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THE EVOLUTION OF PROTOZOA

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1. INTRODUCTION

In considering the evolution of protozoa, two of the first questions to come to mind are 'Where did the first protozoon come from?', and 'How are the 120,000 or so species related to each other?'. We are not yet able to answer either question with any confidence and there is no consensus as to how protozoa evolved. None-the-less, the last 20 years have witnessed a significant accumulation of knowledge. This has allowed some less worthy hypotheses to be rejected, led to new ideas and insights, it has elucidated evolutionary patterns of some groups of protists, and generally created an atmosphere of optimism that an understanding of protozoan evolution is within our grasp. The 'new developments' of the last two decades form the subject of this paper.

Consideration is restricted to the origins and relationships of the major types of protozoa. I treat classification schemes as reflections of prevailing concepts of phylogeny. Taxa lapse as they are shown not to be monophyletic, and entire schemes of classification may be rejected as new information or ideas render them inconsistent with ideas about genealogical relationships.

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2. CHANGING APPROACHES TO THE PROBLEM

The influence of the new developments may be seen by comparing our present understanding with that of the mid-1960's - what we might call the 'traditional understanding'. Most taxonomic treatments of that period categorised protozoa into four types (flagellates, amoebae, sporozoa, and ciliates) - retaining all within a single phylum (e.g., Honigberg et al., 1964). This implied that the protozoa were closely inter-related and that their diversity could be rationalised by assuming four major lineages of evolution. This understanding of the major evolutionary trends among the protozoa had changed little from the days of Bütschli (Bütschli, 1887-1889). Viewed with hindsight, discussions of protozoan phylogeny of this period appear so bereft of appropriate data that even formulating sensible questions appeared difficult (e.g., Kudo, 1966; Raabe, 1964).

The late 1960's witnessed two phenomena which initiated the new developments. The first was the application of ultrastructural techniques to descriptions of protistan diversity (most significantly by Hibberd and Mignot). This approach is still widely used, and protozoa can be classified in robust and informative groupings (see Section 2 below) using results of this technique. As these groupings are stable, electron-microscopy may be said to have replaced an inadequate means of describing protistan diversity (that of light-microscopy) with an adequate means. Ultrastructural (as opposed to light-microscopical or molecular) descriptions are probably effective because protists, as single celled organisms, have primarily undergone adaptive radiation at the level of organelles - and electron-microscopy is the technique best suited to describe the resulting diversity.

The second development came slightly later - being the promulgation and acceptance of the symbiotic theory of the evolution of plastids (Margulis, 1970; Taylor, 1976). Plastid evolution by

this means probably occurred on several occasions, and plastids were also probably lost from a number of lineages. Evolutionary lines have thus crossed over the traditional but artificial boundaries of 'algae' and 'protozoa' on numerous occasions. Protozoan evolution can no longer be considered independent of algal evolution. The consequent adoption of a protistan perspective has removed a major hurdle which prevented a comprehensive picture of protozoan evolution being developed.

Despite these developments, relationships among the major protistan lineages (those with distinctive ultrastructural identities) are still proving difficult to resolve. This is due to an insufficiency of appropriate data rather than being to poor analysis (Smith & Patterson, 1986). More data, either more ultrastructural data, or data of a new kind, are needed. Since the first major synthesis on molecular approaches to the problems of protistan evolution (Ragan & Chapman, 1978), there has been an increase in enthusiasm for the use of molecular data in addressing phylogenetic problems. Emphasis is now placed on sequences of bases in nucleic acids. Until recently, such data have been too sparse to do more than corroborate conclusions from other approaches, but sequence data are now beginning to provide new insights not previously available from ultrastructural studies (Hori & Osawa, 1987; Sogin et al., 1986; Vossbrinck & Woese, 1986).

Palaeontological and stratigraphic studies have not yet provided any significant insights into the origins and evolution of major protistan lineages.

3. ULTRASTRUCTURAL IDENTITIES

The ultrastructural account of protistan diversity has proven to be both robust and informative. The approach is robust in that groups delineated on ultrastructural grounds are subsequently corroborated by data from other techniques. The approach is informative in that it has allowed many more groups of protozoa to

be discriminated than were recognised on the basis of light-microscopy.

The technique is informative not only for systematists. Ecologists benefit from insights into structural adaptation to niches, and cytologists benefit from the greater variety of expressions of various phenomena. The techniques are relatively simple, they generate data on a wide variety of characters, they may be applied to single cells - avoiding the need for cultured material (Patterson, 1985b). Consequently, electron-microscopy often remains the technique of choice in many areas of descriptive protistology.

The varying morphologies of selected organelles form the basis of ultrastructural identities. Most widely used are: the appearance of mitochondrial cristae (which may be bleb-shaped, tubular, discoid, plate-like, or ribbon-like - Patterson & Brugerolle, 1988); nucleation and deployment of spindle microtubules and the behaviour of the nuclear envelope during nuclear division (Heath, 1986); flagella and flagellar anchorage systems (Moestrup, 1980). Other cellular components which exist in varying states include extrusomes (Hausmann, 1978), contractile vacuoles (Patterson, 1980), plastids, cell wall materials, and cytoskeletal materials (Grain, 1987). Ultrastructural identities are based on a part of the complete ultrastructure. Non-discriminatory features such as the ultrastructural appearance of microtubules, and structures showing much variation within a given group, are not used. The ultrastructural identity of different lineages may be based on a differing selection of organelles.

Electron-microscopy, being more informative and more discriminatory than light-microscopy, has revealed a previously unrecorded diversity within the protists. The same approach has revealed relationships among previously remote taxa. I will illustrate these developments in the next sections using specific examples in which I have had a particular interest.

4. THE REVELATION OF DIVERSITY - CRYPTIC PROTISTS

Many of the taxa accepted in the early 1960's have since been shown to contain organisms with widely differing ultrastructural identities, and are therefore believed to be polyphyletic. One of the best documented cases relates to the heliozoa. These are amoeboid organisms, distinguished by having stiff arms radiating from a central body. Ultrastructural studies indicate that the heliozoa are a polyphyletic 'ecological' group, having evolved from several evolutionary sources but adopting the same body form in order to occupy similar evolutionary niches. This is illustrated by a comparison of actinophryid heliozoa and centroheliozoa: members of these groups differ in mitochondrial cristae, extrusomes, the packing patterns and nucleation of the microtubules which support the radiating arms; and siliceous products (Dürrschmidt & Patterson, 1987; Smith & Patterson, 1986). They differ to an extent that they can no longer be seen as being sister groups derived from the same common ancestor (Bardele, 1977, Smith & Patterson, 1986). Adopting similar criteria for other heliozoa, the groups appears to include species derived from six evolutionary sources (actinophryids, centrohelids, desmothoracids, dimorphids, gymnosphaerids, taxopodids).

Stephanopogon is a ciliated organism that was considered to be the most primitive representative of the Ciliophora. This view had to be relinquished as a direct result of ultrastructural studies which showed that S. apogon is very dissimilar to the Ciliophora (Lipscomb & Corliss, 1982; Patterson & Brugerolle, 1988).

These two cases demonstrate that much of the diversity of protists had not been detected by light-microscopy. There are two further processes which are bringing cryptic protists out into the open. The first is the resurrection of unfamiliar taxa which have remained hidden in the literature; and the second is the revelation of previously undescribed taxa from nature.

Generally speaking, taxa resurrected from the literature have

often remained hidden because they are rare and protected from scrutiny. Examples of taxa which have been lifted from literary oblivion to a position of high taxonomic status in virtually a single step include Chlorarachnion (Hibberd & Norris, 1984), and Phalansterium (Hibberd, 1983).

Similarly, many free-living taxa abound in understudied habitats - such as marine sediments (Fenchel & Patterson, 1986, 1988; Larsen & Patterson, 1988; Patterson et al., 1988), and even parasitic protozoa with unassignable patterns of organisation are still being described (Ahne, 1980).

5. TAXONOMIC DEMOLITION AND FOUNDATIONS OF A NEW EDIFICE

Traditional schemes of protozoan classification (e.g., Honigberg et al., 1964) have proven themselves to be inadequate vehicles for the new insights of diversity or of relationships (see below). The period from 1960's to 1980's saw massive taxonomic fragmentation and hierarchical inflation of the protozoa as evidenced by the transition from single phylum schemes to multiphylum schemes (e.g., Krylov et al., 1980; Levine et al., 1980). With the rejection of protozoan and algal perspectives in favour of a integrated protistan approach, the protozoa have become incorporated within 'classifications' of all protists (e.g., Corliss, 1984; Margulis & Schwartz, 1982; Sleigh et al., 1984).

It is not only the taxon 'Protozoa' that is falling into disuse because it is an artificial and therefore misleading concept, but the melding of algae and protozoa, and the electron-microscopical affirmation of cryptic diversity has led to the demise of many familiar taxa. Groups that now have little more than historical curiosity because they do not depict natural relationships, are (within the 'old' protozoa): Protozoa, Sarcomastigophora, Sarcodina, Rhizopoda, Heliozoa, Mastigophora, Phytomastigophora, Zoomastigophora, Sporozoa, and (outside the 'old' protozoa) the Mastigomycotina, and the Chrysophyta (for example).

This uncomfortably destabilising process is accompanied by two constructive trends. The first is that robust natural taxa (those with a common ultrastructural identity) are now recognizable and these can be presented as a list (Table 1). The table is explained below, but '<u>sedis mutabilis</u>' is used to indicate an unresolved polychotomy. The number of problems remaining at an unresolved polychotomy is the number of branches minus two. If our task is to understand the relationships of major groups as defined by ultrastructural identity, then Table 1 would indicate that there are less than 60 major problems of resolving relationships between the members of the list. The number of problems increases as more crytpic diversity is revealed, and decreases as relationships are resolved.

The second cause for optimism is that relationships among taxa which were previously considered obscure, are being resolved.

The opalinids provide an example of this. Members of this group are parasitic organisms from the intestines of cold-blooded vertebrates. As a result of ignorance of their affinities, they were subjected to a process of taxonomic isolation and hierarchical inflation that elevated the Family Opalinidae to phylum status (Patterson, 1985a). Recent re-investigation of opalinids has shown that the cortex and flagella of these protists have much in common with the same parts of proteromonad flagellates - i.e., they have overlapping untrastructural identities (Patterson, 1985b, 1988a). <u>Proteromonas</u> has tripartite tubular hairs either on the flagella or on the body surface, indicating a relationship with heterokont chromophyte algae which also have this character (Patterson, 1988b).

The flagellated parasite <u>Perkinsus marinus</u> (= <u>Dermocystidium marinum</u> = <u>Labyrinthomyxa marina</u>) has cortical alveloli, an apical complex, and micropores similar to those of apicomplexan sporozoa (Perkins, 1976). It is presumably related to these sporozoa. <u>Perkinsus</u> also has flagellar hairs, and these and oter flagellar characters may lead us to identify the flagellate affinities of the

apicomplexan sporozoa.

As these insights are rather fragmentary, there is still no general agreement about the patterns of protistan evolution. However, sufficient information is available to speculate.

6. THE FIRST PROTOZOA AND MAJOR EVOLUTIONARY TRENDS

As chloroplasts almost certainly developed from ingested photoynthetic organisms, it follows that some 'protozoa' existed before any eukaryotic alga. The first protists must have been protozoan, and they were also the first eukaryotes. The problem of resolving the question 'What was the first protozoon?' is not merely a protistological question, but is also a cytological question of great importance ('How did eukaryotic cells evolve?'), thereby addressing what many think of the most dramatic transition during evolution of life - that from prokaryote to eukaryote.

A variety of taxa have been proposed as most closely resembling the first protozoa - usually because they lack one or more features that are found in the majority of eukaryotic cells. Raikov (1978) and Dodge (e.g., 1971) hold that dinoflagellates are the most primitive eukaryotes primarily because the nuclei lack histones. Members of this taxon have a full complement of other eukaryotic organelles making them unlikely to be primitive - an interpretation corroborated by sequence data (Hori & Osawa, 1986). Margulis has long argued that the microaerophilic amoeba Pelomyxa palustris is the most primitive eukaryote - arguing that it lacks mitosis, mitochondria, and flagella (Margulis & Sagan, 1986). Early descriptions of this amoeba did include accounts of mitosis, and the species does have flagella (Griffin, 1979). Indeed has enough in common with the mastigamoebae - particularly in anchorage of flagella and the presence of similar endosymbionts (van Bruggen, 1986) - to justify their joint inclusion within one group - as part of the Archamoebae of Cavalier-Smith, 1987; or as the totality of the Pelobiontida -Table 1. The origin of the Pelobiontida must have

followed the origin of flagella. Cavalier-Smith (1981) favoured the 'higher' fungi as the most primitive eukaryotes because they lack flagella. Others (e.g., Möhn, 1984) used the same argument to grant the red algae the honour of representing the most primitive eukaryotes. Neither of these ideas now attract much support. Recently Cavalier-Smith (1986, 1987) has favoured anaerobic protozoa (his Archezoa) as being primitive. One group of Archezoa, the Microsporidia, are now also favoured on molecular grounds (Vossbrinck & Woese, 1986; Vossbrinck et al., 1987). This proposal is the most attractive from a cytological perspective, as microsporidia lack a variety of eukaryotic organelles (such as flagella, mitochondria, dictyosomes, etc.) (Larsson, 1986). Approaching the problem from the other end, but also using molecular tools, it has been proposed that the eukaryotes stem from thermophilic sulphur-metabolizing prokaryotes (Lake, 1986; Wolters & Erdmann, 1986).

Figure 1 presents a possible skeletal scenario of evolution based on microsporidia as the earliest identifiable lineage of eukaryotes. Clearly, the first eukaryotes did not look like modern-day microsporidia - all of which are obligatory parasites of eukaryotes. They would have been free-living organisms, distinguishable from prokaryotes by having an endomembrane system and a mitotic apparatus. They probably obtained nutrients by absorption (given that parasitic microsporidia show no signs of pinocytosis or phagocytosis - Canning & Lom, 1986;

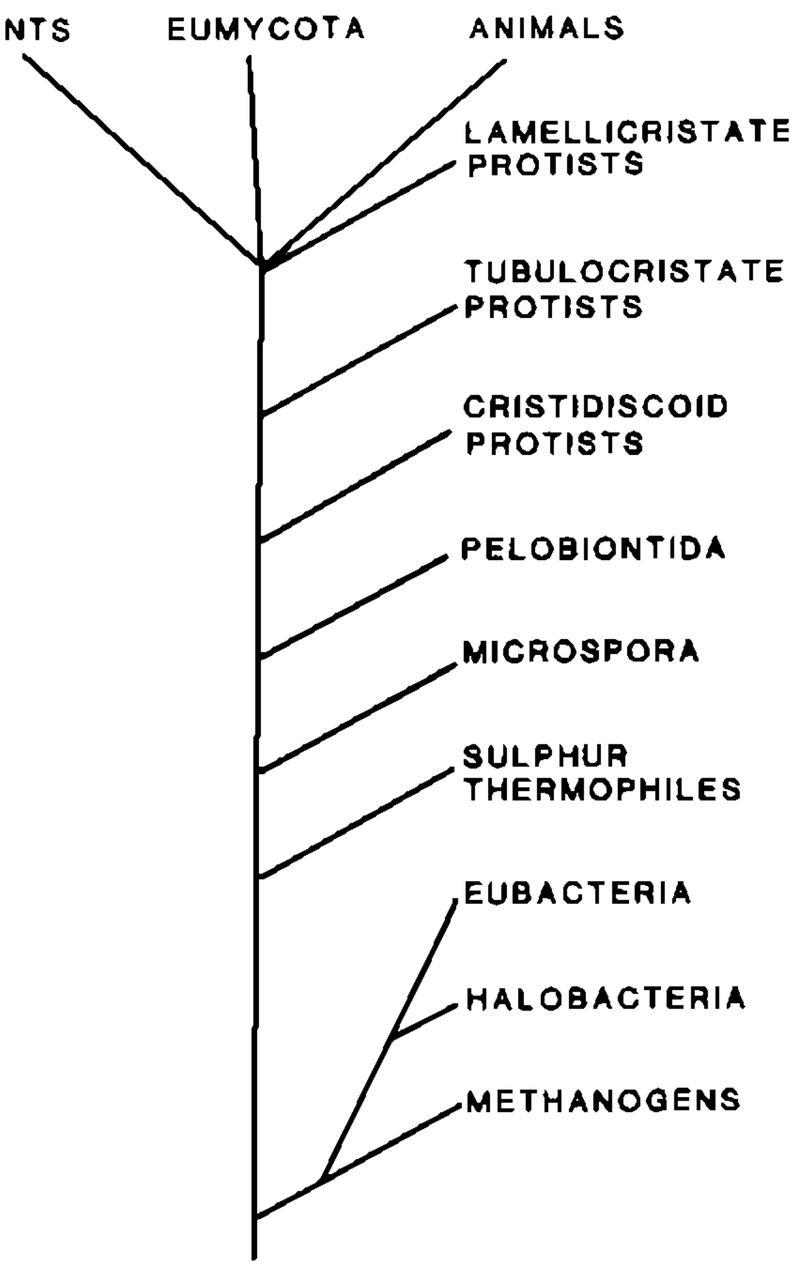

Fig. 1. Hypothetical sequence of derivation of major groups

Larsson, 1986). It seems probable that early stages of evolution were characterized by an enhancement of cytotic activity. This would permit phagocytosis, and facilitate the acquisition of symbionts - a process critical to the evolution of protists. It would also be expected to lead to fragmentation of the endomembrane system and the segregation of membrane-bound compartments. I suggest that the next identifiable line of evolution is that giving rise to the Pelobiontida, marked particularly by the development of flagella, but also containing symbiotic bacteria to supplement metabolic pathways. Given that microtubules were already in use by cells during nuclear division, it is likely that the flagellum derived from the mitotic apparatus and did not involve a symbiotic event as suggested by Margulis (e.g., Margulis & Sagan, 1986). The acquisition of mitochondria was most likely by symbiosis (Gray & Doolittle, 1982). There is little agreement as to what happened next in the cristate (with mitochondria) protists (e.g., Kumazaki et al., 1983; Sogin et al., 1986; Wolters & Erdmann, 1986). None-the-less, most recent publications tend to favour the taxa with discoid cristae (represented by the Kinetoplastida and Euglenida) as the first representatives of cristate eukaryotes. Later, taxa having tubular cristae separate (some suggest this involved an independent symbiosis - Stewart & Mattox, 1984). Early within the tubulocristate lineage came the appearance of tripartite tubular hairs on the flagella. This combination provided the means for extensive diversification leading to the organisms referred to as stramentopiles of Table 1. Ciliates and dinoflagellates form part of the tubulocristate protists - but their locations, like that of the Lobosea (amoebae), are unclear. The remaining protists were those with flattened plate-like or ribbon-like cristae, from which (sequence data suggest) the familiar multicellular groups derive.

This scenario is only one of the many that may be

constructed on the basis of available evidence. Given the uncertainty over what really happened, hypotheses like these should not be used as the basis for classification schemes. We must recognise that any classification scheme will be held to represent prevailing views on relationships, and we therefore have the problem of how best to classify protists while in ignorance of their true relationships.

6. TOWARDS A NEW CLASSIFICATION

A scheme of classification should aspire to be comprehensive, rational, stable, and - most importantly - natural (= phylogenetic). No ideal system for protists can be attained at this time; our ignorance precludes a natural system, and the continued acquisition of knowledge creates instability. Schemes presently available predictably reveal undesirable traits of incompleteness, instability, or irrationality; but in my view some of these traits are not necessary.

Partisan achemes that consider only algae or only protozoa (Levine *et al.*, 1980) are no longer acceptable as they misrepresent present knowledge and understanding, and cannot be phylogenetic.

Of the protistan schemes, those of Möhn (1984) or Cavalier-Smith (1981, 1983, 1986) are particularly idiosyncratic in that many groupings are based on hypotheses - not knowledge. This renders them unstable and unpalatable. The defence that hypotheses are the only option when in a state of ignorance is not - in my view - defensible (see below).

Other schemes (e.g., Corliss, 1984; Krylov *et al.*, 1980; Margulis & Schwartz, 1982; Sleigh *et al.*, 1984) minimise the hypothetical element and present the diversity primarily in the form of a list of high ranking taxa. Generally, these schemes are little more than a crude amalgam of phycological and protozoological schemes. They are incomplete (much of previously

cryptic diversity is not revealed), and they are inaccurate (all schemes referred to above retain a taxon for all heliozoa, whereas the view that this group is polyphyletic has been long held - Bardele, 1977). In such schemes, ideas of relationships are generally obscured by the apparently arbitrary assignment of rank, and the lack of an effective hierarchical structure.

The last cited type of classification is usually acceptable to the biological community, as it has much in common with traditional schemes. However, lack of detail makes the inclusion of new taxa difficult, and the lack of an effective hierarchical structure means that schems such as these have no effective mechanism for incorporating changing perceptions of relationships. Yet it is these two trends which characterise, and will continue to characterise, systematic protistology. A good taxonomic scheme should be designed to accommodate both of these developments.

One further issue deserves attention, that of rank and hierarchical structure. Hierarchical structure is used to depict relationships, rank (at best) indicates some kind of subjective measure of distinctiveness. The intrusion of discriminatory techniques causes greater variety to be perceived and this leads to inflation of rank. As rank is also used to indicate position within the hierarchy, such inflation destabilises the entire classification. This element of destabilisation can be avoided by avoiding the use of rank, or restricting its use to somehow indicating phylogenetic relationships.

It is my view that our concepts of protistan diversity are undergoing changes of such magnitude that major taxonomic changes are to be expected, and these should be preceeded by a discussion of the means of depicting diversity.

The primary (and in situations of conflict - the dominant) requirement is that taxa should be monophyletic. This has several consequences. Where relationships are not known, this ignorance

should be accurately depicted - and not misrepresented. Ultrastructural heterogeneity within a group may be used as an initial criterion for polyphyly. The second requirement of the system is that it must be flexible enough to reflect new concepts of relationships. Finally, the system must be capable of accommodating new types of organization without any profound disturbance to the overall structure.

Phyletic sequences satsify these demands (Patterson 1985a), and here the conventions indicated in that paper are applied to a broader range of protists (Table 1). Ranks are not an essential part of such a scheme and have been excluded.

Table 1 is incomplete and is included primarily for the purposes of discussion. It emphasises those taxa with distinctive ultrastructural identities. Many minor taxa distinguished primarily by light microscopy alone, and often monotypic, have not been included. Taxa in parentheses are to be ignored and are included for ease of 'reading'. Inverted commas indicate paraphyletic groups, and taxa that may be derived from multicellular animals are indicated by question marks. Some associations suggested here are not widely accepted, Some are new but are introduced for the purposes of discussion. Diagnosis of novel combinations are not offered, their characteristics may be established from their composition.

In a phyletic sequence, each group is the sister-group to all taxa following at the same level of indentation. Relationships within a side-branch of evolution are indicated as an indented sequence. This kind of scheme can faithfully depict current ideas about relationships. It also can faithfully depict ignorance - for example by using '<u>sedis mutabilis</u>' to indicate an unresolved polychotomy. A scheme like this can be made more detailed to include smaller groups of species - and so may be comprehensive. As new taxa are recognised (whether species or larger assemblages), they may be included within the list at the

TABLE 1. Major groups of protists distinguished by ultra-structural identity, grouped by phyletic sequence

Microspora
Pelobiontida
Mastigamoebidae
Pelomyxidae
Acantharea sedis mutabilis
Apicomplexa sedis mutabilis
Perkinsidae
Sporozoea
Aulacomonas sedis mutabilis
Apusomonas sedis mutabilis
Centrohelida sedis mutabilis
Cercomonas sedis mutabilis
Chlorarachnion sedis mutabilis
Choanozoa sedis mutabilis
Choanoflagellida
Porifera
Chytridiomycetes sedis mutabilis
Ciliophora sedis mutabilis
Colponema sedis mutabilis
Cristidiscoidida sedis mutabilis
Neucleariidae
Pompholyxophryidae
Cryptophyta sedis mutabilis
Desmothoracidae sedis mutabilis
Dimorphidae sedis mutabilis
Dinophyta sedis mutabilis
Ebriida sedis mutabilis
Eumycetozoa sedis mutabilis
Protostelidae
Myxomycetozoa
Gymnosphaerida sedis mutabilis
Haplospora sedis mutabilis
Lobosea sedis mutabilis
Centramoebidae sedis mutabilis
Acanthopodina sedis mutabilis
Dictyosteliidae sedis mutabilis
Stereomyxidae sedis mutabilis
Euamoebida sedis mutabilis
Himatismenida sedis mutabilis
Leptomyxida sedis mutabilis
Testacealobosea sedis mutabilis
Metamonadida sedis mutabilis
Retortomonadida
Diplomonadida
?Myxozoa? sedis mutabilis
Oxymonadida sedis mutabilis
Parabasalia sedis mutabilis
Monocercomonadidae
Trichomonadida
Hypermastigida
?Paramyxea? sedis mutabilis
Phaeodarea sedis mutabilis
Phalansterium sedis mutabilis
Plasmodiophoromycetes sedis mutabilis
Polycystinea sedis mutabilis
Prodiscea sedis mutabilis
Heterolobosea sedis mutabilis
Schizopyrenida
Acrasida
Stephanopogon sedis mutabilis

CONTINUED ...
(Stephanopogon sedis mutabilis)
Euglenozoa sedis mutabilis
Kinetoplastida
Plicostomatida
Diplonema
Euglenida
Prymnesiophyta sedis mutabilis
Pseudodendromonadidae sedis mutabilis
Pseudodendromonas
Cyathobodo
Rhodophyta sedis mutabilis
Spongomonadidae sedis mutabilis
Spongomonas
Rhipidodendron
Stramentopila sedis mutabilis
Actinomonadida sedis mutabilis
Pedinellales
Actinophryida
Bicosoecida sedis mutabilis
Pseudobodonidae
Bicosoecidae
Chrysophyta sedis mutabilis
Bacillariophyceae sedis mutabilis
Chrysophyceae sedis mutabilis
Ochromonadales
Sarcinochrysidales
Phaeophyta
Eustigmatophyta sedis mutabilis
Microglena sedis mutabilis
Paraphysomonadaceae sedis mutabilis
Pelagococcus sedis mutabilis
Raphidophyceae sedis mutabilis
Rhizochromulina sedis mutabilis
Stylococcaceae sedis mutabilis
Synuraceae sedis mutabilis
Xanthophyta sedis mutabilis
Granuloreticulosea sedis mutabilis
Labyrinthulacea sedis mutabilis
Thraustochytrididae sedis mutabilis
Diplophrys sedis mutabilis
Labyrithulidae sedis mutabilis
Sloomycota sedis mutabilis
Oomycetes sensu stricto
Hyphochytridiomycetes
Slopalinida sedis mutabilis
Proteromonadidae
Opalinidae
Thaumatomastixidae sedis mutabilis
Thaumatomastix
Protaspis
Vampyrellida sedis mutabilis
Arachnula
Vampyrella
Viridiplantae sedis mutabilis
Prasinophyceae
Chlorophyceae
Xenophyophorea sedis mutabilis

appropriate level of indentation, without any significant change to the overall structure of this list. Newly perceived relationships can be indicated by erasing the '<u>sedis mutabilis</u>' and/or moving the taxon to the appropriate place in the list.

This scheme has the drawback that it is not suitable for teaching diversity of protists to novices. It is inherently ambiregnal (incorporates taxa that fall under the jurisdiction of the botanical code with others that fall under the zoological code) - a situation that is a source of suite of problems which still need to be addressed (Patterson, 1986). Phyletic sequencing without ranks also runs counter to some requirements of the nomenclatural codes - especially with regard to typification of suprafamilial taxa.

8. <u>CONCLUSIONS</u>

The last twenty years have seen the problem of protistan evolution move from a trivial to a demanding issue, and seen it develop from a vague question to a series of exact questions. We may now look forward to a period during which ultrastructural and molecular approaches work in partnership to resolve those questions. Lineages can be identified because of a common ultrastructural identity, members of different lineages can be distinguished because of discontinuities in patterns ultrastructural organisation. Each discontinuity represents a problem of relatedness that has yet to be resolved. Much of protistan diversity still awaits description by electron-microscopy. The acquisition of these data will reveal some intermediate states of organization and so resolve some problems of relationships. Other problems (unresolved polychotomies) will emerge or will resist resolution with ultrastructural information, and we may expect that these remaining areas of ignorance will be specifically addressed using the increasingly sophisticated molecular techniques.

Evolutionary protistology has moved into a phase of achievement and optimism. Because of the enormous extent of the errors embedded within traditional approaches, new insights will be very destabilising. However, we should not be misled into believing that the change is random. Change is directed towards creating hypotheses that are more widely accepted because they are more in accord with observations. We are fortunate to be contemplating dramatic changes at a time when we have a rich variety of evolutionary theory at our disposal and can experiment in the search for the classificatory structure that is best able to meet the 'new developments'.

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