite tubular hairs, was found to be the labyrinthulids, followed by the bicosoecids. Though not including biocosoecids in the analysis, Honda et al. (1999) confirmed (with sequences of the 18S rRNA gene) a relatively basal position among stramenopiles for labyrinthulids (including thraustochytrids). Leipe et al. (1994) considered the Oomycetes and the chromophytous algae ("autotrophic stramenopiles") monophyletic, with Oomycetes having the probable earlier divergence of the two groups. Among chromophytous algae, diatoms were tentatively found, based on the effect of selective exclusion analysis on bootstap value, to have diverged first after Oomycetes (Leipe et al., 1994), evidence perhaps tenuous enough to merit further consideration. Nonetheless, Cavalier-Smith and Chao (1996), based on SSU rRNA gene sequences, also indicated that diatoms diverged earliest in ochristan succession. Chrysophytes, synurophytes and eustigmatophytes were found to be terminal in the tree diagram of Leipe et al. (1994), with the xanthophyte/phaeophyte lineage positioned in divergence in between these and diatoms (Fig. 2). These lineage orders were basically reaffirmed in Leipe et al. (1996), with other groups added in the analysis (pelagophytes and dictyochophytes were placed, respectively, between the phaeophyte/xanthophyte grouping and bacillariophytes). The dendrograms of Leipe et al. (1994, 1996) require (stated or not) difficult reversals in fully reaching the eustigmatophyte line, e.g., loss of girdle lamellae and, more problematic perhaps, loss of the chloroplast endoplasmic reticulum connection to the nuclear membrane; the eyespot (stigma) would also have to "move," somehow, from an intra- to an extra-plastidal position. Further, Leipe et al. (1994) stated that, "A transitional helix-like structure (a distinctly helical structure or a set of rings distal to the transverse partition) was acquired prior to the divergence of the oomycetes." Though ambiguous, this statement can be interpreted to imply (in the total context of Leipe et al., 1994) that the more complex transitional zone of oomycete flagella (double helix, viz. rings with struts) probably developed early in stramenopile evolution (if Oomycetes diverged before chromophytous algae, as Leipe et al. suggested), and either devolved into the simpler transitional helix typical of chromophytes; or, that some Oomycete with (perhaps relictually retaining) a single helix gave rise to the chromophytous algal line. However, in either case the progression would be implausible; diatoms, allegedly arising immediately after Oomycetes (Leipe et al., 1994), exhibit no transitional zone structure (cf. Preisig, 1989; Van den Hoek et al., 1995)--in other words, the improbable sequence of loss of a (double, or perhaps single) helix (progressing from Oomycetes to diatoms), and regain of a (single) helix (progressing from diatoms to the majority of kinds of chromophytous algae), would seem implicit in the construct of Leipe et al. (1994). Also unlikely, lacking a specific phagotrophic mechanism, is that plastid uptake and maintenance (leading to the chromophyte algae) could succeed in a fundamentally osmotrophic (pseudofungal) line, cf. Leipe (1994).

Van de Peer and De Wachter (1997), using similar SSU rRNA data, but employing distance methods for calculating dendrograms, derived relationships among stramenopile groups broadly comparable to that of Leipe et al. (1994). However, there are two rather striking differences in order in Van de Peer and De Wachter (1997): 1) the bicosoecids are positioned basal relative to the labyrinthulids; 2) among chromophytous algae, the xanthophyte/phaeophyte line is terminal, preceded by chrysophytes/eustigmatophytes, and then diatoms--again indicated as diverging first after Oomycetes and Hyphochytriomycetes. Van de Peer and De Wachter (1997), however, made the cogent point that the exact divergence order among heterokont (chromophyte) algae is "dubious." Nonetheless, among stramenopiles, both Leipe et al. (1994, 1996) and Van de Peer and De Wachter (1997) sequenced diatoms immediately after pseudofungi. Medlin et al. (1997), also based on SSU rRNA coding regions, reached a generally similar conclusion regarding the position of diatoms, relating the possible origin of the diplont life cycle of diatoms to the more complicated diplontic life cycle of Oomycetes. Mann and Marchant (1989), however, proposed an evolution of diatoms (and their siliceous frustules) from a diploid cyst stage of parmalean chrysophytes--which possess substantial siliceous plate segments (see also Lee, 1999). Round and Crawford (1981), Bold and Wynne (1985), and Van den Hoek et al. (1995) indicated an origin of diatoms from scale-bearing monads (perhaps chrysophytes or synurophytes). Daugbjerg and Andersen (1997a) found that bacillariophytes (diatoms), based on parsimony analysis of rbcL sequences, formed a sister group with the Chrysophyceae/Synurophyceae, these lineages being basally flanked by silicoflagellates, preceded by the more basal eustimatophytes. However, Guillou et al. (1999) suggested that the Bacillariophyceae, and their "sister

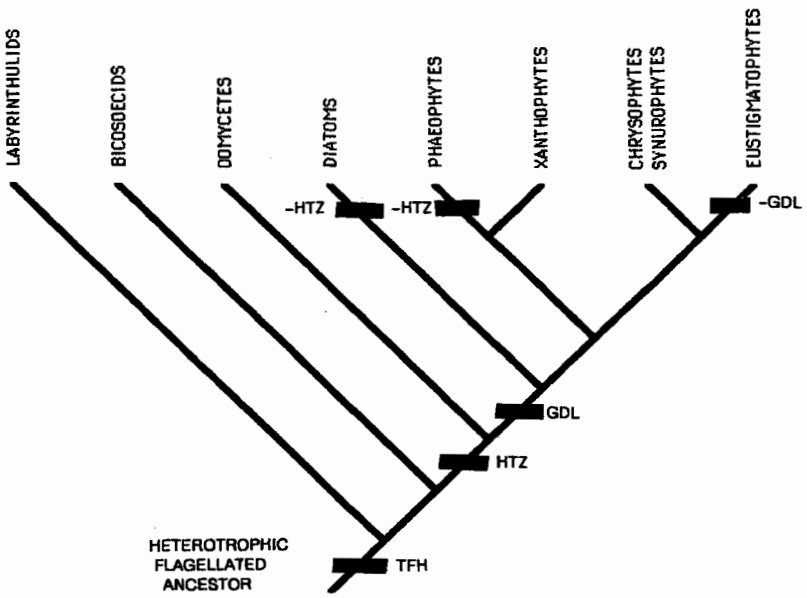

FIGURE 2. Adapted from Leipe et al. (1994). This is part (stramenopile component) of a larger diagram in Leipe et al. which illustrated a putative stramenopile evolutionary sequence in the context of major eukaryote groups (i.e., crown clades). This proposed filiation of stramenopile groups is based on 16S-like ribosomal RNA data; however, certain morphological features were superimposed on the filiation diagram by Leipe et al. (1994), for which we use our own abbreviations (see list for Fig. 6): GDL, chloroplast with girdle lamella; –GDL, loss of girdle lamella of chloroplast; HTZ, helix-like flagellar transition zone; -HTZ,loss of transitional helix; TFH, tubular (usually tripartite) flagellar hairs.

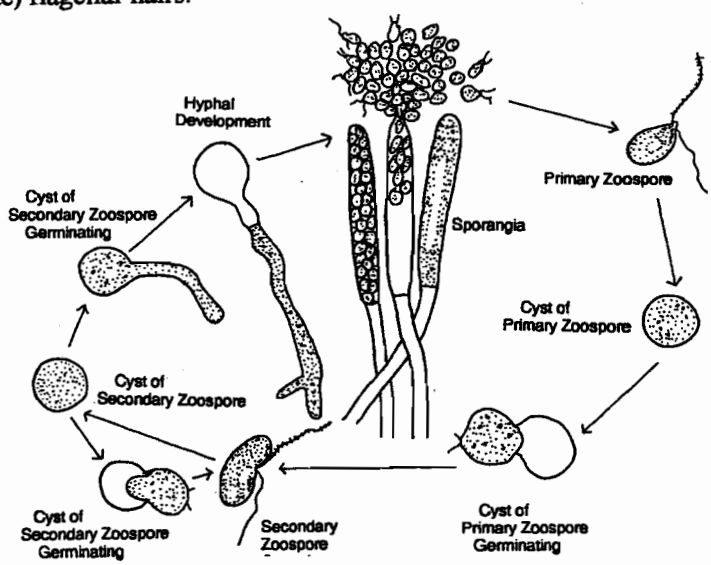

FIGURE 3. Asexual portion of life cycle of Saprolegnia. In this life cycle, primary zoospores (subapically biflagellate) are released from the sporangium. After a brief swimming period, these primary zoospores form primary zoospore cysts. The primary cysts germinate, releasing secondary zoospores (which are laterally biflagellate); after a usually longer swimming interval, these secondary zoospores also encyst. Cysts of secondary zoospores germinate to form either additional secondary zoospores or hyphae; further hyphal development results in the accumulation of mycelium (thallus). The developmental sequence in the life cycle of Saprolegnia, and related Oomycetes, may hold clues to a possible filiation sequence in stramenopiles.

group" the newly described (biflagellate) Bolidophyceae, both arose from biflagellated (heterokont) ancestors. But, Bold and Wynne previously (1985), in discussing possible flagellar reduction, noted the lack of evidence for a second flagellum in the Bacillariophyceae. The question of diatom filiation is thus still open to question. Recent molecular evidence is indicative that certain centric diatoms are more closely related to pennate diatoms than to other centrics (cf. Graham and Wilcox, 2000), further complicating the story of bacillariophyte history.

In discussing some of the above points, Sogin and Silberman (1998) generally concurred with Leipe et al. (1994, 1996) and Van de Peer and De Wachter (1997) that basal stramenopiles were/are heterotrophic. Sogin and Silberman (1998) also appeared to agree with Van de Peer and De Wachter (1997) that stramenopiles and alveolate protozoa are putative sister groups. If, as Sogin and Silberman (1998) suggested, the most basal stramenopiles are heterotrophic; then, as they also intimated, the origin of autotrophy among stramenochromes (autotrophic stramenopiles) is independent of its origin in other groups of organisms (e.g., green algae/green plants or red algae); it is the product of a secondary endosymbiosis (i.e., phagotrophic harvest of an available eukaryotic "algal" cell which already possessed a plastid). The evolutionary radiation of photosynthetic stramenopiles was possible, thus, only after this secondary endosymbiotic event. These statements concerning secondary endosymbiosis and autotrophic stramenopile evolution are essentially in accord with those of Sitte (1993) and Sitte and Eschbach (1992) relating to the origins of complex plastids and "chromophyte algae." This major (secondary) endosymbiotic event in the stramenopile lineage, regardless of the genetics of the plastid (possibly related to red algae, cf. Wee et al., 1996) or the exact period during geologic time in which it occurred, rendered the complex plastids of these "algal" members of the stramenopiles structurally and temporally distinct (set in a different evolutionary path) from plastids of chlorophytes and rhodophytes. By contrast, green algae and red algae do not possess complex plastids (Sitte, 1993), underwent no secondary plastid endosymbiosis, and present a different thylakoid arrangement and pigment combination (cf. Lee, 1989). The secondary endocytosis leading to the plastid of autotrophic stramenopiles is thus lineage demarcating (cf. Sitte, 1993; McFadden, 1999).

Considering comparative approaches (1a, lb), we are nonplused. The morphological (and ultrastructural) approach suggests one order of stramenopile filiation, and the molecular approach another (compare Fig. 1, after Cavalier-Smith, 1989, and Fig. 2, after Leipe et al., 1994). Although both approaches point to labyrinthulids and bicosoecids as basal (with some dispute as to which is the more so), ultrastructural evidence suggests that primitive chromophytous algae are basal to Oomycetes (or to pseudofungi in general), and molecular evidence suggests the reverse. Furthermore, morphological information indicates that, among chromophytous algae, chrysophytes are basal to diatoms; and, again, molecular evidence predominantly suggests the opposite. As pointed out, there are inconsistencies within the molecular interpretations as well, particularly in regard to the sequence of divergence of major chromophytous algal groups (e.g., xanthophytes versus chrysophytes). The general impression may be that either parsimony or distance trees, if based on similar or identical molecular data, will provide similar results (Page and Holmes, 1998). However, if some of the differences in molecular "phylogenies" (gene trees) found in the literature are in fact accounted for by differences in the algorithms used to calculate these dendrograms (e.g., Leipe et al., 1994; compared with Van de Peer and De Wachter, 1997), then this is a problem that clearly should be further addressed. Graham and Wilcox (2000) referred to the "tenuous state of our understanding of ochrophyte evolutionary radiation." About this last point, there can be little doubt. Thus, the remainder of our paper is devoted to additional approaches (paleontological and ontogenetic) which may afford further insight into the determination of the sequence of evolution of chromistan or, more specifically, stramenopile groups. We hope these approaches will illuminate which comparative viewpoint of stramenopile filiation, morphological/ultrastructural or molecular, is more plausible; or, better still, how discrepancies of outcome of these comparative sets of information may be partially or wholly resolved.

APPROACH 2. Paleontology: Given the impasse of interpretation in the two basic comparative approaches discussed (#l a, b), i.e., ultrastructural vs. molecular interpretations, we sought additional information on which to judge the possible order of stramenopile filiation, including evidence from the fossil record. Graham (1993) offered support for paleontological input, in the belief that molecular systematics will not actually replace paleontology; in other words, when fossil evidence is available, it will often be of value in a study. However, Tiffney and Barghoorn (1974), considering Fungi, pointed out that the dearth of a fossil record has been a major reason that evolutionary considerations are often based (mainly or exclusively) on extant forms. But they also caution that "any postulated evolutionary scheme must take fossil.....occurrence into account." The importance

of paleontological information in establishing phylogeny has been more recently reiterated (Marshall and Schultze, 1992; Fox et al., 1999). The possible integration of paleontologic and stratigraphic information with morphological data (Clyde and Fischer, 1997) and with molecular data (Knoll, 1992; Springer, 1995) has been discussed. We agree that the fossil record is important, and have attempted to incorporate evidence available to us from the fossil record on those groups of stramenopiles that present a significant paleontological legacy.

2a. Chromophytous Algae and the Fossil Record. In considering what the fossil record may tell us about stramenopilous (chromophytous) algae, we are, as previously indicated, limited to kinds which have a definitive fossil record--primarily certain chrysophytes (and synurophytes), silicoflagellates (considered by some to be chrysophytes, viz. the Dictyochales of the Chrysophyceae, Bold and Wynne, 1985), and bacillariophytes (diatoms). Other chromophytous algal groups, e.g., raphidophytes, eustigmatophytes, pelagophytes, xanthophytes, and phaeophytes, either lack or have a sparse or debatable fossil record--or one not particularly phylogenetically informative. Tappan (1980) pointed out that compression fossils of brown algae (phaeophytes) are known, but did not elaborate substantially; such macroscopic algal compression fossils are typically of little help in deciphering evolutionary branches (cf. also Graham and Wilcox, 2000, p. 302). A record of a putative Botryococcus-like "xanthophyte" from the Carboniferous (cf. Banks et al., 1967) is questionable, since coccoid algae representative of various algal groups (including chlorophytes) may have a similar appearance, especially as fossils, and no pigment systems remained for analysis. Graham and Wilcox (2000), citing Knoll (1996), reported Vaucheria-like remains ("Paleovaucheria") from deposits in Russia, ca. 900 m.y.b.p.; however, as we discuss under the fossil record of pseudofungi, interpretations of apparent Vaucheria fossils can be subject to question. Tappan (1974), in fact, noted that a number of "protistan" fossils have possibly been misidentified.

The paleontologically significant coccolithophorids (producing calcareous scales or "coccoliths," which are often preserved, cf. Black and Bukry, 1979) present an extensive fossil record. Coccolithophorids, biologically, are a group of planktonic haptophytes (haptophytes are also known as prymnesiophytes), cf. Bold and Wynne (1978, 1985); Lee (1989); Van den Hoek et al. (1995). Haptophytes were considered at least peripherally related to the heterokont algal (stramenopilous) assemblage, both groupings being included within the kingdom Chromista by Cavalier-Smith (1989). However, although some haptophytes possess flagellar hairs, they lack the composite, tubular mastigonemes characteristic of stramenopiles, cf. Graham and Wilcox (2000); also, haptophytes may possess a unique structure, the haptonema (of diverse, sometimes feeding, function). Haptophytes are now believed to be a distinct line based on both fine-structural and molecular evidence (cf. Andersen et al., 1993; Leipe et al., 1994, Bhattacharya and Medlin, 1995; Daugbjerg and Andersen, 1997b; Graham and Wilcox, 2000). However, the fossil record of haptophytes has a bearing on the later development of our discussion of the paleontological history of chromophytous algae.

Until 1980, it appeared that the fossil records of the three groups of chromophytous algae with strong paleontological representation--chrysophytes, silicoflagellates (dictyochophytes), and diatoms (bacillariophytes)--were similar. These three groups apparently first appeared in early to middle Cretaceous time (late Mesozoic); the occurrence of all three extends to the present (Tappan, 1980), although silicoflagellates are greatly reduced in the number of extant taxa (cf. Bold and Wynne, 1985), if not in numbers of individuals (cf. Prescott, 1968). Among diatoms, centric forms (these being predominantly marine) appeared during the early Cretaceous, and pennate diatoms (the dominant form of diatom in fresh water) emerged in late Cretaceous time, not greatly prior to the onset of the Coenozoic (cf. Burckle, 1979; Brasier, 1980; Tappan, 1980). Burckle (1979) considered that individual diatoms occurred in the Jurassic, but, as assemblages, were not manifest until the Cretaceous. Tappan (1980), however, viewed the report of diatoms from the Jurassic as questionable. In short, the available fossil evidence upon which to base an assessment of filiation of chromophyte groups, even those with a substantial fossil record, was quite limited prior to 1980. Nonetheless, an interesting speculation by the paleontologist, Loeblich (1974), was that "the Bacillariophyceae [diatoms] probably diverged from the chrysophycean line before the Jurassic." Independently, Strelnikova (1975) also postulated that diatom predecessors should be sought in pre-Jurassic deposits. One might suspect a siliceous chrysophyte-monad/diatom connection based on morphology of the two groups, and guess that such a connection might have had greater antiquity than the Cretaceous, or even the Jurassic. As we later discuss, some evidence suggests that such a supposition may have been correct.

Soon after 1980 the informational picture of chrysophytes in the fossil record changed dramatically. Allison (1981) reported small siliceous scales from the Lower Cambrian (e.g., Tindir Group) of Northwest

Canada, reminiscent of scales of chrysomonad or synurophyte algae. In followup papers, Allison and Hilgert (1986a,b) expanded this work, concluding that some siliceous scales found were assignable as chrysophyte scales and others as coccolithophorid scales (i.e., as scales of haptophytes). However, coccolithophorid scales (coccoliths) are typically calcareous--composed of calcite, i.e., calcium carbonate (cf. Black and Bukry, 1979; Margulis, 1993). Since no diagenesis, polycrystalline structure, or other evidence of secondary mineral alteration was observed in the Allison and Hilgert scale specimens, it is unlikely (simply on biogeochemical grounds) that haptophytes contributed to these siliceous microfossils. Petrographically, the Allison and Hilgert specimens occurred mainly in angular chert clasts and chert nodules (Allison, 1981); calcareous fossil material (perhaps including coccoliths) is usually dissolved from such structures (Pettijohn, 1975). The haptophyte scenario is made yet more unlikely by the fact that the fossil record of coccolithophorids is only traceable as far back in time as the late Carboniferous (even this being questionable), and not the early Cambrian (cf. Graham and Wilcox, 2000). It is, however, probable that the fossil scales of Allison and Hilgert are of chrysophyte (including synurophyte) origin. Curiously, Graham and Wilcox (2000), while providing astute and accurate (if brief) accounts of the fossil record of several groups of algae, made no reference to the rather striking evidence for chrysophytes prior to the Cretaceous. However, Knoll (1992) considered that the Tindir Group fossils of Allison (1981) indeed resembled scales of chrysophyte algae; Knoll noted that the Tindir fossil-bearing sediments are even older than lower Cambrian, viz. "Neoproterozoic." We note, in favor of the interpretation of the siliceous scales from the Tindir group as chrysophyte material, that if biomineralization in the Chrysophyta occurs, it usually results in cellular deposition of silica, often including formation of mineralized scales (Leadbeater and Barker, 1995).

The scale morphology, ornamentation, aggregation, and remains of scale-processes of the Allison and Hilgert (1986a,b) specimens are suggestive of similar siliceous scales of known chrysophytes, e.g., Paraphysomonas (cf. Leadbeater, 1972; Finlay and Clarke, 1999), and synurophytes, e.g., Mallomonas (cf. Cronberg, 1995; Lavau et al., 1997) and Synura (cf. Leadbeater, 1986, 1990; Kristiansen et al., 1997). Siliceous scale production is not characteristic of all Chrysophyceae (even sensu stricto), but is predominantly associated with the chrysomonad family, Paraphysomonadaceae (Kristiansen, 1986; Preisig and Hibberd, 1986; Leadbeater and Barker, 1995). Paraphysomonas is the main genus of significance among chrysophytes in the cycling of silicon in aquatic systems, on a global basis (Leadbeater and Barker, 1995); Paraphysomonas has a virtually ubiquitous distribution (Finlay and Clarke, 1999), as revealed in sediment examination. There is substantial reason to believe (from the Allison and Hilgert, 1986a,b, specimens) that siliceous chrysomonads and synurophytes were of some importance, geologically, in deposition of silica, even by early Cambrian time. And, there is little doubt of the general identity (as chrysophytes/synurophytes) of the Allison and Hilgert specimens; Lipps (1993), for example, accepted Allison and Hilgert's specimens as representing chrysomonad scales. Potentially very meaningful is the statement of Allison and Hilgert (1986b) that some of the (more disk-like) siliceous scales resemble single valves of certain centric diatom frustules. Cavalier-Smith's (1986) essentially contemporaneous suggestion of a Synura- or Mallamonas-like, scale-bearing, diatom ancestor, is reasonably consistent with Allison and Hilgert's statement, except for questions of scale symmetry (often bilateral in synurophytes). In mentioning a similarity to diatoms, it is important to note that Allison and Hilgert were not saying that these siliceous, chrysophyte-like scales were the remains of diatoms; because of their size, flatness at the margin, specific surface patterns, and uniformly single nature, these scales are implausible as diatom fossils; some merely bear a certain resemblance to half-frustules of centric diatoms. However, this is the very type of evidence that may hold clues to diatom origins. If Loeblich (1974) was correct in his supposition of the origin of diatoms from chrysomonads, then we should continue to search siliceous deposits, of Cambrian to Jurassic age, to ascertain if a filiation of diatoms from chrysomonads may be further supported by the fossil record.

Chrysophytes, however, are probably older even than early Cambrian (as suggested by Knoll, 1992). Siliceous scales, similar to those described by Allison (1981) and Allison and Hilgert (1986a,b), were reported from one-billion-year-old Precambrian rocks in Michigan by Jost (1968)--who noted a resemblance to coccolithophorid scales (i.e., coccoliths), but astutely also noted that coccoliths are, by contrast, calcaerous. The probable conclusion, regarding Jost's (1968) publication, is that the siliceous, scale-like material he examined represents, not coccolithophorid remains, but rather, the remains of chrysophytes. Confusion (in the 1960's) over whether such scale-like remains were those of chrysophytes or coccolithophorids is understandable in that both groups were often combined systematically (as chrysophytes) in paleontological publications of that time (e.g., Black et al., 1967), regardless of the siliceous or calcareous nature of the material.

There is significant fossil evidence for chrysophytes (and synurophytes) in addition to fossil scales. Smol (1995) discussed the importance of both scales and cysts of chrysophytes in paleontological investigations. Chrysophyte cysts--"resting" stages, variously referred to as statocycts, statospores, stomatocysts, and "stoppered" cysts (in reference to an often "plugged" opening)--are a particularly diagnostic feature of this group of organisms (cf. Cronberg, 1986; Cronberg and Sandgren, 1986; Hibberd, 1986; Van den Hoek et al., 1995). Apparent chrysophyte cysts have been reported from chertified stromatolitic parts of the 1.3 billion-year-old Beck Springs Dolomite in California (Cloud, 1976). Cloud considered the cyst morphology observed to be reminiscent of such extant chrysophyte forms as Uroglena. Tappan (1980), in her comprehensive, and generally masterful, treatment of fossil "plant" protists, apparently overlooked Cloud's work. However, Cloud's (1976) photographic illustration (his Plate 5, Fig. A2) of a presumed chrysophycean stomatocyst of Precambrian age is convincing. Lehmann and Hillmer (1983) indicated as well the probable existence of Precambrian chrysophyte cysts.

Thus, what does the fossil record indicate about the filiation of chromophytous algae? Chrysophytes appear to be an early, and relatively basal group, of chromophyte algae. That other groups, such as silicoflagellates (dictyochophytes), might be derived from siliceous chysomonad-like algae, is not a new idea (Fritsch, 1935); such has been tentatively reiterated upon a review of morphological features of chrysophytes and silicoflagelates (Van den Hoek et al., 1995). Based on a number of characters, both groups (chrysophytes and silicoflagellates) have been considered to belong to the Chrysophyta or Chrysophyceae or Chrysomonadida (cf. Moore et al., 1952; Bold and Wynne, 1985; Trainor, 1988; Moestrup, 1995), though not necessarily so at present in the case of silicoflagellates (cf. Guillou et al., 1999; Graham and Wilcox, 2000). Compared with chrysomonads, silicoflagellates are the more specialized, cytologically organized group, with a basket-like "internal skeleton"--actually, external to the plasmalemma, cf. Van den Hoek et al. (1995)--consisting of hollow rods, composed of opaline silica (Tasch, 1980). The "skeleton" appears internal because it is typically obscured by pseudopodia and/or by a viscous envelope surrounding the cell (cf. Prescott, 1968; Tasch, 1980; Trainor, 1988; Van den Hoek et al., 1995). Silicoflagellates also occur much later in the fossil record (Lower Cretaceous) than chrysomonads (late Precambrian), cf. Lehmann and Hillmer (1983). Thus, the fossil record indicates that the origin of chrysophytes occurred first, silicoflagellates originating significantly later. If one group originated from the other, it would seem patent that it was the silicoflagellates which were derived from silica-producing chrysomonads, and not the reverse. However, the closeness of silicoflagellates (Dictyochophyceae) to Chrysophyceae has been questioned on both structural (Hibberd, 1986) and molecular (cf. Bailey et al., 1998) grounds. Saunders et al. (1995), based on 18S rRNA gene sequencing, noted a relationship of the silicoflagellates to the Pelagophyceae and Pedinellophyceae--possibly indicative of an affiliation among certain chromophytous groups with reduced flagellar apparatus; diatoms (also with reduced flagellar apparatus) were considered to be in this lineage as well, though perhaps more questionably so. Daugbjerg and Andersen (1997a) found certain other molecular evidence (i.e., rbcL data) in support of a relationship among silica-producing families of chromophytes, viz., the chrysophytes and synurophytes, the diatoms, and (more distantly) the silicoflagellates. The question of filiation of silicoflagellates is, thus, not clearly answered. However, paleontological evidence (of silicoflagellates originating during the Cretaceous, cf. Moore et al., 1952; Tappan, 1980) contradicts placement of silicoflagellates basal to the much more ancient chrysophytes (a placement suggested in some molecular-based studies, e.g., Saunders et al., 1995; Leipe et al., 1996; Guillou et al., 1999).

An even greater bone of contention when comparing filiation postulates derived from morphological (and ultrastructural) data with those from molecular data (la vs. lb in the previous section) resides with the diatoms (bacillariophytes) versus chrysomonads. Although chrysomonads may have siliceous walls, diatoms (consistently) have the more complex, elaborate, rigid, siliceous wall system--the two valves of the diatom "frustule" together functioning in effect as an "external skeleton." We note, in passing, that diatom valves (regardless of their final external position and function) develop, initially, internal to the plasma membrane in "silica deposition vesicles" (cf. Van den Hoek et al., 1995). Diatoms are the more uniformly adapted, either as planktonic or benthic autotrophs (some specialized auxotrophy may also exist, cf. Bold and Wynne, 1985). The cell specialization and trophic mechanisms in diatoms may be compared with the diverse yet relatively unspecialized, morpho-functional cell plans, and generalized mixotrophy, observed in chrysophytes (Holen and Boraas, 1995). We thus appreciate Loeblich's (1974) speculation that chrysophytes are primitive and gave rise to diatoms, i.e., prior to the Jurassic. Van den Hoek et al. (1995) similarly proposed an origin of diatoms from primitive, siliceous, chrysomonad-like algae. Medlin et al. (1997), from "molecular clock" calculations of SSU rRNA gene sequences, posited an origin

of diatoms (in this case not from chrysophytes) pursuant to the climatic and geochemical upheavals associated with the Permo-Triassic boundary. Although we do not refute the time-line proposed by Medlin et al. (1997) for diatom origin, as such could be a possibility, there is presently no positive fossil evidence to support it. The fossil record indicates that centric diatoms are first found in the early Cretaceous (cf. Strelnikova, 1975), pennate forms appearing in the late Cretaceous (cf. Graham and Wilcox, 2000). Diatoms (whether extant or fossil) are distinctive, with siliceous frustules (double valves) having characteristic ornamentations; when present, diatoms are often abundant, and leave indisputable remains. If diatoms were common in sediments older than Cretaceous, it is doubtful that their remains would have been overlooked. The fossil record, in fact, indicates that chrysomonads substantially preceded diatoms in geologic time (late Precambrian vs. early Cretaceous). Thus, evidence from the fossil record (i.e., bearing upon filiation of chrysomonads and diatoms) contradicts the interpretation based on molecular findings (e.g., Leipe et al., 1994; Van de Peer and De Wachter, 1997; Saunders et al., 1997) that diatoms are basal chromophytes, and supports the morphologically based interpretation that chrysomonads preceded (and were perhaps ancestral to) diatoms in evolution. However, in the interest of not too hastily accepting, totally, that the morphological and paleontological interpretations of diatom origins from chrysomonads are so convincing as to conclude the issue, we should point out that other possibly ancient chromophyte groups, such as xanthophytes (with often bipartite walls, cf. Lee, 1999), not yet eliminated from consideration by evidence from the fossil record, still deserve some consideration in the question of diatom ancestry (diatom walls also being "bipartite"). The evolutionary relationships within diatoms is also not entirely settled. As mentioned, Graham and Wilcox (2000) alluded to molecular evidence apparently supporting a closer relationship of certain centric diatoms to pennate diatoms, than to other centrics. It is possible that paleontological evidence could eventually be brought to bear on the substantiation of this particular claim of an unusual grouping relationship within diatoms. Based on 18S rRNA (i.e., 18S rRNA gene sequences), Cavalier-Smith and Chao (1996) indicated an origin of pennate (i.e., bilaterally symmetrical) diatoms prior to centric forms. In this latter case, however, the fossil record (cf. Loeblich, 1974; Strelnikova, 1975; Brasier, 1980; Lehmann and Hillmer, 1983; Graham and Wilcox, 2000) clearly documents the earlier origin of centric diatoms (this being supported by morphological considerations as well, cf. Allison and Hilgert, 1986b). The rather complicated raphe system, and associated gliding motion, often present in pennate diatoms, is probably an advancement within the pennate line; fossil evidence indicates that araphid pennates preceded pennates possessing a raphe system (Graham and Wilcox, 2000).

2b. Pseudofungi and the Fossil Record. In considering paleontological evidence of pseudofungi, we are on less firm footing than when considering certain groups of chromophytous algae. Since Hyphochytriomycetes have no apparent fossil record, we are confined in discussion of pseudofungi to the Oomycetes. Although Oomycetes present a demonstrable fossil record, it is not as definitive as that of the groups of chromophyte algae emphasized above; yet, since there is a body of fossil evidence bearing on Oomycetes, it should not be ignored.

Both the Oomycetes and the Chrysophyta are unquestionably ancient; but, because of the relative uncertainty of the early oomycete fossil record, we will not attempt to be decisive as to which group is the more ancient (based on information in hand, the nod for greater antiquity would probably go to chrysophytes). Related to our indecisiveness, as to the absolute (or even relative) age of Oomycetes, are some potential sources of confusion. Oomycetes (which we now know to be stramenopiles) were formerly considered to be Fungi, and were placed in the catch-all, fungal category "Phycomycetes" (cf. Sparrow, 1960; Alexopoulos, 1962), along with Chytridiomycetes (true chytrids) and Zygomycetes; the latter two groups are still considered true Fungi (cf. Moore-Landecker, 1996; Purves et al., 1997), and need not concern us further here. However, as regards the fossil record for Oomycetes, in addition to seeking listings for Oomycetes per se, we must search among listings for Fungi in general and, in particular, enumerations of Phycomycetes, to see if some of these references may apply to Oomycetes. Among reports of fossil Phycomycetes, if not specifically indicated, we might suspect the remains of robust, branching, apparently coenocytic tubes, in paleo-aquatic habitats, to be the remains of Oomycetes, perhaps Saprolegniaceae; but, other interpretations are possible; i.e., such microscopic (though comparatively large), tubular structures could as well be the remains of coenocytic or siphonous algae, such as Vaucheria. If reproductive structures (sporangia or oogonia) are preserved, then determination can, of course, be somewhat more precise. In short, it is often more difficult to definitively assess putative Oomycetes fossil material, than is the case with chrysophyte, diatom, or silicoflagellate fossil material.

Probably the report of "Phycomycetes" (and of eukaryotes in general) most ancient in the fossil record was that by Tyler and Barghoorn (1954) from the two billion-year-old Gunflint iron-formation of Canada. Tyler and Barghoorn's report cannot be generally accepted, not so much because of the great antiquity of the geological stratum (early Proterozoic), but because the microfossils found are most certainly the remains of prokaryotes, not eukaroytes (cf. Hofmann and Schopf, 1983; Taylor and Taylor, 1993). Also, the small filaments, the filament-size/"spore"-size differential, and the inexplicable, direct, lateral attachment of the "spores" to filaments, among other features photographically illustrated by Tyler and Barghoorn (1954), would probably rule out Oomycetes--especially free-living forms of the type which might have occurred in a sedimentary environment such as that represented by the Gunflint (now seen to be chert laden and partially stromatolitic). There is no convincing reason to believe that the "spores" illustrated and discussed by Tyler and Barghoorn (1954) actually represent such, as opposed to some other type of cell-like remains, or even some sort of preservation artifact. Later, Knoll and Barghoorn (1975) in effect disavowed statements of eukaryotic organisms in formations as old as the Gunflint, by seriously questioning the existence of eukaryotes in the 900-850 million-year-old Bitter Springs formation of Australia, and in any Precambrian strata of this age or older. This, however, proved to be an overreaction, since "hard-rock" evidence for eukaryotes has been documented from approximately 1.4 billion years before present (Schopf and Oehler, 1976; Vidal, 1984), and is speculated to be older still (cf. Knoll, 1992; de Duve, 1995; Delsemme, 1998). Schopf recently (1999) discussed paleontological evidence, at a date of 1.8 b.y.b.p., for eukaryote appearance.

In the Beck Springs Dolomite of California, dated 1.3 b.y.b.p., Cloud (1976) found (in addition to chrysophyte cysts mentioned previously) robust, branched, filaments or tubes (only rarely septate), some of which he equated to the xanthophyte (he used the term "chrysophyte") Vaucheria. Vaucheria-like filaments have also been reported from 1000 to 900 m.y.b.p. deposits from Siberia (cf. Xiao et al., 1998; Graham and Wilcox, 2000). However, since Vaucheria and some Oomycetes, such as Achlya, have a similar thallus morphology, these tubular or "siphonous" filaments (in the absence of plastids and reproductive structures) could just as well be the remains of saprolegniaceous Oomycetes. Such a suggestion, "phycomycetous fungi," was made (Schopf et al., 1973) for similar filaments from the younger Precambrian of the Grand Canyon, Arizona (Chuar Group, 700-600 m.y.b.p.?). Schopf related these forms to putative "fungal" filaments "Eomycetopsis" from the Bitter Springs formation (ca. 900 m.y.b.p.), cf. Schopf (1968). Taylor and Taylor (1993), however, noted that Eomycetopsis is possibly a cyanobacterial sheath, rather than fungal (or pseudofungal) remains.

Germinated cyst-like structures recovered from the Upper Precambrian ("Rifeen") were alleged to be "Phycomycetes" [Oomycetes] by Timofeev (1970). These structures, however, are scarcely plausible as germinating cysts (oospores) of Oomycetes. The cyst surface is described by Timofeev as "chagrinee-poreuse," and, in addition to this irregular surface granulation and porosity, a large opening may be seen in the photographs on one area of the cyst; this aperture may or may not be associated with a (rather large) germ tube. Oomycete oospores are typically relatively smooth, in free-living forms, and would probably not exhibit the porosity, irregular bumps, or the rather large opening described and illustrated by Timofeev (1970). Also, oospores (and even oogonia) are typically several times smaller than the cyst-like structures examined by Timofeev (cf. Coker and Matthews, 1937). It is, on the other hand, difficult to suggest a probable interpretation of the biological relationship of Timofeev's material, and such would merit further investigation.

With oomycete fossils, we are not really on firm footing until Paleozoic time. Regarding the Paleozoic, accepted fossil finds of saprolegniaceous kinds of Oomycetes are enumerated by Tiffney and Barghoorn (1974) from Silurian (Palaeachlya, Achlyites), Devonian (Palaeachlya), and Pennsylvanian strata (Paleoperone). These reports (all by other workers) are among those accepted by Tiffney and Barghoorn, and seem generally authentic. We also agree with Tiffney and Barghoorn's acceptance of Propythium (Peronosporales) found in upper Pennsylvanian Bryozoa (Elias, 1966); this occurrence suggests a relatively early divergence of primitive Peronosporales from Saprolegniales; a more advanced member of the Peronosporales, Albugo, has been reported (based on a fossil oogonium) from the Upper Carboniferous (cf. Taylor and Taylor, 1993). However, we cannot be sure of the exact time frame of the divergence of Peronosporales from Saprolegniales, nor of that of the divergence of advanced from primitive Peronosporales. In a larger context, the absolute depth of antiquity of Oomycetes must also remain for now a mystery. Oomycetes may be as ancient as chrysophytes, but we cannot be certain with the paleontological evidence in hand. The comparative age of Oomycetes and chrysophytes is one

question, however, where the fossil record, with further collecting and analysis, may eventually provide a reasonably clear answer; this is a question perhaps worthy of the attention of researchers in micropaleontology.

There is another basic question concerning stramenopiles, however, with which the fossil record will probably not be of much assistance. Leipe et al. (1996) used molecular evidence (16S-like rRNA gene sequences) to "confirm" that stramenopiles were initially "protozoan;" i.e., heterotrophic groups such as labyrinthulids and Cafeteria-like bicosoecids are representative of the primitive stock of stramenopiles; and, accordingly, stramenopiles are a "primarily heterotrophic group." On morphological, ultrastructural, and molecular grounds (e.g., O'Kelly and Patterson, 1996; Leipe et al., 1996; Cavalier-Smith and Chao, 1996; Cavalier-Smith, 1997); Guillou et al., 1999; Honda et al., 1999), there is little reason at present to doubt the assessment that stramenopiles were initially a heterotrophic (probably phagotrophic) lineage. However, since these primitive heterotrophs are not among those groups of stramenopiles leaving a significant (or any) fossil record, there would seem to be no meaningful way, paleontologically, to attempt to substantiate this basal-heterotroph theory of stramenopile origin.

APPROACH 3. Ontogeny: We consider here what ontogenetic theory, information, and observed ontogenies suggest about phylogeny, specifically filiation, of stramenopiles. This approach is consistent with Eldredge's (1999) belief that a reexamination should be given to certain pattern phenomena in nature, to ascertain if details of a given pattern may offer further retrospective insight into formative processes, such as evolution. We discuss, then develop in a possibly novel way, the use of a specific ontogenetic method, based on the perception of a particular ontogenetic pattern. Since this methodology is founded in a considerable backdrop of information, some explanation of pertinent concepts and their history seems appropriate. Ontogenetic phenomena, potentially indicative of evolution, or more broadly of phylogeny, are of two basic kinds (attempting a slightly altered, factorial summary of Gould, 1977): (1) neotenic (more inclusively, paedomorphic) and (2) recapitulatory (including atavisms, cf. Thomson, 1988). Neotenic evolution (transformation of the equivalent of the juvenile form of species A into the adult form of species B), well known in a number of animal groups, may also occur in simple organisms, such as stramenopiles, but is difficult to substantiate. This is because, rapidly reproductive, often one-celled organisms, lacking complex mature stages, in a sense never escape juvenility. We focus here therefore on recapitulation, modified conceptually from Haeckel (1866, 1874), and others more recently (e.g., Nelson, 1978).

Haeckel has been criticized for the aphorism attributed to him that "ontogeny recapitulates phylogeny," cf. de Beer (1930), Moody (1970), Gilbert (1991). This "biogenetic law" was interpreted (allegedly by Haeckel) to mean that organisms (e.g., kinds of mammals) pass in their embryonic development through stages comparable to adult stages of their ancestors (i.e., other, presumably precedent, major vertebrate groups), cf. de Beer (1930) Moody (1970), Churchill (1998). There is little doubt that Haeckel considered phylogeny to be, somehow, a driving force of ontogeny (Gould, 1977)--a concept which was appropriately challenged (e.g., Garstang, 1922). However, it is debatable the extent to which Haeckel believed that actual "adult" stages were "recapitulated" during ontogeny (see Moore, 1993). Regardless, since no embryo in reality passes through an adult stage, more favored has been Von Baer's (1828) relatively modest assessment, paraphrased, that embryos (of different kinds of vertebrates) are more similar than are their adult stages (cf. Singer, 1959; Storer et al., 1979; Minkoff, 1983); this is generally true if we don't include the early or late stages of the developmental spectrum, but focus on intermediate stages (such as the "pharyngula" stage), cf. Moody (1970), Smith and Szathmary (1997), Wells (1999). Rather than ontogenetic recapitulation of phylogeny, a more accurate assessment is that descendant ontogenies, in fact, recapitulate ancestral ontogenies (Garstang, 1922; Smith, 1960). However, since the more closely related particular animals or animal groups are to one another, "usually the greater will be the proportion of their ontogenies exhibiting similarities" (Moody, 1970). This being so, then ontogeny, regardless of the precise wording of the recapitulation concept, may provide valuable clues as to ancestral relationships.

Radinsky (1987) cautioned (in reference to evolution of cartilagenous fishes) that recapitulation should not be viewed simplistically. Extrapolating, we suggest that the extent of the recapitulation idea implies more than just recapitulation. For evolution to occur, a descendant ontogeny must undergo modification--developmental change, generationally perpetuated. Beyond providing phylogenetic insight (an anthropocentric viewpoint), ontogenies, of critical importance to evolution, create phylogenies (Garstang, 1922); i.e., ontogeny is the "mechanical cause" of phylogeny (Loevtrup, 1984). Also, rudimentary structures may occur, in descendant ontogenies, which do not fully develop (or perhaps even disappear) in descendant adults; i.e., they are

non-adaptive (Nelson et al., 1967) or vestigial (Strausbaugh and Weimer, 1938), often having little or no adult function. However, such vestigial structures are usually homologous with particular structures in an ancestral ontogeny, which became further developed, persisted, and were perhaps fully functional in an ancestral adult (cf. Lindsey, 1952; Newman, 1939); and in this sense, Haeckel was more correct that he is usually credited with being. There are many human and other vertebrate examples of vestigial structures (cf. Moment, 1967; Moody, 1970; Berra, 1990), such as: the pronephric kidney; the muscles which potentially wiggle the ears; "gill slits" or, more accurately, pharyngeal pouches; the vermiform appendix; the reduced "third eyelid;" the extremely reduced posterior appendages of pythons and whales; the "hand" bones of the horse forelimb; and the second hump on the fetus of a one-humped camel (Caras, 1996). Accurate determination and interpretation of vestigial structures or stages can be of great significance when the attempt is made to apply ontogenetic information to phylogeny.

In considering ontogeny of stramenopiles, in possible relation to phylogeny, it might thus be instructive to search for structures or stages which are apparently vestigial, or which at least invite investigation as such. Since, in stramenopiles, a given stage (semaphoront) in the life cycle is often only a single cell, it is especially important to emphasize the entire life cycle; in this way the developmental origin of such a cell is more fully appreciated. Several investigators (e.g., Arthur, 1997; Bonner, 1962; Nielsen, 1998; Willmer, 1990) have stressed that the whole life cycle of organisms, especially various kinds of invertebrates, should be considered in ontogenetic/phylogenetic investigations, and not just one stage. Arthur (1997) stated that "the interface between life history theory and evolutionary developmental biology is likely to be fertile ground for future advances in our overall understanding of the evolution of developmental systems." McHugh and Rouse (1998) discussed the importance of life history studies to the understanding character transformations, to evaluating (and ordering) character states, and subsequently to reconstructing probable character evolution. Not all writers on the subject have been equally enthusiastic; Smith and Szathmary (1997) believed that future contributions of developmental morphology to evolutionary understanding will be quite limited. Not sharing this last viewpoint, we believe that additional phylogenetic insights may well be obtained from further life cycle examination; we hope to demonstrate this point herein.

Particularly relevant to our consideration of ontogeny (as suggestive of phylogeny) is the reapplication of Haeckel's recapitulation idea to Hennig's (1966) concept of character phylogeny (cf. Nelson, 1978)--i.e., examining the developmental (and presumably evolutionary) change in specific characters as a clue to organismal evolution. In a comparison involving potential character change, organism A might possess a given character in a single state (state 1). In organism B this character state is again seen, then is transformed during development from state 1 to state 2 (i.e., both character states occur in organism B, sequentially). This ontogenetic transformation becomes a clue to character polarity, the direction of evolution, and phylogenetic understanding (viz. a lineage from character state 1 to state 2, and from organism A to organism B). A common example cited (Nelson, 1978) is that of a type of flatfish (organism B) which undergoes a character transformation during ontogeny from one eye on each side of the head (state 1) to both eyes on the same side of the head (state 2). This can be related to the more general condition in which eyes remain on the same side of the head of a presumably antecedent kind of fish (organism A, with character state 1 only). Though a memorable example, one may question if only a single character is involved in the shifting position of the eyes in flatfishes. Regardless, application of such an ontogenetic character state sequence to the determination of character phylogeny (sensu Hennig) has become known as Nelson's Ontogenetic Rule (Wheeler, 1990) or Nelson's Biogenetic Law (De Queiroz, 1985). In actual studies, using types of beetles, Nelson's ontogenetic method proved as accurate in determining character polarity as did out-group comparison methodology (Wheeler, 1990).

We thought that it might be feasible to apply Nelson's (1978) method, not just to characters (and character states), but to stages (each a complex of characters) in the life cycle of a stramenopile (or other comparably simple eukaryote); our approach is thus an extension of Nelson's method. It should be possible to identify an organism of type A, having life cycle stage 1 (only); and also to identify a possibly related organism, B, having stage 1, transformed to stage 2, in the life cycle. We reasoned that, pragmatically, we would probably need to search for putative organism B first, possessing both stages (perhaps in sequence) in its life cycle--using this information as a retrospective clue to identify putative organism A. Regardless of which organism would be determined first, support for our attempt to "step up" the complexity level in analysis (from character states to stages) may be taken from Frost and Kluge (1994) who pointed out that ontogenies are "metaphenomena," not advisedly subject to over-reductionism. Although Frost and Kluge clearly had complex organisms in mind in this statement, we

believe this to an extent to be true even in relatively simple organisms (which, of course, may still exhibit considerable complexity, particularly at the cell level).

With the gist of this approach in mind, we sought to ascertain if recapitulation phenomena had been identified in any eukaryotic microbe, and if such could be used to decipher an ancestor/descendant (tokogenous) relationship. Sleigh (1989) discussed examples of recapitulatory development among suctorial protozoans, which as "adults" are sessile and bear tentacles, but no cilia; nonetheless, suctorians are classified with ciliates. In the life cycle of suctorians, an apparent recapitulatory stage gives evidence of ancestry. The sessile adults bud to produce motile, ciliated "larval" forms, the nature of the ciliature being indicative of a relationship, broadly, as "cyrtophorid" ciliates (Sleigh, 1989). A true metamorphosis occurs (Grell, 1973) as the "larva" settles down, withdraws its cilia, forms a stalk, and tentacles develop. The "adult" retains an infraciliature of a "holotrichous type" (Kudo, 1966); this somewhat irregular "fibrillar network becomes beautifully regular" in the "embryonic stage" (Bonner, 1952). In addition to Suctoria, Dogiel (1965) discussed cases of apparent ontogenetic recapitulation of phylogeny in several groups of Protozoa, including, certain peritrichous ciliates, dinoflagellates, and radiolarians. Citing examples from both chlorophytous and chromophytous algae, Beech et al. (1991) noted that, in passing through more than one life (cell) cycle, changes in flagellar length, form, basal apparatus, and even flagellar type occurred; such flagellar changes are of potential use in providing clues to ancestry. Thus, precedents clearly exist for analyzing potential recapitulatory events among fundamentally unicellular organisms.

We wished, then, to look for developmental sequences in stramenopiles, potentially informative of phylogeny. Among stramenopile groups, a number of developmental cell phenomena might be examined with benefit. One such enticing source of developmental information, at the cell level, is comparative mitosis (cf. Heath, 1986). Gleaning information from several authors (Dodge, 1973; Pickett-Heaps, 1976; Beakes, 1987, 1989; South and Whittick, 1987; Green, 1989; Margulis, 1993; Van den Hoek et al., 1995) on mitosis of certain stramenopile groups, we reach the understanding, expressed simply, that both chrysomonads and diatoms have an open mitosis (with the nuclear membrane breaking down); by contrast, Oomycetes have a closed mitosis with a persistent (intact) nuclear membrane. It has been debated (cf. Green, 1989), without much resolution, whether an open or closed mitosis is the more primitive. Regardless of which type is more primitive, this particular parcel of information (on comparative mitosis) certainly does not speak to a closer relationship of Oomycetes to diatoms, than of chrysomonads to diatoms; in fact, it provides limited justification for our serious questioning of interpretations of molecular evidence (e.g., Leipe et al. 1994, 1996; Cavalier-Smith and Chao, 1996; Van de Peer and De Wachter, 1997) which suggest the filiation of diatoms immediately pursuant to (perhaps from) Oomycetes (or from pseudofungi in general). We should perhaps note here in passing that Hyphochytriomycetes (also belonging to the pseudofungi) possess a persistent mitotic nuclear membrane similar to Oomycetes--yet, in hyphochytrids, polar fenestrae (openings) form in the nuclear membrane, through which spindle fibers develop (Fuller, 1990; Margulis, 1993). This is potentially particularly interesting if primitive chrysophytes (with open mitosis) and hyphochytrids (with polar openings) are, as we might suggest, positioned in filiation (in their respective lines) not greatly removed from the bifurcation of autotrophic and osmotrophic stramenopile lineages.

As intriguing and relevant as comparative mitosis is, we are searching here instead for a particular ontogenetic sequence (i.e., a transformation of stages) within the life cycle of one stramenopilous organism, which may offer, retrospectively, clues to evolutionary polarity or direction (and hence insight into a potential filiation sequence). One possibility is found among the silicoflagellates (dictyochophytes). Two species of Dictyocha may have, each, two motile stages in the life cycle (Moestrup, 1995). The more usual "skeletal" stage, with one functional flagellum, can be preceded by a biflagellate, "nonskeletal," chrysomonad-like stage. This apparent recapitulation event supports our previous discussion that silicoflagellates arose after and were possibly derived from biflagellate, silica-producing, chrysophyte-like monads. Similarly, Hibberd (1971) observed in Chrysamoeba radians (Chrysophyceae) that an occasional "flagellated" stage with a relatively long flagellum would form, in an atavistic manner, mixed among the dominant amoeboid stage (possessing a reduced flagellum). Hibberd suggested an origin from a "permanently motile Ochromonas-like form;" Ochromonas is both biflagellate and heterokont (the anterior flagellum bearing tubular mastigonemes). Wetherbee et al. (1988) found in the chrysomonad, Epipyxis pulchra, that such heteromorphic flagella "evolved" during the course of two or three cell cycles; if, in this case, ontogeny is informative of phylogeny, the primitive state in chrysomonads was not only biflagellate, but also that both flagella were more or less equal, and possibly bore mastigonemes; further, Cavalier-Smith (1989) suggested, based on putative protozoal forerunners, a yet earlier tetraflagellate condition of

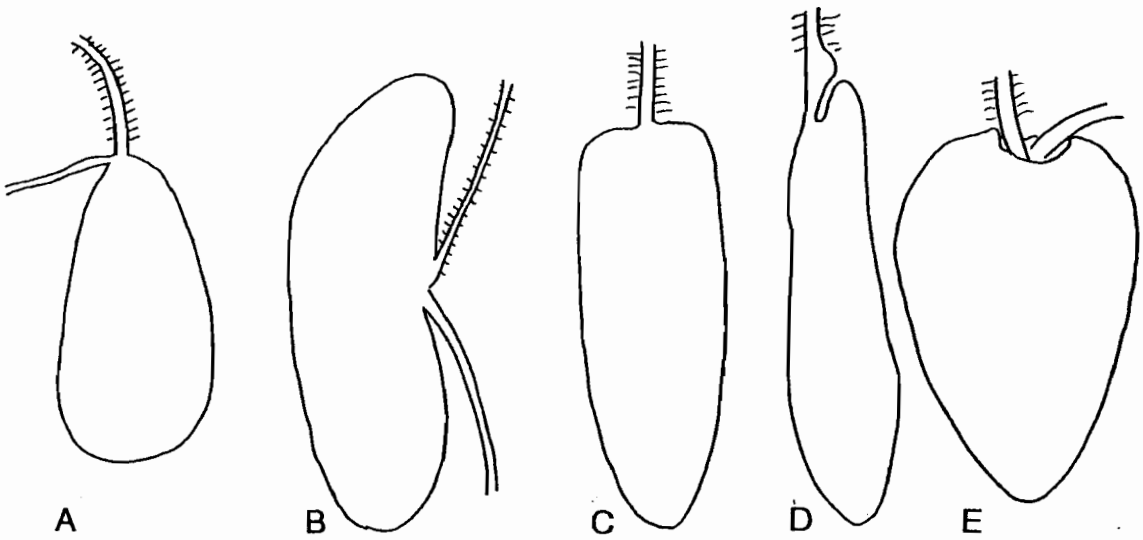

FIGURE 4. Selected comparative motile cells of Stramenopiles. A. Primary zoospore of Saprolegnia. B. Secondary zoospore of Saprolegnia. C. Hyphochytriomycete motile cell. D. Eustigmatophyte motile cell. E. Ochromonas (a chrysophyte monad). Note that the morphological similarity of motile cells C,D, E is greater to A (primary zoospore) than to B (secondary zoospore).

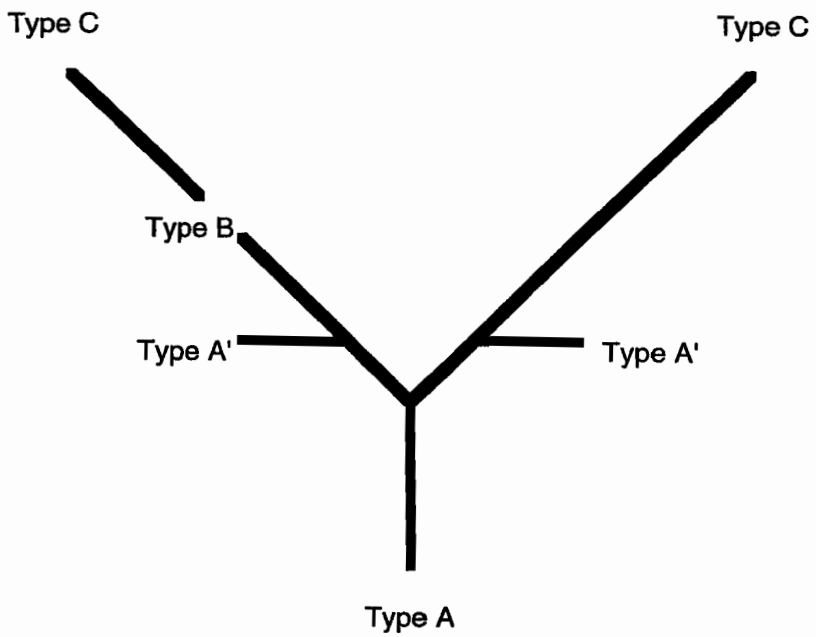

FIGURE 5. Possible evolutionary sequence of structural/functional or positional flagellar changes of zoospores or motile cells of stramenopilous morphological types, based on ontogenetic information.

Type A - Only one morphological motile stage present in the life cycle, this being subapically biflagellate (with one "anterior," i.e., tinsel, flagellum; and one "posterior," often whiplash, flagellum). This subapically biflagellate condition is presumably a relatively primitive state among stramenopiles. Representative organisms (motile cells) of this type are Ochromonas and Cafeteria.

Type A' - Similar in morphology to type A, but the whiplash (second or "posterior) flagellum is reduced, obsolete, or lacking; e.g., motile cells of eustigmatophytes and hyphochytrids.

Type B - Subapically biflagellate and laterally biflagellate motile stages (zoospores) are both present in the life cycle, sequentially; that is, the life cycle of this type of organism contains two distinct types of motile cell stages, in succession; e.g., Saprolegnia (a putatively primitive Oomycete).

Type C - Only a laterally biflagellate motile stage is present in the life cycle; e.g., advanced Oomycetes (left branch), Phaeophyceae (right branch). The development of lateral flagellation in these two branches probably occurred independently (i.e., as a result of parallel evolution).

ancestral chromistans. Regardless of the last point, there is ontogenetic evidence in support of evolutionary reduction from a biflagellate to a uniflagellate condition. For example, Heimann et al. (1995) discovered in Pelagomonas calceolata (thought initially to show no evidence of a second flagellum) that two basal bodies are briefly present, in concurrence with cytokinesis. In addition to pelagophytes, bacillariophytes (diatoms) invite further ontogenetic scrutiny. Diatoms are typically aflagellate; it was not until the work of von Stosch (1951) that it was realized that a few marine, centric diatoms (e.g., Lithodesmium and Biddulphia) possess motile (in this case, uniflagellate) sperm (cf. Bold and Wynne, 1985), these with substantially reduced flagellar apparatus (Andersen, 1991; Van den Hoek et al., 1995). This seeming quasi-vestigial occurrence of motility by flagellation in diatoms suggests that bacillariophytes are derived, possibly from siliceous, chromophyte monads (perhaps flagellated chrysophytes, cf. Fritsch, 1935; Loeblich, 1974; Van den Hoek et al., 1995). As mentioned previously, evidence of a second flagellum in diatoms was not found (cf. Bold and Wynne, 1985). Although, in the centric, Attheya decora, Drebes (1977) indicated that "on the first [of two] meiotic division, during interkinesis, the flagella grow out in pairs near the nuclei;" each flagellum, we assume, becomes associated with its respective spermatocyte. Examples of putative recapitulation among Chrysophyta (sensu lato, cf. Bold and Wynne, 1978, 1985; Moestrup, 1995), and perhaps related groups, provide glimpses of evolution primarily within an assemblage of chromophytous algae, characterized by flagellar (and flagellar apparatus) reduction (cf. Saunders et al., 1995).

A potential ontogenetic marker organism among stramenopiles, with a perhaps more comprehensively informative developmental cycle, is Saprolegnia (Saprolegniales, Oomycetes). Several putatively related genera of the Saprolegniaceae (e.g., Isoachlya, Couchia) could possibly be considered in this regard as well, cf. Powell and Blackwell (1998); but, we will focus this discussion on the well-known genus Saprolegnia. (cf. Seymour, 1970), with a commonly observed life cycle. The asexual phase of the life cycle of Saprolegnia is usually composed of distinct stages (Fig. 3): Primary zoospores are released from the sporangium. Typically, these primaries swim but briefly (Sparrow, 1960), with some difficulty, and soon form primary zoospore cysts. From these primary cysts germinate the secondary zoospores, with greater swimming ability and swarming time. The secondaries eventually also encyst; germination of secondary zoospore cysts can yield additional secondary zoospores or vegetative hyphae. The oogamous (sexual) phase, typical of Oomycetes, need not concern us here.

Holloway and Heath (1977) provided a comparison of primary and secondary zoospores of Saprolegnia. Powell and Blackwell (1995) discussed questions of homology of structures found in such zoospores. The primary zoospore of Saprolegnia is subapically biflagellate (Figs. 3; 4A), often pyriform (or "pip-shaped"), and shows some differences in organellar orientation compared with the secondary zoospore. The secondary zoospore of Saprolegnia is reniform and laterally biflagellate (Figs. 3; 4B), the flagella being inserted in a groove-like area. Barr and Desaulniers (1989) pointed out that the angle described by the kinetosomes (flagellar basal bodies) is consistently greater in the secondary than the primary zoospore; thus, in the secondary zoospore, although both flagella are laterally inserted, one (the tinsel flagellum, with its reverse-thrust-generating, tubular hairs) is directed clearly forward, and the other (smooth, whiplash flagellum) is directed decidedly toward the posterior. This adaptation (greater basal body angle) in the secondary zoospore prevents the two rather different flagella from working against each other. In the primary zoospore, with a substantially lower kinetosome angle, and both flagella directed more or less forward (i.e., the whiplash flagellum is not clearly posterior), there is the probability of a mutual functional interference, particularly since the two kinds of flagella affect the swimming process differently (Sleigh, 1991b). This difference in flagellar orientation (and potential "competitive" flagellar behavior) is the fundamental reason why primary zoospores of Saprolegnia often swim poorly, compared with secondary zoospores. Barr (1983) considered the primary zoospore of Saprolegnia to be a "degenerated" stage, i.e., with vestigial attributes—a stage which does not function particularly well. In contrast, Barr viewed the secondary zoospore a "forward step in evolution," i.e., a stage well-adapted to perform its function. Of the two zoospore stages, there is no question that the secondary zoospore is decidedly the better adapted for swimming, and thus, importantly, for dispersal of the organism.

In the Saprolegniaceae (cf. Sparrow, 1960; Powell and Blackwell, 1998) Saprolegnia and a few other genera (e.g., Couchia, Isoachlya, Leptolegnia) are diplanetic, or more precisely stated, disporic (with two different types of swimming stages, viz. primary and secondary zoospores). However, most genera in the family are in fact functionally monoplanetic (monosporic), the primary zoospores being either quickly suppressed, e.g., Achlya and Aphanomyces, or totally suppressed, e.g., Dictyuchus, Thraustotheca, Calyptrolegnia, and Brevilegnia--yet, the secondary zoospore is fully functional and effective in these genera, as it is in Saprolegnia. The primary zoospore

(at least as a functional stage) is thus seen to be altered or eliminated within the life-history phenomena of the Saprolegniales. In one unusual saprolegniaceous genus, Pythiopsis, only zoospores with a primary type of morphology occur in the life cycle (Sparrow, 1960; Powell and Blackwell, 1998); but, it still remains to be determined whether this condition in Pythiopsis should be considered primitive or derived (cf. Gaumann and Dodge, 1928; Powell and Blackwell, 1998), and, relatedly perhaps, whether the primary zoospore of Pythiopsis is in fact comparable to primary zoospores of other members of the Saprolegniales. In other orders of Oomycetes--the Leptomitales, Lagenidiales, and Peronosporales--possibly more advanced than the Saprolegniales (Barr and Desaulniers, 1989), only (what is apparently) a secondary type of zoospore is to be found. Although, as Barr and Desaulniers (1987) pointed out, in reference to the lagenidiaceous genus, Lagena, if only one kind of zoospore is present, it can possibly be difficult to argue secondary versus primary; based on the flagellar rootlet configuration, their decision was nonetheless tipped in favor of secondary in the case of Lagena. And, there is little question that, in general, the zoospore in putatively advanced groups of Oomycetes exhibits, decidedly, a secondary type of morphology (cf. Ho et al., 1968; Gotelli, 1974; Lunney and Bland, 1976; Barr and Allan, 1985; Randolph and Powell, 1992).

In seeking insight into phylogeny from ontogenetic phenomena, Saprolegnia is a good test case. In the life cycle of this oomycete, a distinct transformation of stages (from primary zoospore to secondary zoospore) may be observed (Figs. 3; 4A,B). Stage 1, the primary zoospore of Saprolegnia, with apparently dysfunctional, perhaps vestigial, attributes, is probably primitive. It is doubtful that the process of natural selection would have fashioned, and maintained, a poorly functional primary zoospore, from a secondary zoospore which functions well. Stage 2, the secondary of zoospore of Saprolegnia, is subsequent in the life cycle, arising transformationally from the cyst of the primary zoospore, and represents (as discussed) a structural and functional advancement compared with the primary zoospore. It is much more plausible that the well-adapted secondary zoospore, with increased basal body angle and more lateral flagellar position, arose from the primary zoospore, than is the reverse proposition; the appearance of secondary zoospores, subsequent to primary zoospores, in the life cycle of Saprolegnia, would also suggest this. Thus, with regard to our permutation of Nelson's ontogenetic method (cf. Nelson, 1978), we have perhaps determined the second half of the equation, i.e., identifying putative organism B, exhibiting a developmental sequence of stages (1 to 2), in one life cycle, with the potential to be phylogenetically informative.

Barr (1983) and Barr and Desaulniers (1989) indicated (based on morphology, flagellation, and internal structure) that the primary, rather than the secondary, zoospore of the Saprolegniales order of Oomycetes, e.g. Saprolegnia (Figs. 3; 4A,B), is more directly comparable to the motile cells of most monadal chromophytous algae (cf. Fig. 4D,E); and, we would add, more comparable as well to the motile cells of Hyphochytriomycetes (Fig. 4C)--as discussed, hyphochytrids are probably related to Oomycetes (together constituting the "pseudofungi," or "Pythiistea," of Cavalier-Smith, 1989). If we examine the apically or subapically flagellate cells of hyphochytrids (cf. Barr and Allan, 1985; Cooney et al., 1985; Fuller, 1990) and of ochromonadalean chrysophytes or eustigmatophytes (cf. Hibberd, 1986; Hibberd and Leedale, 1970, 1972; Lee, 1999), the resemblance of hyphochytrids and monadal chromophytes is (not only to each other but) to the primary zoospore, more than the secondary zoospore, of Saprolegnia (compare the morphology of primary and secondary zoospores of Saprolegnia, not only in our Figs. 3 and 4, but also in Holloway and Heath, 1977). We suggest, thus, that we may have found the first half of the equation among monadal chromophytes and hyphochytrids, i.e., our type A organism (or organisms) with only the untransformed (subapically flagellate) stage (stage 1) in the life cycle (e.g., Fig. 4C, D, E). From our deductions of type A and B representative organisms, we may infer: stage and character changes, polarity, a potential direction of evolution, and even a hypothetical ancestor. Our ontogenetic hypothesis suggests that simple chromophytes and hyphochytrids, variously type A or A', are basal even to relatively primitive Oomycetes such as Saprolegnia, i.e., type B (cf. Figs. 5 and 6); and, further, that the immediate ancestor of the pseudofungal and chromophytous algal lines was probably a subapically biflagellate monad (type A), not necessarily an especially effective swimmer, possibly possessing a helical flagellar transition zone, but not yet a concertina type of organization. The functional/adaptive problem of potentially competitive "apical" flagella of heterokont organisms required solution, however, before major evolutionary advances could occur; the shift to lateral flagellation in the secondary zoospore of Oomycetes represents one sort of solution.

With a major exception of phaeophytes (brown algae), which have laterally biflagellate motile cells, the majority of chromophytous algal groups have apparently solved the problem of potentially competitive "apical"

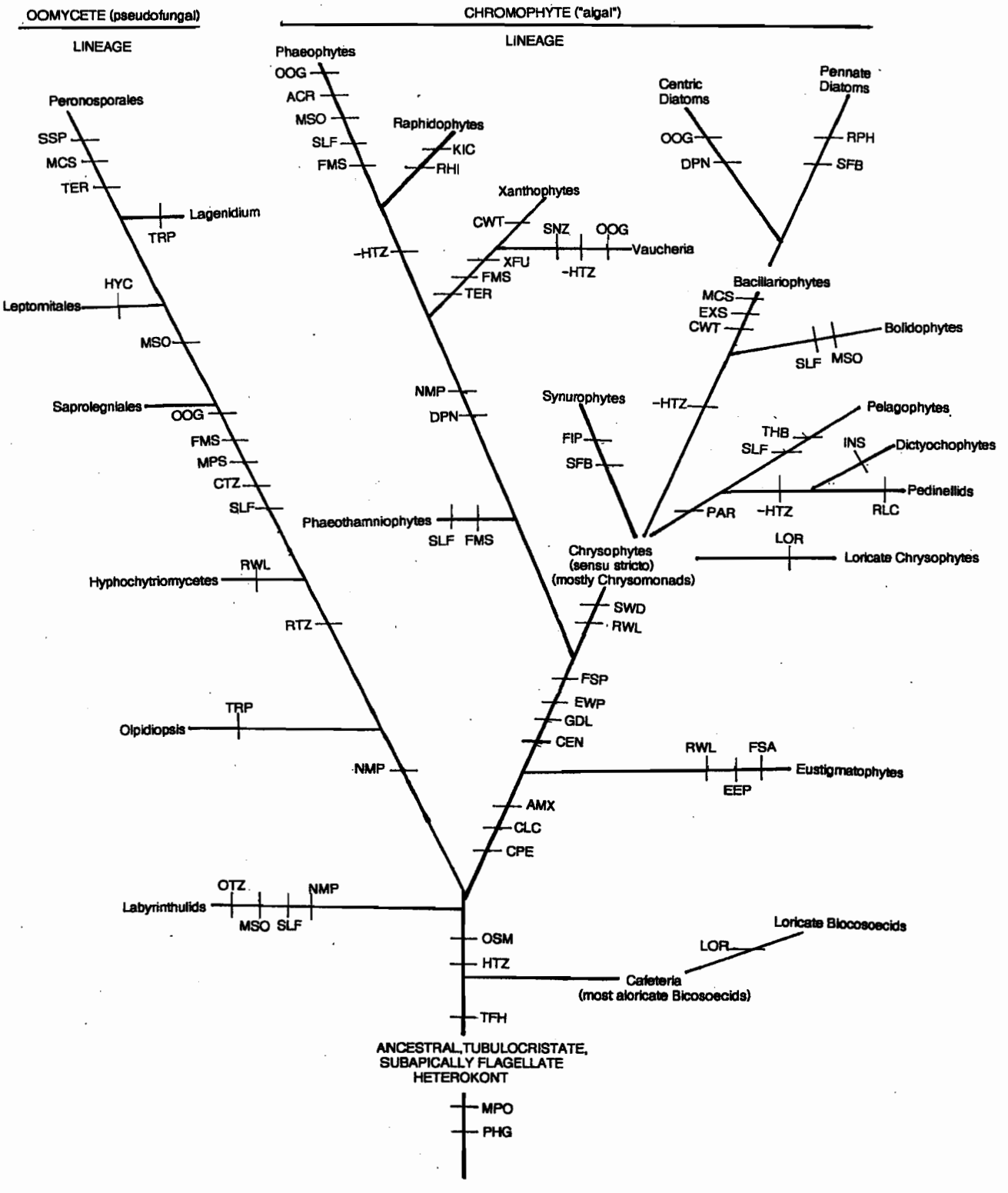

FIGURE 6. Postulate diagram ("platogram") of possible stramenopile group filiation; based on the informally combined input of all sources examined in this study.

For FIGURE 6. Explanatory material: Explanation of diagram ("platogram") of possible filiation of major groups of stramenopiles; based on three major approaches discussed in text: 1. comparative (a. morphological and ultrastructural, and, b. molecular); 2. paleontological; and, 3. ontogenetic.

The comparative approach, both morphological and molecular (not necessarily combined in a particular investigation), is the usual type of investigation seen in the literature on stramenopile phylogeny. Paleontological evidence has not often been included in phylogenetic considerations of stramenopiles, although evidence from the fossil record is indeed available for several major stramenopile groups. The ontogenetic approach, also not often invoked in questions of stramenopile evolution, includes herein our method (utilizing life cycle stages), modified from Nelson's (1978) ontogenetic method of determining character phylogeny, for identifying possible tokogenous (ancestor/descendant) relationships among eukaryotic microbes. Some cladisticians object to combining approaches (e.g., outgroup comparison data with paleontological and/or ontogenetic data) in one analysis. However, our "combined analysis" was merely that of assessing, jointly (and post facto), what the information gained from each of the three approaches has indicated about stramenopile evolution and phylogeny. We then sought to establish an interpretation of putative filiation of stramenopiles based on an awareness of all sets of information. Some published cladograms (or other dendrograms) based on molecular data, depicting stramenopile phylogeny (and filiation sequences), are inconsistent, and at variance with phylogenetic diagrams derived from morphological and ultrastructural evidence. Rather than limit ourselves in yet another computer-generated cladogram, founded on a restricted subset of information (i.e., only those "indisputable" characters and character states which pass present cladistic muster), we developed instead this "platogram" (Fig. 6), or postulate-diagram, based on a mental synthesis of all information and diagrams discussed; though subjective and offered to promote thought and discussion, this "hand-cladogram" is nonetheless inferential from a large, four-fold corpus (counting comparative morphological/ultrastructural, and molecular, investigations, as two of four sets) of accumulated empirical evidence. Molecular and paleontological inputs were strongly represented in constructing our diagram (not necessarily apparent just from the character list for Fig. 6). This "platogram" is intended to represent a common ground, reflective of (but clearly a compromise between) all approaches.

We perceive a fundamentally monophyletic group, stramenopiles (with tubular, usually tripartite, flagellar hairs), in which there was a relatively ancient divergence into a pseudofungal line (Oomycetes and Hyphochytriomycetes) and a chromophytous algal line (e.g., Eustigmatophytes, Chrysophytes, Bacillariophytes, Dictyochophytes, Xanthophytes, Raphidophytes, Phaeophytes). Both lineages (pseudofungal and chromophyte) probably stemmed from a heterotrophic, subapically biflagellate, probably phagotrophic, perhaps Cafeteria-like, monad ancestor. The pseudofungal and chromophyte lineages became separated by nutritional mode difference (i.e., osmotrophy vs. autotrophy). Correspondingly, the "algal" line is structurally demarcated by a "secondary" (probably eukaryotic/eukaryotic) endosymbiosis (endocytosis), resulting in a complex plastid--with four membranes, the outer usually being continuous with the outer nuclear membrane. Our filiation postulate does not require the complete loss of such a plastid (problematical, if connected to the nucleus). In our "phylogeny," there is no impassable concern over which line (pseudofungal or chromophytous) came first, often debated when comparing molecular and ultrastructural presentations. If a choice be required, the slight edge of earlier arrival would go to the chromophytous algae, since the flageller transitional helix (usually single), characteristic of several groups of chromophytes, is considered primitive relative to certain other more complex flagellar transition zone structures (e.g., the "concertina" of Oomycetes). But, with greater antiquity (and putative primitiveness), similarities probably outweighed differences; this is perhaps represented today by the similar morphology of hyphochytrid and eustigmatophyte motile cells (Fig. 4). Both pseudofungal and chromophyte lineages, beyond the point of divergence, underwent an increase in thallus and/or cell (including the cell wall) complexity. The problem of potentially (mutually) competitive subapical flagella (i.e., tinsel vs. whiplash flagellum on the same cell, oriented more or less in the same direction, i.e., "primary" morphology) is solved in essentially the same two ways in both pseudofungal and chromophyte lineages: 1) by shift of the two flagella to a lateral flagellar position (Oomycetes and Phaeophytes), concomitantly increasing the angle between the respective flagellar basal bodies; or, 2) by reduction, obsolescence, or loss of the whiplash ("posterior") flagellum (Hyphochytrids and Eustigmatophytes). Morphological similarities between pseudofungal and chromophyte lineages (e.g., laterally biflagellate zoids of most Oomycetes and Brown Algae; oogamy in Oomycetes and the unusual Xanthophyte, Vaucheria) are probably the result of parallel evolution. Labyrinthulids (including Thraustochytrids) apparently represent an early stramenopile experiment with lateral flagellation.

For FIGURE 6: Character, habit, or habitat trends utilized (in addition to molecular and paleontological data which were considered) in developing the "platogram;" listed by alphabetical abbreviation.

ACR - Both flagella acronemate (with slender, often hair-like tips).
AMX - Autotrophy and mixotrophy.
CEN - Chloroplast endoplasmic reticulum membrane (outer) and nuclear membrane usually connected, including in motile cells, if present.
CLC - Chlorophyll c (plus chlorophyll a, but not b).
CPE - Complex plastids, resulting from endosymbiosis.
CTZ - Double helix or "concertina" (with struts) flagellar transition zone.
CWT - Cell wall of two overlapping parts.
DPN - Trend toward numerous discoid plastids per cell.
EEP - Eyespot (stigma) external to the plastid.
EWP - Eyespot (if present) contained within the plastid.
EXS - "External," rigid, cell wall ("skeletal") support.
FIP - Flagellar insertions nearly parallel to each other.
FMS - Filamentous, multicellular, or multinucleate (siphonous) thallus trend.
FSA - Flagellar swelling (related to eyespot) on the anterior, tinsel (often the only) flagellum.
FSP - Flagellar swelling (related to eyespot), if present, on the posterior flagellum.
GDL - Chloroplast typically with a girdle lamella.
HTZ - Helix-like (usually single) flagellar transition zone.
-HTZ - Loss of transitional helix.
HYC - Hyphal constrictions evident at regular intervals.
INS - "Internal" cell ("skeletal") support, by siliceous rods.
KIC - Distinct kinetochores (centromeres) evident on chromosomes.
LOR - Development of a lorica.
MCS - Motile (flagellated) cell suppression in most or in advanced members.
MPO - Motile cells or zoospores with primary morphology only (subapically flagellate).
MPS - Motile cells or zoospores primary and secondary.
MSO - Motile cells or zoospores of secondary morphology only (laterally flagellate).
NMP - Nuclear membrane persists (at least till late mitosis); mitosis more or less "closed."
OOG - Oogamous sexual reproduction.
OSM - Osmotrophy.
OTZ - Obconic (bell-shaped), ring-like, flagellar transition zone.
PAR - Paraxonemal flagellar rod present.
PHG - Phagotrophy.
RHI - Rhizostyle present.
RLC - Radial (cytological) symmetry around long axis of cell.
RPH - Raphe (and gliding motion) development in some taxa.
RTZ - Ring-like flagellar transition zone. (not obconic).
RWL - Reduction (trend) of whiplash ("posterior") flagellum.
SFB - Siliceous scales or (as the case may be) frustules develop bilateral symmetry.
SLF - Shift (from subapical) to lateral flagellation.
SNZ - Synzoospory (zoospores remain united).
SSP - Trend toward sporangia with only a single spore.
SWD - Trend toward siliceous wall or "skeletal" development.
TER - Increasingly terrestrial.
TFH - Tubular (usually tripartite) flagellar hairs.
THB - Tubular flagellar hairs bipartite.
TRP - Thallus reduction, internal parasitic habit.
XFU - Lack of fucoxanthin, among chromophytous algae.

flagellation in a different way than Oomycetes--by the reduction or elimination of the whiplash (i.e., the "posterior") flagellum. All species of the Eustigmatophyceae, save two, possess just one functional flagellum, the second (probably former whiplash) flagellum usually being represented by only a basal body (Hibberd and Leedale, 1970, 1972; Hibberd, 1990). A number of chrysomonads also exhibit reduction (or functional reduction) tendencies in the whiplash flagellum, e.g., in Anthophysa vegetans (Belcher and Swale, 1972a), in Ochromonas tuberculatus (Hibberd, 1970), and to a greater extent in Chrysococcus rufescens (Belcher, 1969) and Chrysococcus cordiformis (Belcher and Swale (1972b). In fact, in more traditional classifications (cf. Bold and Wynne, 1978), the order Chromulinales of the Chrysophyceae was recognized on the basis of possessing just a single functional flagellum (i.e., the tinsel flagellum), rather than the two "heterodynamic" flagella characteristic of the Ochromonadales (also belonging to Chrysophyceae). However, some species in the Chromulinales were found to have two flagella (cf. Preisig, 1995), although the second (i.e., whiplash) flagellum may be greatly reduced. Members of the Chromulinales and Ochromonadales were, accordingly, usually combined in more recent classifications, variously as Chromulinales (Presig, 1995) or as Ochramonadales (Bold and Wynne, 1985). Interestingly, the Hyphochytriomycetes exhibit a flagellar solution similar to that of chromophytous algae such as Eustimatophyceae; hyphochytrids possess a single anterior tinsel flagellum, the "second flagellum" being represented only by a basal body or centriole (Barr and Allan, 1985; Cooney et al, 1985; Fuller, 1990).

Thus, in this monophyletic, yet perhaps early bifurcating, group of stramenopiles (the pseudofungi and the chromophytous algae), we observe two modes ("alternative solutions," cf. Radinsky, 1987) by which the evolutionary "mistake" of having two counter-acting, more or less apical flagella (placed side-by-side, and attempting to function at the same time) is corrected: (1) by repositioning of the flagella to a lateral position, concomitantly increasing the basal body angle (with one flagellum, i.e., the tinsel flagellum, directed forward, and the other, i.e., the whiplash flagellum, directed backward); consequently, physically separating the disparate functions of these potentially competitive flagella (e.g., Oomycetes; and, independently, Phaeophyceae, among chromophytous algae); or (2) by functionally, even structurally, eliminating one of the two mutually interfering flagella, viz. the whiplash flagellum (e.g, hyphochytrids, and several groups of chromophytous algae). We note that both modes of "correcting the mistake" of competitive subapical flagella occur in the chromophytous algal line and in the pseudofungal line, probably independently; but, we should recall that the entire stramenopilous assemblage appears to be monophyletic (e.g., Leipe et al., 1996; Cavalier-Smith, 1997; Honda et al., 1999). This case of "alternative solutions" within a monophyletic gene pool is perhaps in contrast to more usual cases of "similar alternative solutions" which occur in convergence of relatively unrelated groups (e.g., wings of bats and birds, cf. Radinsky, 1987). However, such a case of comparative morphological solutions (to the same fundamental problem) among stramenopiles seems reasonably analogous to that of "multiple solutions" to a particular problem in groups of cephalopod molluscs (cf. Minkoff, 1983; Moore et al., 1952), in this latter instance the "solutions" involve various morphologically based balancing strategies in an often unstable oceanic water column. Regardless, the connected stramenopilous assemblage of pseudofungi and chromophytous algae--containing subapically biflagellate, laterally biflagellate, and apically or subapically uniflagellate forms--is most likely traceable to a common, biflagellate ancestor, as was suggested independently for Hyphochytriomycetes (Cooney et al., 1985) and for chrysophytes (cf. Hibberd, 1986; Preisig, 1995); on developmental evidence (e.g., Saprolegnia), we assume this ancestor to have been apically to subapically, rather than laterally, flagellate.

An interesting possibility for an organism perhaps resembling the ancestral monad of the chromophyte algal/pseudofungal lineage is the heterotrophic flagellate, Cafeteria roenbergensis, determined to belong to the Bicosoecida (Fenchel and Patterson, 1988; O'Kelly and Patterson, 1996, and recent web-sites). Cafeteria has subapical, heterokont flagellation (with tubular hairs on the more anteriorly directed flagellum); a forked rhizoplast (fibrous flagellar root); three microtubular roots; a single golgi body anterior to the nucleus near the flagella bases; and other features suggestive of "chromulinalean chrysophytes" (O'Kelly and Patterson, 1998 web-site abstract). Cafeteria is a phagotrophic nanoflagellate, consuming bacteria and very small eukaryotes with a temporary cytostome apparatus, or "feeding basket," which would permit intake of plastids if available. Additional taxa of small heterotrophic (often phagotrophic) flagellates have now been described, most appearing to have affinity with basal stramenopile lineages; some are considered relatives of Cafeteria. This particular heterotrophic assemblage is interesting, not only in indicating that basal stramenopiles were "protozoan"-like (Leipe et al., 1996), but in testing certain suppositions found previously in the literature. Cavalier-Smith (1986) suggested that tubular mastigonemes had a "single origin," and, as a structural singularity, are possibly of great

significance as a phylogenetic marker. Such simple (aloricate) bicosoecids as Cafeteria (Fenchel and Patterson, 1988), Acronema (Teal et al., 1998), Siluania (Karpov et al., 1998), and Symbiomonas (Guillou et al., 1999) possess tubular flagellar hairs; but the related Caecitellus (O'Kelly and Nerad, 1998) does not. Subsequent to proposing a single origin of tubular mastigonemes (1986), Cavalier-Smith (1989) posited that "at least a transitional helix was present in the ancestral heterokont....." Where known, the majority of bicosoecids do not have a transitional flagellar helix (or other special transition zone marker); Siluania, however, apparently does exhibit a helical structure in the transition zone (Karpov et al., 1998). The aloricate bicosoecids are, thus, a candidate group among which to search for the origin of tubular flagellar hairs and the flagellar transitional helix. Certain primitive, heterotrophic (usually phagotrophic), stramenopile-like nanoflagellates (not necessarily bicosoecids) appear taxonomically isolated, e.g., Commation (Thomsen and Larsen, 1993) and Pirsonia (Schnepf and Schweikert (1996/97). The human parasite Blastocystis, though lacking flagella, is tentatively placed with primitive stramenopiles on a balance of evidence (Silberman et al., 1996). Other primitive stramenopilous heterotrophs may provide particular clues to ancestry: The very small (picoplanktonic-size) flagellate, Picophagus (Guillou et al., 1999), has been suggested (based on SSU rDNA analyses) for placement at the base of the combined Chrysophyceae and Synurophyceae lineage. Developayella (Tong, 1995) has been determined by similar molecular data to be related to Oomycetes (Leipe et al., 1996); the double helix of the flagellar transition zone of Developayella is also suggestive of a relationship to Oomycetes (Tong, 1995); a similar helical configuration has recently been described in Wobblia (Moriya et al., 2000). The relatively primitive bicosoecid, Acronema, possibly has relationships with both Oomycetes and labyrinthulids (Teal et al., 1998); additionally, the somewhat acronemate (slender-tipped) flagella of Acronema are superficially suggestive of the flagellar morphology of zoids of phaeophytes (cf. Van den Hoek et al., 1995); Acronema, however, is less obviously laterally flagellate than brown algal zoids. Regardless, origins of pseudofungi and chromophytes (and stramenopiles generally) may perhaps be found among such primitive heterotrophs as those we have discussed.

In molecular-based dendrograms, the relative basal position ascribed to labyrinthulids and bicosoecids has been contradictory (cf. Leipe et al., 1994, 1996 vs. Van de Peer and De Wachter, 1997). If we consider that the laterally biflagellate motile cells of labyrinthulids (including thraustochytrids) uniformly possess tubular flagellar hairs on the anterior flagellum, and a distinctive flagellar transition region (an apparently obconic prototype of an Oomycete concertina), we are prone to comparatively consider bicosoecids--usually possessing, but rarely (perhaps significantly) lacking, tubular mastigonemes; and lacking particular internal flagellar transition zone structure, or else with a simple transitional helix--more basal than labyrinthulids. The subapical biflagellation of bicosoecids, e.g., Cafeteria, is also probably primitive. The "advancement" of lateral biflagellation of labyrinthulids was perhaps one of the earlier adaptations, in stramenopile evolution, representing (as we have indicated) a more effective flagellar positioning strategy. This strategy (shift to lateral position of the two flagella, often accompanied by an increased angle described by the two basal bodies, and thus functional flagellar separation) was, as also discussed, exercised more than once during stramenopile radiation, e.g., labryrinthulids, oomycetes, bolidophytes, pelagophytes, phaeothamniophytes, phaeophytes, and "chrysomeridalean" algae. Such a "repetitious" evolution of a similar adaptation (in this case, lateral from apical or subapical flagellation), perhaps independently, but within a fundamentally monophyletic group, is suggestive of the phenomenon of "iterative evolution," discussed by Minkoff (1983) based on cephalopod data of Moore et al. (1952).

Returning to the filiation of the pseudofungal and chromophyte lineages of stramenopiles, a reapplication of Nelson's ontogenetic rule (Nelson, 1978) to the Saprolegnia life cycle--with two morphologically (and to a large extent, functionally) different zoospore stages (Figs. 3; 4A,B)--suggests a stage polarity and, as well, a phylogenetic sequence among stramenopiles. Stage 1 (the subapically flagellate primary zoospore of Saprolegnia) is a form morphologically comparable to the motile cells (subapically to apically flagellate) of many simple chromophytous algae and Hyphochytriomycetes (cf. Fig. 4A,C,D,E). Interpreted thus, most chromophytous algae (clearly excepting brown algae), and also hyphochytrids, possessing only stage 1 in the life cycle, are generally organisms representative of type A (or A') in our proposed motile cell sequence (Fig. 5). Saprolegnia (and several related members of the Saprolegniaceae) would qualify as an organism of type B, in which stage 1 (subapically flagellate primary zoospores) and stage 2 (laterally flagellate secondary zoospores) are both evident, in succession, in the life cycle. To this evolutionary scenario we should add a type C organism (Fig. 5), with stage 2 only; i.e., an organism which produces, as functional zoids, zoospores of secondary morphology only (typically laterally biflagellate). Category C would include advanced members of the Saprolegniales (e.g.,

Dictyuchus, Thraustotheca, Brevilegnia), in which primary zoospores are suppressed, and members of the alleged higher orders of Oomycetes, viz. Peronosporales, Leptomitales, and Lagenidiales, which exhibit no primary zoospore stage. Most Peronosporales, which include such well-known, economically important taxa as Pythium (Lunney and Bland, 1976) and Phytophthora (Ho et al., 1968), exhibit secondary zoospores; however, in some peronosporacean taxa (e.g., Peronospora, Bremia), all motile stages are suppressed. Lagenidialean taxa such as Lagenidium (Gotelli, 1974) and Myzocytium (personal observation) generally present a secondary zoospore morphology (here we recall the mild note of caution of Barr and Desaulniers, 1987, in applying the terms secondary or primary when only one zoospore type occurs in the life cycle). Phaeophycean algae, also with laterally biflagellate zoospores only (category C), probably developed this morphology independently of pseudofungi since, among many other differences, there is no comparability between the flagellar transition zone of brown algae and Oomycetes (cf. Preisig, 1989).

Overall directions of stramenopile filiation suggested, by the above ontogenetic scenario, are: from type A to B to C progressing through the oomycete line (left branch of Fig. 5), and from type A to C progressing to phaeophytes (right branch of Fig. 5). Such is in sync with collective evidence pointing to relatively ancient divergence in this combined (stramenopile) lineage, into generally autotrophic (chromophytous algal) and osmotrophic (pseudofungal) clades (Fig. 6); and is a reasonable match as well with Cavalier-Smith's (1997) proposed, mostly autotrophic phylum, Ochrophyta, and the related heterotrophic phylum, Bigyra--these within his "infrakingdom" Heterokonta. Cavalier-Smith (1997) also recognized (within Heterokonta) a heterotrophic phylum, Sagenista, which is essentially consistent with our basal placement of the heterotrophic groups, bicosoecids (often phagotrophic) and labyrinthulids (osmotrophic), cf. Fig. 6. The simple flagellar transitional helix found in chromophytous algae is probably primitive to the ring-like transitional structure of hyphochytrids, and to the yet more complex concertina of Oomycetes (Cavalier-Smith, 1989). Thus, the secondary endosymbiosis leading to complex plastids and autotrophy in chromophytous algae perhaps slightly preceded the divergence of hyphochytrids, this being followed, in the pseudofungal line, by the sequential steps of evolution of Oomycetes--the Saprolegniales being basal within the Oomycetes per se. Though numerous, relatively minor modifications were required to complete the chromophytous algal lineages (Fig. 6), excepting perhaps the greatly increased complexity of the thallus, and often complex life cycles, of many phaeophytes, and the unusual cell wall systems, wall elaborations and associated specializations (e.g., raphe system) found among bacillariophytes.

In summary, we have in this section (i.e., approach 3) sought to assess what it is that ontogenetic information may tell us about the evolution of stramenopiles, and, correspondingly, about the relationships between, and the filiation of, stramenopile groups. Our efforts here have been relatively limited in examples, if not scope. This is because we focused primarily on organisms exhibiting a distinct, potentially phylogenetically informative, succession of stages in the life cycle. Some emphasis has been given to chrysophyte (sensu lato) life cycles; greater emphasis has been accorded Saprolegnia, as a possibly pivotal organism (and life cycle) in deciphering a plausible polarity of characters and stages, and a putative evolutionary sequence, among stramenopiles. Although our ontogenetic inquiry has been a mostly modest, and largely theoretical, endeavor, it nonetheless rather pointedly indicates that the subapically biflagellate condition among stramenopiles (e.g., Cafeteria, Ochromonas) is primitive to the uniflagellate (reduced) condition, e.g., Bacillariophyceae, Dictyochophyceae; and is also primitive to the laterally biflagellate state (as seen in Oomycetes and in Phaeophyceae). The findings of our ontogenetic analysis are generally supportive of phylogenetic interpretations of Barr (1983), Barr and Allan (1985), Barr and Desaulniers (1989), and Cavalier-Smith (1986, 1989), based primarily on comparative morphological and ultrastructural evidence. Also, these ontogenetic findings are not in the least conflictual with the paleontological evidence considered. Our conclusions, i.e., based on development and life cycles, are to an extent, however, contradictory of molecular-based outcomes (e.g., Leipe et al., 1994, 1996; Potter et al., 1997; Van de Peer and De Wachter, 1997; Honda et al., 1999)--in that, Oomycetes could scarcely be basal to simple, primitive chromophytous algal groups (e.g., chrysomonads); nor, could apparently derived chromophyte groups (e.g., diatoms and silicoflagellates) be primitive in comparison to certain other chromophyte groups not obviously derived (e.g. again, chrysomonads). But, if the timing difference of filiation of possibly primitive pseudofungi (viz. Hyphochytriomycetes) and putatively primitive chromophytous algae (e.g., chrysomonads or eustigmatophytes) is viewed as relatively insignificant (i.e., both lineages perhaps having arisen from a similar organismal pool, in the same general geological time frame), then conflict resolution, between morphological and molecular interpretations of the overall stramenopile lineage, may be achieved (cf. Fig. 6).

This last consideration underscores the cogency of a statement by Cavalier-Smith (1997) that "the Pseudofungi... branch within the Ochrophyta close to the base of the ochrophyte radiation." Cavalier-Smith (1989, 1997) maintained his position that pseudofungi, and certain other colorless heterokonts (e.g., Oikomonas, Cavalier-Smith et al., 1995/96), were derived from primitive chromophytous algae (by chloroplast lost); however, he reversed positions on diatoms (bacillariophytes), considering them first (1986, 1989) as derived (perhaps from chrysomonads or raphidomonads), but later (e.g., 1997) as basal among chromophytous algae. Nonetheless, Cavalier-Smith's ideas of filiation are consistent, in general, with our supposition of a monophyletic but deeply bifurcated stramenopilous lineage, and, as well, a perhaps slightly greater antiquity of the chromophytous (than the pseudofungal) clade. It would indeed appear, as suggested by Cavalier-Smith (1997), that the chromophytes and pseudofungi are clearly connected by ancestry, regardless of the exact timing of the divergence of these particular lineages; and, that the most primitive stramenopiles were heterotrophic (probably phagaotrophic).

SUMMARY AND CONCLUDING STATEMENT

Attempting to determine the relative filiation of stramenopile groups (a major objective of this investigation) eventually led us to a three-fold discovery approach, not unlike that outlined by de Queiroz (1985), and much earlier by Agassiz (1857), viz.: comparative-systematic, paleontologic, and ontogenetic. This came about when we realized that results from the two fundamental comparative systematic approaches, i.e., morphological/ultrastructural (e.g., Cavalier-Smith, 1989) and molecular (e.g., Leipe et al., 1994), were often not in agreement in certain important ways (cf. Figs. 1,2). There is no substantial disagreement with the notion that there are three, general, connected lineages of Stramenopila: a basal, heterotrophic, protozoan-like group (with some phagotrophic members); a mostly autotrophic, algal-like assemblage ("chromophytous algae"); and a heterotrophic, fungal-like group (members of these "pseudofungi" being osmotrophic). As discussed in the text, major questions have centered around the relative filiation position of the two latter assemblages (chromophytes and pseudofungi), and, as well, the filiation order of groups within the chromophytous algae. Some additional consideration has also been given to relative group placement within the basal, heterotroph assemblage. Filiation postulates derived from molecular data were often significantly in conflict with those founded on ultrastructure and morphology. In molecular investigations, Oomycetes and Hyphochytriomycetes (together, the pseudofungi) were generally determined to be basal to chromophytous algae, and, among chromophytes, diatoms were placed basal to chrysophytes (and usually to other chromophyte groups as well). The reverse of this had earlier been deduced from ultrastructural information, with primitive chromophytes considered basal to pseudofungi, and, within chromophytes, chrysomonads (and perhaps other possibly primitive groups, e.g. eustigmatophytes) considered basal to diatoms and most other chromophytous algae. Some additional inconsistencies are found among the various molecular-based dendrograms ("gene trees"), such as the relative position of bicosoecids and labyrinthulids (cf. Leipe et al., 1994, 1996 vs. Van de Peer and de Wachter, 1997).

Since comparative investigations of stramenopiles (or extant organisms in general) are usually divisible into those which are predominantly morphological and those predominantly molecular, we have, in actuality in this study, examined four distinct, yet related, sources of information--here numbered as per text section headings: (1a) morphological/ultrastructural, (1b) molecular, (2) paleontological, and (3) ontogenetic. We have invoked information from both of the last two sources, i.e., the fossil record, and ontogenetic theory (and information), in an attempt to assess, and possibly resolve, inconsistencies in interpretations from molecular data with those previously derived from morphology and ultrastructure. In short, inferences based on both the paleontologic record and on ontogeny are in general more corroborative of filiation proposals derived from morphology and ultrastructure (cf. Fig. 1, after Cavalier-Smith, 1989), than of those based on molecular data (cf. Fig. 2, after Leipe et al., 1994) --as briefly discussed below--although a measure of resolution of morphological and molecular outcomes is plausible.

Paleontological evidence supports the morphologically based view that chrysomonads (chrysophytes) preceded diatoms (bacillariophytes) and silicoflagellates (dictyochophytes) in filiation, and not molecular-based results which generally favor the reverse. The apparent time differential between the appearance of chrysophytes in the fossil record, and that of diatoms and silicoflagellates, is immense (middle to late Proterozoic versus early to middle Cretaceous). However, the reading of the fossil record is presently less conclusive regarding the comparative antiquity of chrysophytes (or chromophytous algae in general), i.e. the generally

autotrophic lineage, and that of Oomycetes, belonging to the osmotrophic (pseudofungal) lineage; both lineages probably trace to the latter Precambrian, but less certainly so for Oomycetes. Thus, if forced to a decision now, the evidence in hand marginally favors an earlier origin of Chrysophyceae than Oomycetes. Future collecting and examination of microfossils may eventually answer this particular question. Unfortunately, however, no certain fossil record is available for Hyphochytriomycetes (nor is it likely to be), which of course, if existent, might bear significantly on the issue of the relative and absolute age of the pseudofungal line.

Ontogenetic observation and reasoning suggest that an organism, such as the oomycete, Saprolegnia--exhibiting a transformation of motile stages (cf. Figs. 3; 4A,B; 5; 6), from motile-cell stage 1 (subapically flagellate) to motile-cell stage 2 (laterally flagellate), in its life cycle--may be interpreted as preceded in filiation by organisms possessing only stage 1 in their life cycles. Possibilities for the precedent type of organism (with only stage 1) are suggested by motile cells of hyphochytrids and putatively primitive types of chromophytous algae (such as chrysomonads and eustigmatophytes), cf. Fig. 4C,D,E. Additional ontogenetic information suggests that simple, biflagellate (heterokont) chrysomonads preceded more complex, ornately silicified, cytologically complex, uniflagellate or aflagellate froms such as diatoms and, the typically uniflagellate, silicoflagellates. However, the apparently primitive, often phagotrophic, aloricate bicosoecids, e.g. Cafeteria (subapically biflagellate, as are primitive chrysomonads such as Ochromonas), are probably precedent to all of these. Saprolegnia may be further interpreted, again extrapolating from life cycle progression, as followed in evolution (cf. Figs. 5, 6) by organisms with only motile stage 2 (laterally biflagellate motile cells), i.e., advanced Oomycetes (e.g., members of the Perenosporales producing motile cells, such as Pythium and Phytophthora). Independently of Oomycetes, the Phaeophyceae (brown algae), also with laterally biflagellate motile cells, appear derived within the chromophytous algal lineage (Figs. 5, 6). That our developmentally founded conclusions indicate that Oomycetes are not likely to be basal to simple chromophytous algae in filiation, is counter to most molecular interpretations of stramenopile lineage sequence (cf. Fig.2, after Leipe et al., 1994).

A reductionist prediction of some years ago, e.g. Schaffner (1969), that biology (including evolution) would eventually be factored down to physics and chemistry (i.e., molecular biology), has not, at least in the case of stramenopiles, yet come to pass. It is very clear from our review that, in deciphering stramenopile filiation, we cannot ignore morphologically based input. In fact, the meaningful integration of molecular and morphological data is "one of the challenges of contemporary phylogenetics" (Marshall, 1992). Cognizant of potential inconsistencies between molecular and morphological information data bases, and, as well, between sets or subsets of molecular data, Sogin and Silberman (1998) stated that "the success of this [molecular] approach is contingent upon whether or not the molecular sequences selected for analysis reflect the historical evolution of the studied organisms," or, similarly, the "biology of the considered organisms" (Sogin et al., 1993). Such statements would seem to imply that we should make the attempt to adapt molecular information to (or at least review such in the light of) our overall understanding of the evolution of various groups--the related implication being, that we should not attempt to found phylogenies just upon molecular data, i.e., simply generate gene trees. In cases of apparently conflicting evidence (i.e., morphological vs. molecular), Doyle (1992) offered the suggestion (seemingly a wise one), that in order to keep from overemphasizing molecular data (especially if contradictory), we should treat such (at least a given type of molecular data, e.g., 16S-like rRNA gene sequences) as a single multistate character, in an overall analysis (perhaps cladistic analysis) involving many kinds of characters: morphological, ultrastructural, physiological, molecular, and so forth. Various authors, e.g., Marshall and Schultze (1992), have stressed, additionally, the importance of incorporating the fossil record (where possible) in evolutionary investigations of extant organisms. Based on our present study, we endorse, furthermore, the idea of infusing ontogenetic information, where feasible, into an investigation of phylogeny. As indicated, we have attempted to assess the bearing of multiple approaches on the understanding of stramenopile evolution. Our filiation diagram (Fig. 6) is purposely not intended as a cladogram; neither should it be received by the reader as a cladogram, in the current (strict) sense of the cladist. Ours is a postulate diagram representing the examination of an extensive literature from several major, distinct, (though perhaps interfacing) sources. As such, this "thought diagram," or "platogram," though based on a great deal of evidence and reflection, is nonetheless, ultimately, a subjective synthesis. It is aimed both at providing new insights into and promoting further thought and research on stramenopile filiation; additionally, there is the goal of improving understanding of, and possibly resolving input (including discrepancies) from, all available information sources. As these are the intentions, we hope indeed that these will be, as well, the effects.

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