

PERSPECTIVE

Protists are microbes too: a perspective

David A Caron¹, Alexandra Z Worden², Peter D Countway¹, Elif Demir² and Karla B Heidelberg¹

¹Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA and

²Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA

Our understanding of the composition and activities of microbial communities from diverse habitats on our planet has improved enormously during the past decade, spurred on largely by advances in molecular biology. Much of this research has focused on the bacteria, and to a lesser extent on the archaea and viruses, because of the relative ease with which these assemblages can be analyzed and studied genetically. In contrast, single-celled, eukaryotic microbes (the protists) have received much less attention, to the point where one might question if they have somehow been demoted from the position of environmentally important taxa. In this paper, we draw attention to this situation and explore several possible (some admittedly lighthearted) explanations for why these remarkable and diverse microbes have remained largely overlooked in the present 'era of the microbe'.

The ISME Journal (2009) 3, 4–12; doi:10.1038/ismej.2008.101; published online 13 November 2008

Keywords: protists; eukaryotic genomics; species concept; diversity; biogeography

Introduction

Environmental science has clearly entered the 'era of the microbe' during the past decade. At no other time in our history have we so completely grasped the immensity of microbial diversity or its significance. Evolutionary biologist Stephen J Gould (1996) offered a cogent argument for this realization by noting that we have always existed on a microbially dominated planet. He emphasized that macroorganismal evolution has yielded some interesting and notable evolutionary forms but, for the most part, Earth's history has been a history of microbes (Gould, 1996). Environmental microorganisms encompass an incredible diversity of physiologies and behaviors that allows them to exploit virtually all habitats on our planet, including many regions that seem uninhabitable such as those characterized by low and high pH or temperature, and high pressure. In these 'extreme' environments, as well as in more hospitable realms, microbes carry out a bewildering array of biochemical processes that are crucial for sustaining and defining life on Earth. The present spotlight on microbes and microbial processes is justified and overdue.

The upsurge in the study of microorganisms has focused largely on bacteria and more recently on the archaea and viruses, and in no way is this commentary meant to detract from the considerable importance of these microbial taxa. Strangely, however, the entrainment of single-celled eukaryotic organisms (the protists) into this explosive growth of

research activity has lagged behind that of other microbes despite their rather impressive contributions to microbiological history, microbial diversity and biogeochemical processes. Protists were some of the first microbial taxa visualized and described by Anton van Leeuwenhoek and other early microbiologists of the seventeenth century. The description and cataloging of a vast diversity of forms and functions among the microbial eukaryotes developed throughout the following centuries. Among these descriptions, the illustrations of Haeckel (Haeckel, 1899, 1904) during the nineteenth century constitute some of the most beautiful scientific illustrations ever made—focusing largely on protists belonging to the Radiolaria.

Early descriptive studies expanded logically into investigations of the ecological roles of protists. Enormous scientific progress during the latter half of the last century has revealed that there are many roles played by these species. For example, some protists have been identified as important human parasites and pathogens. *Plasmodium*, the cause of malaria in humans, directly or indirectly results in up to 2.7 million deaths per year (USAID 2008 statistics). On the other hand, some unicellular eukaryotes, such as yeasts, have tremendous and well-known beneficial attributes for application in the food industry. Photosynthetic protists have been recognized as major contributors to the standing stock of biomass and primary production in nearly all aquatic ecosystems. Equally important roles for phagotrophic protists that consume bacteria and algae have been established for water and soil environments (Sherr and Sherr, 2000). Microbial ecological research during the 1960s and 1970s culminated in the incorporation of phagotrophic forms (also known as protozoa) into classical aquatic

Correspondence: DA Caron, Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, Los Angeles, CA 90089-0371, USA.
E-mail: dcaron@usc.edu

food webs as the paradigm of ‘the microbial loop’ emerged (Pomeroy, 1974; Azam *et al.*, 1983). Studies during the following decade firmly established the contribution of protozoa to the remineralization of major, minor and micronutrients (Sanders *et al.*, 1992; Caron, 1994; Sherr and Sherr, 1994; Calbet and Landry, 2004).

Nevertheless, not everyone today consciously recognizes that these organisms form an important component of the microbial world. A common tendency has emerged among many researchers to use the words ‘microbe’ or ‘microorganism’ when referring exclusively to bacteria and archaea. Microbiologists often speak of these latter taxa as though they exist in vacuum; other types of microbes present in nature are ignored, or perhaps cursorily acknowledged as ‘higher organisms’. This oversight regarding the existence, ecological roles and biogeochemical importance of microbial eukaryotes is a bit disturbing.

Is this exclusion mere oversight? Perhaps it is simply that the study of bacteria and archaea has witnessed such an expansion in recent years that the sheer number of these investigators has diluted the contribution of eukaryote microbial researchers? Alternatively, a decrease in the number of graduate students trained in eukaryotic microbiology may have led us to similar end results. Whatever the reason might be, it is unfortunate that we have arrived at a situation where protists are often dismissed by microbiologists as unimportant, uninteresting or too complicated to study. Indeed, many microbial ecologists show little recognition of the fact that a significant fraction of the biomass and activity within ‘microbial communities’ is actually composed of and conducted by eukaryotes and that protistan primary producers in aquatic ecosystems are ultimately the source of much of the organic matter used by bacteria, archaea and multicellular eukaryotes. Moreover, heterotrophic/phagotrophic protists are major forces of mortality. Our appreciation of the populations performing these different functional roles is further hampered by the fact that relatively few molecular genetic studies have been conducted on heterotrophic and autotrophic protists. Is it reasonable that protistan research has been allowed to lag behind the study of other microbes? In this opinion paper, we politely point out (albeit a bit tongue-in-cheek) that protists are indeed microbes too, we offer possible explanations for why these species have had trouble staying in the mainstream of modern ecological research, and we point out the necessity for their inclusion in the great microbe revolution.

Perceptions and realities

First impressions are lasting impressions?

Our history as ‘microbiologists’ can be traced to the development of the earliest microscopes more than

350 years ago. These allowed the first glimpses of the nature’s smallest organisms. Most of the species described by van Leewenhoek at that time were protists (not bacteria), a point of great pride among some microbial ecologists. It is perhaps unfortunate, however, that van Leewenhoek chose to name his

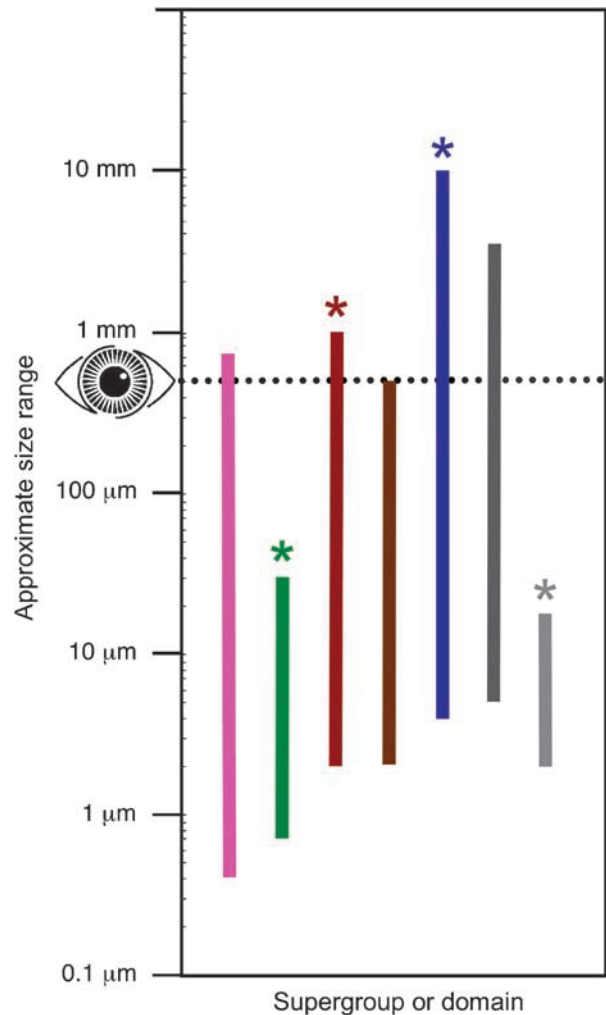


Figure 1 Approximate size ranges for protists as currently placed within the six eukaryotic supergroups, as well as bacteria. Colored columns represent approximate size ranges among taxa within each group. The dotted line indicates the approximate limit of resolution of the human eye. Note that the overall range of single-celled eukaryotic organisms spans several orders of magnitude and can be smaller than 1 μm. Examples of small and large organismal sizes within each supergroup are (columns from left to right): bacteria, *Pelagibacter ubique* and *Thiomargarita namibiensis* (pink); Archaeplastida, *Ostreococcus tauri* and *Chlamydomonas* sp. (green); Chromalveolata, *Cafeteria roenbergensis* and *Stentor roeseli* (red); Excavata, *Bodo saltans* and *Euglena* sp. (brown); Rhizaria, *Bigeloviella natans* and *Hastigerina pelagica* (blue); Amoebozoa, *Platyamoeba* sp. and *Pelomyxa palustris* (dark gray); Opisthokonta, *Encephalitozoon intestinalis* and *Diaphanoeca grandis* (gray). Asterisks indicate the existence of colonial forms within these supergroups (for example, *Volvox*, a member of the Archaeplastida, which can be 2 mm). New protists are discovered each year, we have only molecular marker sequences and no morphological characterization for some of these; therefore this figure is meant only to provide a rough overview of cell sizes. Note the log y-axis scale.

newly discovered creatures ‘animalcules’. Did this awkward moniker that semantically links protists with large multicellular organisms somehow immediately and irrevocably lend a non-microbial status to these species?

Likewise, the term ‘protist’ refers back to a classification system proposed by Whittaker (1969) in which all unicellular eukaryotic organisms (from algae to heterotrophic flagellates) were placed into a single kingdom (Protista), with the exception of some larger fungi. Whole treatises have been written on the naming of unicellular eukaryotes, such as Margulis’s (1971) revisions to Whittaker’s five kingdom system and Rothschild’s (1989) ‘Protozoa, Protista, Protoctista: What’s in a Name?’. The colloquial use of the term ‘protist’ remains today to describe single-celled eukaryotes, despite the fact that all eukaryotes (from microbial to charismatic megaf flora and megafauna) are now grouped into a single domain (Eukarya) and despite the unending reorganization of single-celled eukaryotic taxa into numerous kingdom-level divisions (Burki *et al.*, 2007; Lane and Archibald, 2008). One reason for its current usage is that the term ‘protist’ has several advantages over other colloquial terms for single-celled eukaryotes. The term ‘protozoa’ (literally ‘the first animal life’) does not recognize the strictly phototrophic or mixotrophic ability of many protists, and ‘microalgae’ does not recognize that many phototrophic protists are mixotrophic, and not ‘micro’ but rather ‘pico’, ‘nano’ or even ‘meso’ (Sieburth *et al.*, 1978). The term ‘lower eukaryotes’ that is sometimes employed ignores the fact that some protists, such as the choanoflagellate *Monoisiga brevicolis*, are phylogenetically closer to ‘higher eukaryotes’ such as *Homo sapiens* than they are to most other single-celled eukaryotes. Misconceptions, misnomers and reluctance to discard improper terms do not help the cause to have protists (excuse the term) recognized as microbes!

Size does matter, and that is exactly the point

Strictly speaking, microorganisms are defined by their size; that is, organisms that are smaller than can be resolved with the naked eye (perhaps a few hundred micrometers to half a millimeter, depending on your age). If we define microbes by cell size, then most protists qualify as microbes. A few single cells and numerous colonial forms exist that are visible to the unaided eye, but the vast majority are microscopic (Figure 1). Similarly, most bacteria and archaea are indeed microscopic, but there are exceptions here as well. In fact, a very large number of protistan taxa are much smaller than the largest bacteria (Figure 2).

An example of the preconception that bacteria are microbes and protists are ‘something else’ can be found in the sturgeon fish symbiont, *Epulopiscium*. This organism was originally classified as a protist (Fishelson *et al.*, 1985) and referred to as such in its description. Later analyses correctly reclassified it

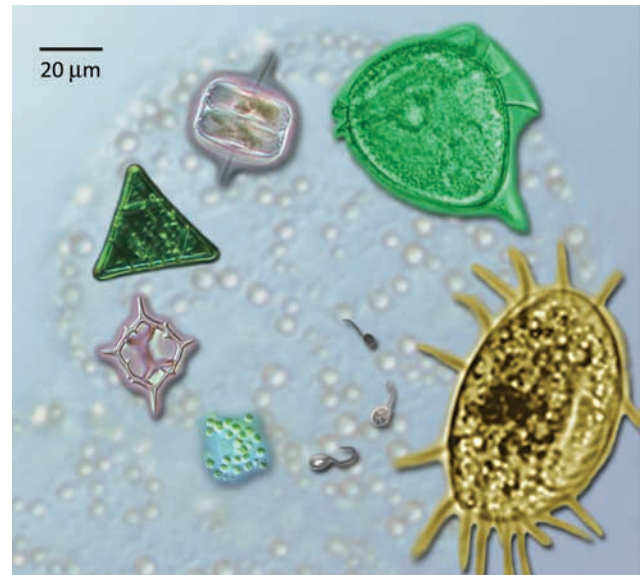


Figure 2 A single cell of the largest described bacterium, *Thiomargarita namibiensis* (diameter $\approx 180\ \mu\text{m}$) forms the backdrop for a variety of phototrophic and heterotrophic protists that are shown at the same scale as *T. namibiensis*. Counterclockwise from the lower right, the protists include a ciliate, a dinoflagellate, two diatoms, a silicoflagellate, a colony of small chlorophytes and three minute heterotrophic flagellates. Photograph of *T. namibiensis* by Heide Schulz-Vogt.

as a bacterium (Angert *et al.*, 1993). One can question whether, in its reclassification, it mysteriously changed from a non-microbial species to a microbial species. Probably not, but its nomenclature did; in correcting the classification of this gargantuan bacterium, Angert *et al.* (1993) referred to the former protist as a ‘microbe’ once again. This is a trivial example, but this kind of ‘size bias’ is pervasive. Although researchers are careful to highlight the need for multiple criteria to be met when identifying fossils and developing hypotheses for the appearance of eukaryotes (Knoll *et al.*, 2006), one of those criteria is based on the preconception that organismal size larger than a typical bacterium might indicate the eukaryotic state (Knoll *et al.*, 2006). This position is hard to support, given the minute size of many eukaryotes (Figures 1 and 2) and the large size of some bacteria (Figure 2).

Enter the viruses, exit the protists?

Heterotrophic protists have somehow been demoted as the primary agents of bacterial mortality in most natural aquatic ecosystems. Research during the twentieth century established that protists account for most of the removal of bacterial productivity (Sanders *et al.*, 1992; Sherr and Sherr, 2002). To some degree, the seeming dismissal of phagotrophic protists as consumers of bacteria (and presumably consumers of archaea, although there is only little known about the latter) stems from the recognition of the large numbers of the smallest entities within

microbial communities—viruses. Studies of viral production/turnover have hypothesized that bacterial mortality by viral lysis must be great to produce the numbers of viral particles observed in many ecosystems. Indeed, there has been a dramatic increase in studies examining viruses and viral activities in numerous habitats (Weinbauer, 2004; Breitbart *et al.*, 2008). There has even been a calculation of how far the viruses on our planet would stretch if they were lined up end-to-end (and it is great; ≈ 100 times the diameter of our galaxy!) (Suttle, 2005). These are exciting findings, but they do not preclude an important role for protists as agents of mortality.

Most aquatic ecologists recognize that the assumptions and calculations made to estimate bacterial mortality by viruses are not infallible, but these caveats are generally lost in summary slides of seminars and discussion sections of papers. A common finding that is oft neglected is that one can filter water through a 0.8- μm pore size filter (to remove protists but presumably not viruses) before incubation, and the number of bacteria will always increase dramatically over time. Marine bacteriologists are well acquainted with this technique for producing 'seawater cultures'. The interpretation of such experiments is not completely straightforward because modifications in dissolved and particulate organic carbon take place due to cell breakage during filtration, and it is possible that selection takes place in these cultures for bacterial taxa that will grow on these compounds but for which no lytic viruses exist in the water sample. It is striking, however, that the establishment of seawater cultures consistently produces higher abundances of bacteria than were present in the original water samples. One clear interpretation is that protists play an important role in regulating the overall bacterial abundance in seawater and that their removal relieves this control on bacterial proliferation.

The possibility that viruses constitute the bulk of bacterial mortality has been questioned by some (Bettarel *et al.*, 2004), but, in general, few direct comparisons are being conducted. Alas, studies of bacterial grazing by protists are not 'new and hot', they are difficult to perform, and therefore this mortality term is often not examined, considered or even funded any more. The somewhat limited methodological approaches employed to examine protistan bacterivory have not been improved during the last few decades. Nevertheless, the few direct comparisons that have been conducted provide internally conflicting data; specifically, bacterial mortality arising from these two sources—viral lysis and predation by protists—often accounts for more than 100% of the bacterial productivity! This conundrum has gone largely overlooked or ignored. Viral activity is probably a significant factor for shaping bacterial community composition in many ecosystems and may even be the primary source of bacterial mortality in some habitats. Nevertheless, it

is clear that protists constitute an important mortality factor that constrains bacterial abundance and results in dramatically different organismal fates and food web linkages than viral lysis, which simply releases dissolved organic carbon and particulate organic carbon. A more integrated ecosystem approach is needed to study bacterial (microbial!) mortality to tease apart these different 'predatory' losses.

As a side note, the importance of protists as consumers of phytoplankton (both cyanobacteria and phototrophic eukaryotes) also appears to have suffered from a reduction in research activity during the past decade. Studies conducted during 1970–1990s firmly established a major role for unicellular eukaryotes as sources of mortality for primary producers (Sherr and Sherr, 2002) and the importance of heterotrophic protists as food for metazoan zooplankton (Stoecker and Capuzzo, 1990; Gifford and Dagg, 1991). Yet, many zooplankton ecologists appear to have lost sight of these important findings and have slipped back into an acceptance of the phytoplankton–zooplankton trophic connection as the major (sole?) source of nutrition for metazoan zooplankton. In turn, this tendency has relegated phagotrophic protists to the role of 'bacterial consumers' in the plankton, despite several recent reviews reiterating their importance as herbivores (Sherr and Sherr, 2002, 2007; Calbet and Landry, 2004). Coupled with the recent upsurge in the viral research community that has eroded the role of protists as the primary source of bacterial mortality, these findings tend to downplay the overall activities of phagotrophic protists to the point where one might think that the ecological role of these taxa has somehow diminished substantially in aquatic ecosystems.

History repeats itself

One practical reason for including protists in discussions of 'microbes' is that similar discoveries, and arguments, that have raged through bacterial and archaeal ecology are central in protistan ecology as well. Advances in protistan ecology have closely mirrored those in bacterial/archaeal ecology, albeit with a lag. If there were no other reasons to consider protists alongside their microbial cohorts, then it could be the simple fact that they share highly analogous problems and that scientific studies of these different groups have taken similar trajectories in developing our understanding of natural microbial communities. A few examples follow.

Diversity. The application of culture-independent molecular approaches to the study of natural assemblages of protists has revealed a 'hidden world' of microbial eukaryotic diversity. Such studies started with the use of plastid-targeted primers, both for the plastid-derived 16S rRNA genes (Rappé *et al.*, 1997) and *rbcl* genes (Pichard

et al., 1997). These ground-breaking studies were followed by a series of subsequent publications in the present decade that have established the presence of a large number of 'undescribed, uncultured' taxa and whole lineages in natural protistan assemblages (Massana *et al.*, 2002; López-García *et al.*, 2003; Romari and Vaultot, 2004; Groisillier *et al.*, 2006; Countway *et al.*, 2007; Not *et al.*, 2007; Cuvelier *et al.*, 2008). These findings have raised questions regarding how well we understand the overall diversity of protists. Interestingly, these discoveries are highly analogous to discoveries in aquatic bacteriology during the 1990s.

Applications of refined molecular tools such as specific fluorescence *in situ* hybridization and quantitative PCR probes are also shaping how we view protistan diversity and community structure, a pathway that has proven useful for studies of other microbial taxa (Amann *et al.*, 2001). In particular, the application of these approaches has significantly improved our understanding of the ecologies of small morphologically non-descript protistan taxa (for example, Not *et al.*, 2005; Countway and Caron, 2006; Demir *et al.*, 2008).

Biogeography. Many protists may be ubiquitously distributed on our planet, but it is presently a topic of rich debate (Fenchel and Finlay, 2004; Foissner, 2006). This argument is highly analogous to debates regarding the distribution of bacteria. The microbiologist's credo that 'everything is everywhere' is strongly held by many microbiologists but strongly refuted by others. Emerging high-throughput sequencing techniques are beginning to address this debate for bacteria (Sogin *et al.*, 2006; Mou *et al.*, 2008). These approaches, and methods adapted from population genetics (for example, Rynearson and Armbrust, 2004), are now finding their way into protistan ecological research, where they will improve our ability to generate and test hypotheses regarding protistan biogeography.

The species concept. A central issue in these debates of ubiquity/endemism is the variable species concept for protists. Traditionally, bacterial species have been described based largely on physiological abilities, whereas protistan species identifications have been based primarily on morphological features. Both taxonomies have their shortcomings, and the debate over 'cryptic species' of protists (morphospecies of protists that contain strains possessing different physiological abilities or mating incompatibilities) is highly analogous to the debate over the significance of bacterial 'ecotypes'. Many would argue that bacterial ecotypes represent essentially species-level distinctions, whereas others accept the significant physiological variability within a bacterial 'species'. What should we think about a single protistan species for which two strains with >97% rDNA identity only share 90% of their genes (Worden *et al.*, unpublished)? In short,

the species concept is no less muddled and no less debated for protists than it is for other microbes.

Where have all the protists gone?

The elephant in the room

Given the obvious overlap in organismal sizes, ecological roles, theoretical concepts and technological approaches common to all microbes, why have many investigators lost sight of the protists within microbial ecology (and is not this last point the needed proof that they are indeed microbes!)? We suggest that a major contributing factor has been limitations in sequencing and computational technologies for dealing with protistan genome size and complexity. More than any other factor, this issue has significantly retarded the entrainment of these species into the mainstream of the present microbial revolution. Technologies are advancing at a head-spinning rate, and the '-omics era' is an exciting period for anyone associated with the study of microorganisms. New genomic techniques, presently applied primarily to bacteria and archaea, are revolutionizing our understanding of the evolution and phylogeny of these species and are providing powerful new tools to investigate their ecologies and biochemistries. The ecologically relevant microbial eukaryotes have not yet garnered the attention or level of effort bestowed upon bacteria and archaea (with a few notable exceptions; for example, Armbrust *et al.* (2004); Derelle *et al.* (2006)). On the basis of their biogeochemical and ecological significance, however, several US and international funding agencies have recently prioritized sequencing of aquatic eukaryotic microbes, particularly the US Department of Energy (http://genome.jgi-psf.org/mic_home.html). Nevertheless, progress on eukaryotes has lagged behind efforts such as the DOE Joint Genome Institute's Genomic Encyclopedia of Bacteria and Archaea and the Moore Foundation Microbial Genome Sequencing Project (bacterial/archaeal sequencing initiative of the Gordon and Betty Moore Foundation).

A significant challenge highlighted above is that eukaryotic microbial genomes can be very large (Figure 3). For instance, dinoflagellate genomes reportedly range from 3000 to 215 000 Mb (Hackett *et al.*, 2004), which is much larger than that of *Homo sapiens* (~3000 Mb). Thus far, only intracellular parasites that cannot grow or divide in the absence of their hosts, such as the microsporidian parasite *Encephalitozoon cuniculi* (~2.9 Mb) (Katinka *et al.*, 2001), present less-challenging protistan sequencing targets. A number of genomes representing biomedically relevant protists have been undertaken (for example, the parasites *Plasmodium*, *Giardia*, *Cryptosporidium*, *Trypanosoma*), but the high priority given to these species is derived from their importance in human health. Relatively few, free-living, ecologically important groups of

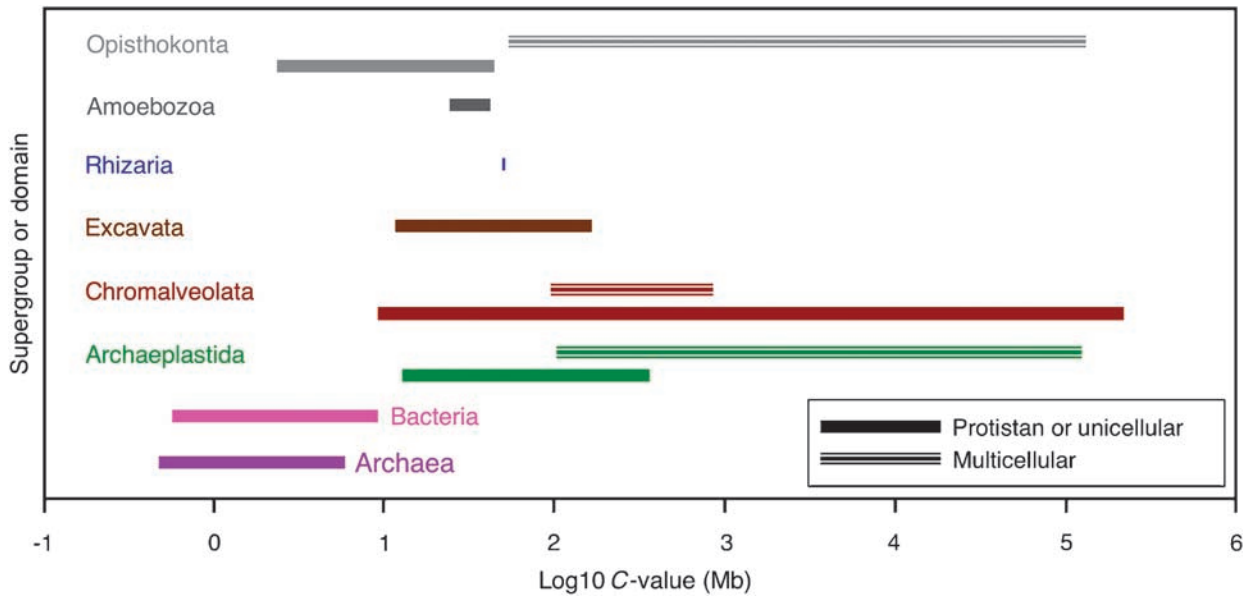


Figure 3 Genome size ranges of taxa within the six eukaryotic supergroups as well as from the domains bacteria and archaea. Protists, bacteria and archaea are shown as solid lines, whereas multicellular eukaryotes are represented by split lines. A range of genome sizes within each group was considered, and bars represent that range from smallest to largest genome. The ranges for smallest to largest genome sizes for protistan taxa within each supergroup are represented here by literature values for Archaeplastida, *Ostreococcus tauri* and *Mesostigma viride*; Chromalveolata, *Cryptosporidium hominis* and *Prorocentrum micans*; Amoebozoa, *Entamoeba histolytica* and *Naegleria gruberi*; Rhizaria, *Bigelowiella natans* (estimate from J. Archibald, personal communication); Excavata, *Giardia lamblia* and *Trichomonas vaginalis*; Opisthokonta *Encephalitozoon intestinalis* and *Monosiga brevicolis*. C-values are as in Gregory (2005); that is, log base 10 of the genome size in megabases (Mb) as opposed to picograms of DNA. Ranges for multicellular eukaryotes, bacteria and archaea are from Gregory (2005). Supergroups are as defined in Lane and Archibald (2008). Data on genome size ranges change rapidly. The information shown depicts the relatively few cultured taxa on which measurements have been made, and these ranges may be biased because many projects focus on small(er) genomes.

phototrophic protists have been sequenced. The heterotrophic and mixotrophic flagellate taxa, represented by many lineages among several protistan supergroups (for example, the heterotrophic chrysomonads), have been almost completely overlooked (Boenigk *et al.*, 2005) until new projects were announced in summer 2008. Only a single representative of a small, free-living, heterotrophic flagellate has been published to date, the fascinating choanoflagellate *Monosiga brevicolis* (King *et al.*, 2008). However, this genus has been targeted primarily because the choanoflagellates occupy a key phylogenetic position with respect to mammals and provide insights into the development of multicellularity. The genomes of many key groups of ecologically relevant protists await exploration.

Genome complexity, in addition to sheer genome size, makes the inclusion of many protistan species in the microbial genomic revolution challenging. For starters, the genomic DNA sequence can be difficult to assemble. The green alga *Chlamydomonas reinhardtii* has been termed ‘a sea of repeats’ referring to the many repetitive sequence regions that make ‘finished’ assembly of the genome virtually impossible under current technologies (Merchant, 2007). Furthermore, eukaryotic gene sequences are often interspersed with abundant non-coding regions (introns) and they can exhibit multiple mRNA variants (alternative splicing), making them far more challenging than their bacterial

and archaeal counterparts for genomic and bioinformatics analysis. The relationships between gene sequence, transcript, various forms of mature mRNA and final protein product are complex. Research on marine diatoms has highlighted weaknesses in homology-based approaches to gene predictions (an understandable outcome, given that much of the homology is related to a database dominated by mostly mammalian and bacterial genomes). Using tiling arrays, Mock *et al.* (2008) identified over 3000 additional genes to the original 11 390 predicted computationally in the genome of *Thalassiosira pseudonana* (34 Mb). Even ‘small’ protistan genomes, such as *Ostreococcus* (12–13 Mb) and *Micromonas* (21–22 Mb), thwart many current gene-calling algorithms (Derelle *et al.*, 2006; Palenik *et al.*, 2007; Worden *et al.*, unpublished). In these cases, the assembly of DNA sequence has been successful but gene predictions have been problematic due to the high numbers of overlapping genes. In the end, interpretation of genome sequences (DNA sequence, gene complement and relationship to the ecological niche) may require understanding of modulation and redirection by the RNA world (regulation)—rather than just the gene-centric view of ‘capabilities’.

Despite these significant hurdles, protistan genomics has yielded unique insights into eukaryotic microbes. Therefore, it constitutes a highly informative complement to bacterial/archaeal genome studies.

Several genome projects of free-living protists are underway at this time (for example, *Aureococcus anophagefferens*, *Emiliania huxleyi*, *Phaeocystis antarctica* and *Dunaliella salina*) or planned for the immediate future, and these studies will undoubtedly add much to our understanding of protistan (and general eukaryotic) genomics, physiology and ecology. In short, when it comes to eukaryotes, unicellular may not mean 'simple', but the unique perspective provided by these studies will be well worth the effort.

Increasing the level of protistan genome sequencing will also improve our ability to exploit databases comprised of gene surveys and metagenomic studies of natural microbial assemblages, as our bacteria-studying colleagues have done so effectively in recent years. The Environmental Shotgun sequence database from the Sargasso Sea (Venter *et al.*, 2004) encompasses sequences from eukaryotic microbes (Worden *et al.*, 2006) and a recent study has addressed these data from the eukaryotic perspective (Piganeau and Moreau, 2007). However, without broad taxonomic representation within reference genome collections and appropriate sequence coverage, assembly of such sequences into something resembling the genome of a protist is still a dream, even with the development of much more powerful bioinformatic approaches. The development of approaches to solve or circumvent such problems is essential to being able to glean useful genetic information on eukaryote species from environmental databases. Just as importantly, these breakthroughs will be required to place molecular biological studies of protists on a more equal footing with their presently much-more-amenable microbial counterparts and thus regain some level of recognition among microbial ecologists.

A light at the end of the tunnel

The last decade has indeed been that of the microbe, but with a slightly unintended side effect. Initial research has been strongly focused on bacteria, given fundamental advances that have facilitated their study. The unexpected consequence of technical obstacles to studying microbial eukaryotes has forced them temporarily to the sidelines. This trend must be reversed because it leaves us in the dark about a large and ecologically important subset of microscopic species. The ecological roles of phototrophic and heterotrophic protists are not minute, albeit the sizes of these species (many of them, at least) clearly are. With respect to simple issues discussed herein, such as terminology, Pace recently proposed a remedy to this situation. He suggested the use of the term 'microbe', which he noted includes '...the poorly acknowledged microbial eukaryotes...' as more appropriate than other terms derived from earlier misconceptions (Pace, 2006).

We are in accordance with this attitude that embraces protistan taxa as true microbes.

Despite the challenges raised in research involving eukaryotic organisms, there is a vital need to reintegrate these species within studies of microbial ecology. Protistan lineages represent one and a half billion years of evolution on Earth (Knoll *et al.*, 2006) and comprise the bulk of eukaryotic phylogenetic diversity as well as myriad life forms. Protists provide the foundation for understanding the origins and developmental innovations underlying the evolution of multicellular taxa (Baldauf, 2003). They also constitute several essential components of global food webs. Given current climatic scenarios, it is critical that we develop a mechanistic understanding of microbial interactions—viruses, bacteria, archaea and microbial eukaryotes! It is the only way that we can hope to construct predictive models of Earth's biogeochemical cycles.

Advances in technology, paired with access to traditional and new sequencing platforms, and advances in bioinformatics are beginning to provide many opportunities that make the microbial eukaryotic field ripe for exploring. Baseline genomic information for ecologically relevant protists is hardly less than 5 years old and is still emerging in public databases. The development of tools and resources for the microbiology community will accelerate the rate of research on these species starting now. Single-cell/population genomics, arrays for the investigation of unpredicted genes (for example, tiling arrays), nanoSIMs tied to FISH, studies of genetic expression through transcriptomics and many other innovations present us with a rapidly expanding array of tools with which to study the eukaryotic fraction of the microbial community. The time is right to avail ourselves of these tools and reinvigorate the exciting field of protistan ecology.

The dictionary defines a 'microorganism' as '...a tiny organism such as a virus, protozoan or bacterium that can only be seen under the microscope...'. We find it significant that 'bacterium' is listed last, because in many parlances within our community, it is considered the sole definition of the word. We implore microbiologists to reincorporate the broader definition into their vocabulary and recognize that unicellular eukaryotic organisms are both 'organisms' and 'micro', and thus worthy of consideration and exploration. The definition above does raise one potentially sticky issue, however: now that we have made a case for protists to be included among the microbial species, someone else can try to decide what viruses are!

References

- Amann R, Fuchs BM, Behrens S. (2001). The identification of microorganisms by fluorescence *in situ* hybridisation. *Curr Opin Biotechnol* **12**: 231–236.

- Angert ER, Clements KD, Pace NR. (1993). The largest bacterium. *Nature* **362**: 239–241.
- Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH *et al.* (2004). The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. *Science* **306**: 79–86.
- Azam F, Fenichel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. (1983). The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* **10**: 257–263.
- Baldauf SL. (2003). The deep roots of eukaryotes. *Science* **300**: 1703–1706.
- Bettarel Y, Amblard C, Sime-Ngando T, Carrias J-F, Sargos D, Garabétian F *et al.* (2004). Viral lysis, flagellate grazing potential, and bacterial production in Lake Pavin. *Microb Ecol* **45**: 119–127.
- Boenigk J, Pfandl K, Stadler P, Chatzinotas A. (2005). High diversity of the ‘Spumella-like’ flagellates: an investigation based on the SSU rRNA gene sequences of isolates from habitats located in six different geographic regions. *Environ Microbiol* **7**: 685–697.
- Breitbart M, Middelboe M, Rohwer F. (2008). Marine viruses: community dynamics, diversity, and impact on microbial processes. In: Kirchman DL (ed). *Microbial Ecology of the Oceans*, 2nd edn. John Wiley and Sons, Inc.: Hoboken, New Jersey. pp 443–479.
- Burki F, Shalchian-Tabrizi K, Skjaeveland Å, Nikolaev SI, Jakobsen KS, Pawlowski J. (2007). Phylogenomics reshuffles the eukaryotic supergroups. *PLoS One* **8**: E790.
- Calbet A, Landry MR. (2004). Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol Oceanogr* **49**: 51–57.
- Caron DA. (1994). Inorganic nutrients, bacteria and the microbial loop. *Microb Ecol* **28**: 295–298.
- Countway PD, Caron DA. (2006). Abundance and distribution of *Ostreococcus* sp. in the San Pedro Channel, California (USA) revealed by qPCR. *Appl Environ Microbiol* **72**: 2496–2506.
- Countway PD, Gast RJ, Dennett MR, Savai P, Rose JM, Caron DA. (2007). Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western N. Atlantic (Sargasso Sea and Gulf Stream). *Environ Microbiol* **9**: 1219–1232.
- Cuvelier ML, Ortiz A, Kim E, Moelig H, Richardson DE, Heidelberg JF *et al.* (2008). Widespread distribution of a unique marine protistan lineage. *Environ Microbiol* **10**: 1621–1634.
- Demir E, Coyne KJ, Doblin MA, Handy SM, Hutchins DA. (2008). Assessment of microzooplankton grazing on *Heterosigma akashiwo* using a species-specific approach combining quantitative real-time PCR (QPCR) and dilution methods. *Microb Ecol* **55**: 583–594.
- Derelle E, Ferraz C, Rombauts S, Rouze P, Worden AZ, Robbens S *et al.* (2006). Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *Proc Natl Acad Sci USA* **103**: 11647–11652.
- Fenichel T, Finlay BJ. (2004). The ubiquity of small species: patterns of local and global diversity. *Bioscience* **54**: 777–784.
- Fishelson L, Montgomery WL, Myrberg AA. (1985). A unique symbiosis in the gut of a tropical herbivorous surgeonfish (Acanthuridae: Teleostei) from the Red Sea. *Science* **229**: 49–51.
- Foissner W. (2006). Biogeography and dispersal of microorganisms: a review emphasizing protists. *Acta Protozool* **45**: 111–136.
- Gifford DJ, Dagg MJ. (1991). The microzooplankton-mesozooplankton link: consumption of planktonic protozoa by the calanoid copepods *Acartia tonsa* Dana and *Neocalanus plumchrus* Murukawa. *Mar Microb Food Webs* **5**: 161–177.
- Gould SJ. (1996). *Planet of the Bacteria*. Washington Post Horizon: Washington, DC. pp 344.
- Gregory TR. (2005). Synergy between sequence and size in large-size genomics. *Nat Rev Genet* **6**: 699–708.
- Groissillier A, Massana R, Valentin K, Vaulot D, Guillou L. (2006). Genetic diversity and habitats of two enigmatic marine alveolate lineages. *Aquat Microb Ecol* **42**: 277–291.
- Hackett JD, Anderson DM, Erdner DL, Bhattacharya D. (2004). Dinoflagellates: a remarkable evolutionary experiment. *Am J Bot* **91**: 1523–1534.
- Haeckel E. (1899). *Kunstformen der Natur*. Prestel Verlag, München.
- Haeckel E. (1904). *Kunstformen der Natur*. Prestel Verlag, München.
- Katinka MD, Duprat S, Cornillot E, Méténier G, Thomarat F, Prensier G *et al.* (2001). Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature* **414**: 450–453.
- King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J *et al.* (2008). The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**: 783–788.
- Knoll AH, Javaux EJ, Hewitt E, Cohen P. (2006). Eukaryotic organisms in Proterozoic oceans. *Philos Trans R Soc Lond B Biol Sci* **361**: 1023–1038.
- Lane CE, Archibald JM. (2008). The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol Evol* **23**: 268–275.
- López-García P, Philippe H, Gail F, Moreira D. (2003). Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. *Proc Natl Acad Sci USA* **100**: 697–702.
- Margulis L. (1971). Whittaker’s five kingdoms of organisms: minor revisions suggested by considerations of the origin of mitosis. *Evolution* **25**: 242–245.
- Massana R, Guillou L, Diez B, Pedros-Alio C. (2002). Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. *Appl Environ Microbiol* **68**: 4554–4558.
- Merchant SS. (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* **318**: 245–250.
- Mock T, Samanta MP, Iverson V, Berthiaume C, Robison M, Holtermann K *et al.* (2008). Whole-genome expression profiling of the marine diatom *Thalassiosira pseudonana* identifies genes involved in silicon bioprocesses. *Proc Natl Acad Sci USA* **105**: 1579–1584.
- Mou X, Sun S, Edwards RA, Hodson RE, Moran MA. (2008). Bacterial carbon processing by generalist species in the coastal ocean. *Nature* **451**: 708–711.
- Not F, Gausling R, Azam F, Heidelberg JF, Worden AZ. (2007). Vertical distribution of picoeukaryotic diversity in the Sargasso Sea. *Environ Microbiol* **9**: 1233–1252.
- Not F, Massana R, Latasa M, Marie D, Colson C, Eikrem W *et al.* (2005). Late summer community composition and abundance of photosynthetic picoeukaryotes in Norwegian and Barents Seas. *Limnol Oceanogr* **50**: 1677–1686.

- Pace NR. (2006). Time for a change. *Nature* **441**: 289.
- Palenik B, Grimwood J, Aertsd A, Rouzé P, Salamov A, Putnam N *et al.* (2007). The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation. *Proc Natl Acad Sci USA* **104**: 7705–7710.
- Pichard SL, Campbell L, Paul JH. (1997). Diversity of the ribulose biphosphate carboxylase/oxygenase form I gene (*rbcL*) in natural phytoplankton communities. *Appl Environ Microbiol* **63**: 3600–3606.
- Piganeau G, Moreau H. (2007). Screening the Sargasso Sea metagenome for data to investigate genome evolution in *Ostreococcus* (Prasinophyceae, Chlorophyta). *Gene* **406**: 184–190.
- Pomeroy LR. (1974). The ocean's food web, a changing paradigm. *Bioscience* **24**: 499–504.
- Rappé MS, Suzuki MT, Vergin KL, Giovannoni SJ. (1997). Phylogenetic diversity of ultraplankton plastid small-subunit rRNA genes recovered in environmental nucleic acid samples from the Pacific and Atlantic coasts of the United States. *Appl Environ Microbiol* **64**: 294–303.
- Romari K, Vaulot D. (2004). Composition and temporal variability of picoeukaryote communities at a coastal site of the English Channel from 18S rDNA sequences. *Limnol Oceanogr* **49**: 784–798.
- Rothschild LJ. (1989). Protozoa, protista, protoctista: what's in a name? *J Hist Biol* **22**: 277–305.
- Rynearson TA, Armbrust EV. (2004). Genetic differentiation among populations of the planktonic marine diatom *Ditylum brightwellii* (Bacillariophyceae). *J Phycol* **40**: 34–43.
- Sanders RW, Caron DA, Berninger U-G. (1992). Relationships between bacteria and heterotrophic nanoplankton in marine and fresh water: an inter-ecosystem comparison. *Mar Ecol Prog Ser* **86**: 1–14.
- Sherr EB, Sherr BF. (1994). Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microb Ecol* **28**: 223–235.
- Sherr EB, Sherr BF. (2000). Marine microbes: an overview. In: Kirchman DL (ed). *Microbial Ecology of the Oceans*. Wiley-Liss, Inc.: New York. pp 13–46.
- Sherr EB, Sherr BF. (2002). Significance of predation by protists in aquatic microbial food webs. *Antonie Van Leeuwenhoek* **81**: 293–308.
- Sherr EB, Sherr BF. (2007). Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Mar Ecol Prog Ser* **352**: 187–197.
- Sieburth JM, Smetacek V, Lenz J. (1978). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol Oceanogr* **23**: 1256–1263.
- Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR *et al.* (2006). Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *Proc Natl Acad Sci USA* **103**: 12115–12120.
- Stoecker DK, Capuzzo JM. (1990). Predation on protozoa: its importance to zooplankton. *J Plankton Res* **12**: 891–908.
- Suttle CA. (2005). Viruses in the sea. *Nature* **437**: 356–361.
- Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA *et al.* (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science* **304**: 66–74.
- Weinbauer MG. (2004). Ecology of prokaryotic viruses. *FEMS Microbiol Rev* **28**: 127–181.
- Whittaker RH. (1969). New concepts of kingdoms of organisms. *Science* **163**: 150–160.
- Worden AZ, Cuvelier ML, Bartlett DH. (2006). In-depth marine microbial community genomics. *Trends Microbiol* **14**: 331–336.