

Bland J. Finlay · Genoveva F. Esteban

Exploring Leeuwenhoek's legacy: the abundance and diversity of protozoa

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Abstract Towards the end of the 17th century, Leeuwenhoek built “magnifying glasses” that enabled him to see and describe protozoa for the first time. Continued exploration of the natural history of protozoa during the past 300 years has progressed far beyond simply documenting morphospecies (global total probably <20,000). We now realize that protozoan ‘biodiversity’ is multi-faceted (e.g. sibling species, variant genotypes and syntrophic consortia). Realization of their extraordinary abundance has secured for protozoa the position of dominant phagotrophs and regenerators of nutrients within microbial food webs. And studies of protozoa in the natural environment have done much to effect a paradigm shift in our understanding of why specific microbes live where they do and how they got there in the first place. In particular, the hypothesis of ubiquitous dispersal of protozoan species does seem to be supported by the evidence provided by morphospecies, sibling species and even individual genotypes.

Keywords Protozoa · Ciliates · Biodiversity · Ubiquity · A. van Leeuwenhoek

“June 9th, having received, early in the morning, some rain-water in a dish (...) and exposed it to the air, about the third story of my house (...) I did not think I should then perceive any living creatures therein; yet viewing it attentively, I did, with admiration, observe a thousand of them in one drop of water, which were the smallest sort, that I had seen hitherto”.

Leeuwenhoek – from a letter dated October 1676.

Introduction

Thus Antonie van Leeuwenhoek (1632–1723), the son of a Dutch basket-maker, but with a passion for designing and building “magnifying glasses”, was probably the first person to see individual, living, micro-organisms. In particular, he was fascinated by the “very little animalcules” – most of which were obviously algae (“of divers colours”), including *Euglena* and *Volvox*, and protozoa – especially some of the more elaborate ciliates. From his descriptions, he clearly found ciliate species that are easily recognized today, such as the bell-animalcule *Vorticella*, the “oval form with divers incredibly thin feet” (probably *Oxytricha*), and others including *Stylonychia*, *Enchelys*, *Vaginicola* and *Coleps*. Most of his observations were made in the period running from 1674 until the first decade of the 18th century. They were recorded in letters that he sent to the Secretary of the Royal Society in London, where they were translated into English and published in the *Philosophical Transactions* [7,53].

Leeuwenhoek was probably the first to see protozoa, the first to gain insight into their great variety of forms, and the first to discover that they can be naturally very abundant. Nowadays, he is justly credited as the ‘Father of Protozoology’. In a sense, he might also be referred to as the ‘Father of Microbial Ecology’, because the two key features of microbes he witnessed – their abundance and their variety – are now acknowledged as fundamental characteristics of microbial diversity [30] and as factors that underpin the ecological significance of microbes. The purpose of this short article is to illustrate this point using recent examples taken from one large group of microbes – the free-living phagotrophic microbial eukaryotes – also known as ‘protozoa’. In so doing, we will, inevitably, be ‘exploring Leeuwenhoek’s legacy’.

Abundance

Morphology and function are closely linked in protozoa, so when we divide the free-living protozoa into broad

B.J. Finlay (✉) · G.F. Esteban
CEH-Windermere, The Ferry House,
Ambleside, Cumbria LA22 0LP, UK
E-mail: bjf@ceh.ac.uk
Tel.: +44-1539-442468
Fax: +44-1539-446914

morphological groups, we are also allocating them to broad functional groups: the flagellated, amoeboid and ciliated protozoa (Fig. 1). The flagellates are the smallest – many are only 2–4 μm (some are even smaller; e.g. [36]), and almost all are < 20 μm . Most amoebae are 5–50 μm , and most ciliates are 15–200 μm . Exceptionally, some amoebae, such as the larger benthic foraminifera, may reach 2 mm or more [23].

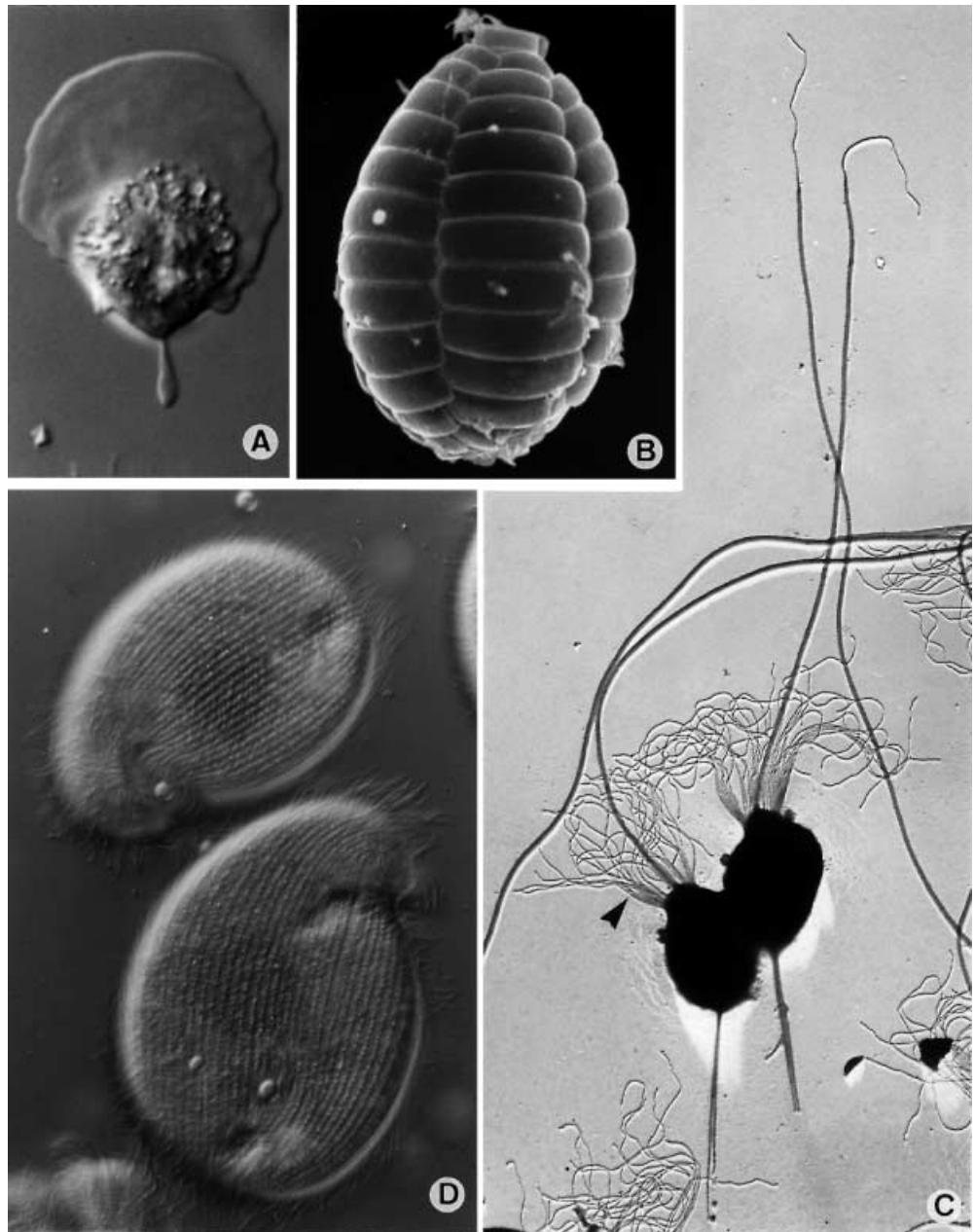
Compared to macroscopic animals, protozoa are extremely abundant; 1 g of soil typically contains around 15,000 naked amoebae [34], and every millilitre of fresh or seawater on the planet supports anything from a minimum of about a hundred to around a million heterotrophic flagellates [3]. A key point here is that smaller species are usually much more abundant than larger ones [45] – a pattern that extends over a

size range covering 20 orders of magnitude (Fig. 2), with a slope of approximately -1 [39,47]. Thus, typical abundances of a mammalian species, a stream invertebrate, and a protozoan species, would be 10^{-5} , 10^{-1} and 10^5 m^{-2} . The message is clear – the typical global abundance of a protozoan species must be astronomically high.

Consequences of abundance – importance as consumers

As protozoa are so small, most of their prey items are other, even smaller microbes. And because they are so abundant, protozoa are the principal consumers of bacteria and other microbes in aquatic environments.

Fig. 1a–d Examples of the diversity of form and function in protozoa. **a** A specimen of the naked amoeba *Vanella* sp. isolated from soil. Approx. size: 30 μm . Nomarski interference contrast. **b** Scanning electron micrograph of *Paulinella chromatophora*, a testate amoeba that appears to live off of its endosymbiotic cyanobacteria. The test is 20–25 μm in length. Specimen isolated from soil (micrograph courtesy of K.J. Clarke, CEH-Windermere). **c** Two individuals of the planktonic, colonial choanoflagellate *Sphaeroeca volvox* after shadowing with Au-Pd for electron microscopy (micrograph courtesy of K.J. Clarke, CEH-Windermere). Individual cells are 5–6 μm long, the collar (arrowhead) is 7–8 μm long and the flagellum is 36–40 μm long. The posterior of the cell is drawn out as a thin filament. **d** The marine anaerobic ciliate *Plagiopyla frontata* feeding on baker's yeast (spherical particles). Nomarski Interference contrast. Approx. length of the ciliate: 80 μm



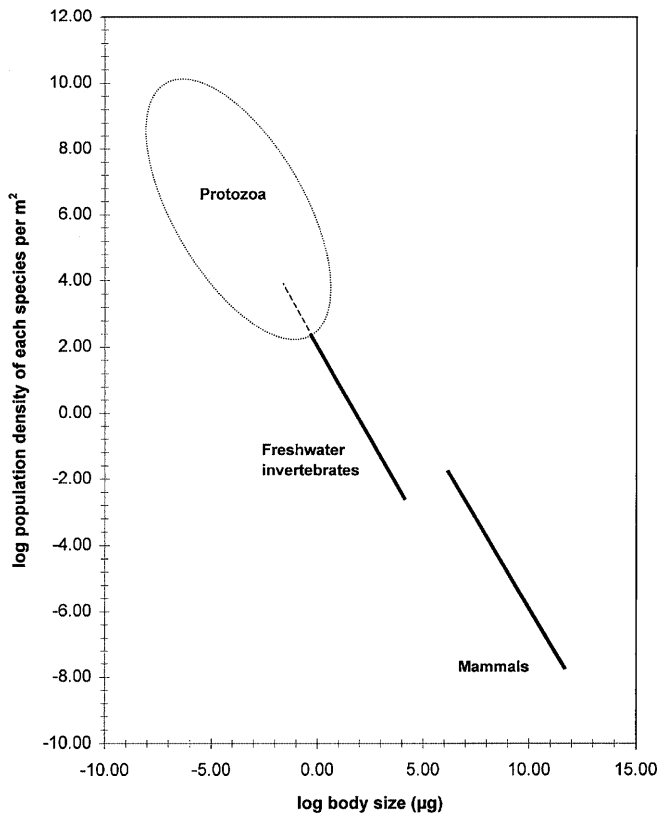


Fig. 2 The numerical density of species increases with the inverse of body mass [45]. Here, our collected data for protozoa (based on those in [26]) and many other sources fit within the *ellipse*, which is located as an extension of the data for metazoans (see [39,47]) to emphasise the extraordinary abundance (up to about 10^9 m^{-2}) of the smallest 'animal-like' organisms. For all taxonomic groups, the slope of the relationship appears to be about -1

They have population growth rates that are similar to those of the microbes on which they feed (doubling times in the order of 1 day), and they are usually able to control microbial abundance within relatively narrow limits [3,14]. Flagellated protozoa alone can probably consume all bacterial production in the plankton.

Protozoan grazing on microbes also stimulates activity of the microbial community in general, in both aerobic and anaerobic environments (e.g. [4,15]). The mechanism involved is not fully understood, although it may operate by increasing the rate of turnover of essential nutrients that would otherwise remain 'locked up' in bacterial biomass. The net effect is that grazing by protozoa stimulates the rate of decomposition of organic matter.

Consequences of abundance – apparent ubiquity of species

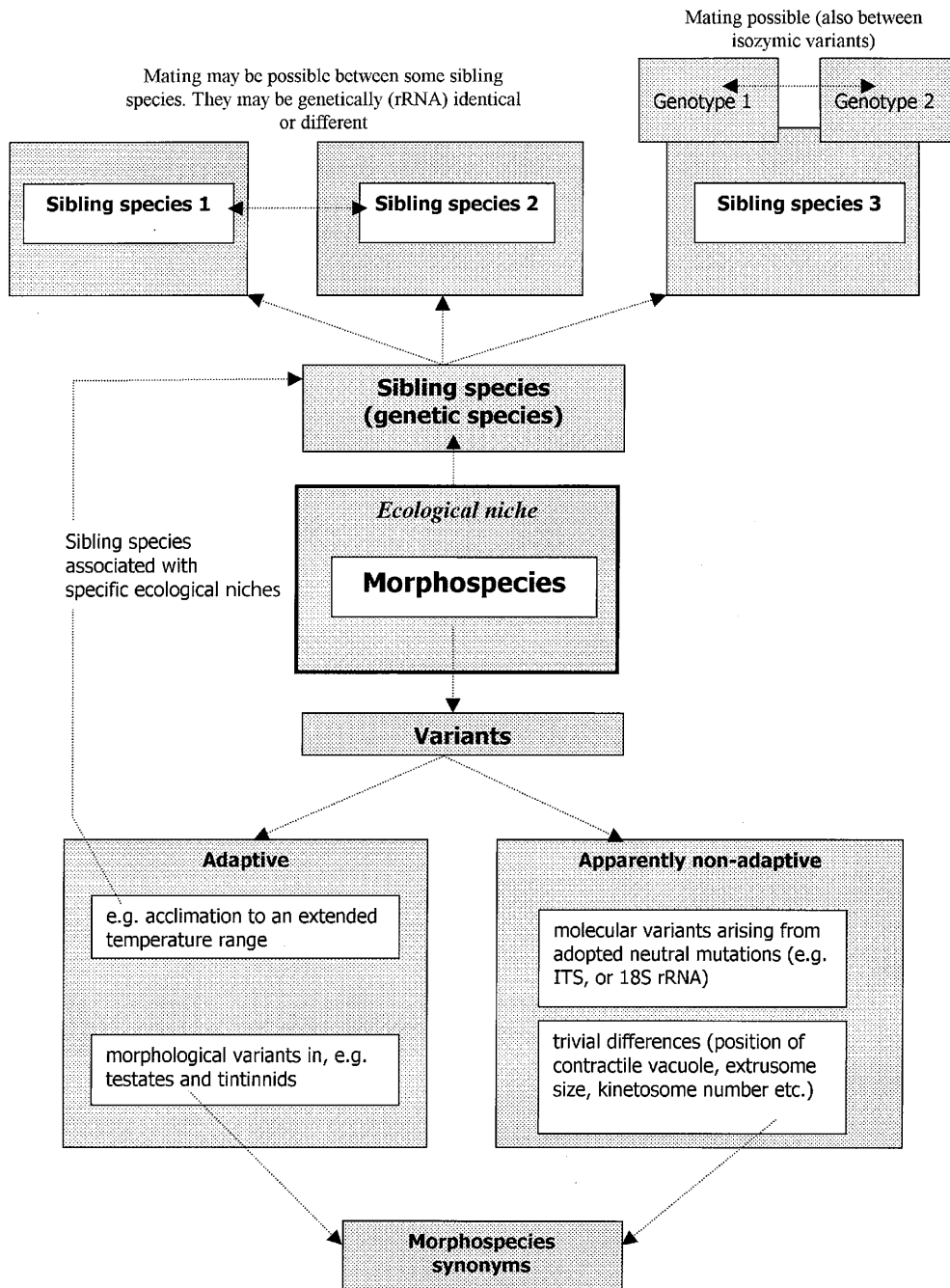
In global terms, the abundance of each protozoan species is very large. One consequence of great abundance is that rates of passive population dispersal must be relatively high. An individual protozoon may not survive

transport through groundwaters, or aerial transport while attached to a duck's foot etc., so at the level of the individual protozoon, there undoubtedly exist significant barriers to dispersal. But for the global population as a whole, great abundance reduces the probability that geographical barriers halt migrations, and given enough time, these barriers become irrelevant. Thus, ubiquitous dispersal of protozoan species (and all other microbial species) seems likely, and driven by high absolute abundance. Not every species is always active everywhere, but a large proportion of microbial species can, with patience (and experimental manipulation [20,29]), probably be found at almost any natural site, although most will usually be encysted or in some other cryptic-dormant state and waiting for conditions suitable for population growth. Any particular species can *exist* in a wide range of habitats, but it is typically *active* in only a few of these – and then, perhaps only occasionally [24,25].

Ubiquitous dispersal has two obvious implications. First, rates of allopatric speciation will be low, and the global number of species will be modest [16]. And second, local species richness will be a large proportion of global species richness. Thus micro-organisms (with ubiquitous dispersal) differ from macro-organisms (with geographically restricted distributions). Differences in the local:global species ratio are particularly marked. We have found, for example, 78% of the global diversity of chrysomonad flagellates (genus *Paraphysomonas*) in less than 1 cm^2 of sediment from a freshwater pond [24,25], but only 0.016% of all described marine bivalves in the two hectares of Nivå Bay in Denmark [16]. This difference, if it is typical (and there is no reason to suppose it is not), has implications for ecosystem function, for it implies that a functional microbial community will be selected in response to any of a wide range of natural perturbations (and indeed this appears to be the case; e.g. [6]). In contrast, the local:global species ratio is considerably lower for all groups of macroscopic organisms (flowering plants, beetles, fish etc.) so the response to local perturbation is either slower (depending on immigration) or impossible (e.g. geographical barriers). Thus, microbes and macro-organisms sustain ecosystem functions in rather different ways [30].

Ubiquitous dispersal of protozoan species – morphospecies

As with most other types of organisms, there is no consensus regarding what constitutes a protozoan 'species', and how its boundaries might be defined (Fig. 3). Nevertheless, the morphospecies is the most popular concept because it is relatively easy to discriminate individual species within the great diversity of protozoa, using body form alone. This is especially useful because of the link between morphology and ecological function. In many protozoa, the size and shape of the organism, in particular the structure of the feeding apparatus,



determine how the organism functions in the natural environment. Thus, the 'form' is assumed to largely determine the ecological niche that the protozoan occupies. We could say that if a protozoan looks the same in different places, then it is the same in different places, although this position may have to be modified by the knowledge that in some morphospecies at least, consistent physiological differences can be demonstrated between clones (e.g. growth rate differences [44]).

Finlay et al. [33] and Esteban et al. [11] set out to test the idea of global ubiquity by focusing on one group, the ciliates, living in a habitat that is relatively isolated and geographically distant from northern Europe (where

most ciliates had been discovered and described by about the year 1935). The habitat was a Holocene volcanic crater-lake in Australia. Ciliates are relatively large and fragile and, in comparison with smaller microbes such as bacteria, they are not particularly abundant. So if ciliate species are globally distributed, it implies that the more abundant smaller organisms have global distributions too. A total of 85 ciliate morphospecies were recorded. None of these was 'new', and all species had already been recorded in northern Europe. The water in the crater was slightly brackish and this created a habitat for some marine species that tolerate brackish water, such as *Tracheloraphis caudata* – a large and fragile

Fig. 3 What is a protozoan ‘species’? The simplest assumption is that a species is a morphospecies, and that it is the sole occupant of an ecological niche. However, some morphospecies, especially those of ciliates, consist of discrete populations (known as ‘syngens’, ‘sibling species’, ‘genetic species’ or ‘biological species’) between which gene flow is restricted or non-existent. The status of these sibling species varies across the genera. Some sibling species of *Paramecium caudatum* have been reported to mate with each other [37] and even their existence has been questioned [49]. Some sibling species in the *Tetrahymena pyriformis* ‘complex’ (= morphospecies) are incapable of mating and are thus excluded from the biological species concept. Some strains of *Tetrahymena thermophila* appear to mate freely and non-specifically [41]; and in *Euplotes crassus* [52] genetic exchange between certain pairs of strains may occur only with the intervention of a third, intermediate strain. Within some sibling species, there exist discrete genotypes and isozymic variants. At least two (often many more) mating types also exist, so that the ciliates can recognize suitable partners. These may have a very wide geographical distribution (as in *Paramecium triaurelia* [48]) but be able to mate with each other and produce viable progeny. Morphospecies also consist of phenotypic variants, some of which are adaptive (e.g. those that have, by acclimatization, extended the temperature range within a genotypically fixed range in which growth is possible) [15]. Others probably have no genetic basis; a testate amoeba for example may use whatever is available to embellish its test, and thus appear morphologically quite variable. Non-adaptive variants would include those arising from mutations that have no impact on the fitness of the organism. Both adaptive and non-adaptive morphological variants exacerbate greatly the problem of synonymy within protozoan morphospecies (see [32]). There is some debate about the ecological significance of sibling species. Nanney et al. [41] propose that each sibling species probably occupies a unique ecological niche but there is no firm evidence for this. Rather, the idea rests on the supposition that if different sibling species co-exist in a habitat, they must be occupying different niches

ciliate that usually lives in the interstitial zone of marine sandy sediments. It probably arrived at the crater-lake from the Southern Ocean, although the mechanism of transport is obscure – particularly as the species does not form a cyst.

Some of the most fragile ciliates belong to the genus *Loxodes*, and the biggest and most fragile is probably *Loxodes rex*. The global abundance of a species is inversely related to its body size (Fig. 2), so the species that are least likely to be ubiquitous are those with relatively small populations – i.e. the largest species. A prime candidate must be *Loxodes rex* (~1.2 mm in length). The species has long been considered to be restricted to fresh waters in tropical Africa but a thriving population has recently been reported in a freshwater pond in Thailand [12]. Thus, even the largest and rarest of ciliates may have a very widespread distribution.

There is now a wealth of evidence indicating that most, if not all, protozoan morphospecies have widespread distribution [22]. Patterson and Simpson [43] could find no evidence of endemism in heterotrophic flagellates from marine and hypersaline sediments in Australia. Tong et al. [51], investigating the diversity of protozoa in the Antarctic, came to the same conclusion. Finlay and Clarke [24,25] found three-quarters of all known *Paraphysomonas* species in a single pond in England. Species that are globally rare or abundant were

likewise rare or abundant in the pond, suggesting that global abundance does influence local abundance. Further evidence can be found in [28].

Ubiquitous dispersal of protozoan species – sibling species and genotypes

The morphospecies is basically an ecological definition of species, but an alternative and widely accepted definition is that a species is a population that shares a gene pool. Within some protozoan morphospecies (e.g. *Tetrahymena pyriformis*, *Paramecium aurelia*), there do indeed exist discrete populations, called ‘sibling species’ or syngens, between which gene flow is restricted. These populations cannot be separated from each other on morphological grounds. The different sibling species within a morphospecies can be genetically identical, or quite different to each other [40,41] – at least with respect to ribosomal RNA sequences. In most cases, they have been defined solely in terms of mutually incompatible mating systems [40], and the best characterized sibling species are ciliates. There is no convincing evidence that these have geographically-restricted distributions (although that inference can be drawn from the results of limited sampling effort). In the genus *Paramecium*, most sibling species are now recognized to have cosmopolitan distributions [42]. Stocks of *Paramecium triaurelia* isolated in North America will conjugate with those isolated in Europe, to produce viable ex-conjugants [48], and the same is true for ciliates in other genera (e.g. *Stylonychia lemnae* [1]). Only three stocks have ever been found of the sibling species *Paramecium tredecaurelia* (‘species 13’; G.H. Beale, personal communication) – one in the River Seine in Paris, one in Madagascar, and one in Mexico – and it is possible to cross all three stocks, although the success rate is low. Doerder et al. [10] could find all seven known mating types of *Tetrahymena thermophila* in all of the ponds they studied in a forest region in Pennsylvania. The indication is that sibling species, like morphospecies, are geographically very widely distributed.

There are, however, some subtle exceptions that interfere with a simple picture of cosmopolitan sibling species. The sibling species may be cosmopolitan, but the same may not be so for the constituent genotypes. *Paramecium triaurelia* has three different (RAPD-fingerprint) genotypes that are widely distributed (in Europe and North America), and these can mate with each other with a high rate of survival of progeny [48]. In contrast, *Paramecium sexaurelia* appears to be an extreme inbreeder. It is widely distributed, and has four constituent genotypes, but each of these seems to be locally restricted in its geographic distribution, possibly because of the low rate of survival of offspring following crosses with other genotypes from other regions. Both sibling species probably have very wide geographic distributions, but only in *P. triaurelia* are the genotypes equally widely distributed [48].

Stoeck et al. [49] have questioned whether some sibling species actually exist. Working with the morphospecies *Paramecium caudatum*, they used DNA(RAPD)-fingerprinting and amplified ribosomal DNA restriction analysis (ARDRA, riboprinting) to investigate 14 strains of 7 putative sibling species. They found that the amplified 18S rRNA products of the different sibling species were identical across all strains, and concluded that there are no sibling species in *P. caudatum* (although the link between ribosomal DNA and sexual behavior is not altogether clear; and note that eight sibling species in the *Tetrahymena americanis* cluster are identical – 190 base sequence of the 23S rRNA [40]).

The real problem of using a biological species concept for protozoa in general is that it is not practical for all but a few easily cultivated protozoa. Most protozoan species have never been cultivated, and neither the frequency nor character of their sexual behaviour (if any) is known, or ever likely to be known. For further discussion of this see [32]. One key question that remains unanswered concerns the ecological significance of sibling species. In morphospecies where the existence of sibling species is not in doubt, they appear to share the same general morphology and perform roughly the same job in the natural environment – e.g. all microstome tetrahymenid sibling species look identical and feed on bacteria in organic detritus in roughly the same way. Many biologists believe that these sympatric sibling species occupy differentiated niches (e.g. [40,41]), but there is no firm evidence to support this. The ecological significance of sibling species has never really been explored.

In recent years, much work has been done to investigate the distribution of specific genotypes of protozoa over large geographical scales. The emerging conclusion is that there is no correlation between geography and genotype. Kusch [38] carried out DNA(RAPD)-fingerprinting of the large ciliate *Stentor coeruleus* living in freshwater ponds across Germany and found no relationship between the genotypes in ponds and the geographical distances between ponds. Bowers et al. [5] examined riboprints (large subunit rRNA gene) of 114 isolates of three species of *Colpoda* from all continents, but could find no geographic pattern in the distribution of any genotype of any species examined. Atkins et al. [2] have shown that flagellates (e.g. *Massisteria marina*) collected from hydrothermal vents separated by thousands of kilometers in the Pacific are genetically identical (small subunit rRNA sequence) to each other and to a strain isolated in northern Europe.

Some of the most striking evidence for the global distribution of protozoan species is provided by the foraminifera [9]. It has long been known that certain morphospecies are present in both Arctic and Antarctic waters. These cold-water provinces have been established for something between 8 and 16 million years, which is certainly long enough for isolated foraminifera to have diverged genetically if they had remained isolated from each other. The authors examined genetic

variation in the small subunit ribosomal RNA gene of three bipolar planktonic foraminiferal morphospecies (*Globigerina bulloides* [d'Orbigny], *Turborotalia quinqueloba* [Natland] and *Neogloboquadrina pachyderma* [Ehrenberg]). Each morphospecies was found to consist of several genotypes, and within each morphospecies, there was at least one genotype that was identical in both Arctic and Antarctic subpolar provinces. This indicated that trans-tropical gene flow must have occurred, and indeed may still be occurring. The mechanism of exchange between cold-water provinces is not absolutely clear. One possibility includes a role for cool boundary currents off the coast of West Africa bringing cool-water genotypes into the seasonally cool sub-tropical upwelling zones, followed by submergence to the cooler water of the thermocline for transfer across the tropics.

As with all other free-living protozoa, the idea of speciation in foraminifera based on geographical isolation seems difficult to sustain, so it is unclear why genetically distinct populations should be maintained within the same water body [9]. The suggestion that genetically distinct populations could actually be cryptic species filling different niches is supported by the observation that specific genotypes are not randomly distributed within the cool-water provinces – rather they may be adapted to specific niches, defined, for example by hydrographic parameters.

In general, protozoan morphospecies (and sibling species, and genotypes) are ubiquitous and apparently cosmopolitan if the habitats to which they are adapted are distributed in different parts of the world. In accordance with this, the global number of protozoan species is indeed relatively modest.

Diversity

The dimensions of global morphospecies richness

One problem with the morphospecies concept is that it is sometimes difficult to decide exactly where a species begins and ends, and morphological variation may often appear to be continuous across a number of species. In some of the large spongiöse spumellarian radiolarians, for example, the skeletal and cytoplasmic morphology used to discriminate species intergrade to such an extent that it may, in some cases, not be possible to ascribe individual radiolarians to nominal species [50].

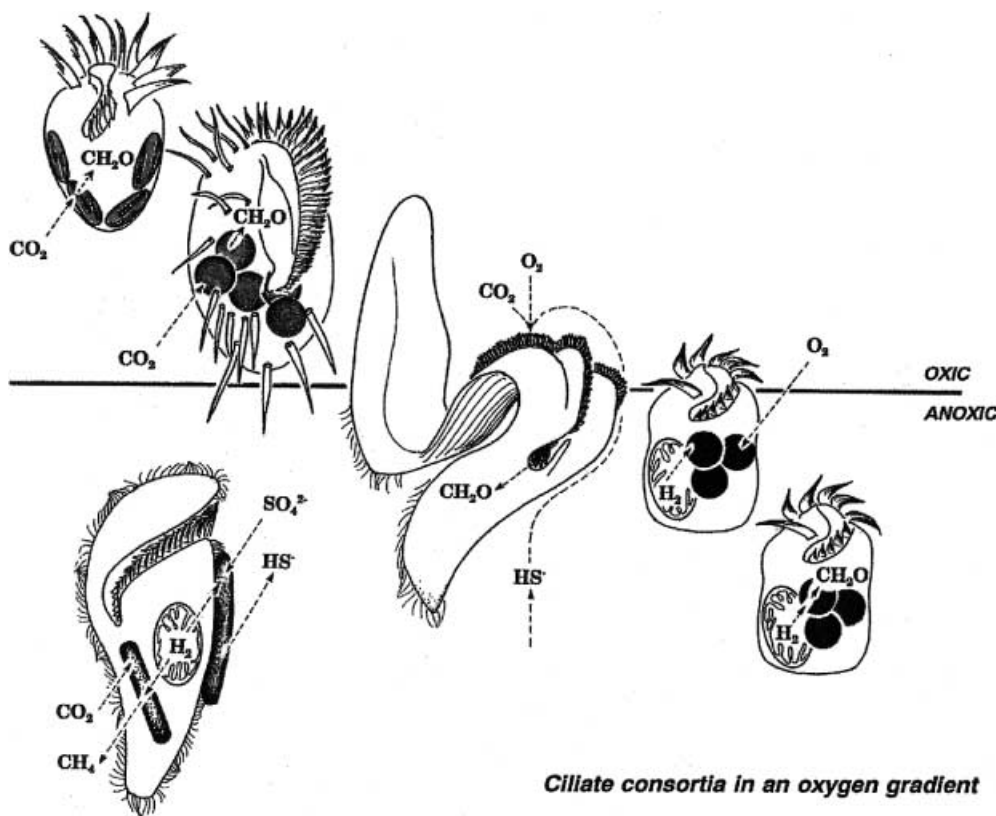
However, the evolutionary process in radiolarians and other protozoa probably works in the same way as it does for other organisms. Phenotypically discrete 'species' exist in niche space that is continuously variable, although the identities of these phenotypes may sometimes be difficult to discern. We use morphological characters to separate species, but there may be additional, underlying phenotypes occupying discrete niches, about which we know very little.

Ubiquitous dispersal of species should ensure that the global species number is relatively low, and this appears to be the case, for the total number of protist species is not particularly great. Two recent, independent estimates have been made for the global number of protozoan morphospecies, defined as extant free-living phagotrophic protists. Finlay [23] put the number at 11,890 (6,570 amoeboid, 2,260 flagellated, and 3,060 ciliated protozoa), although there is uncertainty for some groups e.g. the shallow-water benthic foraminifera which are probably grossly inflated by synonyms. Corliss [8] began with a broader definition of 'protozoa', represented by 83,000 morphospecies. This number was reduced in stages (J.O. Corliss, personal communication) as he converged on the 'Finlay' definition of protozoa above. From his original total, he removed 51,000 fossil species and 10,000 symbiotic/parasitic species. Subtracting a further 3,000 non-pigmented euglenids and dinoflagellates, left a total of 19,000 free-living phagotrophic protozoa. The difference of 7,000 between the two final estimates is almost entirely due to Corliss' acceptance of larger group totals for the rhizopods, radiolarians and ciliates, around which there is, in any case, much controversy. The precise figure is not particularly important, rather the key finding is that there are probably not more than 20,000 protozoan morphospecies. In contrast, the insects (5–10 million species [35]), like most groups of macroscopic animals and plants, tend to have relatively high species numbers – largely generated and sustained by geographical and other physical barriers.

Consortia as clusters of species richness

A large proportion of free-living protozoa have other micro-organisms living inside them or on their external surfaces. These additional microbes are usually bacteria or unicellular algae and they usually have some intimate nutritional or other functional relationship with their 'host'. In many cases the association has evolved alongside other aspects of the life style of the protozoon. For example, many protozoa are microaerophilic, i.e. they seek out habitats such as the oxic-anoxic boundary in the water column of a stratified lake, where the oxygen tension is just sufficient to drive aerobic respiration

Fig. 4 Some common symbiotic consortia involving ciliated protozoa, and their preferred positions in a natural oxygen gradient. From *top left to bottom right*: the oligotrich ciliate *Strombidium viride* containing functional chloroplasts, probably sequestered from diatoms [46]; the ciliate *Euplotes daidaleos* with endosymbiotic photosynthetic green algae (zoochlorellae). These ciliates can reach extraordinarily high densities at the oxic-anoxic boundary in the water column of freshwater lakes [31]; the mouthless ciliate *Kentrophoros* sp. with ectosymbiotic sulphide-oxidising bacteria [18]; an oligotrich ciliate (*Strombidium purpureum*) with endosymbiotic non-sulphur purple bacteria (resembling *Rhodospseudomonas*). The consortium is capable of both oxidative phosphorylation at low oxygen tension in the dark and, using waste H_2 from the ciliate as reductant, of anoxygenic photosynthesis in the light [17,19]. Most marine anaerobic ciliates carry ectosymbiotic sulphate-reducing bacteria. *Bottom left*: an anaerobic ciliate (*Metopus* sp.) from seawater. The ciliate produces H_2 gas which is used by endosymbiotic methanogens and by ectosymbiotic sulphate reducers (see [19]). Modified from Finlay [21]



in the protozoon, but low enough to exclude metazoan competitors and predators. This is also the zone where the inorganic raw materials for microbial growth arrive from opposite directions (e.g. oxygen and light arriving from above, meet carbon dioxide, sulphide and ammonia diffusing up from below) and where there is, therefore, an elevated abundance of microbial food (e.g. [27]). In the water column of a stratified lake, most protozoa will invariably be found close to the oxic-anoxic boundary in the metalimnion. A large proportion will be carrying photosynthetic symbionts – particularly those such as chlorellae that are well-adapted to low light levels [31] – that benefit from elevated CO₂ levels, and which secrete sugars that are metabolised by the protozoon.

Deeper in the lake, in the anaerobic zone, we find a broad diversity of protozoa that are adapted for life in the complete absence of dissolved oxygen [19]. Most use hydrogen-evolving fermentations for energy generation. The hydrogen is used by anaerobic bacteria, especially endosymbiotic methanogens, and methane is released from the protozoan consortia. In some cases, very complex intracellular consortia have evolved. In the anaerobic ciliate *Cyclidium porcatum* [13] there is a tight cluster, about 8 µm in diameter, consisting of bacteria (probably fermenters), methanogens, and hydrogenosomes (=anaerobic mitochondria). There are many other examples of syntrophic consortia involving protozoa, including those with chemolithotrophic bacteria as partners (e.g. see Fig. 4).

All of these consortia involving protozoa represent tightly-integrated functional units. Indeed, the symbionts may be as deeply embedded functionally in the consortium as the protozoon's own organelles. Two points arise from this. The first is that we really have to take a broader view of exactly what a protozoon is and what it does, not least because the consortium is the evolutionary unit on which natural selection will operate. Second, the 'biodiversity' of protozoa in any habitat, when quantified simply in terms of protozoan species richness, will fail to take account of the large supplementary microbial diversity which is, in many cases, specifically adapted for permanent association with the protozoa.

Postscript

Leeuwenhoek was the first to see and describe protozoa, and the first of a long line of scientists (e.g. Müller in the 18th century, Ehrenberg, Dujardin, Stein, Haeckel and Kent in the 19th, to Penard, Fauré-Fremiet, Kahl, Dragesco, Foissner and Patterson in the 20th) who patiently documented and described the greater part of global protozoan diversity. Continued exploration of Leeuwenhoek's legacy in recent decades has progressed beyond simply documenting morphospecies. It has established that the 'biodiversity' of protozoa is multifaceted (Leeuwenhoek knew nothing about sibling species, syntrophic consortia or genetic fingerprinting).

Realization of the extent of their diversity and their extraordinary abundance has secured for protozoa the key position of dominant phagotrophs and regenerators of nutrients within microbial food webs. And finally, studies of protozoa in the natural environment have done much to effect the paradigm shift in our understanding of why specific microbes live where they do. Leeuwenhoek probably believed in spontaneous generation; and in the 20th century biogeography based on physical barriers was borrowed from macroscopic organisms and applied (with little success) to protozoa. This has been replaced in recent years by the hypothesis of ubiquitous dispersal – a purely statistical process driven by great absolute abundance, and providing any microbial species with the potential to live wherever suitable conditions exist.

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