

Molekulare Mikrobielle Ökologie

WS 11/12



Sabine Kleinsteuber



Thomas Fester



Antonis Chatzinotas

Weitere Termine
TF: ab 02.11.2011; SK: ab 13.12.2011

Habitat	Culturability (%)
Seawater	0.001 - 0.1
Freshwater	0.25
Activated sludge	1 - 15
Sediment	0.25
Soil	0.1 - 0.3

Bacterial, protistan and fungal species numbers

Group	Estimated	Cultivated
Bacteria	3.000.000	5.000 (0.2%)
Protists	200.000	40.000 (20%)
Fungi	1.500.000	11.500 (0.8%)

The great plate count anomaly

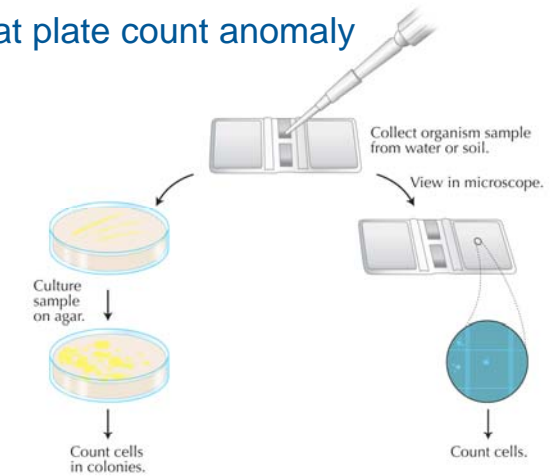
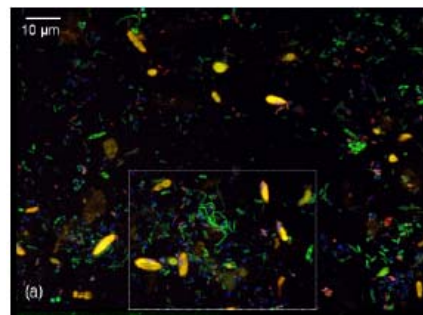
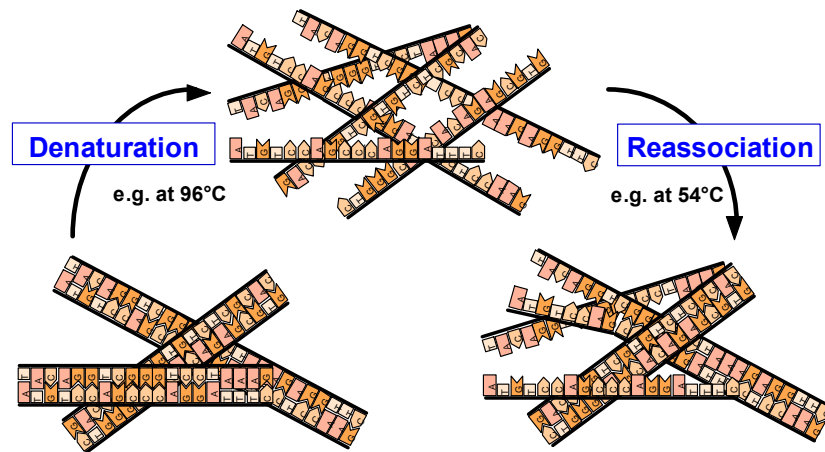
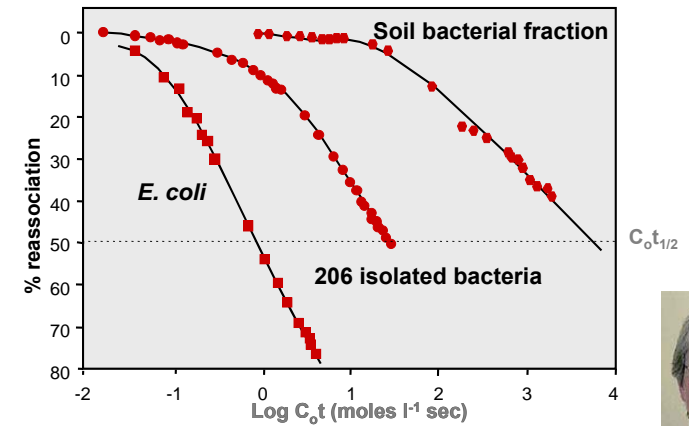


FIGURE 6.12. The great plate count anomaly. Plate counts of cells obtained by cultivation are usually much lower, sometimes by orders of magnitude, than those from direct cell counts under a microscope. Possible reasons are (1) the differing nutritional requirements of the organism, (2) the organism may enter a noncultivable resting state, or (3) the organism may rely on other organisms and thus cannot be cultivated in isolation.

Thermal denaturation and reassociation of DNA



Thermal denaturation and reassociation of DNA



(after Torsvik et al., 1990)

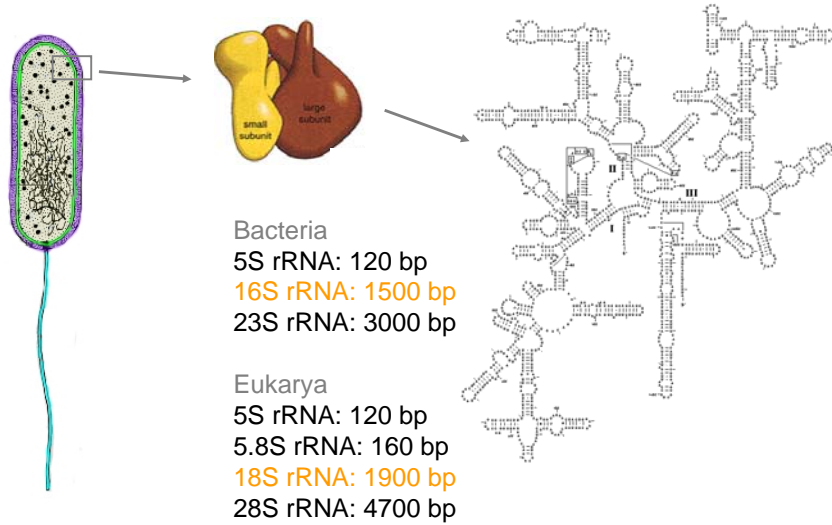
$C_0t_{1/2}$ - values and number of “genomes” for terrestrial and marine environments

DNA Source	$C_0t_{1/2}$	No. of genomes
<i>E. coli</i>	0.79	1
Forest soil 206 isolates	28	35
Forest soil (FS)	4700	6000
Pristine sediment	9000	11400
Fish farm sediment	10	13
Abandoned fish farm sediment	1300	1650
Soil amended with uncontaminated sewage sludge	7800	9900
Soil amended with low metal contaminated sewage sludge	3700	4700
Soil amended with high metal contaminated sewage sludge	1200	1500
Model experiment: agricultural soil	5700	7200
Model experiment: + CH ₄	270	340

(after Torsvik et al.)

Ribosomal RNA: the golden standard in microbial diversity studies

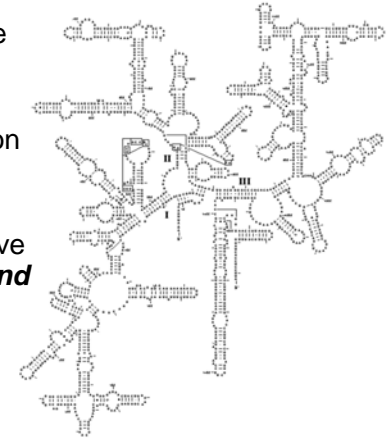
Ribosomal RNA (rRNA)



rRNA-the molecular clock



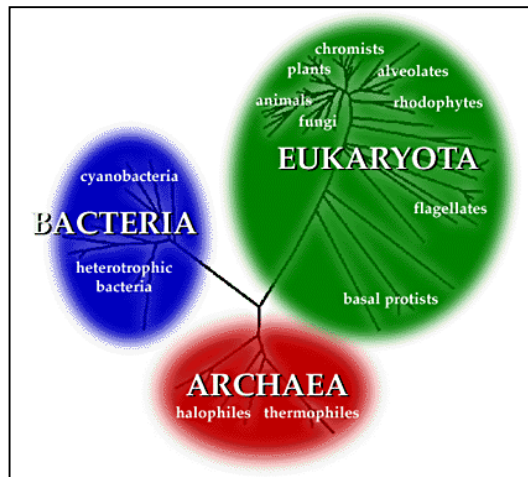
- Present in all organisms with the same essential function
- High copy number (up to 20000/cell)
- Conservation of function > conservation of structure
- Mutations can be “lethal”
- Molecular clock: different regions evolve at different rates > highly conserved **and** hypervariable regions
- Distance Calculations



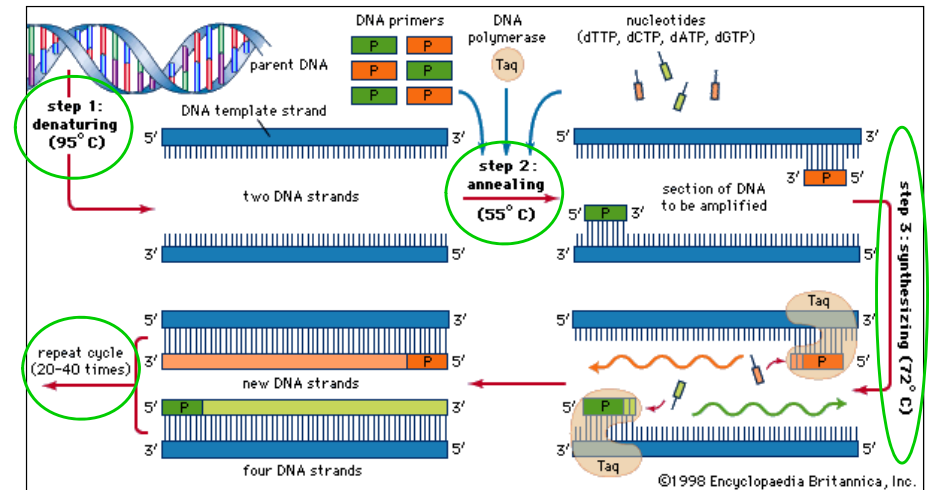
Tree of Life



Carl Woese



Polymerase Chain Reaction (PCR)



Two key innovations for success of PCR

- **Heat-stable DNA polymerase** isolated from bacterium *Thermus aquaticus* which inhabits hot springs

Polymerase **remains active** despite being heated many times

- **DNA thermal cyclers** – a computer that controls repetitive temperature changes required for PCR



The starting material – Dirt & DNA

DNA extraction methods

- **Indirect:** Cells are extracted from soil matrix prior to lysis
- **Direct:** Cells are lysed while still in the soil matrix

Indirect DNA extraction

- Developed in 1975 by D.L. Balkwill et al.
- Cells are extracted from soil matrix
- Lysed by enzymes or the bead beating method
- DNA is extracted and purified. Many purification methods have been used including cesium chloride and other density gradients

Direct DNA extraction

- First described by A. Ogram et al. in 1987
- Cells are lysed in the soil by any number of methods including enzymatic treatment, sonication, sodium dodecyl sulfate (SDS), microwaves and bead beating
- DNA is then extracted and purified

DNA extraction with FastPrep® System

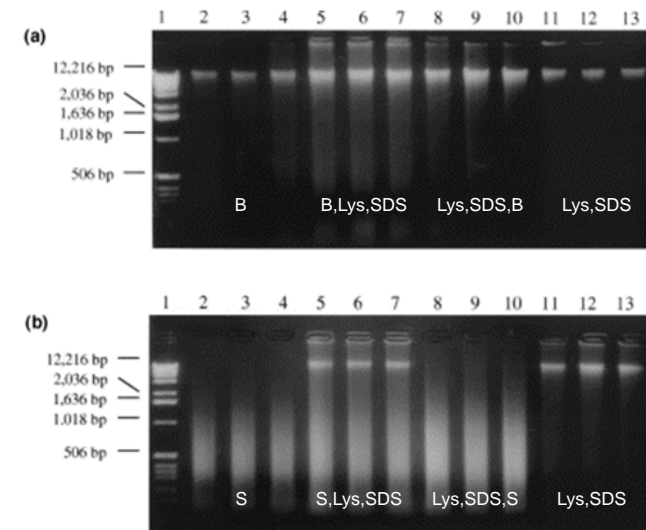


Efficacy of DNA purification procedures as evaluated by DNA recovery and inhibition of the PCR

Purification method	Agricultural soil	Forest soil	Wetland sediment	Inhibition			
				10 ⁰	10 ¹	10 ²	10 ³
No purification	100 ± 7	100 ± 9	100 ± 15	0	0	0	2
SpinBind column	83 ± 3	80 ± 5	41 ± 8	0	1	3	3
Gel electrophoresis	40 ± 12	38 ± 12	10 ± 1	0	2	3	3
Ammonium acetate precipitation	85 ± 4	76 ± 9	69 ± 6	0	0	0	3
Sephadex G-200 column	80 ± 7	95 ± 6	80 ± 8	0	2	3	3

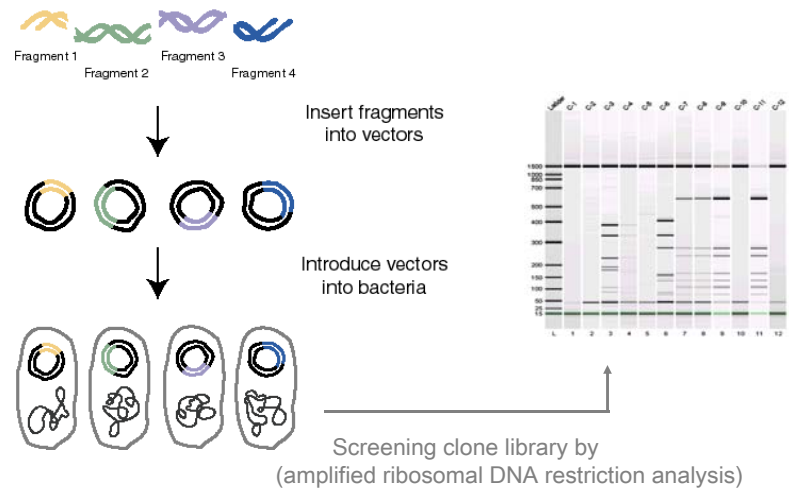
(Miller et al., 1999)

Influence of different lytic treatments on the size of DNA fragments isolated

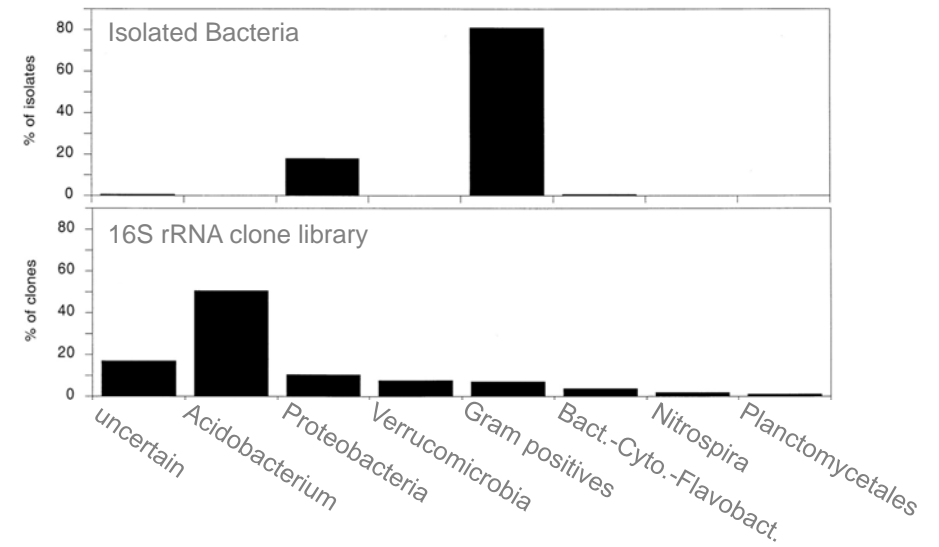


(Krsek and Wellington 1999)

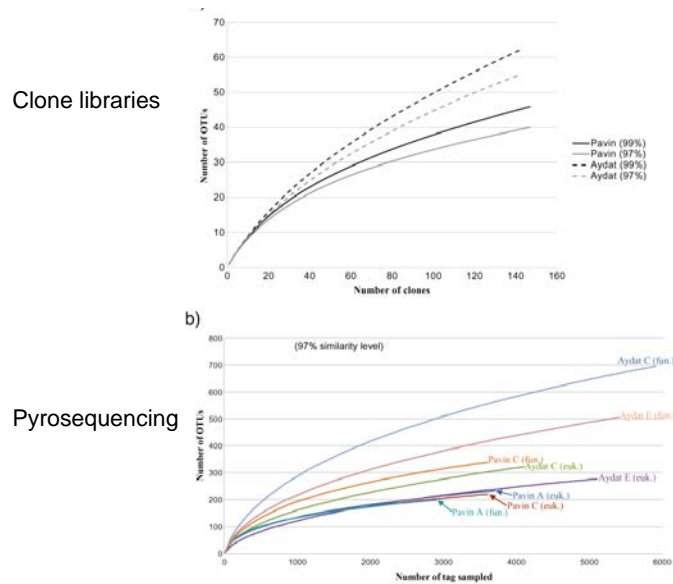
Clone libraries of PCR-amplified rRNA genes



Bacterial diversity in four arid soils

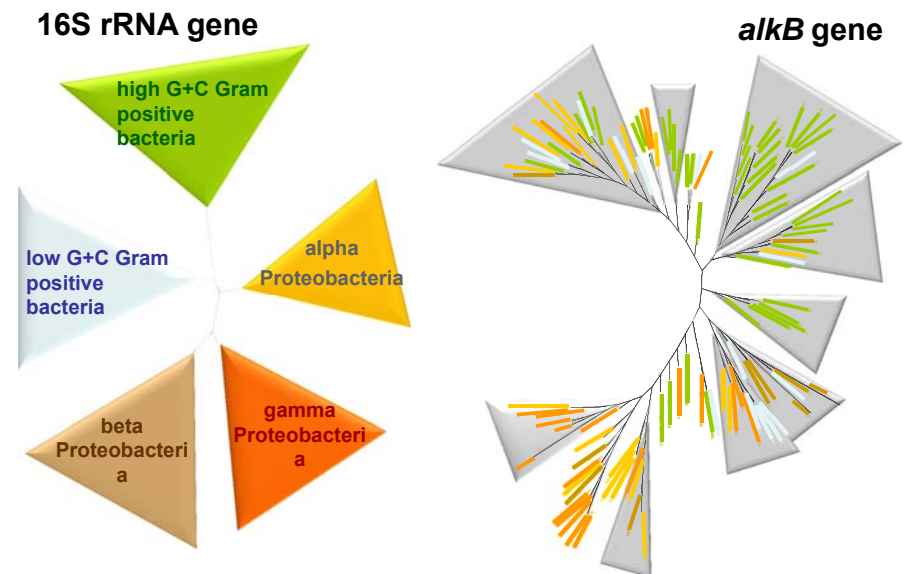


Rarefaction curves: Cloning vs. pyrosequencing



(Monchy et al 2011)

Phylogenetic vs. functional marker genes

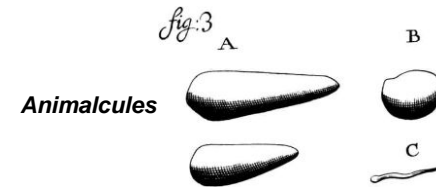


Giebler et al., submitted

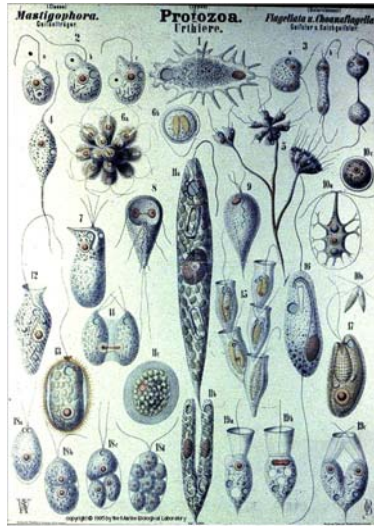
(Micro)eukaryotes - The protists



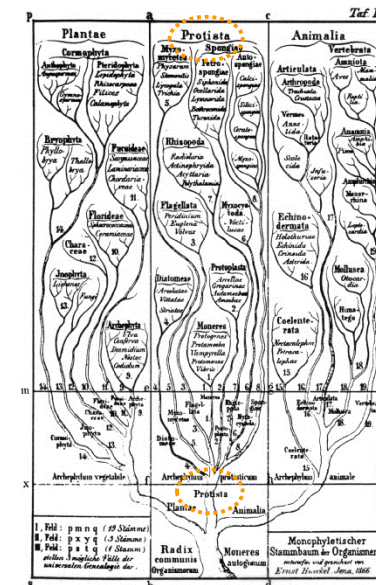
Paramecium humor



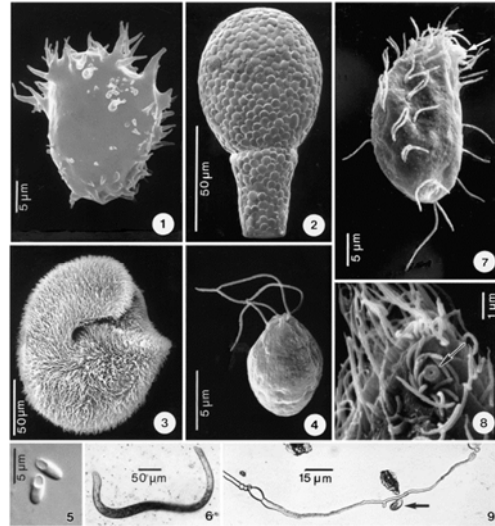
LEEUWENHOEK'S PICTURES OF THE INTESTINAL PROTOZOA OF FROGS
From the engravings in the Dutch edition of *Letter 38* (16 July 1683). X 1½.



Rudolf Leuckart (1822 - 1898)
1869 Professor für Zoology an der Universität Leipzig
<http://www.mblwhoilibary.org/exhibits/leuckart/index.html>



Ernst Haeckel (1834-1919)
1865 Professor für Zoology an der Universität Jena



- Estimated 83.000 protistan species (Corliss 2000)
- **Flagellates** 2-20 μm ; 50 μm^3
- **Amoebae** 5-50 μm ; 400 μm^3
- **Ciliates** 15-200 μm ; 3000 μm^3
- Generation time 2.8-72 h
- Bacteria consumed per cell division 150-12000

Foissner, 1999

The protists - definition

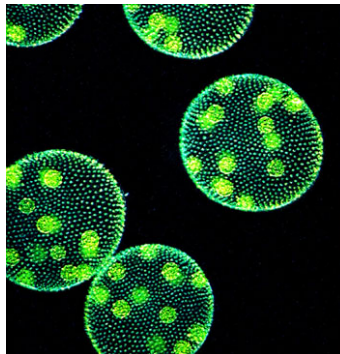
Wikipedia

Protists are a diverse group of eukaryotic microorganisms. Historically, protists were treated as the **kingdom *Protista*** but this group is no longer recognized in modern taxonomy.

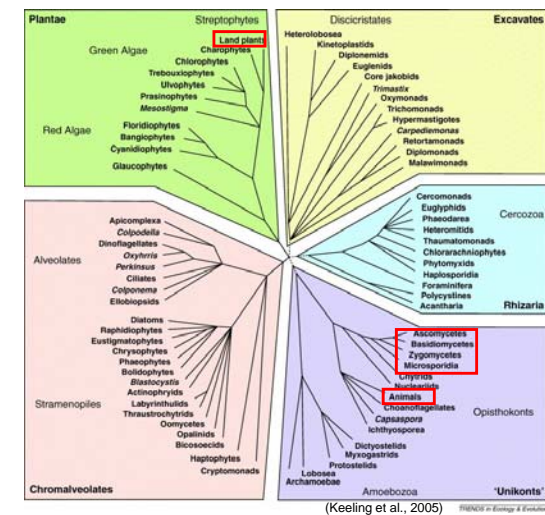
➤ "regarded as a loose grouping of 30 or 40 disparate phyla with diverse combinations of trophic modes, mechanisms of motility, cell coverings and life cycles."

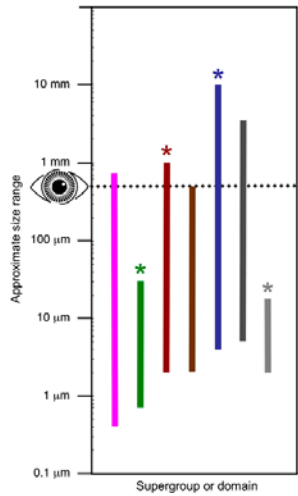
The protists have a relatively simple organization— either they are **unicellular**, or they are **multicellular without specialized tissues**. This **simple cellular organization** distinguishes the protists from other eukaryotes, such as fungi, animals and plants.

Volvox – colonial protist

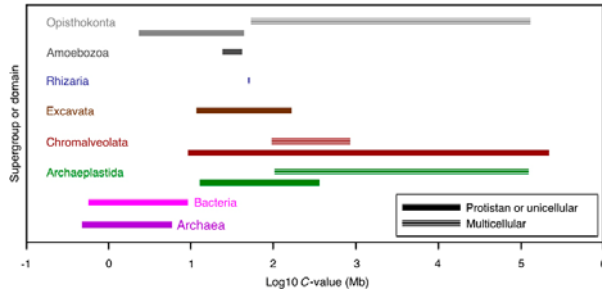


The eukaryotic supergroups



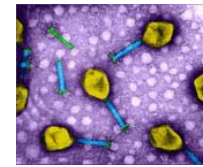


Genome size



e.g. Dinoflagellate genomes: 3000 to 215 000 Mb
 Homo sapiens: ca.3000 megabases (Mb)
 Bacillus subtilis: 4.2 Mb

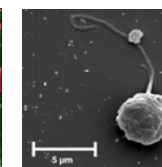
* existence of colonial forms



Viruses
 < 0.2



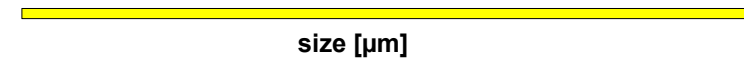
Bacteria



Flagellates



Ciliates
 200

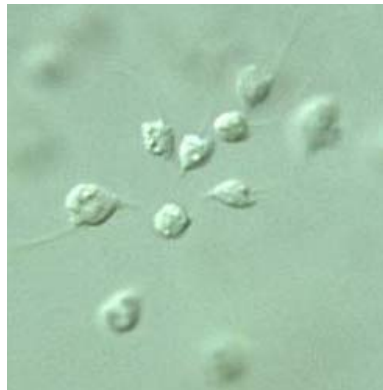


The morphospecies concept

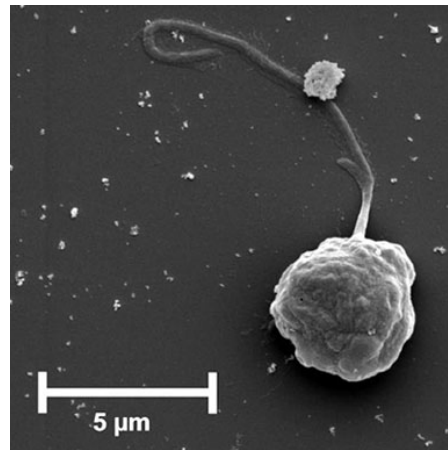
The morphospecies concept

- Species description traditionally on the basis of morphological criteria, > morphological or typological species concept
- Species are groups of individuals that are morphologically similar and clearly distinguishable from individuals of other groups
- Species had traditionally been defined by reference to a morphological type
- Geographic variation among members of the group usually not detected or simply ignored

The morphospecies *Spumella* spp.



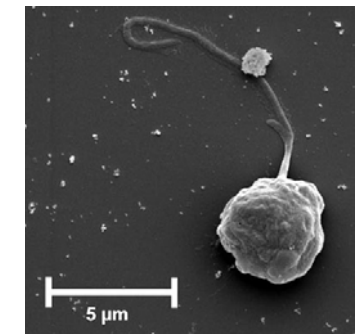
http://protist.i.hosei.ac.jp/Protist_menuE.html



The morphospecies *Spumella* spp.

Monas termo Müller 1773
MONAS gelatinosa.
Mon. GRENSE-MONADEN.
 Animalculum omnium, quæ microscopium simplex offert, minimum, simplicissimum; punctulum gelatinosæ substantiæ; ipsum microscopium compositum eludere viderur, dum ne quidem sub hoc distinctius appareat. Sphæricum, an orbiculare? haud video.
 Gutture aque, in qua maceratio facta est, his corpusculis adeo sæpe repletur, ut ne minimum vacuum distingui liceat, ipsamque aquæ substantiam in aliam minus hyalinam, globularem ex punctis confertissimis omnium calculum superantibus mutam exordies. In hac massa motus, qualem radii solares in statum erodentes. In hac massa motus, qualem radii solares aqua micantes effingere solent, oculis exhibetur, dum animalcula, examinis spum inflat, vehementer commouentur.
 In infusione vegetabilium & animalium. Hujus guttula jam intra vixigi quatuor horas conspicitur quasi massa globularis, et nullis in ea motus nec odor percipitur, brevi vero motus seu fermentatio cum fatore intolerabili infouitur, ac non in omni.

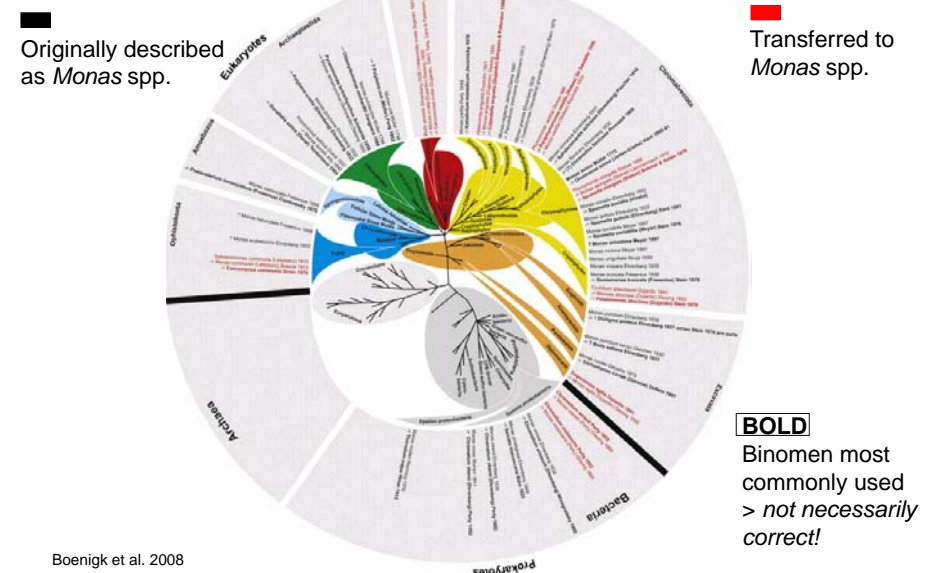
?



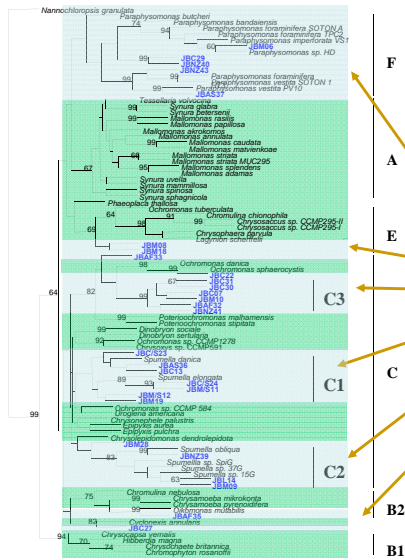
Nanoflagellate taxon diagnoses (*Monas*) since the onset of protistology 1786-1987



Monas – the “oldest” nanoflagellate genus = Spumella?

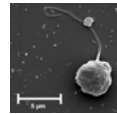


Spumella spp. – 18S rRNA gene analysis



Pfandl et al., 2009

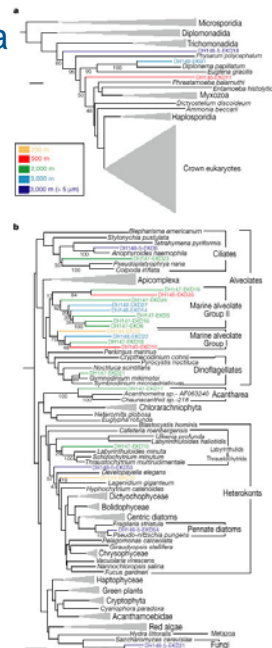
- High molecular variation within nominal flagellate morphospecies
- Divergence among the „Spumella-like“ isolates: up to 10%
- „Spumella-like“ flagellates are polyphyletic



- No biogeographic restriction, e.g. isolates from Austria, China, New Zealand, and Uganda share 100% sequence identity
- Ecotypes??

First rRNA studies in protistan ecology

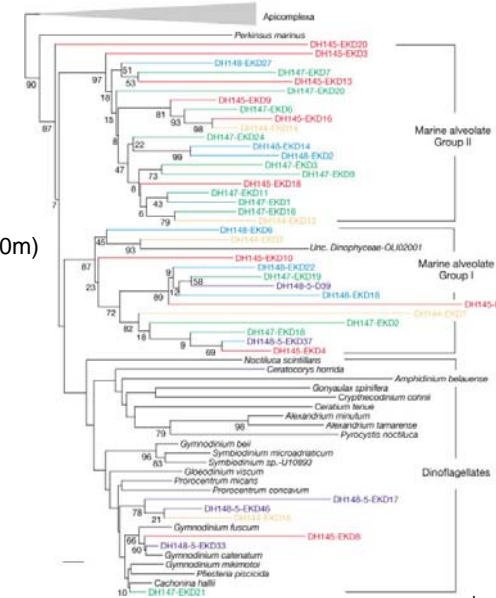
First studies 2001a



- size fraction 0.2-5μm
- aphotic zone (250±3,000m)
- Antarctic polar front.

Lopez-Garcia et al 2001

First studies 2001a



- size fraction 0.2-5μm
- aphotic zone (250±3,000m)
- Antarctic polar front.

Lopez-Garcia et al 2001

Results I

- large diversity
- most frequently retrieved groups: **alveolates**, followed by **heterokonts**
- **Heterokonts**:
 - common in the sea
 - relevant for decomposition processes
 - colonizing faecal pellets
 - also under deep-sea conditions
- Some sequences do not clearly affiliate with any known species > new lineages?

Lopez-Garcia et al 2001

Results II

- **Alveolate** sequences were by far the most diverse
- vast majority grouped in two major clades between dinoflagellates and apicomplexans: **marine alveolate groups I and II**
- mainly retrieved from the smallest planktonic fraction at all depths
- **the diversity found within these groups is equivalent to that displayed by all dinoflagellates known to date**
- No clear diversity fractionation with depth > consistent with the homogeneity of the physico-chemical conditions (poor nutrient concentration, absence of light, average temperature around 2°C)

Lopez-Garcia et al 2001

Results II - conclusions

- Diversity of small eukaryotes at least as important as that of prokaryotes.
- They may reach the deeper parts of the water column by sinking from the upper region, with higher primary production, diversity and nutrient concentration
- However, no sequences corresponding to several phototrophic groups; the new lineages with small planktonic size may be thriving in the deep ocean (cold and oligotrophic waters)

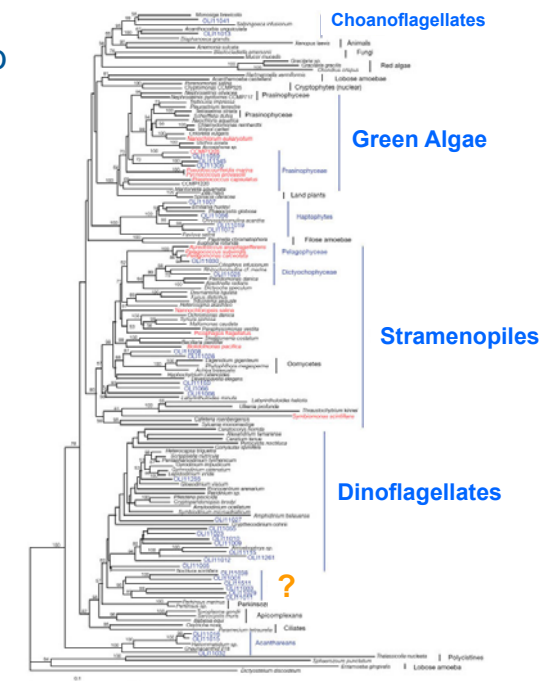
Lopez-Garcia et al 2001

First studies 2001b

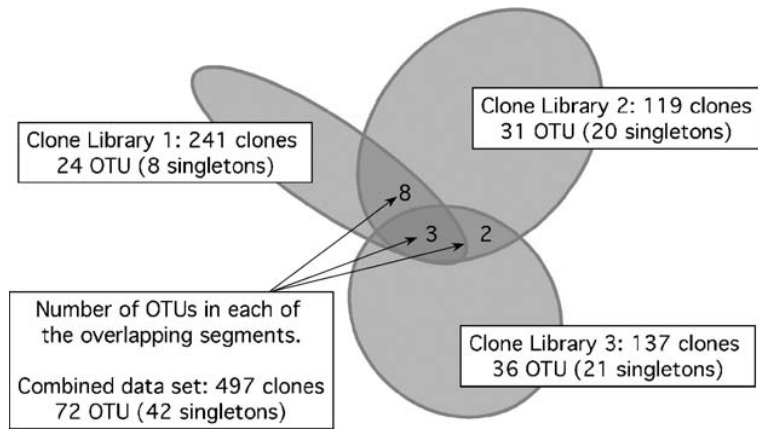
- size fraction <3µm
- 75 m
- Pacific Ocean (equatorial; nutrient-poor)

- Known marine species
- Retrieved sequences

Moon-van der Staay et al 2001

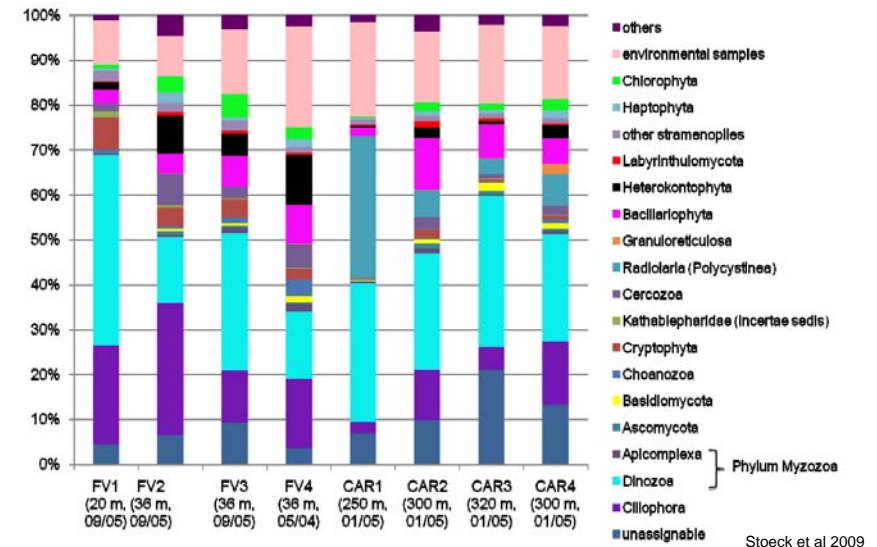


Multiple PCR-primer Approach

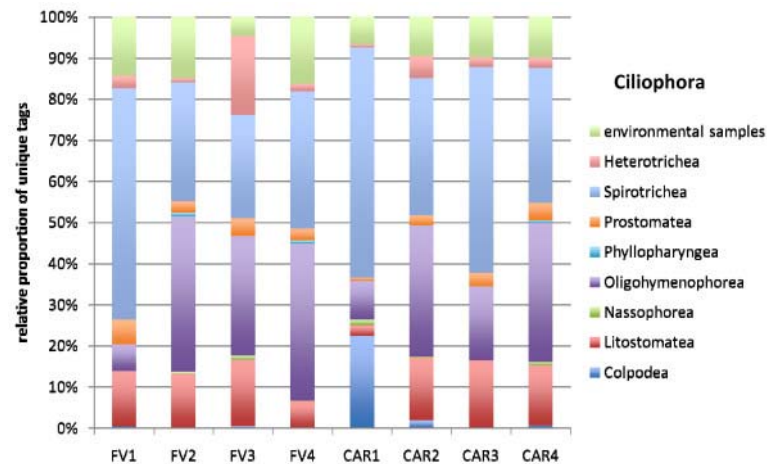


Stoeck et al 2006

Pyrosequencing: Taxonomic distribution (phylum) of protists and fungi in anoxic marine systems

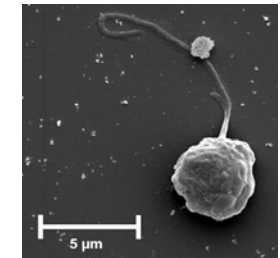
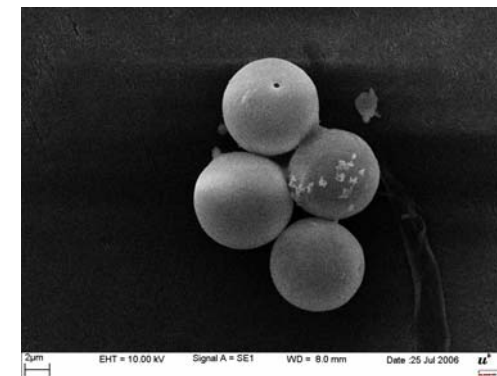


Taxonomic distribution of V9 tags assigned to Ciliophora

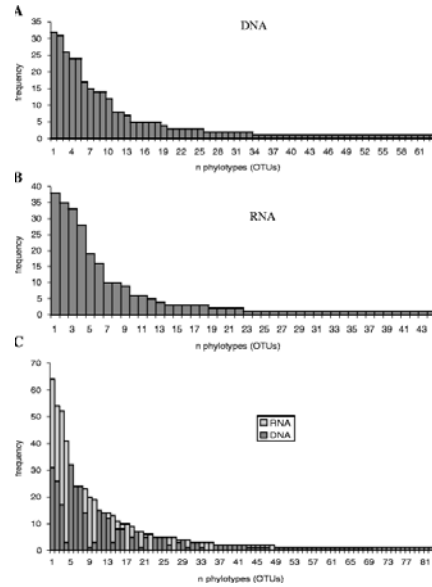


Stoeck et al 2009

Cysts (inactive) vs. vegetative (active)



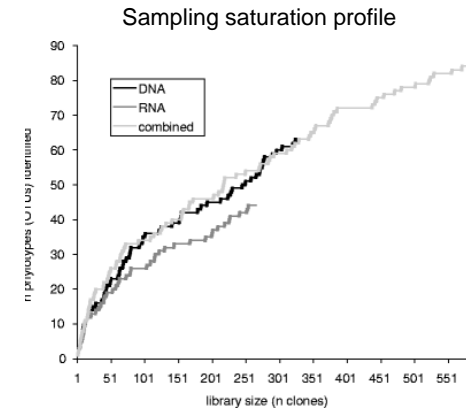
DNA versus RNA



27% in both
25% only in RNA
48% only in DNA

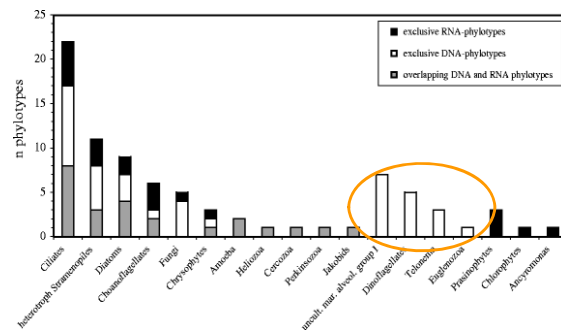
Stoek et al., 2006

DNA versus RNA



Stoek et al., 2006

DNA versus RNA



Dinoflagellates & uncultured marine alveolates I probably non-indigenous members of the anoxic micoreukaryotic community

Stoek et al., 2006

The situation in soil protistan communities

reproduction



active

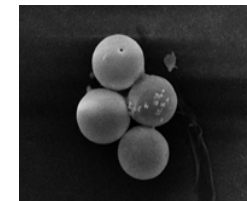
resuscitation



dormancy



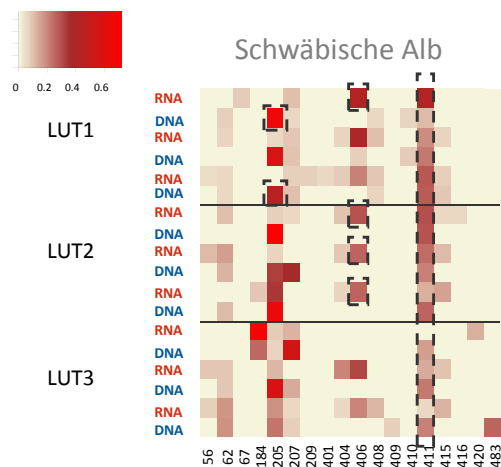
dormant



mortality

mortality

T-RFLP profiles of DNA and RNA pools in soil



Present in DNA and RNA

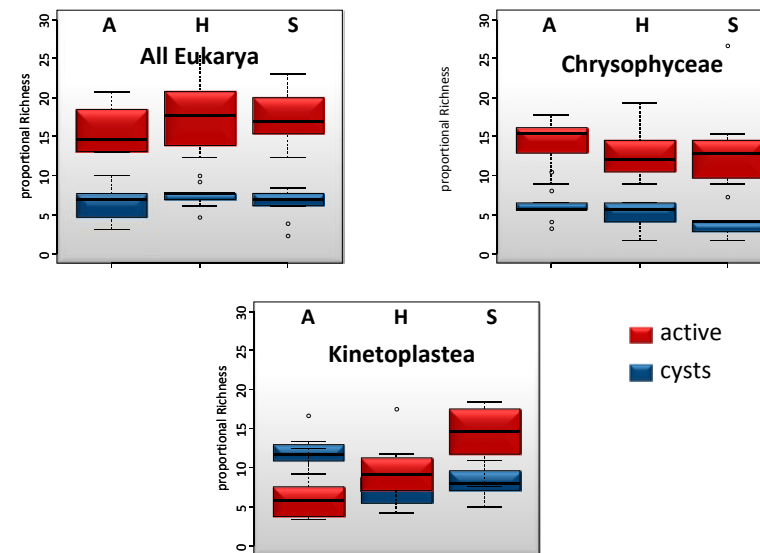
Present only in DNA = cysts

Present only in RNA = rare & active

Eukaryotic 18S rRNA gene pool
April 2009

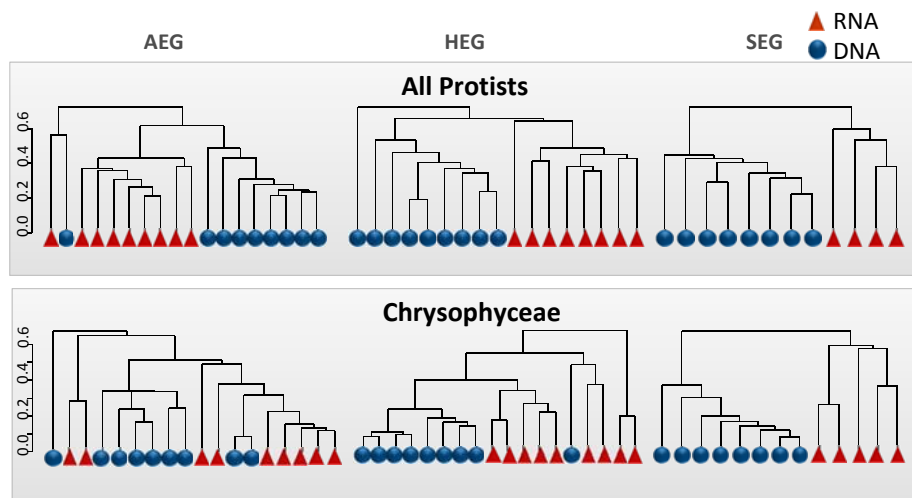
Glaser et al, in prep

Richness of cysts (only DNA) vs active (RNA) communities



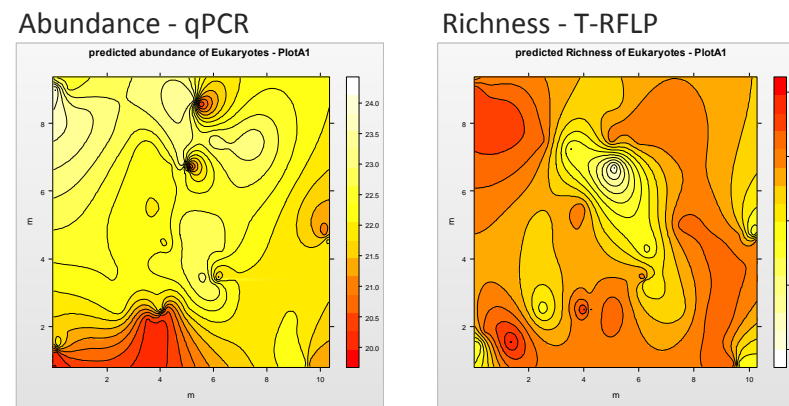
Giebler et al., in prep

Diversity of total (DNA) versus active (RNA) communities

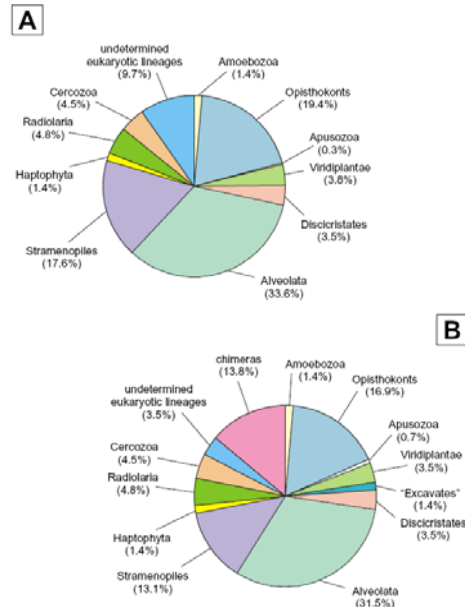


Glaser et al, in prep

Spatial heterogeneity of protist communities in soil

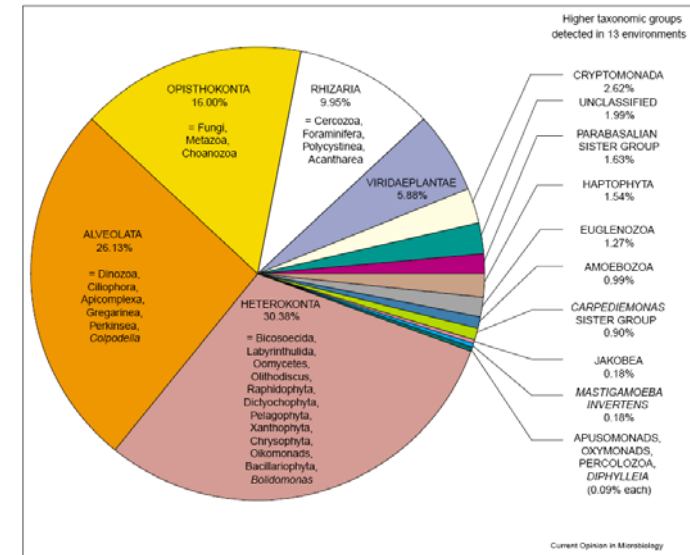


How many novel eukaryotic 'kingdoms'?



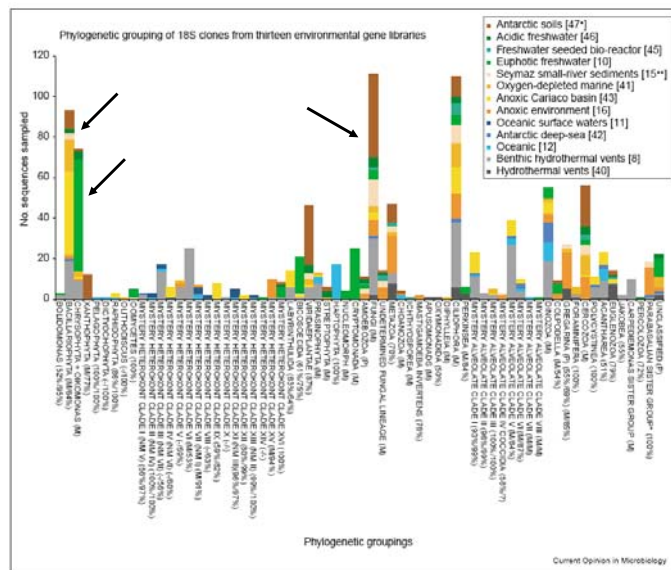
Berney et al., 2004

Meta-analysis of 13 environmental gene libraries



Richards and Bass 2005

Meta-analysis of 13 environmental gene libraries

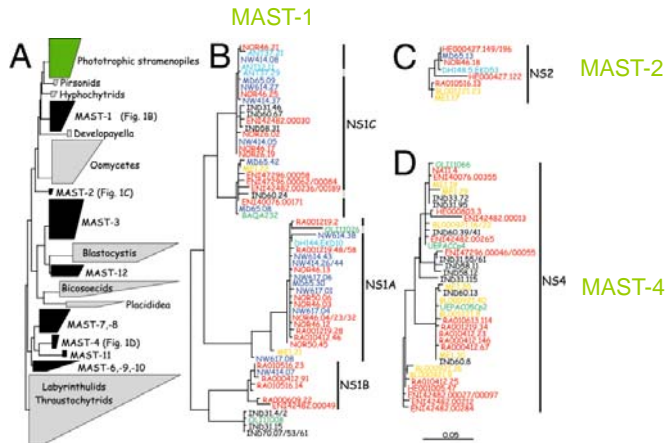


2 only deep sea
8 only freshwater
31 only marine
12 only anoxic

Richards and Bass 2005

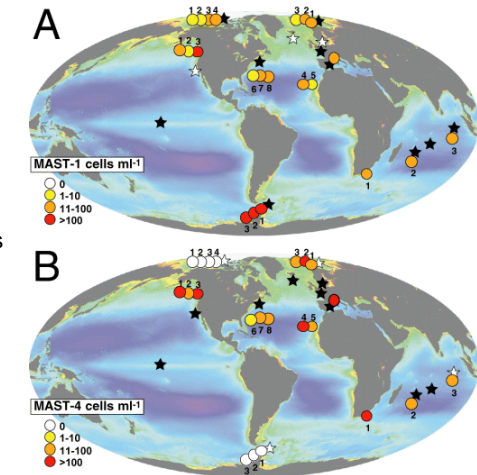
Revealing the organisms behind sequences

Distribution and abundance of uncultured protists in world oceans – marine stramenopiles (MAST)

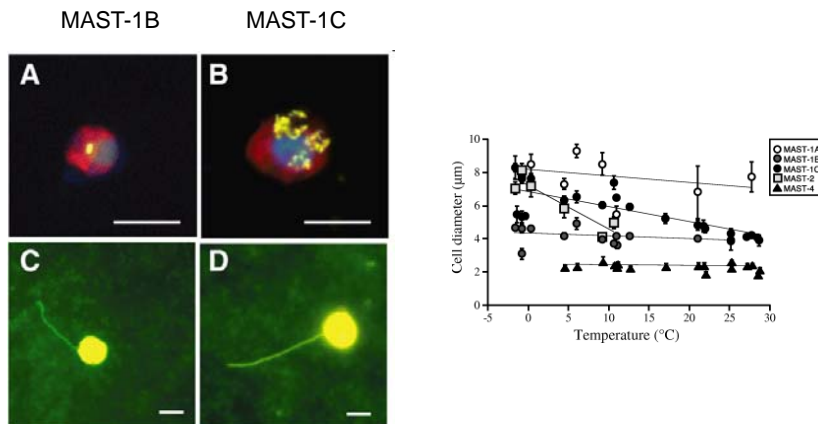


Massane et al., 2006

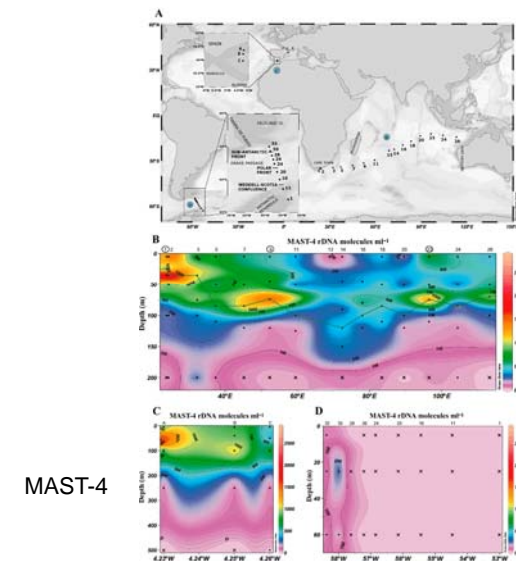
Distribution and abundance of uncultured marine stramenopiles (MAST)



Distribution and abundance of uncultured marine stramenopiles (MAST)

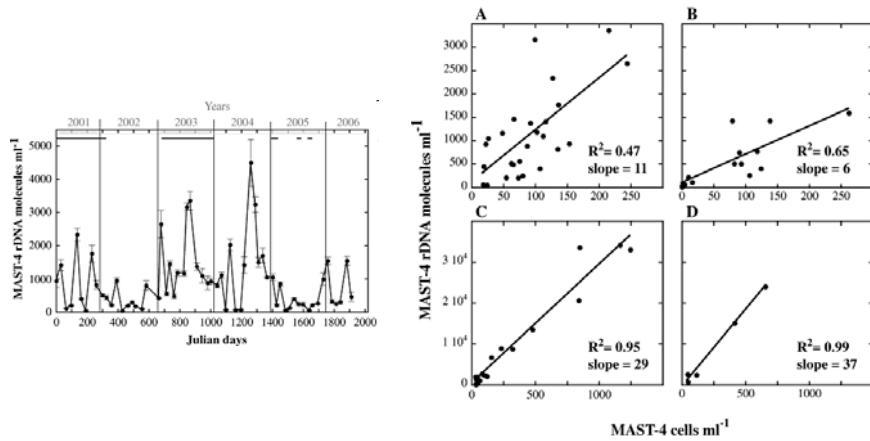


Distribution and abundance of uncultured marine stramenopiles (MAST)



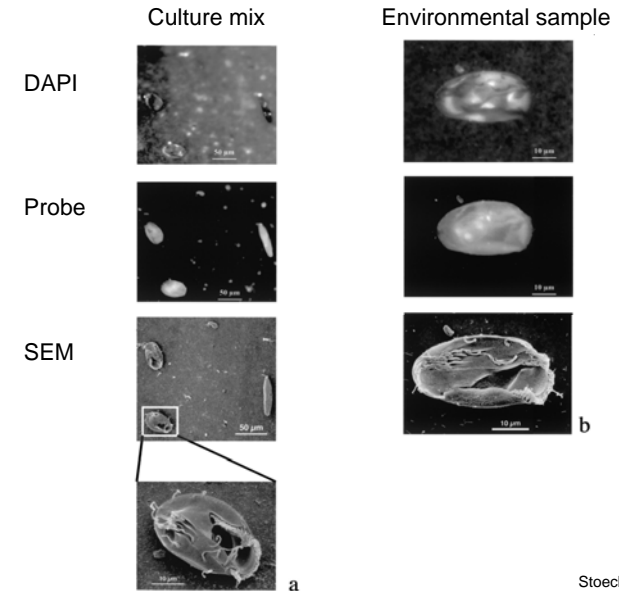
Rodríguez-Martínez et al. 2009

Distribution and abundance of uncultured marine stramenopiles (MAST)



Rodriguez-Martinez et al. 2009

From FISH detection to SEM images



Stoeck et al. 2003

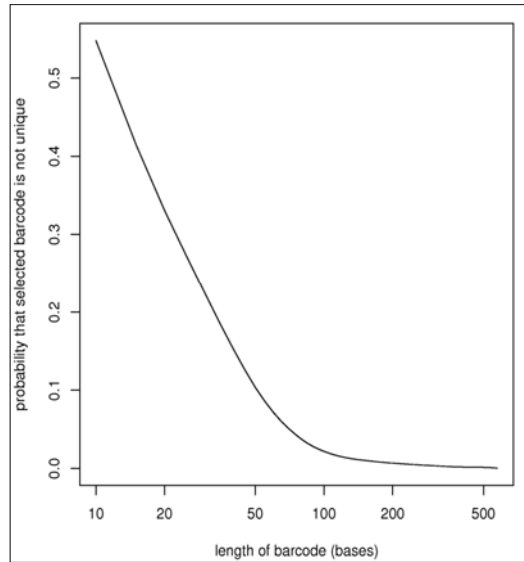
Barcoding

DNA Barcoding – a new way to identify species

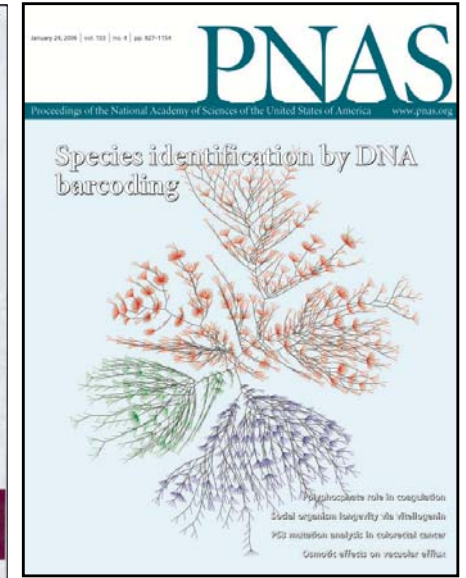
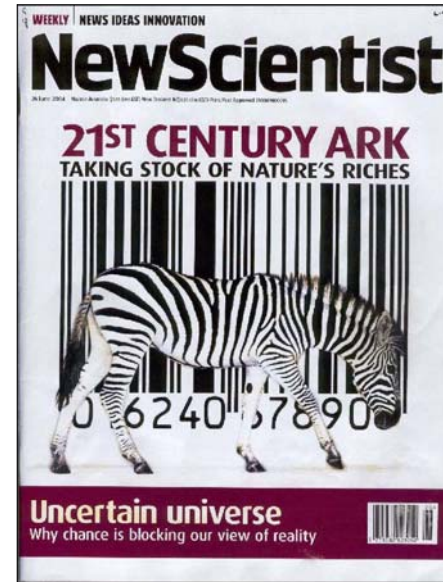
- In most cases an experienced professional taxonomist is needed for morphological identification – Problems if a specimen is damaged
- Uses a very short genetic sequence from the genome
- Barcoding solves these problems, because non-specialists can obtain barcodes from tiny amounts of tissue
- Dual purpose:
 1. new tool in the taxonomists toolbox
 2. innovative device for non-experts for quick identification.
- Standard barcode for almost all animal groups is a 648 bp region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). Highly effective in identifying birds, butterflies, fish, flies
- COI is not an effective barcode region in plants because it evolves too slowly

DNA Barcoding – a new way to identify species

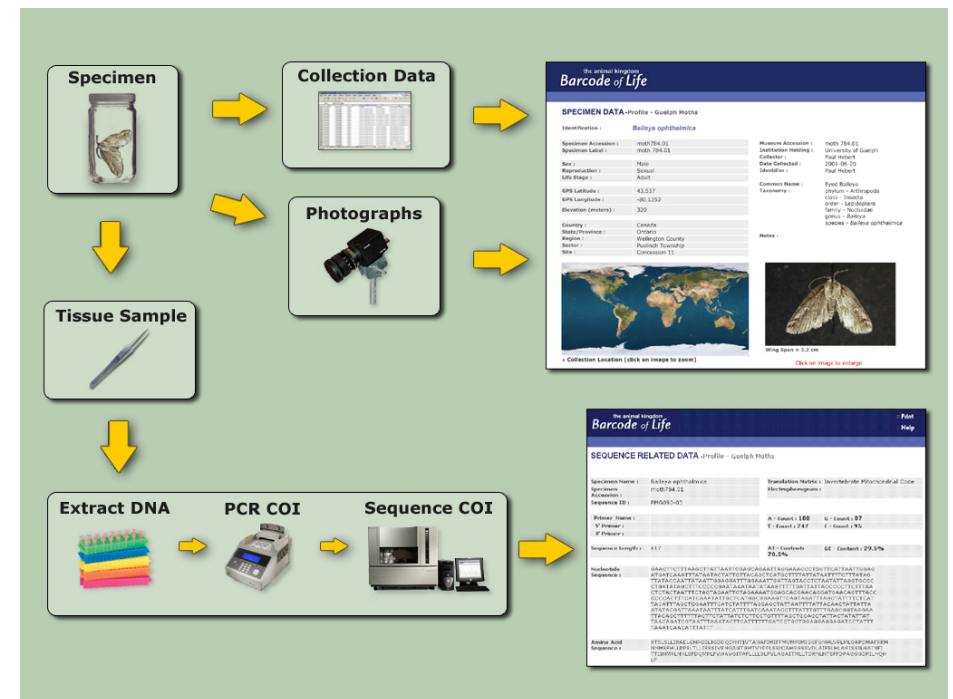
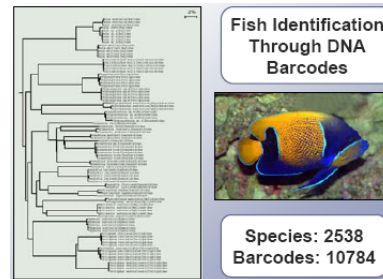
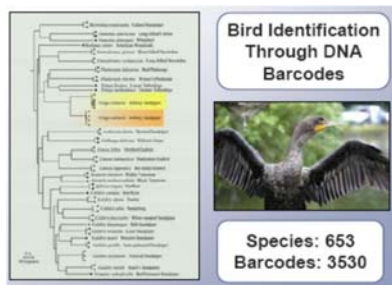
The relationship between barcode length and diagnostic value (Lepidopteran dataset)



DNA Barcoding – a new way to identify species



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DNA Barcoding – a new way to identify species



GoogleGene

GoogleGene Search Clear

Developed by Mehrdad Hajibabaei, Greg Singer, and Donal Hickey

"The goal is to make all of biodiversity Google-searchable".

DNA Barcoding for ciliates – choice of appropriate barcode

International Journal of Systematic and Evolutionary Microbiology (2007), 57, 2412–2425

DOI 10.1099/ijs.0.64865-0

Barcoding ciliates: a comprehensive study of 75 isolates of the genus *Tetrahymena*

Chitchai Chantangsri,^{1†} Denis H. Lynn,¹ Maria T. Brandl,² Jeffrey C. Cole,³ Neil Hetrick³ and Pranvera Ikonimi⁴

Gene	Nucleotide positions (n)	Sequence divergence (%) (mean ± SEM)						
		Overall	Within-genus			Between-genera		
			Genus T	Genus C	Genus G	Genera T and C	Genera T and G	Genera C and G
<i>cox1</i>	689	11.13 ± 0.78	10.47 ± 0.74	12.82 ± 1.46	NC	13.13 ± 0.98	20.31 ± 1.59	21.19 ± 1.80
SSU rDNA	1639 (pairwise)	1.71 ± 0.16	1.39 ± 0.15	3.41 ± 0.45	NC	4.05 ± 0.42	3.48 ± 0.42	3.85 ± 0.39
	1639 (complete)	1.56 ± 0.16	1.24 ± 0.16	3.26 ± 0.44	NC	3.87 ± 0.42	3.41 ± 0.37	3.76 ± 0.42

Biogeography of protists

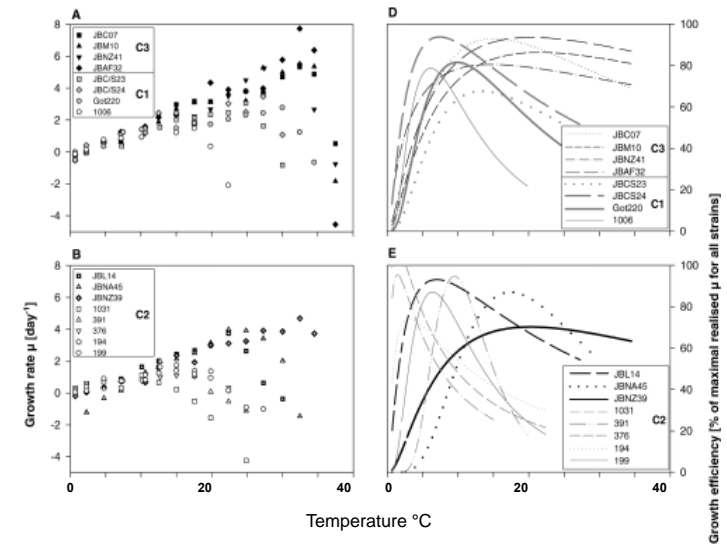
Biogeography

- Is there a geographic restriction to protistan taxa?
- Can protists be found in any given habitat as a function of habitat properties only and not of historical factors?
- Is there a ubiquitous seed bank?

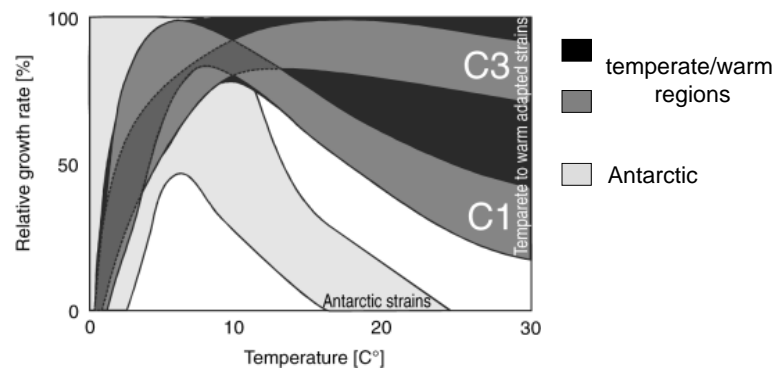
If YES > Geographic barriers do not exist and cannot contribute to evolutionary diversification! Thus, endemic protists do not exist!

TEST – no geographic barriers > correlation between habitat temperature (selects strains from the seed bank) and temperature tolerance independent from geographic location

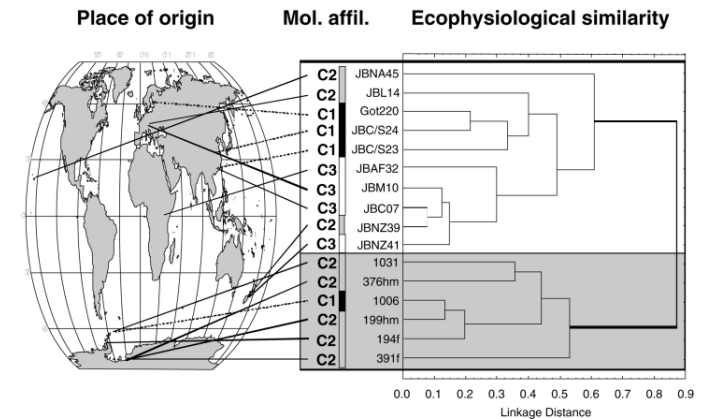
Spumella spp. – Ecophysiology



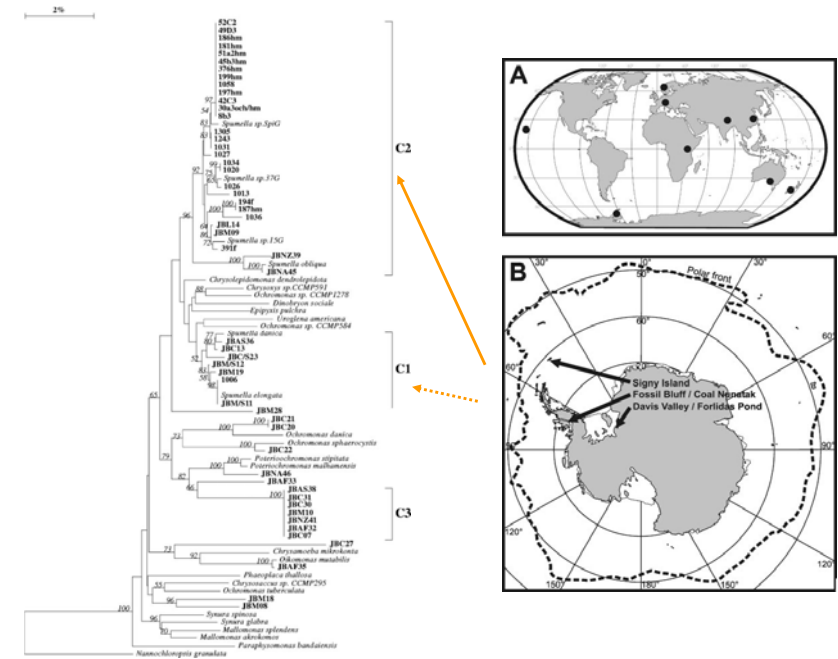
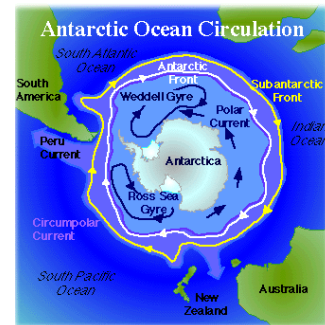
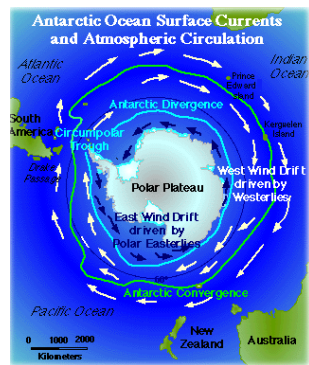
Spumella spp. – Ecophysiology



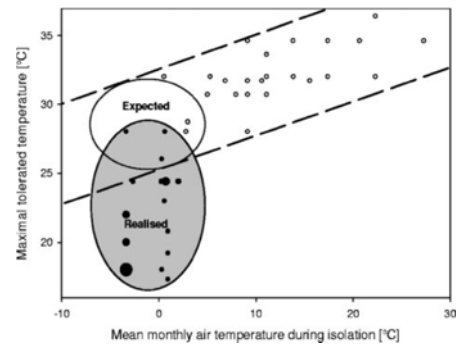
Spumella spp. – Ecophysiology



Antarctica – the place to test biogeography & protist endemism



Spumella spp. – Ecophysiology



- Strains with same morphotype differ considerably with respect to ecophysiology
- Antarctic strains are cold adapted
- Transport to Antarctica is sufficiently restricted to allow the local population to adapt (not only acclimatize) to local conditions
- > “subtle” case of protistan endemism