THE ROYAL SOCIETY

Published online 18 March 2004

Protist taxonomy: an ecological perspective

Bland J. Finlay

CEH Dorset, Winfrith Technology Centre, Winfrith Newburgh, Dorchester DT2 8ZD, UK (bjf@ceh.ac.uk)

This is an exploration of contemporary protist taxonomy within an ecological perspective. As it currently stands, the 'morphospecies' does not accommodate the information that might support a truly ecological species concept for the protists. But the 'morphospecies' is merely a first step in erecting a taxonomy of the protists, and it is expected to become more meaningful in the light of genetic, physiological and ecological research in the near future. One possible way forward lies in the recognition that sexual and asexual protists may all be subject to forces of cohesion that result in (DNA) sequence-similarity clusters. A starting point would then be the detection of 'ecotypes'—where genotypic and phenotypic clusters correspond; but for that we need better information regarding the extent of clonality in protists, and better characterization of ecological niches and their boundaries. There is some progress with respect to the latter. Using the example of a community of ciliated protozoa living in the stratified water column of a freshwater pond, it is shown to be possible to gauge the potential of protists to partition their local environment into ecological niches. Around 40 morphospecies can coexist in the superimposed water layers, which presumably represent different ecological niches, but we have yet to discover if these are discrete or continuously variable. It is a myth that taxonomic problems are more severe for protists than for animals and plants. Most of the fundamental problems associated with species concepts (e.g. asexuals, sibling species, phenotypic variation) are distributed across biota in general. The recent history of the status of *Pfiesteria* provides a model example of an integrated approach to solving what are essentially taxonomic problems.

Keywords: protist; morphospecies; genetic and phenotypic divergence; holistic taxonomy; ecotype

1. INTRODUCTION

A frequently cited but trite definition of taxonomy is that it is what taxonomists do. But it quickly becomes evident that taxonomists are such a diverse crowd, employing a variety of approaches in the pursuit of taxonomy, and with different perspectives on what 'taxonomy' means, that our trite definition is not actually very helpful. It is somewhat easier to focus on what the goals of taxonomy might be. With respect to the protists, we might claim that the goal is twofold—to build and maintain a classification and naming system capable of identifying and grouping all the kinds of protists that exist, and to summarize everything that is known about them, whether morphological, physiological or ecological (based on Simpson (1945) and Blackwelder (1967)).

The protists are a heterogeneous collection of microbial eukaryotes, consisting of the groups commonly referred to as protozoa, algae and slime moulds. They evolved from prokaryotes *ca*. 1.5–2 billion years ago by a process that is not fully understood, although serial endosymbiosis was certainly involved in establishing eukaryotic organelles such as mitochondria and chloroplasts (Margulis 1993; Fenchel 2002; Foster 2003). Most protists are microscopic (less than 0.1 mm) and much is known about the roles they play in the natural environment (Fenchel 1987; Andersen 1992; Finlay 2001; Corliss 2002). The free-living protozoa are phagotrophic and they control the abundance of bacteria and other microbes; the unicellular algae are responsible for most of the carbon fixation in oceans and in the world's freshwaters; and the slime moulds are quantitatively important grazers of bacteria, fungi and other primary decomposers in organic soils and especially in forests. Many phagotrophic protists harbour endosymbiotic algae or functional chloroplasts that they have sequestered from ingested phototrophs, and these protists are usually referred to as 'mixotrophs'. Bacteria (including cyanobacteria), Protozoa, unicellular algae, mixotrophs and micrometazoans (especially rotifers and microcrustaceans), are the major interacting (and interdependent) components of microbial food webs in aquatic environments. As a group, the protists show considerable morphological and ultrastructural diversity, and the morphospecies has remained the dominating species concept for more than 200 years, but the full extent of protist species diversity is not yet known. There is no consensus regarding the phylogeny of protists (Patterson 1999).

This is a probably a good time to look at the current status and future prospects for protist taxonomy, because it now appears that biodiversity at the microbial level may be easier to understand than was previously thought (Finlay 2002). The fundamental reason for this is that the absolute abundance of free-living protist species is huge (typically 10^4–10^7 individuals per m^2) with the consequence that there are probably no completely effective barriers to protist dispersal. Thus, all species are

One contribution of 19 to a Theme Issue 'Taxonomy for the twenty-first century'.

effectively cosmopolitan (Darling *et al.* 2000; Finlay 2002; Finlay & Clarke 1999), with the potential to thrive wherever a suitable habitat exists. Unrestricted dispersal means that the rate of allopatric speciation will be low, so the global species richness of protists is also expected to be low (Fenchel 1993). And if global species richness is of manageable dimensions (Corliss 1999; Finlay 2001), the task of cataloguing them, together with their key biological features, may be tractable. However, the quality of knowledge about protists is very uneven. We know a lot about the morphologically rich species of ciliates, and we even have a good idea of their morphospecies richness at the global scale (Finlay *et al.* 1996*b*) but we know much less about groups such as the amoeboid xenophyophores in the deep-sea benthos, and the 'little green balls' (minute coccoid unicellular algae; Potter *et al.* 1997) in the marine plankton.

This is also a good time to look at the future role of taxonomy because we are now at a crossroads. There is a depressingly large amount of evidence indicating that traditional descriptive taxonomy is going through a difficult period, with funding and other resources remaining generally inadequate, and with taxonomists retiring without being replaced, or training replacements. The problem applies in varying degrees across most taxonomic groups but it is particularly severe for large, heterogeneous and still poorly known groups such as the protists. But perhaps we are justified in sounding a note of optimism—after all, taxonomy, biodiversity and conservation are now topics of serious scientific debate—a debate that is fuelled with concern for diminishing taxonomic resources, in an age of escalating losses of biodiversity and natural habitats (House of Lords Science and Technology Committee into Systematic Biology and Biodiversity 2002; The Royal Society 2003), but a debate that is also driven by the emergence of innovative and optimistic ideas, such as a Web-based unitary taxonomy (Godfray 2002), and Web-based taxonomic servers (Patterson 2003). Developments such as these may also have the potential to unite and rejuvenate the remaining taxonomists, who will raise the status and utility of taxonomy during the twenty-first century.

2. MOLECULAR PHYLOGENIES

Over the past 10–15 years, the decline in traditional protist taxonomy based on morphological characters has coincided with the rapidly expanding application of molecular techniques. During this period, much effort was devoted to constructing molecular phylogenies, and these showed early promise of providing a window of understanding into the origin and evolution of protists. The molecular data did, for example, support hypotheses based originally on ultrastructure and morphology, such as the endosymbiotic origin of mitochondria and chloroplasts (Taylor 1999), and there was some confidence that a molecular phylogeny would provide the scaffolding for an objective classification of the protists. At around the same time, entirely new phyletic assemblages were generated, such as the 'stramenopiles'—containing protists as diverse as diatoms and chrysomonads; and the alveolates (including dinoflagellates, ciliates and the apicomplexans)—introduced on ultrastructural grounds by Cavalier-Smith (1993), then rapidly confirmed by molecular phylogeny.

But it was not long before some bizarre problems appeared. Sequence information from small-subunit ribosomal RNA and from several protein-coding genes indicated that amitochondrial protist groups such as the diplomonads, parabasalids and microsporidia were all primitive. But other trees, based on tubulins, the large subunit of RNA polymerase II and heat shock protein 70 (Roger 1999) indicated that the microsporidia were not deeply branching, but close relatives of the fungi, with a tendency to adopt arbitrary locations in the molecular phylogeny, or even to move from a basal to a crown position depending on which genes were being sequenced (Keeling & McFadden 1998). It is now understood that these problems are due to artefacts or systematic errors caused by the algorithms used for constructing trees. These are based on various evolutionary models which incorporate assumptions that may not be met, and they can generate differing branching patterns from the same dataset. A further problem is that quickly evolving lineages may lead to excessively long branches and incorrect branching patterns.

The discovery of mitochondrion-derived genes in the nuclear genomes of diplomonads, microsporidia and other 'early diverging' groups now suggests that the ancestors of amitochondriate organisms probably had mitochondria. Molecular phylogenies based on multiple-gene analysis will, in the future, almost certainly be refined into useful tools that serve taxonomy, but for the moment, it may be worthwhile taking a step sideways.

3. MORPHOLOGY, ULTRASTRUCTURE AND THE ECOLOGICAL NICHE

Enthusiasm for gene genealogies undoubtedly sidelined ultrastructural information to some extent, although there had been a long history of using morphological and especially ultrastructural features in the attempt to distinguish protist lineages (e.g. Lynn 1991). In passing, Patterson *et al.* (1993) noted that none of the well-supported phylogenies based on morphology had been overthrown by molecular data.

The importance of ultrastructure derives from the unusually rich cytological diversity (e.g. compared with mammalian cells) that is obvious across the diversity of protists. The ultrastructural clues that help establish the identity of a protist are numerous (Patterson 1999), they are all revealed by electron microscopy, and they include features such as: shape of cristae in mitochondria or hydrogenosomes, presence and form of scales on the cell surface, presence of hairs or scales on flagella, the structure of plastids and the number of membranes that bound them, the types of rootlet structures arising from basal bodies, the number of nuclei and types of intranuclear inclusions, the behaviour of the mitotic spindle, and the presence of endosymbiotic prokaryotes and eukaryotes. This list could easily become very long, but the important point is that all of these structures are the products of multiple genes (hence the utility of multiple-gene analysis in phylogenetic analyses; Bapteste *et al.* 2002), and all structures can be observed simultaneously in a TEM section. It is perhaps unsurprising therefore that in some large

taxonomic groups (e.g. the chlorophytes; Melkonian & Surek (1995)) ribosomal sequence data and ultrastructural information are largely congruent. Corliss (1975) attributed the rise of protistology as a discipline to growth in the quantity of ultrastructural information that became available with the rapid improvement in electron microscopy techniques, particularly in the late 1960s and early 1970s. And Patterson (1994) was firmly of the opinion that the most solid development of the previous 30 years had been the use of patterns of ultrastructural organization to distinguish monophyletic lineages of protists. Ultrastructural organization has obvious significance for constructing phylogenies, but it also makes a significant contribution at lower taxonomic levels, and in particular with respect to characterizing, identifying and grouping species of protists.

The morphology and ultrastructure of protists often reflect key aspects of the ecological niches they occupy. At a crude level, we can include the long, ribbon-shaped marine interstitial ciliates such as *Tracheloraphis*, whose gross morphology tells us immediately how the organism manages to have a physical presence within the interstitial habitat. Something similar can be said for the diatom *Asterionella*, whose distinctive stellate morphology equips it to remain buoyant in the water column of lakes. Testate amoebae live predominantly in bogs and soil, but these habitats can dry out, so the shells created by testate amoebae allow them to resist desiccation. Many testate amoebae use local materials (e.g. diatom frustules that can be identified to species level; Ogden & Hedley 1980) to build or reinforce their shells, and this provides additional information about the local environment of the species. Many species interactions between protists—and especially predator–prey interactions—generate morphological adaptations that can be dynamic (e.g. the temporary spine development in *Euplotes* induced by the near presence of a specific predator; Kuhlmann & Heckmann 1994) or simply a symbol of the continuing struggle for existence—as in the marine heterotrophic dinoflagellates that extend a pallium or 'feeding veil' to trap and digest diatoms, although the diatoms have already evolved immensely long spines in response to the threat of 'veil' entrapment (Hansen & Calado 1999).

Some of the clearest links between morphology and the ecological niche of protists are revealed by the filter-feeding protozoa, where the mechanics of feeding have evolved to capture microbial food particles in specific ranges of size and abundance. The choanoflagellates for example often dominate the heterotrophic nanoplankton in seawater. A flagellum drives water through a collar of fine tentacles; the food particles get stuck on the outside and are then swept off by pseudopodia arising from the base of the collar. Fenchel (1986) showed that the free space between neighbouring tentacles in the marine choanoflagellate *Diaphanoeca* was only 0.1–0.3 μm, and that the flow velocity through the filter was therefore relatively low. The consequence for *Diaphanoeca* was that it could trap the smallest of the planktonic prokaryotes, so the 'food niche' of this organism consists of very small prey organisms. By contrast, the filter-feeding helioflagellate *Pteridomonas* has a relatively coarse filter (porosity 1–3 μm) so it cannot retain the smallest food particles that are trapped by the choanoflagellates. Even the functional morphology of a single structure—the filter—guides us to key features of the food niche that is filled by the organism.

Ciliate protozoa, with their more specialized feeding organelles, provide further examples. *Cyclidium* is typical of those ciliates adapted for feeding on the smaller prokaryotes. It has a relatively large, low-porosity filter, consisting of a row of cilia separated from each other by a free distance of *ca*. 0.25 μm; feeding experiments indicate that the lower size limit for retention is *ca*. 0.3 μm (Fenchel 1986). Now compare this with the hypotrich ciliates. Their distinctive morphological feature is a row of membranelles lying along one side of the mouth. The ciliate pumps water through the zone of membranelles, where particles are trapped and transported to the cytostome. These ciliates can trap only those particles that are bigger than *ca*. 1–2 μm (the size of fairly large bacteria), and in hypotrichs such as *Euplotes* the maximum clearance of particles is in the size range of 4–5 μm (because the free space between neighbouring membranelles ranges from *ca*. 2 to 4 μm). So in the ciliates too, even if all we do is look at the structure of feeding filters, we can deduce the sizes of food particles that will be ingested, which in many cases can easily be corroborated by microscopic examination of the food vacuoles. The documented morphological diversity of spines, hooks, suckers, trichocysts, toxicysts, 'nets' and mouths that have evolved in the protozoa to feed on other (usually smaller) organisms is probably nothing more than a pale reflection of the extraordinary number of protist niches that exist in the natural world.

The ecological significance of morphology is also revealed at the ultrastructural level, and this becomes obvious even from a cursory view of a thin TEM section of an unidentified protist. Electron-dense mitochondria with sparse cristae will usually be hydrogenosomes, and often closely associated with methanogenic bacteria (Finlay *et al.* 1993). The organism will, therefore, be an anaerobe. It will almost certainly be a flagellate, a naked amoeba or a ciliate—and TEM should quickly sort that out. If it is a ciliate, there is likely to be material in the food vacuoles that confirms its anaerobic character, such as *Thiopedia* or some other typically large or otherwise distinctive anaerobic bacteria (Guhl & Finlay 1993). The morphologies of any endosymbiotic bacteria, the manner in which they are associated with or attached to the hydrogenosomes, and in particular whether or not they are polymorphic within the host cytoplasm, together with patterns of the host infraciliature that will usually be easily visible in the same thin section, will often allow the ciliate to be identified to within one or two genera, and possibly even to one or two morphospecies. In this particular case, if it is a freshwater ciliate, the creature almost certainly makes a living by grazing bacteria in the superficial anaerobic sediment or anoxic hypolimnion of a lake or pond, where it avoids being eaten by (exclusively aerobic) metazoan predators.

In protists, as in higher animals and plants, the external morphology (and in the case of protists, the internal fine structure too) does reflect aspects of the ecological niche that the species occupies. Unfortunately, we may also recall that for a fairly large number of species—including the many minute scale-bearing forms (e.g. chrysomonads, *Luffisphaera*), the *only* thing we know about them is their morphology, and when and where they were found. Few of them have ever been isolated, cultured and sequenced,

yet the conventional wisdom is that the scale structures are conservative features, which enable discrimination of 'morphospecies'.

The morphological diversity of protists provides a rich fund of information that guides us in our enquiry about the link with role in the environment. But it does provide only a guide, and its taxonomic value varies across taxonomic groups—high for the morphologically rich ciliates, but low for many groups such as the unicellular coccoid green algae. Moreover, it is often the case that we are presented with a striking morphology that has no obvious ecological significance. The filamentous cyanobacterium *Spirulina* lives in soda lakes and has evolved the morphology of a tightly coiled spring. Intuitively, one would think that this reduces the chances of its being eaten, but paradoxically, it is eaten by an unusually broad range of grazers, from ciliates to flamingos (Finlay *et al.* 1987).

4. PROBLEMS WITH MORPHOSPECIES

Most taxonomic effort, whether directed at protists, vertebrates or higher plants, depends on an ability to recognize 'morphospecies'. In some protist groups (e.g. the ciliates, where morphology and ecological function are strongly linked), it is still the species concept of choice used by most researchers, but even for the ciliates, and especially for many other groups, there are problems that need to be addressed. These include the following points.

(a) *Adaptive peaks*

The morphospecies 'concept' dominates most of protist taxonomy, and its core assumption is that each speciation event results in a change in cell morphology (Andersen 1998). This, however, is not always obvious, because natural selection tends to operate by selecting for specific morphotypes that represent adaptive peaks associated with particular sets of environmental conditions. Thus, all sibling species within the *Tetrahymena pyriformis* complex are virtually indistinguishable morphologically (Nanney 1999)—as are the many species of coccoid green algae. Each morphospecies probably supports a range of genotypic and phenotypic variants, but natural selection ensures that the gross morphology remains roughly the same, and probably has done so for the greater part of the history of eukaryotic life on Earth (Schonborn *et al.* 1999; Fenchel 2002).

It is now known that there is a wide variety of morphologically homogeneous taxonomic groups in which it is difficult (for humans) to discriminate the differences (e.g. in cell surface glycoproteins) that may be important to the organisms concerned.

(b) *Little green balls*

Consider the small (less than 3 μm), roughly spherical planktonic green algae that are invariably assigned to the genera *Chlorella* and *Nannochloris*, and that reveal so few morphological characters that it is difficult or impossible to separate and identify them using morphological criteria alone. Even at the ultrastructural level, they yield little information—just a single nucleus, chloroplast and mitochondrion, one or two peroxisomes, and one or two vacuoles (Krienitz *et al.* 1999), although some workers believe that electron microscopy can still be used to differentiate strains (e.g. using the structure of the pyrenoid (Ikeda & Takeda 1995) or the presence or absence of 'spinelets' on the outer surface of the cell wall (Vladimorova *et al.* 2000)). Only when morphological and ultrastructural features are used in combination with biochemical (e.g. production or absence of secondary carotenoids), physiological and molecular (e.g. complete SSU rRNA gene sequences) characters, can the taxa be separated unambiguously (Huss *et al.* 1999). And again, the strikingly high degree of morphological and ultrastructural uniformity across genera presumably reflects convergent evolution towards adaptive peaks in morphotype, as the organisms concerned are known to have evolved from different phylogenetic origins (Potter *et al.* 1997; Krienitz *et al.* 1999).

Whether such morphologically similar organisms fill the same ecological niche in nature is not known, although there is now evidence that discrete groups within morphospecies can differ physiologically and genetically. The cyanobacterium *Prochlorococcus* is probably the most abundant component of the oceanic phytoplankton (Fuhrman & Campbell 1998). Ferris & Palenik (1998) discovered distinct clades that were specifically adapted to either surface or deep water, and Moore *et al.* (1998) found that they could live at different depths because they were physiologically adapted for growth at either high- or low-light conditions. The high-light-adapted organisms were shown to be genetically (16S rRNA) different from those that were low-light adapted (97.5% similarity), whereas physiologically similar organisms from different sites in the ocean shared a higher level of sequence similarity (99.7 and 99.2% for high- and low-light-adapted organisms, respectively).

So here we have organisms that cannot be discriminated morphologically, although they can be divided into physiological variants that apparently fill discrete ecological niches. The different physiological types appear to be correlated with specific genotypes, and it seems that differences of *ca*. 2% in 16S rRNA sequence correspond to ecologically significant diversity within this group. This raises the possibility that the widely recognized high level of genetic variation at the 16S rRNA locus observed in other microbial communities may also reflect physiologically distinct microbial populations. Developments such as these, in concert with the failure of the morphospecies concept (as it now stands) to embrace genetic and phenotypic diversity, have prompted the suggestion from various quarters (Palys *et al.* 1997; Ward *et al.* 1998; Cohan 2002) that a new conceptual framework for defining microbial species—perhaps one based on ecological criteria, would be more useful. Bearing in mind the likelihood that all (or most) protist species are cosmopolitan (the freshwater diatom *Asterionella formosa*, for example, can be found on all continents including Antarctica), it follows that different isolates probably differ physiologically from each other. Current work involving our group and T. Fenchel (personal communication) does indicate that clonal isolates of a single morphospecies from various locations worldwide retain physiological features that reflect the nature of the habitat from which they were taken (e.g. fresh, brackish, sea or hypersaline water). Most of these isolates have considerable capacity for adaptation within broad, genetically fixed ranges, so their individual

physiological performances tend to appear as partly overlapping ranges superimposed on an environmental gradient (e.g. of salinity).

(c) *Arbitrary discrimination*

A further difficulty associated with the practical use of a morphospecies concept is the assumption that adopted morphological changes accompanying speciation events are sufficiently distinctive to be recognized by an observer with a microscope. This of course promotes subjective recognition of characters and character differences, concentration on minor phenotypic features that are probably not linked to the fitness of the organism in the natural environment (e.g. establishing new species on the evidence of the length of mucocysts, number of macronuclear nodes or number of kinetosomes at the base of a cirrus), and to the generation of synonyms. The high rate of synonymy is sustained by a worrying level of undersampling. Take the example of the anaerobic ciliate genus *Metopus*. The 76 nominal species known in 1995 were eventually reduced to 22 morphospecies, but no fewer than 45 of the original nominal species had been reported only once in the literature (Esteban *et al.* 1995). The practice of protist taxonomy is less clearly defined, and in many cases is much more arbitrary than it is for higher organisms—partly because relatively few people work on protists. But perhaps the most serious (historical) problem is that morphospecies have invariably been established following observation of only a few organisms, and sometimes a single one. The facts now emerging from intensive investigation (Finlay *et al.* 2004) indicate that morphospecies contain significant phenotypic (including morphological) variation that can be realized only after examination of clonal cultures and large numbers of organisms.

At what optical magnification should we attempt to discriminate morphospecies—or does it matter? Take the business of identifying a ciliate, using a low-power microscope. Its general body shape will be obvious, and any distinctive swimming or feeding behaviours will be noted. At higher magnifications it may be possible to see the coordinated beating of the cilia, the mouth and the feeding filter and it will be possible to distinguish between different types of cilia and cirri. If the organism is then subjected to one of the silver-staining procedures (which stain the basal body at the root of each cilium), the pattern of oral and somatic infraciliature will be revealed with high-power objectives and it will be possible to identify the ciliate to morphospecies level. By increasing the microscope magnification to the levels possible with the electron microscope, we will see cell surface features (e.g. scales), endosymbionts and cytoplasmic inclusions that are difficult or impossible to resolve with light microscopy. With respect to the ciliates, the consensus is probably that light microscopy, incorporating specialized staining procedures, is what is required, while for most of the larger flagellates, a combination of high-power light microscopy and electron microscopy is required. It is possible, however, that we are dealing with nothing more than a pseudo-problem, especially if morphological detail in protists is fractal—i.e. similarly rich in detail at different spatial scales—say at the scale of a few micrometres, tens of micrometres or hundreds of micrometres. Or, to take a specific example, do we find roughly the same degree of morphological detail in the oral apparatus of a ciliate observed at high magnification as in the whole ciliate observed at low magnification? Such an analysis has probably never been carried out.

In any event, it seems likely that taxonomic practices geared to the morphospecies have evolved in such a way that the choice of spatial scale is largely irrelevant. An illustration of this was provided by a soil habitat that was known to have fractal structure (i.e. with a constant degree of spatial heterogeneity at progressively smaller spatial scales) and was predicted, therefore, to provide relatively constant protist species richness across spatial scales (Finlay & Fenchel 2001). This was confirmed—for in each of the three protozoan size ranges (ciliates, testate amoebae and flagellates), *ca*. 100 morphospecies were recorded. Although different methods and microscope magnifications had been used for the different taxonomic groups, and although the pattern of observation varied from whole cell morphology to selected parts of the organism (such as the oral apparatus), the pattern of morphospecies richness at different spatial scales was consistent with what would be predicted for a fractal environment. The key point here is the irrelevance of the spatial scale selected for the identification of protists.

(d) *Protist consortia*

Many free-living protists harbour symbiotic organisms, which are invariably prokaryotes or other protists. These associations are well studied in the ciliates, where they have reached a high degree of functional integration. The symbiotic partners are often green algae or functional chloroplasts sequestered from algae. Most anaerobic ciliates have endosymbiotic methanogenic bacteria that act as hydrogen sinks, while some marine anaerobic ciliates have ectosymbiotic sulphate-reducing bacteria. In the ciliate *Paramecium*, symbiotic bacteria confer killer status upon the host, and in the mouthless marine interstitial ciliate *Kentrophoros*, only half of the biomass is ciliate—the remainder is a 'coat' of sulphide-oxidizing bacteria, which the ciliate crops by periodically invaginating its cell surface and digesting the bacteria (Fenchel & Finlay 1989).

In all of these consortia, two or more genomes coexist and it is the integrated consortium that fills the ecological niche, and on which natural selection operates. Take the case of the ciliate *Euplotes daidaleos*, which harbours endosymbiotic green algae. It lives at a depth in the water column where there is little if any dissolved oxygen, but where there is just enough light to drive net photosynthesis. The photosynthetic symbionts provide the oxygen required for aerobic respiration, so the consortium becomes an aerobic 'island' surrounded by anoxic water (Finlay *et al.* 1996*a*). By living where it does, the consortium has access to an elevated concentration of carbon dioxide. The algal symbionts fix this into sugars, some of which is then siphoned off by the ciliate partner, and the ciliate controls the supply of inorganic nitrogen to the symbiont.

It could be argued that we are simply dealing with a good example of symbiosis involving two or more species, and indeed, both partners presumably benefit, with an increase in fitness. But the consortium actually achieves something more, for it brings an enhanced level of fitness

to an ecological niche that is denied to each of the individual partners acting alone. Perhaps the *E. daidaleos* example represents a large number of tightly integrated protist consortia (e.g. the *Kentrophoros* consortium cited earlier) that are functionally analogous to prokaryote syntrophic consortia (Fenchel & Finlay 1995). The latter are classified in ways that reflect their roles as ecological species, e.g. '*Chlorochromatium aggregatum*'—consisting of cells of a green photosynthetic bacterium (*Chlorobium*) surrounding an anaerobic organotroph (Overmann & Schubert 2002).

Euplotes daidaleos, like many permanent associations involving at least two complete eukaryotic morphospecies, is a chimera that sits awkwardly within the morphospecies concept. But we do know and understand the nature of the ecological niche that the creature fills, which in this case, at least, makes an ecological species concept rather appealing and probably appropriate.

(e) *Biological species*

Many, perhaps most, protists spend the greater part of their lives as purely asexual creatures, but a significant number reproduce sexually (if infrequently) and can therefore be classified as biological species (i.e. reproductively isolated gene pools—also known as syngens, sibling species or genetic species). For example, the dinoflagellate morphospecies *Crypthecodinium cohni* consists of at least 19 morphologically indistinguishable but genetically isolated biological species (Nanney 1999), and the ciliate *Paramecium aurelia* consists of at least 14 biological species (Sonneborn 1975). But the degree of congruence between biological species and ecological species is not really understood. Is a protist biological species one that occupies a specific ecological niche, and how does this compare with the strength of linkage between a morphospecies and its niche? There is no clear answer to this question. There is tentative evidence of ecological specialization of sibling species of the foraminiferan *Orbulina universa* (de Vargas *et al.* 1999); and the frequent observation of multiple sympatric genetic species (e.g. of the ciliate *Tetrahymena*) may suggest that they are not entirely equivalent with respect to their role in nature. It is believed by some that biological species do probably represent ecologically differentiated evolutionary units. Indeed, it has also been suggested that cryptic ecological specialization may be the evolutionary dynamic generating the biological species in the first place (D. L. Nanney, personal communication). Most biologists probably believe that sympatric sibling species will eventually be shown to occupy differentiated niches. One important task for the future will be to discover if there is any discernible correspondence between biological species, ecotypes and DNA sequence clusters.

Finally, there is no reason to convey the impression that the species concepts applied to protists are less clearly defined and less well supported than those applied to animals and plants, because the latter share many of the problems identified in protists. These include genetic and phenotypic variation within species, continuous variation in form across members of a species with a wide geographical range, and morphospecies that consist of complexes of sibling species. In plants in particular, there are problems with asexual 'species' and speciation resulting from hybridization and polyploidy. Similarly, there is evidence that different ciliate morphospecies (e.g. *Euplotes vannus* and *E. crassus*) mate and exchange genes with each other (Caprette & Gates 1994). In principle, it is likely that the business of applying species concepts to protists is no more complex and difficult than it is for animals and plants.

5. DIVERGENCE, COHESION AND ECOTYPES

Like bacteria, many protists are clonal, insofar as recombination is rare or non-existent. Each cell division in clonal protists is then effectively a speciation event because the daughter cells initiate separate lineages that remain genetically isolated from one another (Fenchel 2002). Each lineage will independently acquire predominantly neutral mutations, so they will diverge genetically, and possibly phenotypically, over time.

According to the 'cohesion species concept' (see Cohan 2002, p. 461), a 'species' is a group of organisms whose 'divergence is capped by one or more forces of cohesion'. In sexual species, the principal cohesive force is genetic exchange and recombination; and even occasional recombination probably limits genetic divergence within species. But asexual species too are subject to a force of cohesion, and most work in this area has been directed at bacteria (Cohan 1994, 2001, 2002; Palys *et al.* 1997). The central thrust of the (still largely theoretical) approach could in principle also apply to asexual protists, and it is therefore set out here.

First, we must attempt to clarify two core terms—'ecotype' and 'ecological niche'—which tend to be interpreted in different ways by people from different backgrounds. The term 'ecotype' first appeared in the botanical world, as a subspecific category in sexual plants (Turesson 1922). Experimental plant taxonomists at that time sought to determine the extent to which visible morphological differences in sexual plants were based on environmental modification, and how much reflected genetic differences. Turesson pioneered the use of cultivation to determine if genetic factors and phenotypic adaptation were both involved, and he discovered that they were. He concluded that every widespread plant species consists of a number of genetically different 'ecotypes' (also referred to elsewhere as 'races'), each of which is phenotypically adapted to a particular environment.

Ciliate protozoa are of course not closely related to higher plants, but they do share the characteristic of being sexual organisms, and, intriguingly, it appears that ciliate morphospecies too may consist of multiple ecotypes—each with a particular genotype and phenotype (Finlay *et al.* 2004). The ciliate *Cyclidium glaucoma*, like ciliates in general, has cosmopolitan distribution, and tentative evidence indicates that any particular ecotype, such as the one associated with hypersaline water, is likely to be found in hypersaline habitats elsewhere, even if these habitats are separated by great distance (e.g. southern Spain and Great Salt Lake in the USA)—a finding for which there is already much scattered supporting evidence (e.g. Darling *et al.* 2000; Montresor *et al.* 2003).

Ecotypes within animal species tend to be defined more loosely. In red deer (*Cervus elaphus*) they have been separated on differences in form and weight of the antlers; and bottlenose dolphin (*Tursiops truncatus*) ecotypes have been

separated on habitat differences (e.g. preference for either inshore or offshore feeding). In the bacteria, 'an ecotype consists of a set of strains that use the same or very similar ecological niches' (Palys *et al.* 1997, p. 1145; Cohan 2002). It is difficult to make the definition more precise because the business of defining any ecological niche, let alone that of a micro-organism, is not a simple task. What qualitative features define the niche? Is it a temporal phenomenon? Do niches abut or overlap? Are they simply arbitrary constructs based on largely anecdotal information, and if so, can we rank the importance of different features? Most important, perhaps: is niche partitioning at the microbial level based, at least partly, on cryptic features that are beyond human powers of discrimination?

Accepting that our knowledge of the character and boundaries of the ecological niches of microbes is imperfect, yet accepting for the moment that belief in a objective reality called 'ecological niche' is not entirely misguided, the process by which distinct microbial ecotypes arise can be described as follows.

A bacterial 'ecotype' is a population of bacteria living in the same ecological niche. A single beneficial mutation appearing within an ecotype might outcompete to extinction all other local strains of the same ecotype. In that case, only the genome associated with the mutant and its descendants will remain. This diversity-purging process is referred to as 'periodic selection' (Atwood *et al.* 1951). Because different ecotypes differ in the resources they use, they are not expected to drive to extinction strains from other ecotypes. Thus, periodic selection eliminates diversity within, but not between, ecotypes. Successive selective sweeps make ecotypes progressively more distinct, and when they are so divergent that they have escaped each other's periodic selection events, they are predicted to be separate and distinct ecotypes.

Recent theory (see Palys *et al.* 1997) also suggests that each DNA sequence-similarity cluster could correspond to an ecotype, but it is not yet clear which level of cluster or subcluster, if any, corresponds to ecotypes, or even if clusters are sufficiently distinct to be resolved. As Cohan (2001) suggests, however, the discovery of correlated ecotypes and sequence clusters is potentially extremely useful for the discovery and identification of new ecotypes. A phylogenetic (and theoretical) perspective on the consequences of periodic selection, and how different ecotypes may become distinct sequence-similarity clusters, is illustrated in Cohan (2001, p. 518).

What are the implications for protists? Many are sexual (e.g. ciliates, diatoms) but for a large number of taxa (e.g. amoebae and many flagellates) sexual behaviour has never been recorded, and in most cases it is possibly rare and probably non-existent. Asexual protists are presumably subject to the same forces of cohesion as bacteria (periodic selection), whereas the sexual protists have cohesion endowed by genetic exchange. In both cases we might expect lineages to terminate in sequence-similar clusters corresponding to ecotypes. How, then, should we circumscribe protist 'species'? We might adopt one of the commonly cited definitions of 'species' for bacteria—for example, that strains belonging to a phenotypically distinct species share 97% identity in SSU rRNA gene sequence (see also Oren 2004). But this immediately becomes unattractive when we consider that the extent of genetic variation in a bacterial species could be similar in scale to that encompassing all mammals. Another commonly cited criterion is that a group of strains should demonstrate at least 70% annealing of genomic fragments in DNA–DNA hybridization if they are to correspond to an ecologically distinct population; but this criterion is entirely unsupported by evolutionary genetic theory (Palys *et al.* 1997).

Animal and plant species, protists and bacteria, all tend to occur in discrete phenotypic and genetic clusters (Sokal & Crovello 1970; Mallet 1995; Melkonian & Surek 1995; Cohan 1995, 2002; Palys *et al.* 1997). In the case of genetic clusters, the theory of periodic selection predicts that ecologically distinct populations will eventually diverge into distinct DNA sequence clusters. Following on from the notable advances in linking bacterial sequence clusters with ecologically distinct populations (Moore *et al.* 1998), it remains to be seen whether such an ecological species concept can accommodate the protists.

It is predicted that future surveys of protein-coding gene sequences will disclose many ecological populations of bacteria about which nothing currently is known (Palys *et al.* 1997). Sequencing of the 16S rRNA gene may not have a major role in identifying ecological differences, because it fails to distinguish ecologically distinct groups of bacteria—hence the switch to sequencing diversity at protein-coding loci which, unlike the ('housekeeping') 16S rRNA gene, evolve relatively quickly. It is not known if the dense SSU rRNA sequence clusters of protists now appearing (Pröschold *et al.* 2001; Marin *et al.* 2003) indicate protist ecotypes.

6. NAMING PROTISTS

The shifting sands of protist phylogenies, the continuing proliferation of nominal protist phyla, the lack of agreement as to how protists should be classified and how names of higher taxa should be selected and applied, plus the absence of agreed nomenclatural codes, all conspire to muddy the waters of protist taxonomy (Vickerman 1992). It is perhaps inevitable therefore, especially while striving for greater clarity, that nomenclature, at least, should provide names that are as informal as possible, while remaining useful. Patterson's (1999) 'rankless informal names' are in common parlance; they are probably as useful as any, and they may serve as a guide for the future (see also Forey *et al.* 2004). Examples include the 'actinophryids' (with radiating stiff arms), the 'alveolates' (containing the ciliates, dinoflagellates and apicomplexa, and all with the shared ultrastructural character of 'alveoli'); 'cryptomonads' (with refractile ejectisomes and flattened cristae in an extensive mitochondrion); 'haptophytes' (marine flagellates with two flagella and an additional feeding organelle, the 'haptonema'); 'red algae' (with chloroplasts containing phycobilins); and 'xenophyophores' (marine amoeboid organisms with an agglutinated test, living in the benthos of the deep sea, with barite in the cytoplasm).

At the 'species' level, the 'morphospecies' is most useful (and ecologically relevant) for morphologically rich groups such as the ciliates, but it is wholly inadequate for 'little green balls' and the like. If it can be shown that the ecotypes suggested by sequence-similar clusters are indeed

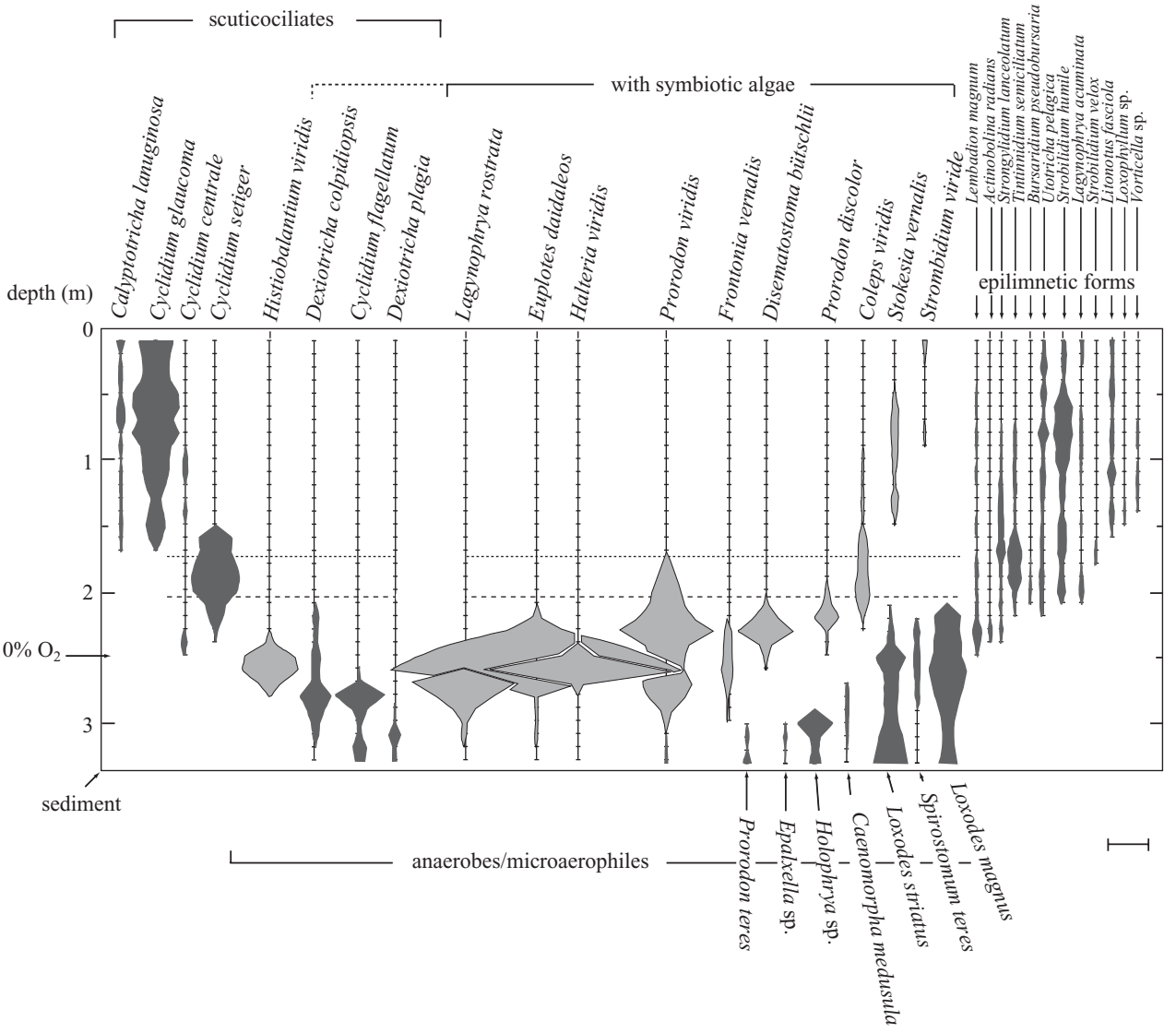

Figure 1. Vertical distribution of ciliate species in the stratified water column of a freshwater pond (Priest Pot, Cumbria, UK; August 1995). Pale-shaded kites signify ciliates with endosymbiotic algae or sequestered chloroplasts. The depth of the oxic–anoxic boundary is indicated by '0% O_2'. The scale bar represents 100 ciliates ml^{-1} for all species apart from *Halteria viridis*, where it signifies 500 ml^{-1}. The total number of ciliate species in the water column on this occasion was 37. (Reproduced from Finlay & Esteban (1998) and reprinted from *Protist*. Copyright © 1998 Gustav Fischer.)

ecologically distinct, and if the clusters can be resolved, then it may be fruitful to adopt the suggestion of Cohan (2002) of recognizing them in a tripartite name. As a bacterial example, Cohan suggests classifying *Neisseria meningitidis* to the ecotype level as '*N. meningitidis* ecotypus *africana*'. An example of a free-living microbe might be the cyanobacterium *Prochlorococcus*—the smallest and most abundant photosynthetic organism in the ocean, classified to ecotype level as '*P. marinus* (high-light-adapted)', and a ciliate example might run as '*Cyclidium glaucoma* (hypersaline)'. Of course, problems and complications will undoubtedly arise. It may be relatively easy to define ecotypes of pathogenic microbes, because the ecological niches they fill are tightly defined, and determined by a specific host. In the case of free-living microbes, the boundaries of the ecological niche are leaky. Natural selection might produce an intermediate-light-adapted *Prochlorococcus*, or a ciliate from a hypersaline lagoon might acquire greater phenotypic flexibility and become able to live at low salinities (which appears already to have happened; T. Fenchel, personal communication). In many cases we will know so little about the natural history of the organism that we cannot say anything sensible about the ecological niche it fills. In other rare cases, we are afforded a tantalizing glimpse of the potential of protists to partition their local environment, and the spatial niches they carve out for themselves.

7. THE (CILIATE) PROTIST NICHE

Consider a freshwater pond at temperate latitude, in a sheltered location, during a prolonged period of calm weather in summer. The surface water temperature is likely to be much higher than at depth, so the water column will become thermally stratified, and vertical mixing will be restricted by a thermal gradient (the thermocline) located at some intermediate depth. The upper water will remain oxygenated, but deep water and sediment will become anoxic, and the region of the thermocline and oxic–anoxic boundary will support steep physical and

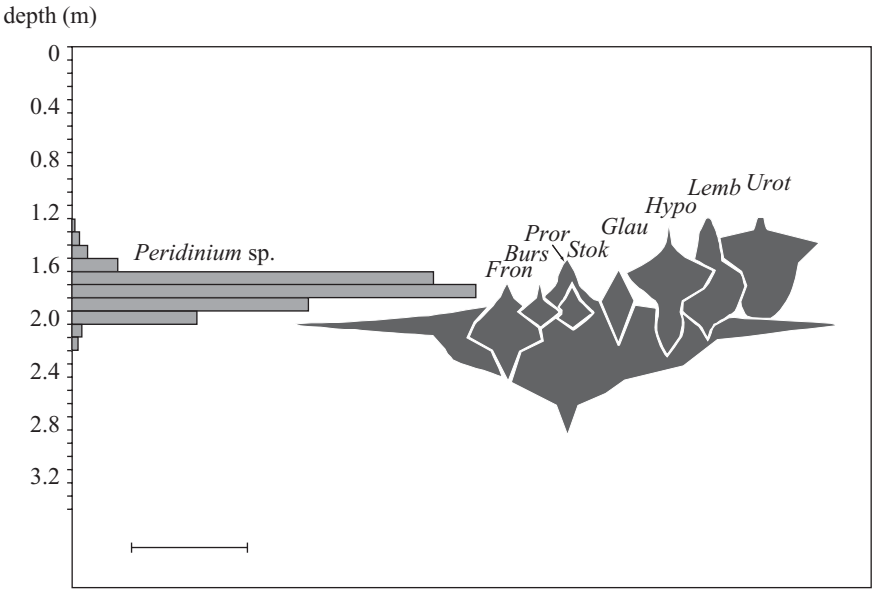

Figure 2. Vertical distribution of ciliate species in the water column of the same pond, on 5 June 1997, when a dinoflagellate (*Peridinium* sp.) formed a dense population around a depth of 1.8 m. The ciliate species arranged themselves in a series of superimposed spatial niches, where they fed on the dinoflagellates. *Urot*, *Urotricha pelagica*; *Lemb*, *Lembadion magnum*; *Hypo*, *Hypotrichidium conicum*; *Glau*, *Glaucoma myriophylli*; *Stok*, *Stokesia vernalis*; *Pror*, *Prorodon viridis*; *Burs*, *Bursellopsis* sp.; *Fron*, *Frontonia* (*vernalis* + *leucas*, principally the former). Small numbers of *Bursaridium pseudobursaria* and *Acaryophrya collaris* were also recorded in the vicinity of, and feeding on, the *Peridinium* peak on this occasion. *Loxodes* (*magnus* + *striatus*) was abundant (100 ml^{-1}) at all depths from 2.2 m down to the sediment, in which zone it fed mainly on sedimenting *Peridinium*. Scale bar represents 1000 *Peridinium* ml^{-1} and 100 ciliates ml^{-1}. On this occasion the water column was anoxic at depths greater than 2.1 m. The total number of species recorded in the water column on this occasion was 48. (Reproduced from Finlay & Esteban (1998) and reprinted from *Protist*. Copyright © 1998 Gustav Fischer.)

chemical gradients. The underwater light climate will change with increasing depth, and the pH will often be significantly higher in surface water, whereas concentrations of dissolved CO_2, inorganic nitrogen and reductants such as sulphide will be much higher at depth. Thus, the heterogeneous vertical profile of the water column provides a series of superimposed microenvironments. With the passage of time, these expand and contract vertically, but they maintain their relative positions in the water column for weeks or months. Very soon after these microhabitats are created, random dispersal, plus taxic and kinetic swimming behaviours (Finlay & Fenchel 1986; Fenchel *et al.* 1989), together with natural selection, ensure that all available habitats are quickly filled with populations of protists selected from the vast local diversity of rare and cryptic species.

Of course, niche width cannot become infinitely small so there must be a limit to the number of niches available in any habitat. With respect to the ciliates, roughly 30–50 morphospecies typically coexist in the water column of a freshwater pond (Finlay & Esteban 1998), and each species tends to find its 'spatial niche' at a slightly different depth. Thus, *Strobilidium velox*, *Loxodes striatus* and *Caenomorpha medusula* are only ever found in oxygenated surface water, the oxic–anoxic boundary and in deep anoxic water, respectively (figure 1). Where gradients are weak, as in the upper water layer, the spatial niche will extend over a relatively wide depth range; and where gradients are steep, as around the oxic–anoxic boundary, the spatial niche will be squeezed into a narrow depth range. Sometimes, the degree of vertical packing of species can be extreme—as when a group of at least eight ciliate morphospecies were attracted to a dense population of the dinoflagellate *Peridinium* (35 000 ml^{-1}) sitting on the oxic–anoxic boundary (figure 2). The eight coexisting and partly overlapping species distributions each formed peak abundances at specific depths, separated vertically by only a few centimetres (Finlay & Esteban 1998).

In the case of the ciliates in the freshwater pond, the spatial niche is also an ecological niche because the 'space' is defined by specific ranges of ecological factors such as oxygen tension, subsurface light and the activities of the other microbes that live in that 'space'. *Lagynophrya rostrata*, for example, occupies an ecological niche that overlaps several other ciliate niches, yet it appears to be the exclusive occupant of an ecological niche, as it forms a population peak that is deeper than that of any other ciliates with photosynthetic endosymbionts.

Individual ciliate morphospecies—whether planktonic oligotrichs, interstitial trachelocercids or the ciliates living in an activated sludge plant—tend to be associated with specific physical–chemical environmental characteristics, i.e. they appear to fill ecological niches, and the same morphospecies are found worldwide, wherever the same habitat type is found (Esteban *et al.* 2000). Ciliate morphospecies might then be resurrected as *bona fide* ecological species, if it were not for the problem that different isolates of the same morphospecies may fill different ecological niches. The morphospecies *Cyclidium glaucoma* is found in freshwater ponds as well as hypersaline lagoons. Isolates from the two habitats can be morphologically identical but the ecological niches they fill are different, and the isolates are physiologically quite different. A truly ecological species concept for ciliates (and other protists)

will have to take account of the finer divisions of morphospecies.

8. THE 'CELL FROM HELL' AND LESSONS FOR THE PURSUIT OF A HOLISTIC PROTIST TAXONOMY

Many protists are polymorphic—whether as flagellated forms of naked amoebae or giant macrostomes of ciliate species, or simply as over-wintering or dessication-resistant cysts. In many species, the polymorphs are poorly documented, and there is no proven course of enquiry by which we might establish the range of polymorphic variants of any particular species. However, a course of events in recent years has shed some light on how we might approach this problem, and it may actually provide a model for how to progress in the future.

Burkholder *et al.* (1992) described a dinoflagellate with 'phantom-like' behaviour. It was associated with fish kills in estuaries, and these events were large scale—'15 million silvery carcasses would carpet the water'. Within a few hours of fish death, it was noticed that the dinoflagellates produced resting cysts, amoeboid stages and various other morphotypes. The dinoflagellate (now referred to as *Pfiesteria piscicida*) was apparently capable of transforming into at least 24 distinct forms, including filose and lobose amoeboid forms, and one form that resembled a heliozoan (Burkholder 1999). The only problem was that this great variety of *P. piscicida* morphotypes had been observed only in field samples that had been transferred to aquaria. The alarm was raised when one of the life cycle stages was identical to the heliozoan *Actinophrys sol*, and another was a representative of the chrysomonad genus *Paraphysomonas*.

Litaker *et al.* (2002) re-described the life cycle of *P. piscicida* that developed from single-cell isolates and found only typical free-living marine dinoflagellate stages, no amoeboid forms and no flagellate-amoeboid transition stages. But the key development was to use molecular methods to test whether the dinoflagellate and the amoeboid forms were genetically identical. Clonal cultures of both amoebae and *Pfiesteria*-like dinoflagellates were established from Chesapeake Bay tributaries, and from aquaria where *Pfiesteria* was thought to be the cause of fish mortalities. In clonal culture, the putative amoeboid stages of *P. piscicida* were studied using light and electron microscopy, plus sequencing of the SSU rRNA gene; and fluorescently labelled nucleic acid probes were developed for *in situ* hybridization. *Pfiesteria*-specific probes failed to react with the amoebae, and vice versa. Clearly, the amoebae were not related to the dinoflagellates, and it has been suggested that the creatures regarded as dinospores transforming into amoebae were actually dying dinospores shedding their shells (Kaiser 2002). However, in terms of taxonomy, the whole business did represent some sort of achievement. An effective and relatively simple method for characterizing polymorphic protist life cycles had been produced. All that was required was a combination of light microscopy, clonal cultivation, sequencing SSU rRNA genes and the development of species-specific fluorescent probes.

Once the taxonomic background was sorted, an intensive research effort could be directed at *Pfiesteria*. It is, for example, still debated whether or not it produces toxins that kill fish (Rhodes *et al.* 2002). Alternatively, the evidence from electron microscopy and videomicrography indicates that dinospores swim towards fish; they then attach to the skin and rapidly denude the epidermis (Vogelbein *et al.* 2002).

The *Pfiesteria* 'stimulus' has led to spin-off developments in the morphological investigation of dinoflagellates. New methods have been developed to visualize the thecal and wall plates in lightly armoured dinoflagellates (Mason *et al.* 2003), and it appears that the molecular characterization of dinoflagellates generally confirms their taxonomy on the basis of external morphology (Steidinger *et al.* 2001). The wider ecological and public health issues are also being addressed. Rublee *et al.* (2001) collected more than 2000 water and sediment samples from estuarine sites along the US Atlantic and Gulf coasts and assayed for the presence of *Pfiesteria* by PCR probing of extracted DNA. They obtained positive results throughout the geographical range extending from New York to Texas. Saito *et al.* (2002) have developed a simple, rapid, species-specific PCR-based detection assay that can detect a single zoospore of *P. piscicida* in 1 ml of water, and it now seems that *Pfiesteria* may be distributed in estuarine waters worldwide. It is, for example, a common inhabitant of many estuaries in New Zealand (Rhodes *et al.* 2002).

The 'cell from hell' has come a long way in a short time—from obscurity, to the focus of intense investigation driven by economic and public health concerns. Unravelling its taxonomy may mean that the creature no longer lives up to its rather graphic epithet—but the process has highlighted the value of multiple integrated approaches to protist taxonomy.

This work was carried out with financial support from the Natural Environment Research Council, UK (M&FMB grant no. NER/T/S/2000/01351). The author is indebted to two anonymous referees for their thorough and constructive reviews.

REFERENCES

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

Andersen, R. A. 1998 What to do with protists? *Aust. Syst. Bot.* **11**, 185–201.

Atwood, K. C., Schneider, L. K. & Tyan, F. J. 1951 Periodic selection in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **37**, 146–155.

Bapteste, E. (and 10 others) 2002 The analysis of 100 genes supports the grouping of the three highly divergent amoebae: *Dictyostelium*, *Entamoeba*, and *Mastigamoeba*. *Proc. Natl Acad. Sci. USA* **99**, 1414–1419.

Blackwelder, R. E. 1967 *Taxonomy*. New York: Wiley.

Burkholder, J. M. 1999 The lurking perils of *Pfiesteria*. *Sci. Am.* **281**, 28–35.

Burkholder, J. M., Noga, E. J., Hobbs, C. H. & Glasgow, H. B. 1992 New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. *Nature* **358**, 407–410.

Caprette, C. L. & Gates, M. A. 1994 Quantitative analyses of interbreeding in populations of *Vannus* morphotype *Euplotes*, with special attention to the nominal species *E. vannus* and *Euplotes crassus*. *J. Euk. Microbiol.* **41**, 316–324.

Cavalier-Smith, T. 1993 Kingdom Protozoa and its 18 Phyla. *Microbiol. Rev.* **57**, 953–994.

Cohan, F. M. 1994 Genetic exchange and evolutionary divergence in prokaryotes. *Trends Ecol. Evol.* **9**, 175–180.

Cohan, F. M. 1995 Does recombination constrain neutral divergence among bacterial taxa? *Evolution* **49**, 164–175.
Cohan, F. M. 2001 Bacterial species and speciation. *Syst. Biol.* **50**, 513–524.
Cohan, F. M. 2002 What are bacterial species? *A. Rev. Microbiol.* **56**, 457–487.
Corliss, J. O. 1975 Nuclear characteristics and phylogeny in the protistan phylum Ciliophora. *Biosystems* **7**, 338–349.
Corliss, J. O. 1999 Biodiversity and numbers of species of protists. In *Nature and human society: the quest for a sustainable world* (ed. P. Raven & T. Williams), pp. 130–155. Washington, DC: National Academy Press.
Corliss, J. O. 2002 Biodiversity and biocomplexity of the protists and an overview of their significant roles in maintenance of our biosphere. *Acta Protozool.* **41**, 199–219.
Darling, K. F., Wade, C. M., Stewart, I. A., Kroon, D., Dingle, R. & Leigh Brown, A. J. 2000 Molecular evidence for genetic mixing of Arctic and Antarctic planktonic foraminifers. *Nature* **405**, 43–47.
de Vargas, C., Norris, R., Zaninetti, L., Gibb, S. W. & Pawlowski, J. 1999 Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. *Proc. Natl Acad. Sci. USA* **96**, 2864–2868.
Esteban, G., Fenchel, T. & Finlay, B. J. 1995 Diversity of free-living morphospecies in the ciliate genus *Metopus*. *Arch. Protistenk.* **146**, 137–164.
Esteban, G. F., Finlay, B. J., Olmo, J. L. & Tyler, P. A. 2000 Ciliated protozoa from a volcanic crater-lake in Victoria, Australia. *J. Nat. Hist.* **34**, 159–189.
Fenchel, T. 1986 Protozoan filter feeding. *Prog. Protistol.* **1**, 65–113.
Fenchel, T. 1987 *Ecology of Protozoa*. Madison, WI: Science Tech.
Fenchel, T. 1993 There are more small than large species? *Oikos* **68**, 375–378.
Fenchel, T. 2002 *Origin and early evolution of life*. Oxford University Press.
Fenchel, T. & Finlay, B. J. 1989 *Kentrophoros*: a mouthless ciliate with a symbiotic kitchen garden. *Ophelia* **30**, 75–93.
Fenchel, T. & Finlay, B. J. 1995 *Ecology and evolution in anoxic worlds*. Oxford Series in Ecology and Evolution (ed. R. M. May & P. H. Harvey). Oxford University Press.
Fenchel, T., Finlay, B. J. & Gianni, A. 1989 Microaerophilic behaviour in ciliates: responses to oxygen tension in an *Euplotes* species (Hypotrichida). *Arch. Protistenk.* **137**, 317–330.
Ferris, M. J. & Palenik, B. 1998 Niche adaptation in ocean cyanobacteria. *Nature* **396**, 226–228.
Finlay, B. J. 2001 Protozoa. In *Encyclopedia of biodiversity*, vol. 4 (ed. S. A. Levin), pp. 901–915. San Diego, CA: Academic.
Finlay, B. J. 2002 Global dispersal of free-living microbial eukaryote species. *Science* **296**, 1061–1063.
Finlay, B. J. & Clarke, K. J. 1999 Ubiquitous dispersal of microbial species. *Nature* **400**, 828.
Finlay, B. J. & Esteban, G. F. 1998 Planktonic ciliate species diversity as an integral component of ecosystem function in a freshwater pond. *Protist* **149**, 155–165.
Finlay, B. J. & Fenchel, T. 1986 Photosensitivity in the ciliated protozoon *Loxodes*: pigment granules, absorption and action spectra, blue light perception and ecological significance. *J. Protozool.* **33**, 534–542.
Finlay, B. J. & Fenchel, T. 2001 Protozoan community structure in a fractal soil environment. *Protist* **152**, 203–218.
Finlay, B. J., Curds, C. R., Bamforth, S. S. & Bafort, J. M. 1987 Ciliated protozoa and other microorganisms from two African soda lakes (Lake Nakuru & Lake Simbi, Kenya). *Arch. Protistenk.* **133**, 81–91.
Finlay, B. J., Embley, T. M. & Fenchel, T. 1993 A new polymorphic methanogen, closely related to *Methanocorpusculum parvum*, living in stable symbiosis within the anaerobic ciliate *Trimyema* sp. *J. Gen. Microbiol.* **139**, 371–378.
Finlay, B. J., Maberly, S. C. & Esteban, G. F. 1996*a* Spectacular abundance of ciliates in anoxic pond water: contribution of symbiont photosynthesis to host respiratory oxygen requirements. *FEMS Microbiol. Ecol.* **20**, 229–235.
Finlay, B. J., Corliss, J. O., Esteban, G. & Fenchel, T. 1996*b* Biodiversity at the microbial level: the number of free-living ciliates in the biosphere. *Q. Rev. Biol.* **71**, 221–237.
Finlay, B. J., Esteban, G. F., Brown, S., De Jonckheere, J. & Fenchel, T. 2004 Multiple cosmopolitan ecotypes within a protist morphospecies. (In preparation.)
Forey, P. L., Fortey, R. A., Kenrick, P. & Smith, A. B. 2004 Taxonomy and fossils: a critical appraisal. *Phil. Trans. R. Soc. Lond.* B **359**, 639–653. (DOI 10.1098/rstb.2003.1453.)
Foster, K. W. 2003 Making a robust biomolecular time scale for phylogenetic studies. *Protist* **154**, 43–55.
Fuhrman, J. A. & Campbell, L. 1998 Microbial microdiversity. *Nature* **393**, 410–411.
Godfray, H. C. J. 2002 Challenges for taxonomy. *Nature* **417**, 17–19.
Guhl, B. E. & Finlay, B. J. 1993 Anaerobic predatory ciliates track seasonal migrations of planktonic photosynthetic bacteria. *FEMS Microbiol. Lett.* **107**, 313–316.
Hansen, P. J. & Calado, A. J. 1999 Phagotrophic mechanisms and prey selection in free-living dinoflagellates. *J. Eukaryot. Microbiol.* **46**, 382–389.
House of Lords Science and Technology Committee into Systematic Biology and Biodiversity 2002 *What on Earth?* London: HMSO.
Huss, V. A. R., Frank, C., Hartmann, E. C., Hirmer, M., Kloboucek, A., Seidel, B. M., Wenzeler, P. & Kessler, E. 1999 Biochemical taxonomy and molecular phylogeny of the genus *Chlorella sensu lato* (Chlorophyta). *J. Phycol.* **35**, 587–598.
Ikeda, T. & Takeda, H. 1995 Species-specific differences of pyrenoids in *Chlorella* (Chlorophyta). *J. Phycol.* **31**, 813–818.
Kaiser, J. 2002 The science of *Pfiesteria*: elusive, subtle, and toxic. *Science* **298**, 346–349.
Keeling, P. J. & McFadden, G. I. 1998 Origins of Microsporidia. *Trends Microbiol.* **6**, 19–28.
Krienitz, L., Takeda, H. & Hepperle, D. 1999 Ultrastructure, cell wall composition, and phylogenetic position of *Pseudodictyosphaerium jurisii* (Chlorococcales, Chlorophyta) including a comparison with other picoplanktonic green algae. *Phycologia* **38**, 100–107.
Kuhlmann, H. W. & Heckmann, K. 1994 Predation risk of typical ovoid and winged morphs of *Euplotes* (Protozoa, Ciliophora). *Hydrobiologia* **284**, 219–227.
Litaker, R. W., Vandersea, M. W. & Kibler, S. R. 2002 Reply to comment on the life cycle and toxicity of *Pfiesteria piscicida* revisited. *J. Phycol.* **38**, 1268–1272.
Lynn, D. H. 1991 The implications of recent descriptions of kinetid structure to the systematics of the ciliated protists. *Protoplasma* **164**, 123–142.
Mallet, J. 1995 A species definition for the modern synthesis. *Trends Ecol. Evol.* **10**, 294–299.
Margulis, L. 1993 *Symbiosis in cell evolution*, 2nd edn. New York: Freeman.
Marin, B., Palm, A., Klingberg, M. & Melkonian, M. 2003 Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist* **154**, 99–145.
Mason, P. L., Vogelbein, W. K., Haas, L. W. & Shields, J. D. 2003 An improved stripping technique for lightly armored dinoflagellates. *J. Phycol.* **39**, 253–258.

Melkonian, M. & Surek, B. 1995 Phylogeny of the Chlorophyta: congruence between ultrastructural and molecular evidence. *Bull. Soc. Zool. Fr.* **120**, 191–208.

Montresor, M., Lovejoy, C., Orsini, L. & Procaccini, G. 2003 Bipolar distribution of the cyst-forming dinoflagellate *Polarella glacialis*. *Polar Biol.* **26**, 186–194.

Moore, L. R., Rocap, G. & Chisholm, S. W. 1998 Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* **393**, 464–467.

Nanney, D. L. 1999 When is a rose?: The kinds of tetrahymenas. In *Species: new interdisciplinary essays* (ed. R. W. Wilson), pp. 93–118. Cambridge, MA: MIT Press.

Ogden, C. G. & Hedley, R. H. 1980 *An atlas of freshwater testate amoebae*. London and Oxford: British Museum (Natural History) and Oxford University Press.

Oren, A. 2004 Prokaryote diversity and taxonomy: current status and future challenges. *Phil. Trans. R. Soc. Lond.* B **359**, 623–638. (DOI 10.1098/rstb.2003.1458.)

Overmann, J. & Schubert, K. 2002 Phototrophic consortia: model systems for symbiotic interrelations between prokaryotes. *Arch. Microbiol.* **177**, 201–208.

Palys, T., Nakamura, L. K. & Cohan, F. M. 1997 Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int. J. Syst. Bacteriol.* **47**, 1145–1156.

Patterson, D. J. 1994 Protozoa: evolution and systematics. In *Progress in protozoology* (ed. K. Hausmann & N. Hülsmann), pp. 1–14. Stuttgart: Gustav Fischer.

Patterson, D. J. 1999 The diversity of eukaryotes. *Am. Nat.* **154**(suppl.), S96–S124.

Patterson, D. J. 2003 Progressing towards a biological names register. *Nature* **422**, 661.

Patterson, C., Williams, D. H. & Humphries, C. T. 1993 Congruence of molecular and morphological phylogenies. *A. Rev. Ecol. Syst.* **24**, 153–188.

Potter, D., Lajeunesse, T. C., Saunders, G. W. & Anderson, R. A. 1997 Convergent evolution masks extensive biodiversity among marine coccoid picoplankton. *Biodiv. Conserv.* **6**, 99–107.

Pröschold, T., Marin, B., Schlösser, U. W. & Melkonian, M. 2001 Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). 1. Emendation of *Chlamydomonas* Ehrenberg and *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. *Protist* **152**, 265–300.

Rhodes, L. L., Burkholder, J. M., Glasgow, H. B., Allen, C. & Adamson, J. E. 2002 *Pfiesteria shumwayae* (Pfiesteriaceae) in New Zealand. *N. Z. J. Mar. Freshwat. Res.* **36**, 621–630.

Roger, A. J. 1999 Reconstructing early events in eukaryotic evolution. *Am. Nat.* **154**, S146–S163.

Rublee, P. A., Kempton, J. W., Schaefer, E. F., Allen, C., Harris, J., Oldach, D. W., Bowers, H., Tengs, T., Burkholder, J. M. & Glasgow, H. B. 2001 Use of molecular probes to assess geographic distribution of *Pfiesteria* species. *Environ. Hlth Perspect.* **109**, 765–767.

Saito, K., Drgon, T., Robledo, J. A. F., Krupatkina, D. N. & Vasta, D. R. 2002 Characterisation of the rRNA locus of *Pfiesteria piscicida* and development of standard and quantitative PCR-based detection assays targeted to the nontranscribed spacer. *Appl. Environ. Microbiol.* **68**, 5394–5407.

Schonborn, W., Dorfelt, H., Foissner, W., Krienitz, L. & Schafer, U. 1999 A fossilized microcenosis in Triassic amber. *J. Eukaryot. Microbiol.* **46**, 571–584.

Simpson, G. G. 1945 The principles of classification and a classification of mammals. *Bull. Am. Mus. Nat. Hist.* **85**, 1–350.

Sokal, R. R. & Crovello, T. J. 1970 The biological species concept: a critical evaluation. *Am. Nat.* **104**, 127–153.

Sonneborn, T. M. 1975 The *Paramecium aurelia* complex of 14 sibling species. *Trans. Am. Microsc. Soc.* **94**, 155–178.

Steidinger, K. (and 15 others) 2001 Classification and identification of *Pfiesteria* and *Pfiesteria*-like species. *Environ. Hlth Perspect.* **109**, 661–665.

Taylor, F. J. R. 1999 Ultrastructure as a control for protistan molecular phylogeny. *Am. Nat.* **154**, S125–S136.

The Royal Society 2003 Measuring biodiversity for conservation. Document 11/03. London: The Royal Society.

Turesson, G. 1922 The genotypical response of the plant to the habitat. *Hereditas* **3**, 211–350.

Vickerman, K. 1992 The diversity and ecological significance of protozoa. *Biodiv. Conserv.* **1**, 334–341.

Vladimorova, M. G., Klyachko-Gurvich, G. L., Maslova, I. P., Zholdakov, I. A. & Bartsevich, E. D. 2000 A comprehensive study of *Chlorella* sp. IPPS C-48 and revision of its taxonomic position. *Russ. J. Pl. Physiol.* **47**, 644–654.

Vogelbein, W. K., Lovko, V. J., Shields, J. D., Reece, K. S., Haas, L. W. & Walker, C. C. 2002 *Pfiesteria shumwayae* kills fish by micropredation not exotoxin secretion. *Nature* **418**, 967–970.

Ward, D. M., Ferris, M. J., Nold, S. C. & Bateson, M. M. 1998 A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol. Mol. Biol. Rev.* **62**, 1353–1370.

GLOSSARY

SSU: small subunit

TEM: transmission electron microscopy