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PROTIST NEWS

Protist Diversity: Estimates of the Near-Imponderable

I present and summarize data which show that biodiversity of free-living ciliates is perhaps one order of magnitude greater than the 3 000 morphospecies proposed by Finlay and Fenchel. Furthermore, there is indisputable evidence of endemism and restricted biogeographical distribution of at least some protozoan species. At present, any estimate of protist species richness is highly speculative because only a tiny fraction of the potential protist habitats of the biosphere has ever been carefully analyzed. I thus suggest that further research should concentrate on alpha-taxonomy, that is, the accurate description of species and their distribution according to habitats and regions.

Avant-propos

Discussion on biodiversity is driven by ecologists because the few taxonomists are over-burdened with routine identification and conservation work, as well as the description of new species they continuously discover (Cotterill 1995; Foissner 1997, 1998). In protists, mainly Finlay and Fenchel, two respected protozoan ecologists, have published on biodiversity. They conclude (Finlay and Fenchel 1999) that the "real figure of total protozoan (phagotrophic microbial eukaryotes) diversity lies somewhere in the range of 12 000–19 000 species", and estimate global diversity of free-living ciliates to be around 3 000 species (Finlay et al. 1996, 1998). This sharply contrasts with my view that there are probably about 30 000 species of free-living ciliate morphospecies (Foissner 1999: Floodplain soils – untouched protozoan biotopes. 3rd European Congress of Protistology, Helsingør, Abstracts, p. 30). Geneticists arrived at a similar number: "Obviously the protists – at least the ciliated protozoa – are greatly underclassified, by at least an order of magnitude, and perhaps by two orders of magnitude" (Nanney et al. 1998). Being mainly an alpha-taxonomist (species describer), and having described hundreds of new taxa during the past 25 years, my estimate is based on two simple facts: (1) I found, on average, about one new species in almost every sample carefully analyzed, irrespective of biotope, and (2) about half of the 2 000 ciliate species I have seen were undescribed (new).

Basically, I think that a reliable estimate of protist species richness is impossible with the knowledge available. So, why did I write this comment? Because it seems that no other protist alpha-taxonomist has responded to Finlay and Fenchel, and I am convinced that their estimates are far too low. If there were as many taxonomists as there are ecologists, we would very likely already know many more than the 3 000 free-living ciliate species proposed by Finlay et al. (1996, 1998). Unfortunately, alpha-taxonomy is strongly declining generally, both in quality and quantity, because it is not a "mainstream science" (Cotterill 1995; Schminke 1994). Thus, there is little hope that we shall know who is right, Finlay and Fenchel or Foissner, within the next 100–200 years! My main paradigm will be the soil ciliates, which I have been studying extensively for 20 years. But I also have evidence from typical freshwater habitats which supports the soil data (Foissner et al. 1999).

There are Protists with a Restricted Geographical Distribution!

I basically agree with Finlay and Fenchel that most free-living protists are cosmopolitan, at least at the morphological level. I am, however, opposed to the biased view they hold in their most recent paper (Finlay and Fenchel 1999).

Of the many examples available for endemism or restricted Gondwanan/Laurasian distribution of certain protist genera and species, I shall discuss only the most important ones and those related to specific statements of Finlay's group. Most of the exam-

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ples concern species with very distinct morphologies ("flagships"), such as large ciliates or testate amoebae, whose shells favour recognizing distinct morphotypes. There is every reason to assume that endemics or species with restricted geographical distribution occur also among the less conspicuous protists. However, this is much more difficult to prove because detailed and reliable distribution data are extremely rare.

(1) Lake Baikal contains, without doubt, several endemic ciliate species, especially evident in the Colepidae, a group of ciliates with a richly structured armour. Obolkina (1995) found four new genera and seven new species which are so distinct that they would certainly have been noticed before, if they were present in other waters. Although some of the new ciliate species described by Gajewskaja (1933) from Lake Baikal were later found in various lakes worldwide, most are still unique and were not encountered in detailed studies on small lakes in Germany (Foissner et al. 1999) and England (Finlay et al. 1988). Very likely, many endemic protists can be found in more detailed investigations, for instance, in Lake Tanganyika, a biodiversity centre, which has never been investigated in detail for heterotrophic protists. Interestingly, the only reliable study available (Dragesco and Dragesco-Kernéis 1991) describes several new ciliate species, among them also a new colepid genus and species, similar to those discovered in Lake Baikal.

(2) There is no doubt among taxonomists that quite a number of testate amoebae have a restricted Gondwanan/Laurasian distribution (Schönborn 1966). For instance, Meisterfeld and Lor-wai Tan (1998) found six of these Gondwanan relicts in a preliminary study of several habitats in Australia. The "flagship" is *Apodera vas*, a species of about 170 µm length with a very distinct morphology. There are, however, also several ciliates which, undoubtedly, have a restricted Gondwanan/Laurasian distribution, for instance, *Neobursaridium gigas*, a gigantic (about 1 mm in length) Gondwanan freshwater species. Several large soil ciliates, such as *Gigantothrix herzogi* and *Bresslauides discoideus*, very likely also have a restricted Gondwanan/Laurasian distribution (Foissner 1987, 1999). In flagellates, restricted distribution is much more difficult to prove because their small size makes most characters recognizable only under the electron microscope. However, there is at least one promising example, *Hemimastix amphikineta*, a highly characteristic soil species, which I have found in about 30 Gondwanan samples, but in none of the 200 samples from Europe.

(3) Finlay et al. (1996) stated: "It is possible that the number of undescribed symbiotic ciliates is not excessively large". This contradicts literature data, for example, astome ciliates from the digestive tract of oligochaetes. In 1954, about 65 species were known, mainly from Europe (Puytorac 1954). This number increased considerably when seven species of glossoscolecoid and megascolecid earthworms, which occur mainly in tropical and subtropical Gondwanan areas, were investigated. Not less than 23 new species belonging to 13 new genera were discovered (Puytorac 1969; Puytorac and Dragesco 1968, 1969). Considering that there are at least 3000 species of oligochaetes worldwide, of which only a minute fraction has ever been investigated for symbiotic ciliates, it is reasonable to assume that most astome ciliates are still undescribed.

Local versus Global Diversity

The ratio of local:global diversity is an important measure in conservation biology because it indicates "hot spots" of biodiversity. In protists, this measure was first used by Fenchel et al. (1997). They found that about 10% of the estimated global diversity of free-living ciliates (3000 species) could be detected in local samples. From this, they conclude that "everything is (almost) everywhere" (Fenchel et al. 1997) and that "all species of freshwater protozoa could eventually be discovered in one small pond" (Finlay and Esteban 1998). At first glance, this conclusion appears reasonable because the ratios of local:global diversity in protozoa are much higher than those generally found in metazoan and plant communities (usually <1%). However, a closer examination reveals that the method provides meaningless results in groups whose full diversity is largely unknown, such as protists. Furthermore, it is highly unlikely, from a general ecological viewpoint, that a single, small pond has the carrying capacity to contain all protists (ciliates). And this is fully confirmed by Finlay's and Esteban's (1998) own data: "Our inventory of ciliate species recorded from a single, one-hectare pond, over a period of several years, currently stands at about 260 species". This is far away from the 3000 species global diversity they estimate.

In monthly samples and with various methods, I found about 160 ciliate species in an about 100 m^2 area of beech forest in Salzburg (Fig. 1). All efforts, to increase this number significantly failed. How does this figure relate to global soil ciliate diversity? In 1987, when only about 250 species of soil ciliates were known (Foissner 1987), 64% of the global diversity occurred at the single site in Salzburg. In 1998, when 643 soil ciliates had been described

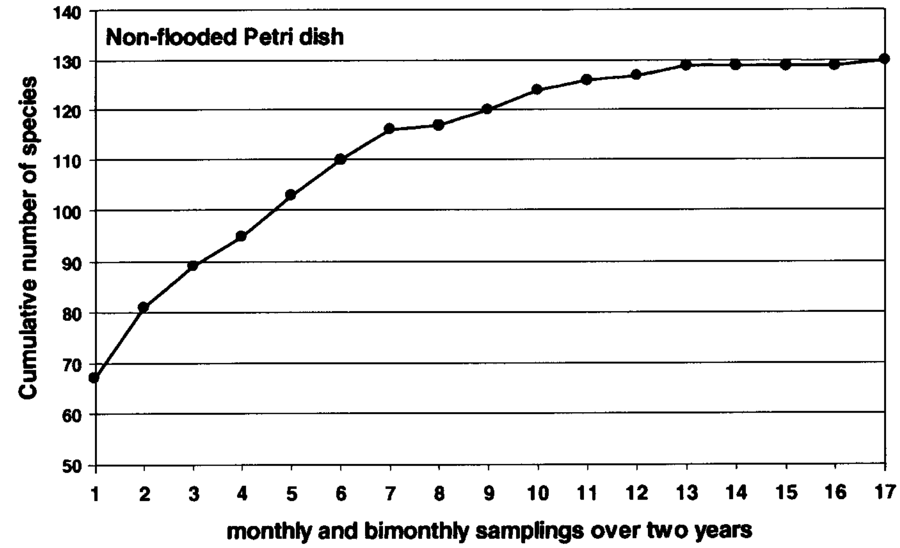

Figure 1. Cumulative number of species obtained with the non-flooded Petri dish method (Foissner 1987) in 17 monthly and bimonthly samplings from an 100 m^2 area of beech forest soil in Austria. The curve flattens distinctly at sample number 13, indicating that further effort hardly will increase species number significantly. However, direct investigation of fresh samples after rainfalls provided 30 further species. Accordingly, the total number approaches 160 species, which is far from the total number (about 1 000) of soil ciliates known. Thus, I do not agree with the hypothesis of Finlay and Esteban (1998) that "all species of freshwater protozoa could eventually be discovered in one small pond".

(Foissner 1998), the percentage dropped to 25%, and when the 500 undescribed (new) species, which I have in my records (Foissner 1998), are added, the percentage drops to about 14%. Finally, when the estimated number of global soil ciliate diversity (up to 2 000 species; Foissner 1997) is taken, the ratio approaches 8%.

These simple calculations show that the ratio of local:global diversity is meaningless for estimating global diversity, as long as the actual global diversity of the group under investigation is unknown. Accordingly, the conclusions by Fenchel et al. (1997) and Finlay and Esteban (1998) lack a reliable basis.

Interestingly, the 8% ratio calculated above is very close to that found by Finlay and Esteban (1998) in various protozoan groups and habitats. However, I do not believe that this has a deeper ecological meaning. It probably only shows that our knowledge (better: ignorance) is similar for all groups and habitats.

Undersampling: The Key to Understand Protist Diversity

As compared to higher animals and plants, protists are extremely difficult to recognize because they are small and encysted most of their life. Only when the appropriate conditions set in, do they excyst and become "visible" (Foissner 1997). Then, however, many of them may be hidden by an abundance of a few ubiquitous and numerically dominant species. Only when comparatively large samples are carefully (!) inspected by an experienced (!) investigator, are these rare species recognized. And it is well known that rare species comprise >80% of the total species in practically all organism communities (Schwerdtfeger 1975). At present, we probably know mainly the more common species.

These problems in recognizing protist diversity become evident if large sample collections from a large region are analyzed (Table 1). Although the individual samples contain only 1 to 3 (4–7%) new species on average, the sample collective shows that up to 50% of the species found are undescribed. A similar picture is obtained when the papers on flagellates by Patterson and co-authors are analyzed. This is an important and impressive fact, which Finlay and Fenchel never recognized because they have studied only individual samples from certain small biotopes. Furthermore, they concentrated on the more common species, possibly leaving the rare species unidentified.

Only a Tiny Fraction of the Potential Habitats has Ever been Investigated for Protists

Finlay et al. (1996) base their estimation of global free-living ciliate diversity on the assumption that the main biotopes of the Earth have already been

Table 1. Data summary on diversity of soil ciliates in Europe, Africa, Australia and Antarctica (from Foissner 1997).

Characteristics	Europe (99 samples)	Africa (92 samples)	Australia (157 samples)	Antarctica (90 samples)
Total number of species[a]	345	507	361	95
Species/sample (mean)	26	35	23	4
Undescribed species	185	240	154	14
Undescribed species (%)	54	47	43	15
Undescribed species/sample (mean)	1.9[b]	2.6	1.0	0.2
Undescribed species/sample (%)	7.2	7.5	4.4	3.9

[a] Each sample collection was considered as a unique entity. Thus, a new species found, for instance, in both Africa and Australia, was classified as 'undescribed' in each of the data sets.
[b] Sample size (n) = 52.

sufficiently investigated. This is, however, not the case. Seen on a global scale, only a tiny fraction of ponds, lakes and rivers has been investigated in sufficient detail, mainly in Europe and North America. From all other habitats and regions, reliable (!) information is extremely scarce. Again, I shall concentrate on a few representative examples.

(1) There are only a dozen comprehensive and reliable studies available on the marine ciliate fauna, and some of these are outdated. Over a hundred new species and many new genera were reported in a few recent studies (Jankowski 1973; Lynn and Gilron 1993; Petz et al. 1995), demonstrating our ignorance of the marine ciliate biota.

(2) Another major biotope, namely floodplain soils, has rarely been investigated for protists. Floodplains cover a significant portion of the world's land area and are known as biodiversity centres. I have studied a few soil samples from floodplains worldwide (Table 2). They contain a wealth of new species and genera and have, as expected, an increased proportion of freshwater species. The two samples each from the Murray River and Amazon floodplain each contained 25 new species!

(3) During the past 20 years, I found about 700 new species of soil ciliates, and further new species are being discovered at a constant rate, indicating that the summit is a long way off (Foissner 1997, 1998). For instance, 10 (!) new species were discovered during the investigation mentioned in the chapter "Local versus Global Diversity", indicating our ignorance of even the European soil ciliates. A similar figure was obtained for heterotrophic flagellates from coastal marine and hypersaline sediments in Australia. Patterson and Simpson (1996) identified 46 species, of which seven were undescribed, and a further seven could not be identified. Of the remaining 39 species, 23 (!) have been described during the past 10 years. This means, if Patterson and Simpson (1996) had studied these sites in 1985, 30 of the 46 species would have been undescribed.

These examples show that practically all large and diverse biotopes are heavily underinvestigated as concerns the ciliate (protist) biota. Of similar importance is the question "What is a habitat for a protozoan?" I think, a square metre of soil is a universe for any protist. Thus, seen on a global scale, there must be millions of potential protozoan habitats. For

Table 2. Diversity and structure of floodplain soil ciliate communities.

Floodplain	Total number of species	Freshwater species[a]	New or supposedly new species
Danube River, Austria (2 samples from close sites)	86	28 (33%)	8 (10%)
Mlambane River, Kruger National Park, South Africa (1 sample from dry river bed)	62	32 (52%)	10 (16%)
Murray River, Australia (2 samples from very close sites)	110	35 (32%)	25 (23%)
Rio Corobici, Costa Rica (1 sample)	87	14 (16%)	5 (6%)
Rio Amazonas, Brazil (2 samples from very close sites)	112	27 (24%)	23 (21%)

[a] Proportion for all soil ciliates: 16% (Foissner 1998).

instance, the rhizosphere of any plant species is a special habitat governed by the root exudates. And there are hundreds of soil types containing thousands of strata with very distinct biological, physical and chemical environments. We do not yet know how many of these potential habitats are actually occupied by specific inhabitants. Usually, however, specific habitats produce specific organisms. It is only man who reduces the great number of potential protist habitats to a few categories, such as soil, pond, benthos, and pelagial. Only in this sense, do I agree with Fenchel et al. (1997) that "everything is (almost) everywhere".

113 or 900 Species of "True" Soil Ciliates?

In their papers Finlay and Fenchel refer to several of my studies in an incomplete and inaccurate way. As an example, I cite some of their statements (Finlay and Fenchel 1999). "The current number of species listed in the 'world soil ciliate fauna' (Foissner 1998) is 643, but the species considered to have completely published descriptions, and that are 'probably found exclusively in true terrestrial habitats' number 113, of which 98 were described by Foissner and co-authors". This calculation is completely wrong and neglects what I wrote (Foissner 1998): "Only 25% (160 out of the 643 species) of the described soil ciliate species have been reliably recorded from both soil and freshwater habitats. Thus, there would appear to be a high proportion (483) of species which occur only or mainly in terrestrial environments and very likely evolved in such habitats". The 113 species calculated by Finlay and Fenchel are mainly those with special adaptations, as I clearly indicated in footnote 7: "Only species with specific food requirements or very characteristic morphological adaptations have been classified as probably found exclusively in terrestrial habitats". This restriction thus limits the number to a very conservative minimum, necessary due to the scarcity of detailed data. However, almost 500 other species, most of which I and others have carefully described or redescribed during the past 20 years, have as yet also only been found in terrestrial habitats! This fact is ignored by Finlay and Fenchel.

Finlay and Fenchel also omit that I clearly stated to have "about 500 new species in my records, whose full description will require years of work" (Foissner 1998). As I am continuously publishing on new soil ciliates (e.g. Foissner 1999), this number is not a fiction. Further, Finlay and Fenchel state: "New species are being described from soil; but their number is increasing relatively slowly, and linearly over time (Foissner 1998)". This statement takes into account neither the 500 undescribed species mentioned above, nor the unfortunate reality that I am, since a decade, practically the only researcher worldwide publishing on taxonomy of soil ciliates.

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