

Zooflagellate Phylogeny and the Systematics of Protozoa

T. CAVALIER-SMITH

Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

In the six kingdom system of life, the kingdom Protozoa occupies a pivotal position between the ancestral kingdom Bacteria and the four derived eukaryotic kingdoms, Animalia, Plantae, Fungi, and Chromista (1). The diversification of the protozoans is fundamentally important for understanding both the early evolution of eukaryotes as a whole and the origins of these higher kingdoms. Recent advances in molecular phylogeny have led to many changes from the earlier protozoan system (2). Archezoa are now treated, not as a separate kingdom (2, 3), but as a subkingdom of the Protozoa, comprising only the two zooflagellate protozoan phyla, Metamonada and Parabasalia (1), which have no mitochondria and are microaerophilic or anaerobic. Parabasalia are clearly secondarily amitochondrial, since their hydrogenosomes probably evolved from mitochondria by the loss of cytochromes and DNA; protein phylogeny ((4); Hasegawa, pers. comm.) suggests that metamonads are also secondarily amitochondrial.

The kingdom Protozoa is currently divided into 13 phyla, of which eight contain zooflagellates (Table I; fuller details of this revised classification are in refs. 1, 5). Microsporidia have been removed from Protozoa and transferred instead to the Fungi; several protein phylogenies suggest that their deep divergence on rRNA trees is a gross artifact of an idiosyncratically high rate of rRNA evolution.

The first eukaryotes were probably zooflagellates similar to the enteromonad or the retortomonad metamonads. The basic backbone of the protozoan phylogenetic tree consists of zooflagellates (phagotrophic flagellates with no plastids) or amoeboid flagellates. All other Protozoa, such as rhizopods,

sporozoans, and ciliates, are derived from them, as are all eukaryotic algae (by the symbiogenetic origin of chloroplasts, or their lateral transfer by secondary symbiogeneses, as in the origin of chromists and chlorarachnean algae) and the four higher eukaryotic kingdoms. Some zooflagellates, notably in the Chromista and Dinoflagellata, have arisen secondarily by independent losses of plastids. Multiple losses of cilia, mitochondria (or their conversion to hydrogenosomes), and peroxisomes have also been important in eukaryote evolution. Spliceosomal introns are currently known only in the more advanced subkingdom Neozoa.

The earliest diverging aerobic phyla on rRNA trees are the Percolozoa and Euglenozoa, both of which have discoid mitochondrial cristae and are therefore grouped as infra-kingdom Discicristata. The order of their divergence is still uncertain, but is important in relation to the origin of the *trans*-splicing that characterizes nuclear genes in Euglenozoa, and stands in contrast with the *cis*-splicing of Percolozoa and the nine higher protozoan phyla. Of these higher phyla, only two consist almost exclusively of flagellates: Dinozoa, which has a mix of zooflagellates and phytoflagellates; and the purely heterotrophic and largely zooflagellate Neomonada (3). Two other phyla with many amoebae (Amoebozoa, typically with lobose pseudopods; and Cercozoa (1, 5) with usually filose or reticulate pseudopods) also include some zooflagellates. The infraphyla Mycetozoa and Archamoebae, grouped together as subphylum Conosa within the Amoebozoa, usually diverge on rRNA trees below the megakaryote radiation (*i.e.*, the explosive radiation in which animals, fungi, plants, and chromists diverged). But most protein trees place Mycetozoa close to the opisthokonta (animals, fungi, and Choanozoa). Several protein trees suggest that the Discicristata may also be placed too low on rRNA trees and may have been part of, rather than occurring prior to, the megakaryote radiation.

The parasitic or predatory zooflagellates *Perkinsus* and

This paper was originally presented at a workshop titled *Evolution: A Molecular Point of View*. The workshop, which was held at the Marine Biological Laboratory, Woods Hole, Massachusetts, from 24–26 October 1997, was sponsored by the Center for Advanced Studies in the Space Life Sciences at MBL and funded by the National Aeronautics and Space Administration under Cooperative Agreement NCC 2-896.

Table I

Revised classification of the Kingdom Protozoa

Subkingdom 1. Archezoa*

- Phylum 1. Metamonada
 - Subphylum 1. Eopharyngia (e.g., *Giardia*, *Trepomonas*, *Chilomastix*, *Retortamonas*)
 - Subphylum 2. Axostylaria (e.g., *Oxymonas*, *Pyronympha*)
- Phylum 2. Parabasalia (e.g., *Trichomonas*, *Trichonympha*)

Subkingdom 2. Neozoa*

- Infrakingdom 1. Discicristata
 - Phylum 1. Percolozoa
 - Subphylum 1. Tetramitia (e.g., *Percolomonas*, *Lyromonas*, *Naegleria*, *Tetramitus*)
 - Subphylum 2. Pseudociliata (*Stephanopogon*)
 - Phylum 2. Euglenozoa
 - Subphylum 1. Plicostoma (e.g., *Diplonema*, *Euglena*, *Petalomonas*, *Peranema*)
 - Subphylum 2. Saccostoma (e.g., *Bodo*, *Rhynchomonas*, *Trypanosoma*, *Leishmania*, *Postgaardi*)
- Infrakingdom 2. Alveolata Cavalier-Smith 1991
 - Superphylum 1. Miozoa Cavalier-Smith 1987
 - Phylum 1. Dinozoa*
 - Subphylum 1. Protalveolata* (e.g., *Colponema*, *Ellobiopsis*, *Colpodella*, *Perkinsus*)
 - Subphylum 2. Dinoflagellata (e.g., *Noctiluca*, *Cryptothecodinium*, *Amphidinium*)
 - Phylum 2. Sporozoa (syn. Apicomplexa Levine 1970)
 - Subphylum 1. Gregarina (e.g., *Monocystis*)
 - Subphylum 2. Coccidiomorpha (e.g., *Eimeria*, *Sarcocystis*, *Paramyxa*, *Plasmodium*, *Babesia*, *Haplosporidium*, *Minchinia*)
 - Subphylum 3. Manubrispora (*Metchnikovella*, *Amphiacantha*, *Amphiamblys*)
 - Superphylum 2. Heterokaryota
 - Phylum Ciliophora
 - Subphylum 1. Tubulicorticata (e.g., *Loxodes*, *Stylonychia*, *Colpoda*)
 - Subphylum 2. Epiplasmata (e.g., *Tetrahymena*, *Paramecium*, *Vorticella*)
 - Subphylum 3. Filocorticata (e.g., *Spathidium*)
- Infrakingdom 3. Sarcomastigota
 - Phylum 1. Foraminifera
 - Subphylum 1. Monothalamia (e.g., *Allogromia*)
 - Subphylum 2. Polythalamia (e.g., *Ammonia*, *Globigerina*)
 - Phylum 2. Cercozoa (Cavalier-Smith 1998a; called Rhizopoda in Cavalier-Smith 1997)
 - Subphylum 1. Phytomyxa (e.g., *Plasmodiophora*)
 - Subphylum 2. Reticulofilosa (e.g., *Chlorarachnion*, *Biomyxa*, *Gymnophrys*)
 - Subphylum 3. Monadofilosa (e.g., *Cercomonas*, *Bodomorpha*, *Heteromita*, *Thaumatomonas*, *Allas*, *Metromonas*, *Euglypha*, *Paulinella*, *Spongomonas*, *Rhipidodendron*)
 - Phylum 3. Amoebozoa
 - Subphylum 1. Lobosa (e.g., *Amoeba*, *Acanthamoeba*, *Arcella*, *Diffugia*, *Multicilia*)
 - Subphylum 2. Conosa [infraphyla Archamoebae (e.g., *Pelomyxa*, *Mastigamoeba*, *Phreatamoeba*, *Entamoeba*) and Mycetozoa (e.g., *Protostelium*, *Physarum*, *Dictyostelium*)]
 - Phylum 4. Neomonada*
 - Subphylum 1. Apusozoa* (e.g., *Apusomonas*, *Ancyromonas*, *Ebria*, *Jakoba*, *Histiona*, *Reclinomonas*)
 - Subphylum 2. Isomita (e.g., *Phalansterium*, *Kathablepharis*, *Nephromyces*)
 - Subphylum 3. Choanozoa* (e.g., *Monosiga*, *Diaphanoeca*, *Corallochytrium*, *Dermocystidium*, *Ichthyophonus*, *Ministeria*, *Nuclearia*)
- Infrakingdom 4. Actinopoda
 - Phylum 1. Heliozoa (e.g., *Actinophrys*, *Acanthocystis*)
 - Phylum 2. Radiozoa
 - Subphylum 1. Spasmaria (e.g., *Acanthometra*, *Sticholonche*)
 - Subphylum 2. Radiolaria (e.g., *Thalassicolla*, *Aulacantha*)

* Probably paraphyletic.

Colpodella are now placed in the subphylum Protalveolata of the Dinozoa, and not with the Sporozoa. The Sporozoa are once again restricted to nonflagellate endoparasites, so the name Apicomplexa is abandoned. Metchnikovellids, because of their sporozoan-like cortical alveoli, are transferred from Microsporidia to Sporozoa, as the subphylum

Manubrispora; they are probably yet another example of the secondary loss of mitochondria and peroxisomes.

The recently created Neomonada comprise three subphyla: Choanozoa (choanoflagellates, *Corallochytrium*, and the parasitic ichthyosporeans, all with predominantly flat cristae); Apusozoa (*Apusomonas* and its biciliate anisokont

relatives, such as *Ancyromonas*); and Isomita (isokont usually biciliate zooflagellates). Apusozoa and Isomita include many of the tubulicristate zooflagellates that were formerly included in the Opalozoa. The latter taxon has been discontinued, because the Opalinata were transferred to the Heterokonta within the kingdom Chromista, and the sarcomonad flagellates (6) and numerous others (e.g., *Spongomonas*) were removed to the Cercozoa. In addition to the sarcomonads, the new phylum Cercozoa includes many filose amoebae, chlorarachnean algae, the reticulose *Gymnophrys*, *Plasmodiophora*, and a variety of zooflagellates (e.g., *Bodomorpha*, *Allas*) not previously classified. Cercozoa are a major novel clade within the megakaryote radiation and, due to their phyletic distinctiveness, might hold many surprises for cell and molecular biologists if they were seriously studied. Cercozoans, though severely neglected organisms, are very diverse and are of major ecological importance in soils, as well as in marine and freshwater habitats.

I have discussed here some of the recent molecular and ultrastructural evidence that supports the substantial changes to the high-level classification of the kingdom Protozoa and the phylogenetic ideas that underlie them. Ongoing molecular and ultrastructural studies will probably necessitate still further redistribution of taxa among the 13 phyla of the present protozoan system and their 26 subphyla. Despite the phylogenetic distinctiveness of the two groups, the phenetic boundary between the Neomonada and Cercozoa is particularly unclear, as are the affinities of the

various heliozoans. Though future studies may also require the transfer of a few minor taxa from the Protozoa to the kingdom Chromista, the circumscription of the kingdom Protozoa as a whole will probably be more stable in the future than in the recent past. However, the branching order of the eight non-alveolate neozoan phyla relative to each other and to the alveolates, plants, and chromists is very uncertain and will be difficult to establish. The ancestral relationship between the neomonad subphylum Choanozoa and the animals and fungi, however, appears to be relatively solid.

Literature Cited

1. Cavalier-Smith, T. 1998a. A revised six-kingdom system of life. *Biol. Rev.* 73: 203–266.
2. Cavalier-Smith, T. 1993. Kingdom Protozoa and its 18 phyla. *Microbiol. Rev.* 57: 953–994.
3. Cavalier-Smith, T. 1997. Amoeboflagellates and mitochondrial cristae in eukaryote evolution: megasystematics of the new protozoan subkingdoms Eozoa and Neozoa. *Arch. Protistenkd.* 147: 237–258.
4. Roger, A. J., S. G. Svård, F. D. Gillin, and M. L. Sogin. 1998. Protists without mitochondria: an update on the status of the Archezoa hypothesis. *J. Eukaryot. Microbiol.* 45: 5A.
5. Cavalier-Smith, T. 1998b. Neomonada and the origin of animals and fungi. Pp. 375–407 in *Evolutionary Relationships Among Protozoa*, G. H. Coombs, K. Vickerman, M. A. Sleigh, and A. Warren, eds. Chapman & Hall, London.
6. Cavalier-Smith, T., and E. E. Chao. 1997. Sarcomonad ribosomal RNA sequences, rhizopod phylogeny, and the origin of euglyphid amoebae. *Arch. Protistenkd.* 147: 227–236.

Discussion

PALMER: When do you think eukaryotes arose?

CAVALIER-SMITH: I would think between about 600 and 1600 million years ago.

PALMER: On what evidence do you base this?

CAVALIER-SMITH: The fossil record. I don't think there is any other source of evidence. About 1600 million years ago there was a huge increase in cell size. Perhaps that change marked the origin of eukaryotes from bacteria. But between that event and the beginning of the Cambrian, about 550 million years ago, there's not a single group of fossils that can be positively identified with any extant eukaryote group. The protist fossil record is consistent with that of animals and plants in suggesting that there was a Cambrian explosion. Basically I equate that with the big diversification of what I am calling Neozoa plus the four higher kingdoms. I think that was about six hundred million years ago. If there really were some earlier groups, they could go back in the period

between 600 million years and 1600 million years ago. There are large fossils that cannot be equated with any modern group: they might have been early eukaryotes, but we just don't really know what they were.

GUERRERO: Gonzalo Vidal put the origin at 1600–800 million years ago.

CAVALIER-SMITH: That is based on that large cell size argument. Some of those early fossils have an increased complexity that might have had something to do with the endomembrane system and secretion of complicated surface structures. I tend to favor an origin for eukaryotes that is earlier than 600 million years ago, but exactly when I wouldn't really know. Probably my more serious guess would be between 1000 and the date that you gave.

ELLINGTON: How do you choose which characteristics to study? Why is it that the number of membranes that a bacterium has is

much more important that the entire chemical composition of that membrane?

CAVALIER-SMITH: Whether the number is more important or less is debatable. That is one reason why, at one time, I did accept Archaeobacteria as a separate kingdom rather than just as an infrakingdom. There is not a hard and fast answer to your question. The number is important because it is very conservative in evolution. If something is very conservative and correlates with other characters, then one attributes great importance to it; so that character correlates with, for instance, the molecular trees on the whole. Each of the major clades on the ribosomal RNA tree has either one membrane or two. Therefore, that number hasn't changed within a clade; it changes very rarely.

Another problem is how the number of membranes changes. What mutational mechanism might lead to such a change? I've put forward a mechanism that would reduce the number from two to

one: that is, producing more peptidoglycan between the membranes and throwing off the outer one. However, no one, as far as I know, has suggested a biophysical mechanism that would create a second membrane on the outside of the initial one, while meeting the need to target proteins and lipids. Concerted evolution of many components is necessary to create two membranes rather than one.

JAHNKE: Regarding the timing of the origin of eukaryotes, there is some very excellent evidence in the organic molecular record for steranes in the 1500 to 1600 million year range.

CAVALIER-SMITH: That is definitely a reason for favoring the 1500. In most of my published work, that is the date that I have used, partly for that reason and partly for the increase in cell size. But we cannot be sure whether that event coincided with the origin of the eukaryotes, and that's why I have left this broad window of uncertainty.