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Biodiversity of aquatic protists – what can we learn from bacteria and *Daphnia*?

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Abstract

This article discusses the current uncertainty in the assessment of the biodiversity of aquatic protists. By comparison with prokaryotes (bacteria) and asexually reproducing metazoans such as *Daphnia* (water fleas) it is illustrated that morphological and some genetic species demarcations may be too conservative to identify functionally different ecotypes. Intraspecific variation is extensive and has been demonstrated by ecophysiological experiments, in particular, for ciliates. The significance of the clonal differences for the biodiversity of aquatic protists needs to be addressed in a conceptual framework.

Introduction

It is currently assumed that aquatic microbial eukaryotes are globally distributed and have a remarkable ability to adapt to wide ranges of ecologically important factors (e.g., Finlay 2002). Local adaptation and genetic radiation are, therefore, considered to be of minor importance. A corollary of this view favoured by some authors is that the biodiversity of ciliates and other aquatic protists is thought to be low. Biodiversity, in particular the so called alpha-diversity at a single site (e.g., Perlman and Adelson 1997), is typically measured in terms of either species richness or species richness combined with equitability. The species concept used is, therefore, crucial for the assessment of biodiversity. These issues are reflected in the summary of the 3rd European Congress of Protistology, when Finlay and Fenchel (1999) addressed three fundamental questions for future research: (1) “What is a species?”, (2) “how many species are there?” and (3) “are there limits to the geographical distributions of species?”

The most widely accepted Biological Species Concept cannot be applied to asexually reproducing taxa such as bacteria and many protists; the morphospecies concept favoured by taxonomists has no solid theoretical foundation, and some highly theoretical species concepts are not operational. Even worse, biologists do not agree if a species is a taxonomic category only, i.e. an abstract concept, or an evolutionary unit ('real' species, see Claridge et al. 1997, Bachmann 1998, Hey 2001). Using examples from prokaryotes, I will demonstrate, in the first part of this article, that estimates of biodiversity are highly sensitive to arbitrary species definitions. Examples from metazoan taxa such as *Daphnia* (water fleas) show, in the second part, that the functional ecological variability may be large even within one well defined species. In the third part, I will apply the knowledge gained from the previous discussion to planktonic protists and present some examples on their intraspecific variation.

Similar to bacteria and *Daphnia*, there is increasing evidence that morphologically and/or genetically defined protist species may harbour many ecotypes. In particular, using new results obtained with freshwater ciliates, I will demonstrate that local adaptation or narrow ecological niches may be typical for spatially or temporarily isolated habitats. The overall conclusion is that the biodiversity of aquatic protists is much higher than is obvious at the morphospecies level.

I will not describe methods in any detail in this review. The reader interested in a particular method is referred to the original publications cited in this article.

The current dilemma – what is a bacterial species and how to assess bacterial biodiversity?

The global species diversity of prokaryotes is not characterised, mainly because there is still debate about the bacterial species definition and only a small fraction of prokaryote taxa has been isolated and cultured (e.g., Cohan 2001 & 2002, Rosselló-Mora and Amann 2001, Stackebrandt et al. 2002). Isolation and characterization in pure culture, however, is a prerequisite for the recognition of prokaryote species; accordingly, to date less than 6000 prokaryotic species have been accepted by the International Committee on Systematics of Prokaryotes (formerly the International Committee on Systematic Bacteriology) (Rosselló-Mora and Amann 2001, Cohan 2001, www.bacterio.cict.fr/). It is commonly believed that culturable bacteria amount to <0.1% of the species in a given environment (discussed by Cohan 2001), that is, the global number of bacterial species should be in the range of several to many millions (Weisse 2006a). Recent evidence using refined isolation and culturing methods suggests that the fraction of culturable aquatic bacteria may be higher than hitherto assumed (Rappé et al. 2002, Hahn et al. 2003, Hahn 2003)). This does not necessarily reduce the total number of potential bacterial taxa, because the advanced isolation techniques, followed by genetic identification using 16S rRNA sequence analysis, led to the detection of a large number of novel, free-living bacteria (Hahn 2003, Hahn et al. 2003).

The novel RNA and DNA sequence-based approaches to identifying bacterial taxa do, however, not solve the problem, since species circumscription uses empirically derived, arbitrary categories (e.g., Rosselló-Mora and Amann 2001, Stackebrandt et al. 2002). As a solution to the current dilemma, Cohan suggested to replace bacterial species by ecotypes (Cohan 2001 and 2002). Ecotypes are populations of organisms occupying the same ecological niche, whose divergence is purged recurrently by natural selection. A typical named bacterial species may contain many ecotypes, each with the attributes of species sensu eukaryotic taxonomy. Cohan (2002) concludes that a named bacterial species represents more a genus than a species. Using a different approach, Rosselló-Mora and Amann (2001) proposed to describe prokaryote species as a monophyletic and genomically coherent cluster of organisms that show a high degree in many independent genotypic and phenotypic characteristics (see also Stackebrandt et al. 2002). Both approaches agree in that is not enough to define prokaryote taxa solely genetically; what remains crucial for an improved assessment and understanding of functional prokaryote biodiversity is the characterization of their ecological niches.

Two examples, one for heterotrophic and one for autotrophic freshwater bacteria, shall illustrate the rapid progress that is currently being made and some potential pitfalls of the molecular approach:

(1) The knowledge on the diversity and taxonomic composition of heterotrophic freshwater bacteria has dramatically changed over the past 20 years. In contrast to the earlier conjecture, recent evidence obtained by phylogenetic analyses from 16S rDNA sequences suggests that there are typical freshwater bacteria which are commonly not found in soil or in the ocean (Zwart et al. 2002). In 1997, Hirons et al. established from 16S rRNA gene sequence analysis a cluster of hitherto unknown freshwater bacteria. The gene sequences were obtained from 7 mountain lakes located in New York State, USA, and the cluster was later designated the *Polynucleobacter necessarius* cluster (Zwart et al. 2002). This name was chosen according to the most similar available gene sequence, which represented the bacterial species, *P. necessarius.* This species was known exclusively as an obligate endosymbiont of the hypotrich ciliate *Euplotes aediculatus* (Heckmann and Schmidt 1987). Since 1997, >100 environmental 16S rRNA sequences affiliated with the *P. necessarius* cluster have been published, and all but one originated from surface freshwater habitats (Hahn 2003). The closely related bacterial sequences of the *P. necessarius* cluster are found virtually in all inland waters (Hahn 2003) and the respective cells may constitute up to 50% of all bacteria in a lake (Burkert et al. 2003). The recent isolation of >100 bacterial strains belonging to the *P. necessarius* cluster (Hahn 2003, M.W. Hahn, unpubl.) and the undergoing sequencing of the *Polynucleobacter* genome by the Joint Genome Institute) may help characterize the ecological niches of the various taxa.

(2) Recently, Ernst et al. (2003) presented a phylogenetic analysis of picocyanobacteria inferred from 16S rRNA and ITS-1 sequences. These authors suggested that several strain clusters have undergone ecosystem-dependent adaptive radiations within several brackish, freshwater and saline (Antarctic) lake environments. For instance, 10 strains obtained from the subalpine Lakes Constance, Zurich and Maggiore formed the tight 'subalpine cluster I' with >99.4% 16S rRNA gene sequence identity. Ernst et al. (2003) concluded that the marginal differences in the gene sequences pointed to recent adaptive radiation within this cluster, and that many closely related genotypes can coexist in a particular ecosystem. Based upon a refined analysis originating from a larger database (with >20 new rRNA gene sequences), Crosbie et al. (2003) challenged this conclusion by Ernst and co-workers. Ten new 16S rRNA sequences obtained from two Austrian subalpine lakes fell into the subalpine cluster I, confirming the earlier results. Phylogenetic analyses derived from a more variable gene region of the phycocyanin operon (*cpcBA*-IGS, Robertson et al. 2001) yielded, however, a different result; the so-called subalpine cluster I contained isolates obtained from Irish Lough Neagh and several Japanese lakes of very different type from the oligomesotrophic, deep subalpine lakes. Sequences from both gene regions revealed two novel clusters of freshwater picocyanobacteria that do not seem to be exclusive for central European subalpine lakes. Similarly, Ivanikova et al. (2007), who also sequenced the 16S rRNA gene and *cpcBA* PCR amplicons, found two new *Synechococcus* spp. clusters at a pelagic station in Lake Superior; however, at an inshore station in Lake Superior, >95% of all clones reflected the *Synechococcus* diversity described previously throughout the world. Crosbie et al.

(2003) concluded that the bias originating from taxon undersampling and from selective sampling and sequencing of picocyanobacterial strains must be taken into account before safe conclusions regarding the geographical restriction of ecotypes sensu Cohan (2001) can be made.

The foregoing two examples illustrate that the vast majority of natural prokaryote genotypes is as yet undiscovered. The current controversy is primarily on the meaning of the undisputed genotypic variation. The question as to whether or not there is a "biogeography for bacteria" (Fenchel 2003) strongly depends on the level of genetic resolution that is used for the phylogenetic analysis. Several authors found no close match between genotype and phenotype of prokaryotes, if a conservative gene region such as the 16S rRNA was used for the analysis (Ernst et al. 2003, Crosbie et al. 2003, Fenchel 2003 and references therein). In particular, it must be clarified which, if at all, level of genetic and phenotypic variation in bacteria corresponds to species rank of eukaryotes (Weisse 2006a). Since genetic exchange in bacteria is principally different from and much more complex than that of eukaryotes, there may be no universal solution to this problem central for the biodiversity debate.

Genotypic divergence and phenotypic plasticity in *Daphnia*

The genus *Daphnia* (water fleas) of the crustacean suborder Cladocera represents common zooplankters in many freshwater lakes and ponds. Due to their significance in the pelagic food web as consumers of algae and food for various fish species, *Daphnia* spp. have been studied in great detail, both in the field and in the laboratory. *Daphnia* reproduce by obligate or cyclical parthenogenesis, i.e., in the latter case, several parthenogenetic generations alternate with one sexual generation. Sexual reproduction is usually triggered by unfavourable environmental conditions. The parthenogenetic generations stem from females that produce unfertilized eggs which develop into more parthenogenetic females. The eggs of each clutch are genetically identical, i.e. a typical *Daphnia* population in a pond is composed of many clones, and each clone consists of genetically identical individuals. The various clones, however, differ genetically, and the genetic diversity within the population may change locally and seasonally due to shifts in the clonal composition of the *Daphnia* populations (e.g., Weider 1985, Carvalho 1994, DeMeester 1996). The genetic polymorphism of *Daphnia* populations was first studied by allozyme gel electrophoresis (Lynch 1983, Weider 1984) and, following the advent of the polymerase chain reaction (PCR) technique, by various molecular methods such as sequencing of the mitochondrial (mt)DNA (e.g., Weider et al. 1996) and randomly amplified polymorphic DNA (RAPD) analysis (Repka et al. 1999, Weber and Van Nordwijk 2002).

Daphnia clones often show considerable variation in behavioural, morphological and life-history traits (summarized in Carvalho 1994, DeMeester 1996, Declerck and Weber 2003). Coexisting, sympatric clones may, for instance, differ in their swimming speed and diel vertical migration patterns (Weider 1984, DeMeester et al. 1995, Weber and Van Nordwijk 2002), temperature and salinity tolerance (Weider 1993, Mitchell and Lampert 2000) and size of offsprings (Tessier and Consolatti 1989).

In addition to these genetically fixed, intraspecific differences *Daphnia* may exhibit high phenotypic plasticity. The latter is defined as the ability of a single genotype to produce more than one alternative form in response to environmental conditions (Repka et al. 1999 and references therein), i.e. as local adaptation to the environment. Phenotypic plasticity in *Daphnia* has been demonstrated as morphological and physiological adaptation in filter screens and beat rates of their feeding apparatus. *Daphnia* adapted to low food conditions have larger filter screens, finer meshes, enhanced filtering efficiency, and reduced filtering appendage beat rates (Lampert 1994, Lampert and Brendelberger 1996, Repka et al. 1999).

The overall conclusion of the pronounced genotypic and phenotypic variability of *Daphnia* is that analysis of a single clone, often adapted to specific laboratory conditions, does not adequately describe the characteristics of a species (O'Keefe et al. 1998).

Lessons from bacteria and *Daphnia* applied to aquatic protists - Intraspecific variation among planktonic ciliates

Sexual reproduction in aquatic protists has been observed in a few cases only, and for >99% of all extant species it is at present unknown if they outbreed at all. The conventional belief is that sexual reproduction in ciliates and other protists is triggered by deteriorating environmental conditions, in particular, by a decline in the resources available for growth (Dini and Nyberg 1993). If we accept that the vast majority of species *in situ* reproduce obligately or primarily asexually, each lake, pond or river should harbour many sympatric clones, and the number of protist clones in the ocean should be infinite.

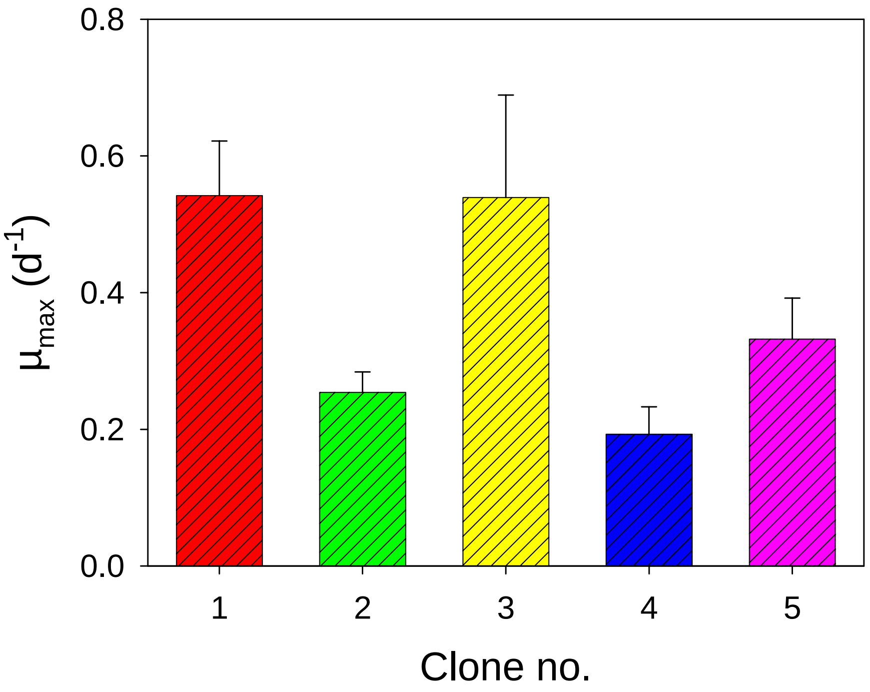

Figure 1: Maximum growth rates of five clones of *Coleps spetai* isolated from Lake Mondsee over a period of six weeks during summer. Growth rates were measured in laboratory cultures with *Cryptomonas* sp. as food. Each experiment was run in triplicate. Error bars denote 1 SD of the mean values (modified from Weisse and Lettner 2002).

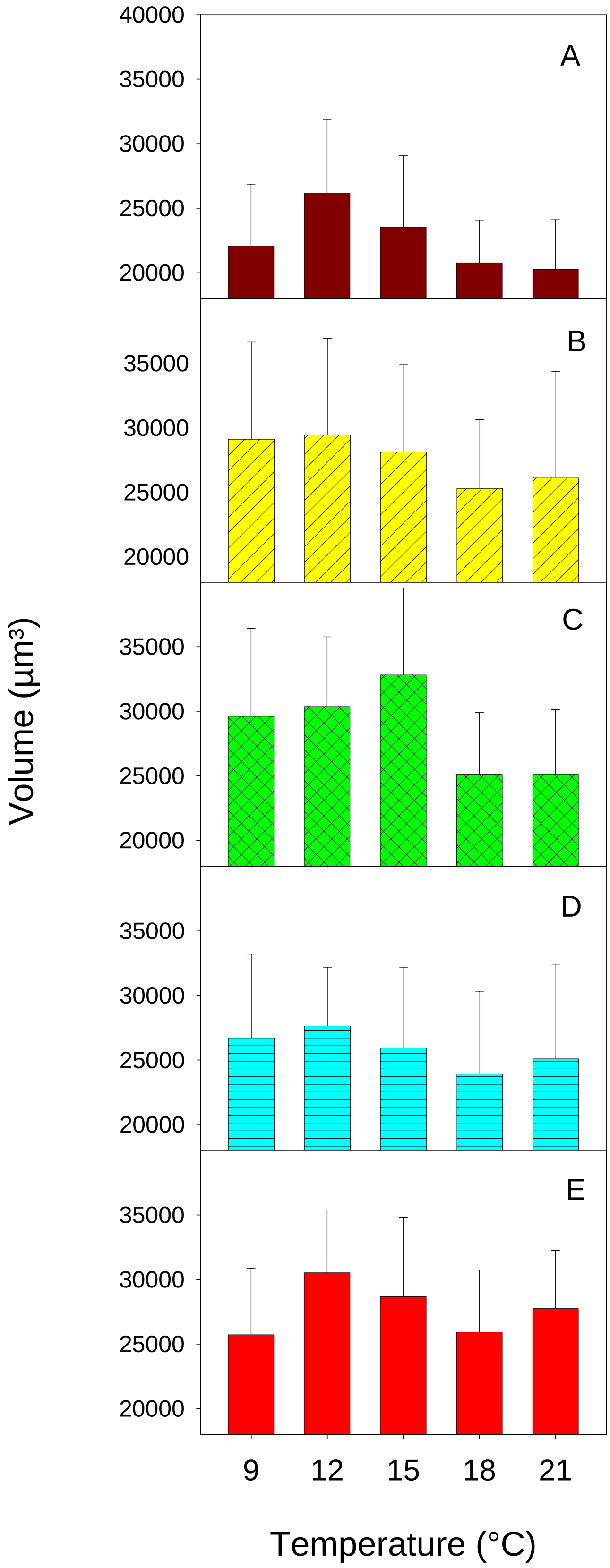

Figure 2: Cell volume of the same five clones of *Coleps spetai* shown in Fig. 1. Error bars denote 1 SD of the mean values (modified from Weisse and Rammer 2006).

With respect to protist biodiversity the crucial issue is the niche width of the clones of a given morphological and/or genetically defined species. If the individual clones overlap to a large extent in their ecophysiological demands, the ecological niche of a species will be stable and can be portrayed, in principal, by studying a single clone in the laboratory. There is increasing evidence that this is not the case; similar to *Daphnia* (see above), intraspecific differences at the clone level are important among asexually reproducing ciliates and other protists (reviewed by Weisse 2002, 2006b). Differences in growth rates of geographically distant clones by a factor of two to three have been demonstrated both for planktonic marine (Pérez-Uz 1995) and freshwater species (Weisse & Montagnes 1998, Montagnes and Weisse 2000, Weisse and Lettner 2002). Note that the physiologically different isolates of the prostome species, *Urotricha furcata,* investigated by Weisse and Montagnes (1998), Montagnes and Weisse (2000) and Weisse et al. (2001) did not differ in a 450 nt large gene region of their 18S rDNA (Bruchmüller 1998 and Buchmüller, unpubl. res.). The intraspecific differences seem to be of comparable magnitude as differences between closely related ciliate species of the same genus or even between similarly sized species belonging to different genera (Weisse and Montagnes 1998, Weisse et al. 2001, Weisse and Lettner 2002). An example for clonal divergence of ciliate growth rates within a lake is shown in Fig. 1. All clones were carefully adapted to the same experimental conditions over several generations, i.e. the results should not reflect phenotypic plasticity. The temperature reaction norm of the clones of *Coleps spetai* is significantly different, as would be expected for different species (Weisse and Rammer 2006, Weisse 2006b). Evidence emerges that intraspecific variability is also common in grazing rates and feeding related parameters such as growth efficiency (Weisse et al. 2001). The latter is correlated to cell volume that is also variable among clones under identical laboratory conditions (Fig. 2).

The geographic boundaries of individual protist clones and their persistence in particular habitats await further research.

Conclusions

It is obvious that aquatic protists, in particular ciliates, show considerable phenotypic clonal divergence. Recent evidence suggests that the variability is genetically fixed (Gächter and Weisse 2006). This conclusion is in contradiction with the assumption that protists are generally able to adapt to very wide ranges of ecologically important factors such as temperature and salinity (Finlay 2002). Based upon many laboratory studies tracing back to the pivotal work of Sonneborn with *Paramecium aurelia* (e.g., Sonneborn 1937, 1939) and later including electrophoretic isozyme analyses, extensive intraspecific variation is a fact in ciliates (reviewed by Dini and Nyberg 1993) and highly likely for other protists. Aquatic protists are thus similar to asexually reproducing metazoa such as *Daphnia*, and it may be misleading to infer the ecological niche of a species from the investigation of a single clone or a particular population (Weisse 2006b). The significance of the observed clonal differences for the biodiversity of aquatic protists needs to be addressed in a conceptual framework; it is not known if genetic and phenotypic divergence are correlated (Finlay 2002). Similar to bacteria, gene sequences of the small ribosomal subunit may be too conserved to reveal ecotypes within a morphologically defined protist species.

References

BACHMANN K (1998) Species as units of diversity: an outdated concept. Theory Biosci 117, 213-230

BRUCHMÜLLER I (1998) Molekularbiologische Charakterisierung und phylogenetische Einordnung heterotropher Nanoflagellaten und prostomatider Ciliaten des Süßwassers Ph.D. thesis, Mathematisch-Naturwiss Fakultät, University of Kiel, Kiel, 196 pp

BURKERT U, WARNECKE F, BABENZIEN D, ZWIRNMANN E, PERNTHALER J (2003) Members of a readily enriched ß-proteobacterial clade are common in surface waters of a humic lake. Appl Environ Microbiol 69, 6550-6559

CARVALHO GR (1994) Evolutionary genetics of aquatic clonal invertebrates: concepts, problems and prospects. In: Beaumont AR (ed) Genetics and evolution of aquatic organisms. Chapman & Hall, London, pp 291-323

CLARIDGE MF, DAWAH HA, WILSON MR (eds) (1997) Species - The units of biodiversity. The Systematics Association Special Volume Series, vol 54. Chapman & Hall, London, 439 pp

COHAN FM (2001) Bacterial species and speciation. Syst Biol 50, 513-524

COHAN FM (2002) What are bacterial species? Annu Rev Microbiol 56, 457-487

CROSBIE ND, PÖCKL M, WEISSE T (2003) Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and *cpcBA*-intergenic spacer sequence analyses. Appl Environ Microb 69, 5716-5721

DECLERCK S, WEBER A (2003) Genetic differenctiation in life history between *Daphnia galeata* populations: an adaptation to local predation regimes? J Plankton Res 25, 93-102

DEMEESTER L (1996) Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. Ecoscience 3, 385-399

DEMEESTER L, WEIDER J, TOLLRIAN R (1995) Alternative antipredator defenses and genetic polymorphism in a pelagic predator-prey system. Nature 378, 483-485

DINI F, NYBERG D (1993) Sex in ciliates. In: Jones JG (ed) Advances in Microbial Ecology, vol 13. Plenum Press, New York, London, pp 129-144

ERNST A, BECKER S, WOLLENZIEN UIA, POSTIUS C (2003) Ecosystem-dependent adaptive radiations of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis. Microbiology 149, 217-228

FENCHEL T (2003) Biogeography for bacteria. Science 301, 925-926

FINLAY BJ (2002) Global dispersal of free-living microbial eukaryote species. Science 296, 1061-1063

FINLAY BJ, FENCHEL T (1999) Divergent perspectives on protist species richness. Protist 150, 229-233

GÄCHTER E, WEISSE T (2006) Local adaptation among geographically distant clones of the cosmopolitan freshwater ciliate *Meseres corlissi*. I. Temperature response. Aquat Microb Ecol 45, 291-300

HAHN MW (2003) Isolation of strains belonging to the cosmopolitan *Polynucleobacter necessarius* cluster from freshwater habitats located in three climatic zones. Appl Environ Microbiol 69, 5248-5254

HAHN MW, LÜNSDORF H, WU QL, SCHAUER M, HÖFLE MG, BOENIGK J, STADLER P (2003) Isolation of novel ultramicrobacteria classified as *Actinobacteria* from five freshwater habitats in Europe and Asia. Appl Environ Microbiol 69, 1442-1451

HECKMANN K, SCHMIDT HJ (1987) *Polynucleobacter necessarius* gen. vov., sp. nov., an obligately endosymbiontic bacterium living in the cytoplasm of *Euplotes aediculatus*. Int J Syst Bacteriol 37, 456-457

HEY J (2001) Genes, categories and species. Oxford University Press, Oxford, 217 pp

HIRONS WD, METHÉ EA, NIERZWICKIBAUER SA, ZEHR JP (1997) Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. Appl Environ Microbiol 63, 2957-2960

IVANIKOVA NV, POPELS LC, MCKAY ML, BULLERJAHN GS (2007) Lake Superior supports novel clusters of cyanobacterial picoplankton. Appl Environ Microbiol 73, 4055-4065

LAMPERT W (1994) Phenotypic plasticity of the filter screens in *Daphnia*: Adaptation to a low-food environment. Limnol Oceanogr 39, 997-1006

LAMPERT W, BRENDELBERGER H (1996) Strategies of phenotypic low-food adaptation in *Daphnia*: Filter screens, mesh sizes, and appendage beat rates. Limnol Oceanogr 41, 216-223

LYNCH M (1983) Ecological genetics of *Daphnia pulex*. Evolution 37, 358-374

MITCHELL SE, LAMPERT W (2000) Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. J Evol Biol 13, 371-382

MONTAGNES DJS, WEISSE T (2000) Fluctuating temperatures affect growth and production rates of planktonic ciliates. Aquat Microb Ecol 21, 97-102

O'KEEFE TCO, BREWER MC, DODSON SI (1998) Swimming behavior of *Daphnia*: its role in determining predation. J Plankton Res 20, 973-984

PÉREZ-UZ B (1995) Growth rate variability in geographically divers clones of *Uronema* (Ciliophora: Scuticociliatida). FEMS Microb Ecol 16, 193-204

PERLMAN DL, ADELSON G (1997) Biodiversity: exploring values and priorities in conservation. Blackwell Science, Cambridge, MA., 182 pp

RAPPÉ MS, CONNON SA, VERGIN KL, GIOVANNONI SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418, 630-633

REPKA S, VEEN A, VIJVERBERG J (1999) Morphological adaptations in filtering screens of *Daphnia galeata* to food quantity and food quality. J Plankton Res 21, 971-989

ROBERTSON BR, TEZUKA N, WATANABE MM (2001) Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int J Syst Evol Micr 51, 861-871

ROSSELLÓ-MORA R, AMANN R (2001) The species concept for prokaryotes. FEMS Microb Rev 25, 39-67

SONNEBORN TM (1937). Sex, sex inheritance and sex determination in *Paramecium aurelia*. Proc. Natl. Acad.Sie. USA 23: 378-395.

Sonneborn TM (1939) Mating types and groupes, lethal interactions, determination and inheritance. Am Nat 73, 390-413

STACKEBRANDT E *ET AL*. (12 co-authors) (2002) Report of the ad hoc committee for the re-evaluatin of the species definition in bacteriology. Int J Syst Evol Microbiol 52, 1043-1047

TESSIER AJ, CONSOLATTI NL (1989) Variations in offspring size in *Daphnia* and consequences for individual fitness. Oikos 56, 269-276

WEBER A, VAN NORDWIJK A (2002) Swimming behaviour of *Daphnia* clones: differentiation through predator infochemicals. J. Plankton Res 24, 1335-1348

WEIDER LJ (1984) Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. Limnol Oceanogr 29, 225-235

WEIDER LJ (1985) Spatial and temporal genetic heterogeneity in a natural *Daphnia* population. J Plankton Res 7, 101-123

WEIDER LJ (1993) A test of the "general-purpose" genotype hypothesis: Differential tolerance to thermal and salinity stress among *Daphnia* clones. Evolution 47, 965-969

WEIDER LJ, HOBÆK A, CREASE TJ, STIBOR H (1996) Molecular characterization of clonal population structure and biogeography of arctic apomictic *Daphnia* from Greenland and Iceland. Mol Ecol 5, 107-118

WEISSE T (2002) The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists. Antonie van Leeuwenhoek 81, 327-341

WEISSE T (2006a) Biodiversity of freshwater microorganisms - achievements, problems, and perspectives. Pol J Ecol 54, 633-652

WEISSE T (2006b) Freshwater ciliates as ecophysiological model organisms - lessons from *Daphnia*, major achievements, and future perspectives. Arch Hydrobiol 167, 371-402

WEISSE T, KARSTENS N, MEYER VCM, JANKE L, LETTNER S, TEICHGRÄBER K (2001) Niche separation in common prostome freshwater ciliates: the effect of food and temperature. Aquat Microb Ecol 26, 167-179

WEISSE T, LETTNER S (2002) The ecological significance of intraspecific variation among freshwater ciliates. Verh Internat Verein Limnol 28, 1880-1884

WEISSE T, MONTAGNES DJS (1998) Effect of temperature on inter- and intraspecific isolates of *Urotricha* (Prostomatida, Ciliophora). Aquat Microb Ecol 15, 285-291

WEISSE T, RAMMER S (2006) Pronounced ecophysiological clonal differences of two common freshwater ciliates, *Coleps spetai* (Prostomatida) and *Rimostrombidium lacustris* (Oligotrichida), challenge the morphospecies concept. J Plankton Res 28, 55-63

ZWART G, CRUMP BC, KAMST-VAN-AGTERVELD MP, HAGEN F, HAN S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 28, 141-155