

**14. Wissenschaftliche Tagung der Sektion Phykologie  
der Deutschen Botanischen Gesellschaft**

26. bis 29. Februar 2012



**&**

**31. Wissenschaftliche Tagung der  
Deutschen Gesellschaft für Protozoologie**

29. Februar bis 03. März 2012



Wuppertal

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## GRUßWORT

Liebe Mitglieder der Sektion Phykologie der Deutschen Botanischen Gesellschaft,  
liebe Mitglieder der Deutschen Gesellschaft für Protozoologie,  
liebe Kolleginnen und Kollegen,

wir freuen uns, Sie zu den Tagungen der beiden Gesellschaften im Bergischen Land begrüßen zu können.

Wie Sie dem Programm entnehmen können, werden beide Tagungen im für die jeweiligen Gesellschaften gewohnten Rhythmus ablaufen. Hier in Wuppertal werden die Phykologen und die Protozoologen das erste Mal an einem gemeinsamen Tagungsort stattfinden. Im Anschluss an die Phykologen tagen die Protozoologen. Beide werden durch einen Workshop am Mittwochnachmittag miteinander verknüpft.

Der erste Teil des Workshops wird sich in einem Vortrag über aktuelle Bemühungen zum BioCode mit einem nicht nur für Taxonomen und Phylogenetiker aller Richtungen bedeutenden Thema befassen. Anschließend gibt es sechs Vorträge zum DNA-Barcoding mit Podiumsdiskussion, in der auch gerne über Ihre Erfahrungen diskutiert werden kann.

Wir hoffen, dass es dadurch über die Fachgesellschaften hinaus zu inspirierenden und ergebnisreichen Begegnungen mit zahlreichen neuen wissenschaftlichen Erkenntnissen kommt!

Gela Preisfeld

Präsidentin der Deutschen Gesellschaft für Protozoologie e.V.

## TAGUNGORT

CVJM-Bildungsstätte  
Bundeshöhe

Bundeshöhe 7  
42285 Wuppertal

## TAGUNGSORGANISATION

Prof'in Dr. Gela Preisfeld  
Dr. Monika Steinhof  
Anna Kaminska  
Lehrstuhl Zoologie & Biologiedidaktik  
Bergische Universität  
Gaußstr. 20  
42119 Wuppertal



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Deutsche Gesellschaft zur Förderung  
des wissenschaftlichen Nachwuchses  
in der Protozoologie



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# 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

**Sonntag, 26.2.2012**

## ANREISE

ab 13.00      Registrierung  
Anbringen der Poster im Kaminzimmer

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## ERÖFFNUNG DER TAGUNG

15.00-15.20      Begrüßung durch den 1. Sprecher der Sektion Phykologie:  
**Prof. Dr. Peter Kroth**  
Begrüßung durch die Gastgeber:  
**Prof'in Dr. Gela Preisfeld**

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## SESSION I: Physiology of Algae      CHAIR: Gela Preisfeld

15.20-15.40      **Claudia Büchel**, Frankfurt  
FUNCTIONAL DIFFERENT FUcoxANTHIN CHLOROPHYLL PROTEINS IN DIATOMS

15.40-16.00      **Frederik Barka**, Frankfurt  
KNOCK DOWN OF A PUTATIVE TRIACYL GLYCEROLE (TAG)-LIPASE LEADS TO LIPID ACCUMULATION IN *PHAEODACTYLUM TRICORNUTUM*

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16.00-16.30      Tee- und Kaffeepause mit Kuchen

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## SESSION II: Algal Biotechnology      CHAIR: Monika Steinhof

16.30-16.50      **Dieter Hanelt**, Hamburg  
IS LIGHT A LIMITING FACTOR IN PHOTOBIOREACTORS?

16.50-17.10      **Opayi Mudimu**, Kiel  
BIOTECHNOLOGICAL SCREENING OF MICROALGAL STRAINS

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## POSTER SESSION I

17.10-18.00      Die verantwortlichen Autoren stehen bei ihrem Poster im Kaminzimmer für Fragen bereit

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## ICEBREAKER

ab 18.30      Get together mit Umtrunk und Stehimbiss

ab 21.00      Die Pinte ist geöffnet für Nachtschwärmer

## 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

Montag, 27.2.2012

8.00-9.00 Frühstück

**SESSION III: Terrestrial and Soil Algae** CHAIR: Regine Jahn

9.00-9.20 **Fabian Faßhauer**, Göttingen  
BIODIVERSITY OF TERRESTRIAL MICROALGAE IN TROPICAL MOUNTAIN  
RAIN FOREST HABITATS IN PODOCARPUS NATIONAL PARK (ECUADOR)

9.20-9.40 **Christine Hallmann**, Göttingen  
TERRESTRIAL GREEN ALGAE UNDER DIFFERENT LAND USES AND  
MANAGEMENTS: A CULTURE-INDEPENDENT APPROACH

9.40-10.00 **Ladislav Hodač**, Göttingen  
PHYLOGENY AND ECOLOGY OF SOIL ALGAE FROM GRASSLANDS AND  
FORESTS IN GERMAN BIODIVERSITY EXPLORATORIES

10.00-10.20 **Dominik J. Patzelt**, Göttingen  
COMPARATIVE ASSESSMENT OF CYANOBACTERIAL DIVERSITY USING  
MOLECULAR METHODS: EXAMPLES FROM THREE DIFFERENT  
GEOGRAPHICAL REGIONS

10.30-11.00 Tee- und Kaffeepause mit Kuchen

**SESSION IV: Algae and Climate Change** CHAIR: Ulf Karsten

11.00-11.20 **Miriam Koblöfsky**, Rostock  
DARK SURVIVAL OF POLAR BENTHIC DIATOMS

11.20-11.40 **Malin Teegen**, Bremerhaven  
COMBINED EFFECTS OF TEMPERATURE AND CO<sub>2</sub> ON GROWTH AND  
PHOTOSYNTHESIS OF TWO MARINE RED ALGAE: *PALMARIA PALMATA* AND  
*CHONDRUS CRISPUS*

11.40-12.00 **Mark Olischläger**, Bremerhaven  
EFFECTS OF OCEAN ACIDIFICATION ON DIFFERENT STAGES IN THE LIFE-  
CYCLE OF THE KELP *LAMINARIA HYPERBOREA*

12.00-12.20 **Sandra Heinrich**, Bremerhaven  
COMPARISON OF GENE EXPRESSION UNDER UV RADIATION AND  
DIFFERENT TEMPERATURES IN *SACCHARINA LATISSIMA* (PHAEOPHYCEAE)  
FROM FIELD AND CULTURE

## 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

12.30-14.00      Mittagspause mit Buffet

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### SESSION V: Cell Biology of Green Algae      CHAIR: Dieter Hanelt

- 14.00-14.20      **Frauke Peschek**, Kiel  
UVB SENSITIVITY OF PHOTOSYNTHESIS AND DNA IN GREEN  
MACROALGAE RELATED TO DEPTH DISTRIBUTION
- 14.20-14.40      **Steffen Storck**, Mainz  
EXPLORING THE FUNCTION OF LOROXANTHIN IN LIGHT-HARVESTING  
COMPLEX II OF GREEN ALGAE
- 14.40-15.00      **Andreas Holzinger**, Innsbruck, Österreich  
COMPARISON OF OSMOTIC POTENTIAL AND PLASMOLYSIS EFFECTS IN  
*KLEBSORMIDIUM* SP. AND *ZYGNEMA* SP. (STREPTOPHYTA)
- 15.00-15.20      **Paul Christian Wieners**, Kiel  
DESICCATION-INDUCED NON-RADIATIVE DISSIPATION PROTECTS GREEN  
LICHEN ALGAE AGAINST PHOTOINHIBITION
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15.30-16.00      Tee- und Kaffeepause mit Kuchen

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### POSTER SESSION II

16.00-18.00      Die verantwortlichen Autoren stehen bei ihrem Poster im  
Kaminzimmer für Fragen bereit

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18.30-19.30      Abendessen

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19.30              Mitgliederversammlung der Sektion

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ab 21.00         Die Pinte ist geöffnet



# 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

Dienstag, 28.2.2012

8.00-9.00 Frühstück

**SESSION VI: Cell Biology of *Chlamydomonas* CHAIR: Peter Kroth**

- 9.00-9.20 **Maria Mittag**, Jena  
CHARACTERIZATION OF AN ANIMAL-LIKE CRYPTOCHROME IN *CHLAMYDOMONAS REINHARDTII*
- 9.20-9.40 **Georg Kreimer**, Berlin  
PHOTOTROPIN, A NOVEL PLAYER IN EYESPOT DEVELOPMENT AND CONTROL OF PHOTOTAXIS IN *CHLAMYDOMONAS REINHARDTII*
- 9.40-10.00 **Burkhard Becker**, Köln  
THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII*: MODULATION OF THE CV ACTIVITY ALLOWS CELLS TO ADAPT TO A WIDE RANGE OF OSMOTIC CONDITIONS
- 10.00-10.20 **Karin Komsic-Buchmann**, Köln  
THE SEC6 PROTEIN IS REQUIRED FOR FUNCTION OF THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII*

10.30-11.00 Tee- und Kaffeepause mit Gebäck

**SESSION VII: Evolution of Algae CHAIR: Burkhard Becker**

- 11.00-11.20 **Tatyana Darienko**, Kiew, Ukraine  
THE GENUS *JAAGICHLORELLA* REISIGL (TREBOUXIOPHYCEAE, CHLOROPHYTA) AND ITS CLOSE RELATIVES: AN EVOLUTIONARY PUZZLE
- 11.20-11.40 **Maria Schmidt**, Leipzig  
BIODIVERSITY AND EVOLUTION OF SYNCHROMOPHYCEAE AND RELATED AMOEBOID HETEROKONT PROTISTS
- 11.40-12.00 **Lenka Caisová**, Köln  
DO CBCs OF ITS2 CORRESPOND TO THE SPECIES LEVEL? A CASE STUDY OF THE ITS2 EVOLUTION IN THE ULVALES
- 12.00-12.20 **Klaus Valentin**, Bremerhaven  
GENOME EVOLUTION IN EUCARYOTES INFERRED FROM THE WHOLE GENOME SEQUENCE OF *CHONDRUS CRISPUS* (RHODOPHYTA)

## 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

12.30-14.00      Mittagspause mit Buffet

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### SESSION VIII: Ecology - Primary Production and Nutrients      CHAIR: Kerstin Hoef-Emden

14.00-14.20      **Jana Wölfel**, Rostock  
ARCTIC MICROPHYTOBENTHOS PRIMARY PRODUCTION IN KONGSFJORDEN (SVALBARD, NORWAY) COULD GAIN FROM GLOBAL WARMING - IN SITU MEASUREMENTS AND MODELLED CHANGES

14.20-14.40      **Susanne Dunker**, Leipzig  
INTERSPECIFIC EFFECTS ON GROWTH AND PRIMARY PRODUCTION IN MIXED CULTURE OF THE GREEN ALGA *OOCYSTIS MARSONII* AND THE CYANOBACTERIUM *MICROCYSTIS AERUGINOSA*

14.40-15.00      **Carolin Paul**, Rostock  
NUTRIENTS AND PHYTOPLANKTON IN MESOKOSMS

15.00-15.20      **Julia Rottberger**, Konstanz  
NUTRITIONAL REQUIREMENTS AND METABOLIC CHARACTERISTICS OF HETEROTROPHIC, MIXOTROPHIC AND AUTOTROPHIC FRESHWATER CHRYSOPHYTES

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15.30-16.00      Tee- und Kaffeepause mit Kuchen

***Deadline zur Abgabe der Stimmzettel für Posterpreise***

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### EXKURSION

15.30-17.30      Fahrt mit der Wuppertaler Schwebbahn inkl. Stadtführung

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### TAGUNGSDINNER

ab 19.00      **Geselliger Abend mit Verleihung der Posterpreise**

# 14. WISSENSCHAFTLICHE TAGUNG DER SEKTION PHYKOLOGIE

## Mittwoch, 29.2.2012

8.00-9.00 Frühstück

### SESSION IX: Ecology of Algae CHAIR: Claudia Büchel

9.00-9.20 **Magdalena Mayr**, Wien, Österreich  
FIGHTING PLANKTIC ALGAE WITH BENTHIC ALGAE: A PILOT STUDY AT THE HEUSTADELWASSER IN VIENNA

9.20-9.40 **Anne Jungandreas**, Leipzig  
THE ADAPTATION OF THE DIATOM *P. TRICORNUTUM* TO RED AND BLUE LIGHT CONDITIONS

9.40-10.00 **Kristin Sauer**, Göttingen  
ALGAL PIRATES - SEARCHING FOR OPTIMAL ANTIFOULING TEST SPECIES

10.00-10.20 **Lothar Krienitz**, Stechlin  
ALGAL FOOD OF LESSER FLAMINGOS

10.30-11.00 Tee- und Kaffeepause mit Gebäck

### SESSION X: Phylogeny and Cell Biology CHAIR: Stefan Pärschke

11.00-11.20 **Markus R. Gruber**, Wien, Österreich  
PHYLOGENY, MORPHOLOGY AND PHYSIOLOGY: COMPARISON OF *BOTRYOCOCCUS BRAUNII* ISOLATES

11.20-11.40 **Martin Lohr**, Mainz  
CHARACTERIZATION OF PROTEINS OF THE EXTENDED VIOLAXANTHIN DE-EPOXIDASE (VDE) FAMILY FROM THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

11.40-12.00 **Carolina Río Bártulos**, Konstanz  
MITOCHONDRIA ISOLATION FROM DIATOMS

12.00-12.20 **Thomas Leya**, Potsdam-Golm  
FUNKTIONELLE UND MOLEKULARE CHARAKTERISIERUNG EINIGER EISSTRUKTURIERENDER PROTEINE (ISP) AUS PSYCHROPHILEN SCHNEEALGEN

12.30-14.00 Mittagspause mit Buffet

**FAREWELL - Abreise der Teilnehmer der Sektion Phykologie oder Teilnahme am Barcoding Workshop**

**Mittwoch, 29.2.2012**

**ANREISE DER TEILNEHMER DER DGP**

ab 13.00            Registrierung  
                         Anbringen der Poster im Kaminzimmer

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**KEYNOTE LECTURE                    CHAIR: Gela Preisfeld**

14.15-14.45        **Regine Jahn**, Berlin  
                         NOMENKLATUR IM GRENZBEREICH VON PHYKOLOGIE UND  
                         PROTOZOLOGIE: MARGESCHNEIDERTE CODES ODER EIN UNIVERSELLER  
                         BIOCODE?

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**BARCODING WORKSHOP                CHAIR: Birgit Gemeinholzer**

14.45-15.45        **Christina Bock**, Essen  
                         ASSESSMENT OF SPECIES DIVERSITY WITHIN THE CHLORELLACEAE USING  
                         MORPHOLOGICAL AND MOLECULAR DATA (BARCODES)

**Thomas Friedl**, Göttingen  
ASSESSING DISTRIBUTION PATTERNS IN TERRESTRIAL ALGAE FROM  
VARIOUS HABITATS: A DNA-BARCODING APPROACH

**Kerstin-Hoef-Emden**, Köln  
PITFALLS OF DNA BARCODING METHODS: THE CRYPTOPHYCEAE AS A  
TEST CASE

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15.45-16.15        Tee- und Kaffeepause mit Kuchen

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16.15-17.15        **Thomas Pröschold**, Rostock  
                         ITS-2 AS UNIVERSAL DNA BARCODE MARKER FOR PROTISTS  
**Thorsten Stoeck**, Kaiserslautern  
                         ASSESSING THE HYPERVARIABLE D1-D2 REGION OF THE LSU rDNA FOR  
                         CILIATE BARCODING

**Jonas Zimmermann**, Berlin  
DIATOM DNA BARCODING REVISITED

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17.15-18.00        Podiumsdiskussion

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**ICEBREAKER**

ab 18.30            Get together mit Umtrunk und Stehimbiss

ab 21.00            Die Pinte ist geöffnet für Nachtschwärmer

**Donnerstag, 1.3.2012**

8.00-8.45 Frühstück

**ERÖFFNUNG DER TAGUNG**

8.45-9.00 Begrüßung durch die Präsidentin der Gesellschaft:  
**Prof'in Dr. Gela Preisfeld**  
Grußworte

**KEYNOTE LECTURE CHAIR: Gela Preisfeld**

9.00-9.30 **Julia Walochnik**, Wien, Österreich  
AMOEBIA GENOMICS – FREE-LIVING VERSUS PARASITIC TRAITS

**SESSION I: Aspects of Ecology I CHAIR: Thomas Weisse**

9.30-9.50 **Wolf-Henning Kusber**, Berlin  
WAS BEDEUTET DAS GBIF-NETZWERK FÜR DIE BIODIVERSITÄTS-  
FORSCHUNG IN DER ALGENKUNDE UND PROTOZOLOGIE?

9.50-10.10 **Bettina Sonntag**, Mondsee, Österreich  
DO CILIATES SUFFER FROM SUNBURN?

10.10-10.30 **Martin Schlegel**, Leipzig  
MOLEKULARE UND MORPHOLOGISCHE ANALYSE RÄUMLICHER UND  
ZEITLICHER BIODIVERSITÄTSMUSTER VON CILIATEN IN  
PFLANZENKLÄRANLAGEN

10.30-11.00 Tee- und Kaffeepause mit Gebäck

11.00-11.20 **Stefan Geisen**, Köln  
IDENTIFICATION, FUNCTIONAL ROLES AND ECOSYSTEM SERVICES  
OF PROTOZOA IN SOIL

11.20-11.40 **Sebastian Dirren**, Zürich, Schweiz  
NUCLEARIA SP. AUS DEM ZÜRICHSEE MIT LOS SYMBIONTOS LIVE IN  
CONCERT

**POSTER PRESENTATION I**

11.40-12.30 Präsentation der im Kaminzimmer ausgestellten Poster in  
Kurzvorträgen im Saal (1-10)

12.30-13.30 Mittagspause mit Buffet

**POSTER SESSION I**

13.30-14.00 Die verantwortlichen Autoren stehen bei ihrem Poster im Kaminzimmer für Fragen bereit

**SESSION II: Cell Biology I**

**CHAIR: Thorsten Stoeck**

14.00-14.20 **Helmut Plattner**, Konstanz  
CALCIUM SIGNALING IN CLOSELY RELATED PROTOZOAN GROUPS (ALVEOLATA): NON-PARASITIC CILIATES (*PARAMECIUM*, *TETRAHYMENA*) VS. PARASITIC APICOMPLEXA (*PLASMODIUM*, *TOXOPLASMA*)

14.20-14.40 **Karin Komsic-Buchmann**, Köln  
THE SEC6 PROTEIN IS REQUIRED FOR FUNCTION OF THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII*

14.40-15.00 **Ulrike Günzler**, Kaiserslautern  
ANALYSIS OF ENDOGENOUS REGULATORY SHORT RNAs INVOLVED IN THE MUTUAL EXCLUSIVE GENE EXPRESSION

15.00-15.20 **Miriam Cheaib**, Kaiserslautern  
RNAi-REGULATED CHROMATIN-MODIFICATIONS ENABLE MUTUAL EXCLUSIVE TRANSCRIPTION

15.30-16.00 Tee- und Kaffeepause mit Kuchen

**POSTER PRESENTATION II**

16.00-17.00 Präsentation der im Kaminzimmer ausgestellten Poster in Kurzvorträgen im Saal (11-22)

17.00-18.30 Mitgliederversammlung der DGP

anschließend Versammlung der DGPF (ggf. Fortsetzung nach Abendessen)

ab 18.30 Abendessen

**Freitag, 2.3.2012**

8.00-9.00 Frühstück

**SESSION III: Cell Biology II CHAIR: Thomas Posch**

9.00-9.20 **Atef Omar**, Salzburg, Österreich  
 ONTOGENESIS OF *LEPTOPHARYNX COSTATUS COSTATUS* (CILIOPHORA, MICROTHORACIDA) AND ITS PHYLOGENETIC SIGNIFICANCE

9.20-9.40 **Michael Schweikert**, Stuttgart  
 ENDOCYTOBIOSIS IN DINOFLAGELLATES

9.40-10.00 **Helmut Plattner**, Konstanz  
 MICRODOMAIN FORMING STOMATIN PROTEIN SUPERFAMILY IN THE CILIATED PROTOZOAN, *PARAMECIUM TETRAURELIA* – MOLECULAR STRUCTURE, LOCALIZATION AND FUNCTION

10.00-10.20 **Martin Simon**, Kaiserslautern  
 EVOLUTION OF THE SURFACE ANTIGEN MULTIGENE-FAMILY IN *PARAMECIUM TETRAURELIA*

10.30-11.00 Tee- und Kaffeepause mit Gebäck

**SESSION IV: News on Phylogeny and Taxonomy I CHAIR: Bettina Sonntag**

11.00-11.20 **Sabine Agatha**, Salzburg, Österreich  
 MORPHOLOGIC DATA UNRAVEL THE PARAPHYLY OF THE TINTINNID GENUS *FAVELLA* IN SSU rRNA TREES (CILIOPHORA, SPIROTRICHEA, TINTINNINA)

11.20-11.40 **Alexander Kudryavtsev**, Genf, Schweiz  
 PHYLOGENETIC RELATIONSHIPS OF THE HIMATISMENIDA AND TAXONOMY OF AMOEBOZOA

11.40-12.00 **Willhelm Foissner**, Salzburg, Österreich  
 REDISCOVERY OF *PARAMECIUM CHLORELLIGERUM* KAHL, 1935, A SECOND ZOOCHLORELLAE-BEARING *PARAMECIUM* SPECIES BELONGING TO THE *P. NEPHRIDIAM* CLADE

12.00-12.20 **Anna Maria Fiore-Donno**, Greifswald  
 MYXOMYCETES (AMOEBOZOA) PHYLOGENIES

12.30-13.30      Mittagspause mit Buffet

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**Poster Session II**

13.30-14.00      Die verantwortlichen Autoren stehen bei ihrem Poster im  
Kaminzimmer für Fragen bereit

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**SESSION V: Molecular Biodiversity      CHAIR: Klaus Eisler**

- 14.00-14.20      **Micah Dunthorn**, Kaiserslautern  
A MARINE ENVIRONMENTAL PYROSEQUENCING VIEW OF TRADITIONALLY  
TERRESTRIAL AND FRESHWATER COLPODEAN CILIATES
- 14.20-14.40      **Sabine Filker**, Kaiserslautern  
ENVIRONMENTAL SELECTION OF PROTISTAN PLANKTON COMMUNITIES IN  
HYPERSALINE ANOXIC DEEP-SEA BASINS, EASTERN MEDITERRANEAN SEA
- 14.40-15.00      **Anna Gimmler**, Kaiserslautern  
MOLEKULARE MARKERGENE ZUR UNTERSUCHUNG BIOGEOGRAPHISCHER  
MUSTER VON DIATOMEEN AM BEISPIEL VON *PINNULARIA VIRIDIS*  
(BACILLARIOPHYCEAE)
- 15.00-15.20      **Alexandra Stock**, Kaiserslautern  
NOVEL ACTIVE KINETOPLASTIDS ASSOCIATED WITH HYPERSALINE ANOXIC  
BASINS IN THE EASTERN MEDITERRANEAN DEEP-SEA
- 

15.30-16.00      Tee- und Kaffeepause mit Kuchen

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**SESSION VI: Aspects of Ecology II**    **CHAIR: Renate Radek**

- 16.00-16.20    **Bettina Eugster**, Zürich  
*OBERTRUMIA AUREA* (CILIOPHORA) AND ITS TOXIC FOOD, THE  
FILAMENTOUS CYANOBACTERIUM *PLANKTOTHRIX RUBESCENS*
- 16.20-16.40    **Barbara Kammerlander**, Innsbruck  
CILIAE COMMUNITY COMPOSITION IN TWO LAKES OF DIFFERENT  
TURBIDITY RESULTING FROM GLACIER RETREAT
- 16.40-17.00    **Stefanie Moorthi**, Wilhelmshaven  
INTERACTIVE EFFECTS OF DISSOLVED NUTRIENTS AND PREY ON  
POTENTIALLY HARMFUL MIXOTROPHIC DINOFLAGELLATES

***Deadline zur Abgabe der Stimmzettel für Posterpreise***

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**TAGUNGSDINNER**

- ab 19.00    **Geselliger Abend mit Verleihung der Posterpreise**

**Samstag, 3.3.2012**

8.00-9.00 Frühstück

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**SESSION VII: News on Phylogeny and Taxonomy II CHAIR: Anja Scherwaß**

9.00-9.20 **Thomas Pröschold**, Rostock  
THE SYSTEMATICS OF "ZOOCHLORELLA" REVISITED EMPLOYING AN INTEGRATIVE APPROACH

9.20-9.40 **Jie Huang**, Kaiserslautern  
EXPANDING CHARACTER SAMPLING FOR THE MOLECULAR PHYLOGENY OF EUPLOTID CILIATES (PROTOZOA, CILIOPHORA) USING THREE MARKERS, WITH A FOCUS ON THE FAMILY URONYCHIIDAE

9.40-10.00 **Frank Nitsche**, Köln  
CRYPTIC DIVERSITY WITHIN THE CHOANOFLAGELLATE MORPHOSPECIES COMPLEX *CODOSIGA BOTRYTIS* – PHYLOGENY AND MORPHOLOGY OF ANCIENT AND MODERN ISOLATES

10.00-10.30 **Sebastian Hess**, Köln  
SHEDDING LIGHT ON VAMPIRES:  
THE PHYLOGENY OF VAMPYRELLID AMOEBAE REVISITED

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10.30-11.00 Tee-und Kaffeepause mit Gebäck

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**SESSION VIII: Morphology and Phylogeny CHAIR: Hans-Werner Breiner**

11.00-11.20 **Renate Radek**, Berlin  
OXYMONADEN IN DER GÄRKAMMER NIEDERER TERMITEN

11.20-11.40 **Jürgen Strassert**, Marburg  
'*CANDIDATUS ANCILLULA TRICHONYMPHAE*', A NOVEL LINEAGE OF ENDOSYMBIOTIC ACTINOBACTERIA IN TERMITE GUT FLAGELLATES

11.40-12.00 **Stefan Pärschke**, Wuppertal  
UNRAVELLING THE RDNA OPERON OF EUGLENOZOAN FLAGELLATES

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12.30-13.30 Mittagspause mit Buffet

**FAREWELL - Abreise der Teilnehmer**







**ABSTRACTS**

**TALKS AND POSTERS**

In alphabetical order by first author



## **KNOCK DOWN OF A PUTATIVE TRIACYL GLYCEROLE (TAG)-LIPASE LEADS TO LIPID ACCUMULATION IN *PHAEODACTYLUM TRICORNUTUM***

Frederik Barka, W. Lorenzen, H. Bode and C. Büchel,  
*Institute of Molecular Biosciences, Goethe Universität Frankfurt am Main*

Today, microalgae are in focus due to their capacity to accumulate high amounts of storage lipids as an energy reservoir. They are believed to be the most promising future feedstock for biofuel production and furthermore several algal species possess very high amounts of polyunsaturated fatty acids (PUFA) that play an important role in human nutrition and health. The diatom *Phaeodactylum tricornutum* produces high amounts of the PUFA eicosapentaenoic acid (EPA) that is an antiinflammatory agent and prevents atherosclerosis as well as various carcinomas.

Algal cells store lipids in the chemical form of triacyl glycerol (TAG). In times of demand TAG is activated and subsequently catabolized into glycerol and free fatty acids which are further utilized to produce chemical energy after  $\beta$ -oxidation. The initial step of this breakdown of storage TAGs is catalyzed by the enzyme class of TAG-Lipases that have been characterized in model organisms like *A. thaliana* and *S. cerevisiae*.

Despite the great interest in algal lipids, information about these key enzymes of lipid metabolism is still sparse for microalgae. By sequence homology we could identify a potential TAG-Lipase gene in *Phaeodactylum* (*put\_lip*). The expression of an antisense RNA complementary to the *put\_lip* mRNA results in a strong accumulation of TAG in the mutant. In addition to higher lipid content, the mutant also exhibits an altered fatty acid composition compared to the wild type. Our study identifies *put\_lip* as an essential gene of the lipid metabolism in *Phaeodactylum* and demonstrates a possible way to increase the lipid content in microalgae by genetic engineering.

## **THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII*: MODULATION OF THE CV ACTIVITY ALLOWS CELLS TO ADAPT TO A WIDE RANGE OF OSMOTIC CONDITIONS**

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Cells of *Chlamydomonas reinhardtii* contain two contractile vacuoles (CV) involved in osmoregulation. At the end of diastole the contractile vacuole of *Chlamydomonas* has a spherical shape, expels the liquid into the medium and the CV fragments into smaller vacuoles (systole). During diastole these smaller vacuoles swell and fuse with each other to form again the spherical shaped vacuole at the end of a cycle. Modulation of the CV activity allows *Chlamydomonas* to adapt to changes in the



osmolarity of the environment. We have investigated these changes and the role of aquaporins in CV function, using an MIP1-GFP fusion protein and quantitative PCR. We now show that modulation of the CV activity involves changes in the contraction interval of the CV, the CV size and the CV membrane permeability.

### FUNCTIONAL DIFFERENT FUcoxANTHIN CHLOROPHYLL PROTEINS IN DIATOMS

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Diatoms harvest light with intrinsic antenna proteins, so-called fucoxanthin chlorophyll proteins. These FCPs functionally fall into three groups, the main light harvesting proteins (Lhcf), the photosystem I associated polypeptides (Lhcr) and a group which is involved in photoprotection (Lhcx). Whilst the three major groups of FCPs are common to all diatoms known so far, the Lhcf proteins between pennate and centric diatoms differ. In the first part of the talk we will report on subpopulations of the main FCP complexes from *Phaeodactylum tricornutum* and compare it to the FCP complexes of *Cyclotella meneghiniana*. In contrast to *P. tricornutum*, where the main trimeric FCP complexes consist of Lhcf proteins only, some of the trimeric complexes in centric diatoms are composed of Lhcf and Lhcx. The mechanism how the Lhcx polypeptides are involved in the process of non-photochemical quenching is still not known in detail. In the second part of the talk we will report on *in-vitro* studies demonstrating the effect of protein interaction, diatoxanthin and pH on these trimeric FCPs of *C. meneghiniana*, demonstrating their involvement in non-photochemical quenching.



## DO CBCs OF ITS2 CORRESPOND TO THE SPECIES LEVEL? A CASE STUDY OF THE ITS2 EVOLUTION IN THE ULVALES

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The Second Internal Transcribed Spacer (ITS2) is a relatively short but variable fragment of the nuclear-encoded rDNA operon, located between 5.8S and 28S RNA genes. Based on crossing experiments in the volvocalean algae, it has been proposed that the presence of already one Compensatory Base Change (CBC) in the conserved regions of two ITS2 helices between two taxa correlates with their sexual incompatibility. Since this time, the ITS2 marker has become a widely used tool to delimit putative boundaries of biological species, especially in taxa that are morphologically almost indistinguishable or in which sexual reproduction is unknown. However, no comprehensive study of ITS2 evolution has been performed in the green algal order Ulvales. This situation inspired us to focus in detail on comparative investigations of ITS2 sequences covering five families of this order. We propose a general consensus secondary structure and introduce a new universal numbering system of ITS2 nucleotides. To obtain more insight into ITS2 evolution, a detailed comparative analysis of all substitutions has been done. By plotting CBCs on the phylogenetic tree, it has been revealed that CBCs in the ITS2 of the Ulvales do not correspond with the species level. Furthermore, it has been demonstrated that most CBC 'clades' sensu Coleman are paraphyletic. Our current investigations of ITS2 sequence evolution in four orders of the Chlorophyceae, have confirmed these conclusions and thus question the use of CBCs in ITS2 for species delimitation in green algae which appears to have been often based on ill-defined secondary structures.





## THE GENUS *JAAGICHLORELLA* REISIGL (TREBOUXIOPHYCEAE, CHLOROPHYTA) AND ITS CLOSE RELATIVES: AN EVOLUTIONARY PUZZLE

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The genus *Chlorella* (in traditional sense) is polyphyletic and belongs to at least twelve independent lineages of the Trebouxiophyceae and Chlorophyceae. Most of the aquatic species belong to the genera *Chlorella* and *Parachlorella* (within the so-called *Chlorella*-lineage of the Trebouxiophyceae), or to *Scenedesmus* and *Mychonastes* (within the DO-group of the Chlorophyceae) according to phylogenetic analyses of the SSU and ITS rDNA sequences. In contrast to the aquatic species, the terrestrial strains investigated so far form a monophyletic lineage (*Watanabea*-clade) within the Trebouxiia-lineage of the Trebouxiophyceae. To the *Watanabea*-clade belong several genera with *Chlorella*-like morphology (*Chloroidium*, *Heterochlorella*, *Watanabea*, *Kalinella*, and *Viridiella*).

We studied 22 strains isolated from soil, bark, and artificial hard substrates, which have been traditionally identified as *Chlorella luteoviridis*. To clarify the taxonomical status and intrageneric diversity of this group, we used an integrated approach (molecular phylogeny of SSU and ITS rDNA sequences, secondary structures, DNA barcoding, morphology, and polyol production) including the ecological distribution and ecophysiological properties, which could provide survival strategies and successful development in extreme biotops. All strains investigated produce ribitol as osmolytic active substance, and showed a low phenotypic plasticity, but a surprisingly high genetic diversity, which could be only resolved in complex evolutionary models based on secondary structures. Based on these results, we re-established the genus *Jaagichlorella*, and proposed new species of *Jaagichlorella*, *Kalinella*, and *Watanabea*.



**INTERSPECIFIC EFFECTS ON GROWTH AND PRIMARY PRODUCTION IN MIXED CULTURE OF THE GREEN ALGA *OOCYSTIS MARSONII* AND THE CYANOBACTERIUM *MICROCYSTIS AERUGINOSA***

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A classical approach to study the influence of allelopathic effects is cross-culturing of algae with cell-free filtrates of emitter organism. But two main disadvantages of this approach are: 1.) The decreasing ratio of allelopathic compounds per cell during the growth experiment. Therefore, this method does not mimic the natural situation where the cells are exposed to constant or increasing concentrations of allelochemicals. 2.) The ecological benefit for emitter-organism cannot be understood in detail because only growth of target organism is considered. For this reasons we compared growth in mono-algal with bi-algal culture. To exclude nutrient and light limitation which could lead to a competitive advantage of one organism we used natural Chl a-concentration. Flow cytometry and single cell analysing techniques allowed to get a closer look to growth performance of both organism. Interestingly the study resulted in the surprising finding that the emitter organism *Microcystis aeruginosa* (cyanobacteria) not only suppressed the target organism *Oocystis marsonii* (green alga) but also grew better compared to the mono-algal control after several days of co-cultivation. The macromolecular composition of *M. aeruginosa* in bi-algal culture shifted to a high protein to carbohydrate ratio, which was also detectable under mixotrophic growth condition. Therefore it is concluded that the emitter organism of allelopathic compounds could profite twofold: 1) by the inhibition of growth of competitive species and 2) a better growth performance with released organic compounds of the inhibited organism.

**BIODIVERSITY OF TERRESTRIAL MICROALGAE IN TROPICAL MOUNTAIN RAIN FOREST HABITATS IN PODOCARPUS NATIONAL PARK (ECUADOR)**

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Tropical mountain rain forests are one of the most important but also most endangered biodiversity hotspots worldwide. In this ongoing project, the diversity of green algae from various terrestrial habitats of the Podocarpus National Park in Ecuador is investigated. Goals of the project are 1) to test whether tropical mountain rain forests are also hotspots for the diversity of terrestrial green algae, 2) to



investigate whether the terrestrial green algae in the tropical mountain rain forest are different from comparable habitats of temperate regions, 3) to test whether terrestrial green algal diversity changes along an altitudinal gradient, and 4) to develop novel isolates of terrestrial green algae.

Samples of epiphytic crusts and soils were collected at 1000m a.s.l. (Bombuscaro), 2000m a.s.l. (SanFrancisco Valley) and 3000m a.s.l. (Cajanuma). The DNA was extracted directly from the samples of tree bark, leaves and soil followed by PCR using green algae specific primers, cloning and sequencing of an rDNA stretch that reaches from the 3'-end of 18S to the ITS2 region using two reactions. The ITS2 is used as DNA barcode while the 18S allows a phylogenetic assessment.

23 green algal phylotypes at the generic level from three classes of Chlorophyta were identified so far. Except two phylotypes (*Phyllosiphon* and *Heveochlorella*), all were already known from temperate regions. No dependence of biodiversity from altitude was found so far. Using ITS2 several new groups within *Apatococcus*, *Heveochlorella* and the Scenedesmaceae were identified, but also some already known species from temperate regions.

#### PHYLOGENY, MORPHOLOGY AND PHYSIOLOGY: COMPARISON OF *BOTRYOCOCCUS BRAUNII* ISOLATES

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*B. braunii* is a colonial green algae within the Trebouxiophyceae and well known for its ability to produce high amounts of long-chained hydrocarbons. Up to date, scientists have mainly focused on physiological and genetical aspects of this species to explore its potential as renewable energy source. We followed a more comprehensive approach and compared the phylogeny (SSU rDNA, ITS-1, 5.8S rDNA and ITS-2), morphology and hydrocarbons (GC/MS) of seven different *B. braunii* isolates. Phylogenetic analyses revealed that the examined *Botryococcus*-strains belong to four sub-clades within a monophyletic lineage of the Trebouxiophyceae; the hydrocarbon analyses propose once more the presence of three chemical races and considerable variation within the B-race. Morphological analyses disclosed a significant positive correlation among length-width ratios, stratified mucilage and phylogeny based on ITS2 sequences (Mantel-test,  $r=0.56$ ,  $p=0.04$ ). Our results suggest that the investigated *Botryococcus* strains represent several species, however, this need further studies.



## TERRESTRIAL GREEN ALGAE UNDER DIFFERENT LAND USES AND MANagements: A CULTURE-INDEPENDENT APPROACH

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Algal communities, present in large amounts in the top few centimeters of soil, may be highly influenced by environmental factors. Therefore, we aim at exploring the genetic diversity of green algae and their variation with respect to various soil types, different land use (grassland and forest) and management intensities. Using green algae-preferring PCR primers, 18S rDNA clone libraries were established from jointly sampled soil cores of 57 defined research plots within the three so-called German Biodiversity Exploratories ([www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de)). To identify the recovered algal clones, their sequences were grouped into OTUs (Operational Taxonomic Units), next closest relatives determined by comparisons with already available sequences and phylogenetically analyzed. Grassland soils were found more OTU rich than forest soils which may be explained by differences in pH. In forest soils Trebouxiophyceae (37 OTUs) was the predominant group whereas it was the Chlorophyceae (31 OTUs) in grassland soils. Certain gradients in the algal communities of both vegetation types were revealed by multivariate statistics, but could be explained only for the Schwäbische Alb Exploratory by management intensity. With respect to differences between the three Exploratories, grassland soil algal communities of the Hainich and Schwäbische Alb Exploratories were rather similar to each other while the Schorfheide Exploratory differed significantly. In addition, tree bark algal communities of the Schwäbische Alb Exploratory were investigated. There only members of Trebouxiophyceae were found, the number of OTUs (13) was reduced compared to soils. All tree bark OTUs were also found in soils, but less frequent there.

## IS LIGHT A LIMITING FACTOR IN PHOTOBIOREACTORS?

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In nature the organisms are well adapted to their respective ecosystem, whereas a closed bioreactor system is a quite artificial system with usually not very favorable conditions for growth. For high biomass production natural sunlight is needed in high quantities, however, much less than 10% of the sun radiation is used for C-fixation, so that light becomes a limitation factor in a dense algal cultures. However, the large proportion of radiation not used for electron transport can provoke temperature stress to the algae, which caused a decrease of the production rates. The talk will



present first results from outdoor bioreactor culture and will focus on algal photosynthetic performance. The different reactions of photosystem II and I and its acclimation to light limitation as well as light stress will be discussed. Results show that with high algal density photoinhibition does not occur as light is not more saturating but temperature stress occurs, whereas under low density it is vice versa. In winter season irradiances to perform photosynthesis with optimal rates is even high enough in North Europe, however, the length of day light is too short to compensate C-loss by dark respiration during night periods. Additional irradiation by LED-lamps during winter season did not solve this problem.

### COMPARISON OF GENE EXPRESSION UNDER UV RADIATION AND DIFFERENT TEMPERATURES IN *SACCHARINA LATISSIMA* (PHEOPHYCEAE) FROM FIELD AND CULTURE

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Macroalgae of the order Laminariales (kelps) are important marine coastal primary producers and also a valuable source of food, biochemical and pharmaceutical compounds. Geographical distribution as well as the determination of vertical patterns of kelps is constrained by abiotic factors such as light, including UV and temperature. Therefore global environmental changes, e.g. global warming, might have a negative impact on the performance and survival of kelp species.

Comparative studies of stress acclimation to in laboratory and field grown are rare, and it is still unknown, in what extend results from laboratory can be used to predict environmental effects in the field. In order to study gene expression in *Saccharina latissima* from field and culture under UV radiation and temperature stress a cDNA microarray approach was applied.

Large differences in the number of regulated genes were observed, furthermore simultaneous regulated transcripts in culture and field sporophytes showed differences in the level of expression fold change in response to similar stress conditions. A higher sensitivity to UVR and a higher oxidative stress level at 12°C was observed in field compared to culture sporophytes, culture plants on the contrary must make stronger efforts of acclimating to UVR at 2°C than field plants. Our results demonstrate the influence of growth conditions on the acclimation to stress on the transcriptional level, and underscore the importance of conducting experiments with field material, when predicting biological and environmental effects, of changing abiotic factors in the field.



## PHYLOGENY AND ECOLOGY OF SOIL ALGAE FROM GRASSLANDS AND FORESTS IN GERMAN BIODIVERSITY EXPLORATORIES

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The composition of algal communities from grassland and forest soils has been investigated using a culture-based approach. In order to link changes in algal diversity and the landscape management intensity, correlations with relevant physico-chemical parameters of soils were taken into account. Soil core samples were obtained from 57 research plots within three areas in Germany, the so called Biodiversity Exploratories. For taxa identification microscopy and 18S/ITS2-rDNA sequencing of isolates have been used. A total of 56 genera of eukaryotic algae were identified with the majority of them from the Chlorophyta, i.e. Trebouxiophyceae and Chlorophyceae. Several of the new green algal isolates were phylogenetically and morphologically close to genera recently described only from tropical regions. Several other isolates have not become available from culture collections so far. Among all three Exploratories, there was a general pattern of the differences between forest and grassland algal communities. The algal communities in grassland soils exhibited larger species richness than those in forests. This was combined with a more frequent occurrence of filamentous Xanthophyceae, pennate diatoms and nostocalean cyanobacteria in grassland soils. Algal communities of forest soils were characterized by a considerably higher abundance of chlamydomonads (Chlorophyceae) and genera also inhabiting tree-bark. The intensity of land use was not reflected by significant changes in algal communities because the soil algal diversity was almost the same at intensively managed and unmanaged plots. In contrast, changes in the soil algal communities were significantly correlated with physico-chemical parameters such as pH and the carbon/nitrogen ratio.



## COMPARISON OF OSMOTIC POTENTIAL AND PLASMOLYSIS EFFECTS IN *KLEBSORMIDIUM* SP. AND *ZYGNEMA* SP. (STREPTOPHYTA)

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The osmotic potential was determined in different arctic and antarctic *Zygnema* sp. and alpine *Klebsormidium* sp. by incubation in 200 mM - 1000 mM sorbitol solutions. Incipient plasmolysis was found in *Klebsormidium crenulatum* at 800 mM sorbitol, *K. nitens* at 600 mM sorbitol<sup>1</sup>, in the arctic *Zygnema* strain B at 600 mM sorbitol, *Zygnema* strain G at 800 mM sorbitol and the Antarctic *Zygnema* strains D, E at 400 mM sorbitol. These are astonishingly high osmotic values (water potentials,  $\Psi$  -1.3 to -2.1 MPa) for green algae, which translate into, and are likely related to their aeroterrestrial lifestyle.

In plasmolysed *Zygnema* sp. massive Hechtian strands were observed by light microscopy, emerging from the retracted protoplast mainly towards the cross walls. By transmission electron microscopy, organelles appear condensed and small cytoplasmic portions remain in contact with the cross walls, which lack plasmodesmata. Occasionally the cross walls were not fully closed

Photosynthetic oxygen production was measured by a Presens Fibox oxygen optode in untreated as well as plasmolysed samples. While photosynthetic oxygen production was higher in *Zygnema* sp., *Klebsormidium* sp. showed a lower light compensation point and higher values for alpha (initial slope in the light-limited range). The oxygen production was drastically reduced in 800 mM sorbitol at 500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  to about ~20-40% of the initial values in *Zygnema* sp., while this treatment reduced oxygen production in *Klebsormidium* sp. only to ~60-80% of the initial values. This suggests that the investigated *Zygnema* sp. react more sensitive to osmotic water loss than *Klebsormidium* sp.



## THE ADAPTATION OF THE DIATOM *P. TRICORNUTUM* TO RED AND BLUE LIGHT CONDITIONS

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Diatoms represent about 40 % of the marine primary productivity and are therefore one of the most important groups of phytoplankton. In contrast, the knowledge about processes associated with light perception in diatoms is very limited. The genome of *P. tricornutum* contains sequences of red light-perceiving phytochromes as well as cryptochromes and aureochromes which are blue light-receptors. To reveal the impact of different light qualities on the cellular physiology of *P. tricornutum*, cultures were grown at a similar quantum flux density under red and blue light-conditions. Hence, all cultures absorbed the same amount of quanta, which excluded possible effects induced by differences in the light quantity. Both cultures showed identical rates of cell division and biomass production per day. In contrast, the increased protein content in the blue light-adapted culture points to changes in the biomass composition in response to light quality. Furthermore, the red light-adapted culture possessed a lower activity of alternative electron pathways and a lower capacity of non-photochemical quenching. Thus, the physiological adaptations of *P. tricornutum* observed under low irradiance with blue light are comparable to the physiological response usually known from experiments under high light conditions. This interpretation is also supported by the finding of a larger pool of xanthophyll cycle pigments in blue light-adapted cultures. Light shift experiments from red to blue light-conditions and vice versa revealed differences in the kinetics of adaptation to red and blue light, respectively.

## DARK SURVIVAL OF POLAR BENTHIC DIATOMS

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The highly productive microphytobenthos of polar shallow water zones is dominated by diatoms which have to cope with long periods of darkness between late autumn and early spring. Since the dark survival strategies of these organisms are almost unstudied, experiments were carried out under controlled conditions in the laboratory with three representative species isolated in the Arctic Adventfjord, *Navicula* cf. *perminuta*, and two *Surirella* species.

Cell biological parameters (membrane integrity, intracellular activity and incorporation of silica), growth activity after re-irradiation and photosynthetic performance (PI-curves and respiration) were measured after different dark-





incubation periods up to five month. At the same time the amount of storage products, such as cellular neutral lipids and carbohydrates, was examined. Because the influence of climate change is expected to be highest in arctic regions, it is important to find out whether a raised temperature has effects on the ecological function of benthic diatoms. Therefore two temperatures (1,5°C as normal, 6,5°C as raised temperature) were chosen for the experiments.

### **THE SEC6 PROTEIN IS REQUIRED FOR FUNCTION OF THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII***

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Contractile vacuoles (CVs) are key players of osmoregulation in many protists. To investigate the mechanism of CV function in *Chlamydomonas*, we isolated novel osmoregulatory mutants. 4 isolated mutant cell lines carried the same 33,641 b deletion rendering the cell lines unable to grow under strong hypotonic conditions. One mutant cell line (Osmo75) was analyzed in detail. Mutant cells contained a variable CV morphology (multiple small CVs (major phenotype), enlarged 1 or 2 CVs or no light microscopically visible CVs at all). These findings indicate that the mutant is impaired in homotypic vacuolar and exocytotic membrane fusion. Furthermore the mutants displayed a long flagella phenotype. One of the affected genes is the only SEC6 homologue in *Chlamydomonas* (CreSEC6). The SEC6 protein is a component of the exocyst complex required for efficient exocytosis. Transformation of the Osmo75 mutant with CreSEC6GFP construct rescued the mutant completely (osmoregulation and flagellar length). Rescued strains overexpressed CreSEC6 and displayed a modified CV activity. CVs were significantly larger, whereas the CV contraction interval remained unchanged leading to increased water efflux rates. These results indicate that the CreSEC6 is essential for CV function and required for homotypic vesicle fusion during diastole and water expulsion during systole. In addition CreSEC6 is not only necessary for CV function, but possibly influencing the CV cycle in an indirect way and flagellar length control in *Chlamydomonas*.



**PHOTOTROPIN, A NOVEL PLAYER IN EYESPOT DEVELOPMENT AND CONTROL OF  
PHOTOTAXIS IN *CHLAMYDOMONAS REINHARDTII***

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The eyespot of *C. reinhardtii* is a complex multi-layered organelle that allows the cell to perceive environmental light signals and to precisely phototax. We here describe that the eyespot size in this alga is strain specific and dynamically regulated by light. The blue-light sensory photoreceptor phototropin (Phot) has a central role in this regulation. A *C. reinhardtii* strain in which the Phot gene was deleted by homologous recombination loses light regulation of the eyespot size, which is restored by over-expression of full-length Phot or its C-terminal kinase part. Inactivation of both photoreceptor LOV domains in the full length construct or of the kinase domain prevents this complementation. Over-expression of the LOV-domains alone also affects the eyespot size. The role of Phot in eyespot size regulation is additionally confirmed by over-expression analyses in different wild-type strains. Further, Western analyses under different conditions reveals that Phot is important for adjusting the levels of Channelrhodopsin 1 (ChR1), the primary receptor for phototaxis. Phot affects the ChR1 starting levels at the onset of illumination as well as the steady state level during the light period. The phototactical behaviour of strains over-expressing the kinase domain and LOV domain constructs suggests that Phot is involved in adaptational processes and might integrate the overall light level by modulating the transduction of the photocurrents from the eyespot to the flagella. Our data also clearly show for the first time that the Phot LOV domains do have a distinct function besides regulating the kinase activity.



## ALGAL FOOD OF LESSER FLAMINGOS

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Lesser Flamingos are character birds of alkaline-saline lakes and pans of Africa and India. They have a highly specialized diet consisting preferably of planktonic and benthic cyanobacteria and algae. However, the availability of food algae is subjected to considerable alterations. The dominance of the main food resource, the oscillatoriacean cyanobacterium *Arthrospira fusiformis*, is interrupted at irregular intervals and replaced partly by populations of nostocalean cyanobacteria or by the picoplanktonic chlorophyte *Picocystis salinarum*. Lesser Flamingos can respond to fluctuations of algal food supply by migrating to other lakes. Furthermore, the last two decades have witnessed increasing episodes of Lesser Flamingo die-offs in East Africa. This presentation explores the link between sudden flamingo fluctuations and deaths and the algal food quantity and quality. Based on phycological studies at three soda-lakes of Kenya (Bogoria, Nakuru and Oloidien) in the period 2001-2011, the challenges facing the flamingos are discussed and compared with the situation at habitats in southern Africa and north-western India.



## FUNKTIONELLE UND MOLEKULARE CHARAKTERISIERUNG EINIGER EISSTRUKTURIERENDER PROTEINE (ISP) AUS PSYCHROPHILEN SCHNEEALGEN

Thomas Leya

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Eisstrukturierende Proteine (ISP) sind bereits seit längerer Zeit aus einer Vielzahl von Mikroorganismen, Tieren und Pflanzen bekannt. Unter den Algen sind sie an marinen Diatomeen beschrieben und seit kurzem auch an einer Chlamydomonade aus dem hohen Intertidal der Antarktis.

Aus unserer Stammsammlung CCCryo (<http://cccryo.fraunhofer.de>) konnten wir bisher in 15 psychrophilen Schneeealgenstämmen eine ISP-Aktivität nachweisen. Sie wurden von verschiedenen Arten der Gattungen *Chloromonas*, *Chlamydomonas* und cf. *Desmotetra* aus unterschiedlichen evolutiven Linien unter Standardkulturbedingungen bei 2-4 °C produziert und direkt an das Kulturmedium abgegeben. Fakultative Schneeealgen produzieren keine ISP. Eine großskalige Gewinnung der ISP bzw. Aufkonzentrierung aus dem Kulturmedium konnte durch Ultrafiltration über 10 kDa Filter erfolgen. Eine Aufreinigung war für die Wirksamkeit nicht notwendig. Eiskristallwachstum wurde bei Proteinkonzentrationen von 50 µg mL<sup>-1</sup> signifikant unterdrückt. Bei aus dem Kulturmedium aufgereinigtem ISP betrug die notwendige ISP-Konzentration 2,5 µg mL<sup>-1</sup>. Die ISP waren unproblematisch lagerfähig. Bei Raumtemperatur oder 4 °C verloren die ISP ihre Wirksamkeit im Verlauf von 7 Tagen nicht, selbst kurzzeitiges Erhitzen auf 75 °C (10 min) führte zu keiner Aktivitätsminderung.

Homologien der ISP auf Nukleotid- bzw. Aminosäureebene scheinen es zwischen den einzelnen evolutiven Linien nicht oder kaum zu geben und selbst in einzelnen *clades* deutete sich eine unvermutet hohe Diversität an. Dabei ist es erstaunlich, dass die eigentliche Funktion und Fähigkeit, nämlich an Eiskristalle zu binden und deren Morphologie in Form und Größe aktiv zu beeinflussen, trotz molekularer Unterschiede allen ISP gemein ist. Je nach Algenstamm lagen die Molekülgrößen etwa zwischen 14 und 27 kDa. An den Stämmen CCCryo 257-06 (cf. *Desmotetra* sp.) und 273-06 (*Chloromonas* sp.) konnten bisher insgesamt die Sequenzen von 5 ISP-Isoformen bestimmt werden. Auffällig war dabei das verstärkte Auftreten von Threonin-Motiven (TxT) an beta-Faltblattstrukturen. In Homologievorhersagen zeigte die Tertiärstruktur dadurch einen tunnelartigen Aufbau mit den TxT-Motiven an der Außenseite der Faltblätter, die vermutlich für die Bindung an die Eiskristallstrukturen notwendig sind. Neben biologischen Anwendungen sind industrielle Anwendungen für ISP u.a. in Bereichen der Tiefkühl-Lebensmittel oder Kryokonservierung denkbar.



## CHARACTERIZATION OF PROTEINS OF THE EXTENDED VIOLAXANTHIN DE-EPOXIDASE (VDE) FAMILY FROM THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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The nuclear genome of the diatom *P. tricornutum* encodes four homologues of the enzyme violaxanthin de-epoxidase (VDE) from higher plants that is involved in the photoprotective xanthophyll cycle. Based on *in silico* analyses, the diatom protein with highest sequence similarity to plant VDE was designated as a putative VDE, two others with lower similarity as VDE-like (VDL1 and VDL2), and the protein with lowest similarity as VDE-related (VDR). Aiming at the functional characterization of the four proteins, we cloned the corresponding genes from cDNA preparations of *P. tricornutum*. A bioinformatic analysis of the deduced protein sequences indicated the presence of bipartite targeting signals at the N-terminus of VDL2 and VDR and tripartite targeting signals at the N-terminus of VDE and VDL1 suggesting that they localize to the chloroplast stroma and thylakoid lumen, respectively. After heterologous expression in *E. coli*, only the product of the putative VDE gene was able to convert the substrates violaxanthin and diadinoxanthin to zeaxanthin and diatoxanthin, respectively. We tested the other three proteins with different carotenoid substrates and detected enzymatic activity for one of them. We will present data on the identity of the product and on the biochemical properties of this enzyme.



## FIGHTING PLANKTIC ALGAE WITH BENTHIC ALGAE: A PILOT STUDY AT THE HEUSTADELWASSER IN VIENNA

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Anthropogenic eutrophication in surface waters causes serious problems, such as fish kills, mass development of algae, and cyanoprocarvites, which facultatively produce severe toxins. Radical hydrologic changes caused the eutrophication at the Heustadelwasser, which was faced with a grain filter in 2007. One strategy for reducing planktic algae is nutrient removal. The nutrient reduction can be achieved through the ability of self-purification in streams, which is particularly done by the biofilm. We therefore installed artificial stream beds (algae turf scrubbers) on the grain filter, which were periodically supplied with surface water. For removing the nutrients the grown algal biomass was harvested. Three growth periods from June to September 2011 were conducted. To characterize the biomass weekly measurements of biomass, nutrients, fatty acids and chlorophyll-a were done. Maximal removal rates were highest in August with about 19 mg total phosphorous  $\text{m}^{-2} \text{d}^{-1}$ . In June and September the maximal removal rate was lower with about 10 mg total phosphorous  $\text{m}^{-2} \text{d}^{-1}$ . Further use of the biomass is possible, such as fertilizer, fermentation or biodiesel production. The pilot study showed that the algae turf scrubber technology has great potential and provides an effective and ecologically sustainable way to remove nutrients from surface waters.

## CHARACTERIZATION OF AN ANIMAL-LIKE CRYPTOCHROME IN *CHLAMYDOMONAS REINHARDTII*

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The circadian clock is entrained by environmental stimuli such as the daily light-dark cycle, involving different photoreceptors. In the green alga *Chlamydomonas* it was shown that light of different qualities resets the phase of its circadian phototaxis rhythm including blue light. After genome sequence data were available, homology searches revealed that *Chlamydomonas* has a plant and an animal-like CRY. aCRY was expressed heterologously in *E. coli*. The protein was shown to bind FAD in its oxidized form in vitro. Upon illumination with blue light, the neutral radical of flavin was formed. The absorption spectrum of the radical shows characteristics of a



member of the animal type II cryptochrome and (6-4) photolyase family. For in vivo studies, an insertional mutant library of 25.000 lines was screened. An *acry* mutant was identified that has one insertion of the selection marker, localized in an intron of the *acry* gene. This results in a reduction of aCRY protein down to about 20% in comparison to wild type. In the *acry* mutant, blue light regulation of certain genes encoding enzymes of metabolic pathways or clock-relevant components is altered.

### BIOTECHNOLOGICAL SCREENING OF MICROALGAL STRAINS

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Microalgae represent an enormous natural resource to generate a large number of substances. They can have pharmacological activity, be used as additives in food, feed and cosmetics or can be used for bioenergy production. The variety of potential agents and the use of microalgae biomass for the production of these substances are little investigated and not exploited for the existing market. Because of the enormous biodiversity of microalgae, they show great promise for new products.

A large number of microalgae strains of the Culture Collection of Algae at Göttingen University (SAG) are investigated within the presented project of the Centre of Excellence of Biomass (CE Biomass) in Schleswig-Holstein. In this study, a broad range of different microalgal species was screened for hydrogenase activity, carbon dioxide absorption capacity, biogas production, carotenoid and chlorophyll contents, tocopherols, tocotrienols, fatty acids and antibacterial effects. The result of the current study showed that microalgae are able to generate a large number of substances in different quantities. In addition, between closely related species and even among multiple isolates of the same species, the productivities may be rather variable.



## EFFECTS OF OCEAN ACIDIFICATION ON DIFFERENT STAGES IN THE LIFE-CYCLE OF THE KELP *LAMINARIA HYPERBOREA*

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The objective of this study was to evaluate the influence of preindustrial and future pCO<sub>2</sub> (280µatm and 700µatm pCO<sub>2</sub>, respectively) on different life-cycle stages of the kelp *Laminaria hyperborea* from Helgoland. Zoospore germination, gametogenesis, vegetative growth, sorus formation and photosynthetic performance in vegetative and fertile tissue were addressed. The importance of the external carbonic anhydrase (exCA) on net-photosynthesis and the Chl*a*- and phlorotannin content were examined. Zoospore germination and sorus formation were not affected by pCO<sub>2</sub>, whereas female gametogenesis and vegetative growth of sporophytes were significantly enhanced under future pCO<sub>2</sub>. rETR(max) and net-photosynthesis of young vegetative sporophytes displayed a trend towards increased performance. The trend towards elevated net-photosynthesis vanished after inhibition of the exCA. In young vegetative sporophytes a trend towards an elevated phlorotannin content was evident. The results are discussed within a physiological and ecological context and indicate that the stimulation of photosynthesis caused the faster vegetative growth of *L. hyperborea* at future pCO<sub>2</sub>.

## COMPARATIVE ASSESSMENT OF CYANOBACTERIAL DIVERSITY USING MOLECULAR METHODS: EXAMPLES FROM THREE DIFFERENT GEOGRAPHICAL REGIONS

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The cyanobacterial diversity of soils of the Atacama Desert (Chile) was investigated using 16S rDNA cloning/sequencing directly from soil samples and 16S rDNA sequencing from cultures. Within the hyper-arid Atacama Desert, one of the driest parts of the world, 10 plots were sampled along a total air line distance of about 1100 km. The obtained sequences were compared to corresponding sequences from cyanobacterial cultures and 16S rDNA clones established from soils of temperate regions in Germany, Hainich National Park and Schorfheide-Chorin biosphere reserve, as well as from a saline arid region of Black Sea coast of Ukraine. The sequences were grouped into OTUs (97% cut off). A total of 32 OTUs were recovered





from all regions. A high similarity among the different sampling sites within the Atacama Desert was observed. Members of Oscillatoriales were most abundant there. Most cyanobacterial OTUs formed clades distinctly separating South-American and the European sequences. However, also a few OTUs included sequences from South-America as well as Europe, i.e. there was one Nostoclean OTU at even a 99% cut off that was comprised of rather similar sequences from all three regions. However, there is observable inner genetic variability for these OTUs which include sequences from different regions. Using standard PCR cyanobacterial primers, also a considerable range of other bacterial phyla were amplified and analyzed. Some non-cyanobacterial phyla demonstrated a wide geographical distribution, but most of the OTUs assigned to these phyla occurred distinctly either in the Atacama or Europe.

### NUTRIENTS AND PHYTOPLANKTON IN MESOKOSMS

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Fluctuating parameters like temperature, salinity and light are known as some of the most important influences on the yearly periodic of the phytoplankton in the Darss-Zingst Bodden Chain, an estuary of the Baltic Sea. Following the “Intermediate disturbance hypothesis” reduced or absent changes, like in this study, should lead to a lower diversity in species. Independent of abiotic conditions, irregular dynamics in the species system are also supposed to be a driving force for biodiversity and interactions between the organisms. Therefore we used four mesokosms in a long-time experiment to examine for the development and diversity of planktical phototrophical organisms under constant laboratory conditions. In that way, advantages for temperature- and light specialists could be excluded in these experiments. With no additional input of nutrients over all months, nutrient flow was only possible by internal microbial food webs between zooplankton, bacteria and phytoplankton. We could find out that the four parallel mesokosms were similar in nutrient content and species diversity during time of experiment. Cyanobacteria dominated the phytoplankton nearly all months. The species composition of the whole plankton in detail showed some differences to the natural ecosystem. The calculated mean net production of  $5 \text{ mg O}_2 \text{ m}^{-2} \text{ day}$  is also known from natural summer conditions, but from a much higher biomass. After the breakdown of the mass development of phytoplankton a high nutrient release was measured. Possible predator-prey relationships and connections between biomass and nutrient development could be proofed in different periods of time.



## UVB SENSITIVITY OF PHOTOSYNTHESIS AND DNA IN GREEN MACROALGAE RELATED TO DEPTH DISTRIBUTION

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Although ultraviolet B (UVB) radiation only comprises less than 1% of the incoming solar radiation it has a multitude of negative effects on plant performance. Absorption of this high energy quanta occurs primarily by DNA and aromatic amino acids and leads to structural and biochemical changes in these molecules. Thereby UVB radiation impairs physiological processes like DNA replication, transcription and photosynthesis. The physiological process that is most susceptible will determine the UVB sensitivity of the investigated plants.

The green macroalgae *Ulva intestinalis*, *Cladophora sericea* and *Bryopsis hypnoides* grow at different depth in the littoral zone and therefore experience different UVB intensities. Accordingly, one would expect the highest UVB resistance in *U. intestinalis* which dominates the upper eulitoral. Surprisingly, *U. intestinalis* is lacking a major resistance mechanism, the screening of UVB radiation. We hypothesize that this species compensates the missing UVB screening by increased UVB tolerance. Tolerance is determined among others by repair capacity. Therefore we evaluated the repair of DNA lesions and of PSII damage after a strong UVB treatment in all species. The results will be discussed with respect to the natural depth distribution of the species.

## MITOCHONDRIA ISOLATION FROM DIATOMS

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Diatoms belong to the stramenopiles, a diverse group of organism which include photoautotrophic and heterotrophic species. Stramenopiles evolved by secondary endocytobiosis, the uptake of a photosynthetic eukaryote into an eukaryotic host cell. This evolutionary process increased the complexity of the resulting cells by combining two nuclear and three organellar genomes. Extensive gene transfers and genomic reorganization had strong implications on physiology and biochemistry of the resulting cells. Stramenopile genome projects reveal an interesting distribution of metabolic pathways. One surprising finding is that all members of the stramenopiles investigated so far (including several diatom and oomycete species) possess nuclear encoded mitochondrial isoforms of enzymes of the second half of glycolysis, the oxidation of triosephosphate to pyruvate. This was unexpected as glycolysis in all other eukaryotes investigated so far does not take place in the mitochondria. To date, the knowledge on mitochondria of stramenopiles is limited. To characterize the physiological properties of diatom mitochondria we have established an isolation protocol for the mitochondria of *Thalassiosira pseudonana*.



## NUTRITIONAL REQUIREMENTS AND METABOLIC CHARACTERISTICS OF HETEROTROPHIC, MIXOTROPHIC AND AUTOTROPHIC FRESHWATER CHRYSOPHYTES

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Chrysophytes or golden algae are geographically widespread and abundant in freshwaters, marine waters and soils. Many of the golden algae have adapted their nutritional mode in a way that they are between purely heterotrophic and purely phototrophic (mixotrophic). Despite more than fifty years of research on mixotrophy in phytoplankton overall survival strategies, metabolic regulation and molecular biology of mixotrophs are not yet understood. To relate mixotrophic chrysophytes to each other and to algae with other nutritional modes, we performed comparative physiological studies for several species under the same laboratory conditions. We compared the freshwater chrysophytes *Spumella* sp. JBM10 (heterotrophic), *Poterioochromonas malhamensis* (mixotrophic), *Dinobryon divergens* (mixotrophic) and *Mallomonas annulata* (photoautotrophic) concerning light regime, photosynthetic capacity, growth on different carbon sources and nutritional limitations in the media. We also took into account the brackish water diatom *Phaeodactylum tricornutum* as phylogenetic relative and sequenced model organism. We show, that even at the same conditions, the two mixotrophic strains behave differently. For example, *D. divergens* shows light-dependent growth whereas *P. malhamensis* can grow in complete darkness. To gain deeper insight into regulation and existence of common (or uncommon) metabolic pathways in chrysophytes, we established transcriptome datasets for the four chrysophytes. We are currently annotating and comparing the data to proceed with expression studies (quantitative Real-Time PCR) for mRNAs of key enzymes from several C-, N- and P-pathways.



## ALGAL PIRATES - SEARCHING FOR OPTIMAL ANTIFOULING TEST SPECIES

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Prevention of fouling on artificial substrates like ships, bridges and platforms is a significant problem. Extensive ocean immersion testing is generally considered the “gold standard” for evaluating the performance of marine antifouling coatings, but it is time consuming and expensive.

The EU-funded R&D project *IATS* aims to develop an innovative, completely automated antifouling test system for professional examinations of marine coatings. Contrary to ‘marine’ laboratories these fouling bioassays will use freshwater algae species that have been isolated from typical freshwater biofilms and proven biofouling capacity on different substrates under standard lab conditions. Out of over 300 isolates of cyanobacteria, diatoms and green algae 55 strains showed high biofouling capacity on glass beads. Among those are genera like *Stigeoclonium* and *Klebsormidium*, which are known to include metal tolerant species. Today up to 95% of marine coatings are still based on biocides, which often contain Cu and Zn as algaecides. The selection of test specimen for *IATS* therefore focus on strains that are tolerant to such biocides but show typical fouling on modern foul-release silicone coatings as well. Short time freshwater assays using potential test algae (e.g. *Stigeoclonium*, *Klebsormidium*, *Navicula*, *Phormidium* and some coccal green algae) showed fouling results on industrial coatings that were comparable with exposition for up to 2 years in professional marine test facilities.

## BIODIVERSITY AND EVOLUTION OF SYNCHROMOPHYCEAE AND RELATED AMOEBOID HETEROKONT PROTISTS

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Synchromophyceae are a new, amoeboid class of heterokont algae (Ochrophyta) with the type species *Synchroma grande*. Numerous new isolates and a new species, *S. pusillum*, have been identified recently, which all possess the key morphological feature of this class, the chloroplast complex. Here we present two possible new members of the Synchromophyceae and their closest related species, which share most features with the Synchromophyceae, like the sessile, amoeboid and floating cell stages, interaction of cells in a meroplasmodium, binary division inside a lorica with subsequent hatching and pigment composition. However, their plastids are randomly distributed in the cytoplasm, showing no sign of a chloroplast complex formation. Preliminary 18S rDNA data show that the new members *Chrysopodocystis*



*socialis* and *Chrysopodocystis* sp. Tf09 are related to the Synchromophyceae, but form a clade of their own and possess unusual nucleotide insertions compared to all other Synchromophyceae studied. Different evolutionary scenarios for this morphological and molecular heterogeneity include (i) the secondary loss of plastid complexes for *Chrysopodocystis* species (ii) the invention of plastid complexes after the split of the genera *Synchroma* and *Chrysopodocystis* and (iii) possibly additional endocytobiotic events. The existence of 2 closely related clades of amoeboid algae with and without plastid complexes could improve our understanding of the plastid complex function and evolution by physiological, biochemical and molecular comparisons. A first approach lies in the sequencing of these plastid genomes, preceded by plastid DNA enrichment and amplification.

## EXPLORING THE FUNCTION OF LOROIXANTHIN IN LIGHT-HARVESTING COMPLEX II OF GREEN ALGAE

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The carotenoid loroxanthin (19-hydroxy-lutein) is present in many green algal species. In *Chlamydomonas reinhardtii*, loroxanthin partially replaces lutein in the light-harvesting complexes of photosystem II (LHCII), but the biological significance of this exchange is still unknown. Here, we observed an increase of the loroxanthin/lutein ratio in *C. reinhardtii* and other green algae at higher temperatures suggesting that loroxanthin may contribute to thermal stabilization of LHCII. We pursued this idea by *in vitro* reconstitution of recombinant LHCII from *C. reinhardtii* with mixtures of chlorophyll a, chlorophyll b, neoxanthin, and either lutein or loroxanthin. Interestingly, the resulting complexes were virtually identical in terms of pigment stoichiometries, absorbance properties, fluorescence excitation spectra, circular dichroism spectra and thermal stability. A major difference, however, was a 15-20 % higher fluorescence emission of loroxanthin-containing LHCII. Notably, *in vitro* reconstitutions of recombinant LHCII from the vascular plant *Pisum sativum* showed similar effects although loroxanthin is not present in higher plants. In agreement with the *in vitro* results, native LHCII from *C. reinhardtii* with different loroxanthin/lutein ratios showed a higher fluorescence quantum yield for preparations with increased loroxanthin content. Thus, we suggest that the modulation of the loroxanthin content may allow green algae to adjust the light-harvesting efficiency of LHCII to the capacities of the electron transport chain and Calvin cycle under varying environmental conditions.



## COMBINED EFFECTS OF TEMPERATURE AND CO<sub>2</sub> ON GROWTH AND PHOTOSYNTHESIS OF TWO MARINE RED ALGAE: *PALMARIA PALMATA* AND *CHONDRUS CRISPUS*

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Ocean acidification and temperature increase are two major global change factors concurrently acting in coastal ecosystems. We tested the assumption that future CO<sub>2</sub> increase will be beneficial for uncalcified seaweeds if acting in combination with elevated sub-optimal temperatures. In two-factorial experiments we measured the response of relative growth rate RGR, photosynthesis and pigment concentrations against a combination of preindustrial (280 µatm) and future (700 - 1200 µatm) pCO<sub>2</sub> and optimal (10 and 15°C, respectively) or elevated sub-optimal temperatures (18 and 24°C, respectively) in two North Atlantic red algae, *Palmaria palmata* and *Chondrus crispus*. In both species pCO<sub>2</sub> and temperature interacted significantly on RGR and photosynthesis while pigment concentration was dependent on temperature only. The responses were species-specific. In *P. palmata* RGR was enhanced at 800 µatm pCO<sub>2</sub> in optimal and elevated temperatures (10 and 18 °C) compared to preindustrial CO<sub>2</sub> concentrations. The interaction with temperature became obvious at a pCO<sub>2</sub> of 1200 µatm. At this CO<sub>2</sub> concentration growth decreased significantly at 10°C, but not at 18°C. Compensation for decreased growth rates at elevated temperatures also was observed in *C. crispus* at a pCO<sub>2</sub> of 700 µatm in 24°C. In both species photosynthesis partially showed a slight decrease at future compared to preindustrial pCO<sub>2</sub> which is the oppositional response to the observed growth increase and was enhanced at elevated temperatures. The reaction pattern of both shallow intertidal red algal species show that the combined rise of CO<sub>2</sub> concentrations and temperature that is expected for the future may have the potential to reshape coastal seaweed ecosystems.

## GENOME EVOLUTION IN EUKARYOTES INFERRED FROM THE WHOLE GENOME SEQUENCE OF *CHONDRUS CRISPUS* (RHODOPHYTA)

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Red algae (Rhodophyta) represent a unique lineage of eukaryotes, separated from others for at least 1.2 billion years. Together with Chlorophyta and Glaucophyta they form the primary plastid-containing Plantae. Also red algae donated the plastid to the chromalveolate lineage via secondary endosymbiosis. Finally red algae are one of only 5 lineages to develop complex multicellularity independently, others are animals, fungi, chlorophyta and brown algae; red algae were probably the first to do



this step more than a billion years ago. From a scientific point of view these are more than enough reasons to look in detail into the genome of a multicellular red algae and therefore a genome project for *C. crispus* was initiated in 2006 and recently completed. Here we report on the features of the genome and a detailed comparison to a genome from a unicellular red alga *Cyanidioschyzon merolea*, to chromalveolate genomes, and other genomes. We found that the structure of red algal genes and genomes is different from most other organisms, characterised by gene-dense regions, few introns, metabolic streamlining, and recent transposon invasion.

### DESICCATION-INDUCED NON-RADIATIVE DISSIPATION PROTECTS GREEN LICHEN ALGAE AGAINST PHOTOINHIBITION

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Lichens are tolerant to almost complete desiccation and are able to restore functioning of photosystem II (PS II) upon rehydration within few minutes. In the dry state, Fv/Fm is close to zero, accompanied by a strong reduction of Fo. While this fluorescence reduction is partially due to optical changes within the thallus structure, the larger part is caused by enhanced non-radiative dissipation [1]. It has been hypothesized that the fluorescence quenching protects PS II in the desiccated state when lichens are often exposed to high irradiance but cannot utilize the excitation energy for photosynthesis [2]. To test this hypothesis, we have used isolated green lichen algae with varying ability for desiccation-induced Fo-quenching. *Diplosphaera* spec. and *Coccomyxa* spec. were grown in liquid culture and desiccated after fixation on fiberglass filters. Photoinhibition by exposure to a photon flux density of 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 60 min was higher in the wet than in the dry state. *Diplosphaera* showed a much stronger quenching than *Coccomyxa* and was less photoinhibited. The sugar trehalose, which enhances desiccation resistance in many organisms, reduced photoinhibition without affecting fluorescence quenching, when added to the growth medium. On the other hand, growth in seawater enhanced photoinhibition resistance together with fluorescence quenching. The results strongly support the hypothesis that non-radiative dissipation in the desiccated state is photoprotective. However, other so far unknown mechanisms seem to contribute to photoprotection as well.

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## ARCTIC MICROPHYTOBENTHOS PRIMARY PRODUCTION IN KONGSFJORDEN (SVALBARD, NORWAY) COULD GAIN FROM GLOBAL WARMING - IN SITU MEASUREMENTS AND MODELLED CHANGES

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Arctic microphytobenthic *in situ* primary production was measured as oxygen exchange rates in benthic chambers at three representative sandy sites in Kongsfjorden (Svalbard, 79°N, 12°E) at 3 to 11 m water depth during June 2008. No significant differences, neither in photoautotrophic biomass nor in primary production, were detected between stations and depths. All sites showed low but variable rates of net production from  $-225$  to  $+434$  mg O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> ( $-85$  to  $+163$  mg C m<sup>-2</sup> day<sup>-1</sup>) and gross production from 615 to 1670 mg O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (231 to 625 mg C m<sup>-2</sup> day<sup>-1</sup>), which is comparable to other polar as well as temperate regions. The numerical model of Walsby (1997) was applied to estimate seasonal and regional rates of nearshore production for the Arctic summer, i.e. 15 March to 15 September 2008. Based on the parameters derived from the measured *in situ* P/I curve, chlorophyll concentrations, solar global radiation data and satellite-derived sea surface temperatures, the model estimated highest rates between 1000 and 1500 mg O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (370-556 mg C m<sup>-2</sup> day<sup>-1</sup>) at intermediate water depth where biomass is higher than in the shallower water. Regional production of the entire Kongsfjorden during June 2008 yields 23-27 tonnes O<sub>2</sub> (9-10 tonnes C). The increase in SST by 2 °C would cause a small increase in net production during the Arctic summer between 6 and 20% for a stable stratified and fully mixed water column scenario, respectively.

## CHARACTERISATION OF A PUTATIVE TAG-LIPASE *PUT\_LIP* OF *PHAEODACTYLUM TRICORNUTUM*

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Receiving Triacylglycerides (TAG) as well as special fatty acids from photosynthetic, unicellular Organisms has become more and more important for biotechnological approaches over the last years. Especially TAGs can be used to produce biodiesel and therefore it would be an advantage to have Organisms which contain a high amount of TAG's in order to increase efficiency of biodiesel production.

*P. tricornutum* is a marine diatom and uses TAG's as the major storage component to save energy gained by photosynthesis. The organism further uses TAGs to supply the cell with energy, during inactive photosynthesis in the night, e.g.





The initial mobilisation of TAGs is catalyzed by TAG-Lipases, by converting the TAG into a free fatty acid and a Diacylglyceride (DAG). In *P. tricornutum*, a cDNA sequence was found in an EST database which shows a certain similarity to lipases from other organisms. It could be shown, that a knockdown of the gene expression by RNAi lead to an increased accumulation of TAGs.

In order to prove that the high amount of TAGs in the mutant is a result of the downregulation of the putative lipase, the expression level of *put\_lip* was investigated by qRT-PCR.

Furthermore, a recombinant his-tagged protein (Put\_lip) was expressed in *E. coli* to prove the function of this unknown protein to be a TAG-Lipase.

### **PLASTID LSU rDNA (23S) VARIATION OF *SYMBIODINIUM* (DINOFLAGELLATA) WITHIN *PHYLLODESMIUM* (GASTROPODA)**

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The genus *Phyllodesmium* (Gastropoda, Aeolidida) is well known to host symbiotic photosynthetic dinoflagellates of the genus *Symbiodinium* (Alveolata, Dinzoa).

Although these dinoflagellates mostly occur as freeliving cells in fresh and marine water, some are known to exist as endosymbionts (zooxanthellae) in reef-building corals and other invertebrates, for example in the coral feeding sea slug *Phyllodesmium*. During the digestion of the corals *Symbiodinium* cells remain intact embedded in the cerata tissue of the slug. Even those incorporated *Symbiodinium* maintain fully functional plastids to perform photosynthesis that can be measured by PAM (pulse amplitude modulated fluorometry).

Although distribution of *Symbiodinium* is well studied in corals, very little is known about diversity within the genus *Symbiodinium*. Due to extremely restricted morphological and ultrastructural autapomorphies the genus *Symbiodinium* is divided into clades A to I revealed by molecular data. So far *Symbiodinium* clades have not been isolated and molecularly identified in other Aeolidida than *Pteraeolidia ianthina*. In this study we amplified the plastid LSU rDNA (23S) of different *Symbiodinium* clades from the aeolidian *Phyllodesmium* and performed phylogenetic analyses.



## MODULATION OF CAROTENOID QUALITY AND QUANTITY BY GENETIC ENGINEERING

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Carotenoids are produced by all photosynthetic organisms and take part in light harvesting as well as in photoprotection. Their antioxidative character and other functions are reasons for their important role in the humans' nutrition. The high costs of synthetic carotenoid production give reason to find suitable hosts for natural biosynthesis.

For a proof-of-principle we use the model organism *Phaeodactylum tricornutum*, a unicellular diatom occurring in salt and brackish water. Since rather little is known about the enzymes involved in carotenoid biosynthesis in diatoms we focus here on four endogenous genes, (*Psy*, *Zep1-3*), coding for phytoen-synthase and zeaxanthin-epoxidases 1-3, respectively. *Psy* catalyses the entry and limiting reaction of the carotenoids biosynthesis. *Zep1-3* are coding for the enzymes responsible for the conversion of zeaxanthin to violaxanthin.

To investigate these four putative enzymes in *P. tricornutum* we cloned cDNA and genomic DNA in order to identify their functions by complementing systems in *E. coli*.

An interesting step for industrial purposes is the carotenoid over expression. Therefore we will transform *P. tricornutum* with an inducible *Psy*-construct to overcome the limiting entry reaction.

Moreover, an additional gene (*bkt*), coding for a ketolase, is cloned to set a bypass for astaxanthin synthesis without disturbing the physiology.



## LIVING WELL WITH A SCRAMBLED METABOLISM: CO<sub>2</sub> FIXATION AND CARBOHYDRATE PATHWAYS IN DIATOMS

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Diatoms are responsible for up to 20% of the global carbon fixation, while the mechanisms of CO<sub>2</sub> fixation in these organisms are still unclear. Diatoms have evolved by secondary endosymbiosis, which apparently led to subcellular reorganization of metabolic pathways within the new organism. In the genome of the diatom *Phaeodactylum tricorutum* we have identified a number of genes for enzymes which are important for operating a C4-like pathway including several carboxylases. However, we could not yet identify plastid localized decarboxylases. Such enzymes would be important for a release of CO<sub>2</sub> from malate or oxaloacetic acid in close proximity to RubisCO. The only decarboxylases identified so far apparently are targeted to the mitochondrial matrix which is separated from the plastid stroma (and thus the RubisCO) by six membranes. Therefore it is unclear yet whether *P. tricorutum* might operate either a biochemical or a biophysical carbon concentrating mechanism (CCM) or a combination of both.

To study individual enzymes of the photosynthetic carbon fixation, we are using a “reverse genetics” approach including silencing of genes which are involved in the biochemical CCM via RNAi. Mutants have been designed with sense-antisense constructs for silencing phosphoenolpyruvate carboxylases (PEPC1 & PEPC2), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate carboxylases (PYC1 & PYC2) and NAD malic enzyme (ME1). To control expression of the silencing constructs we have decided for a nitrate reductase (NR) promoter which can be switched on and off depending on the nitrogen source in the medium. The obtained mutants will be tested for silencing efficiency Western Blots. Physiological investigations will be conducted under e.g. different CO<sub>2</sub> and light concentrations to learn more about their adaptation abilities due to future environmental changes.



## IF CHEMISTRY IS RIGHT – HOW CHEMICAL MARKERS CAN COMPLEMENT THE TAXONOMY OF COCCOID GREEN MICROALGAE (TREBOUXIOPHYCEAE)

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Cocoid green microalgae colonize almost every habitat worldwide and are also used for biotechnological purposes, as is the case for *Chlorella vulgaris*. However, the numerous, little round green balls' are difficult to identify because they lack distinct morphological features. Molecular analyses of 18S rDNA and the ITS-region indicated an affiliation of the cocoid species to different lineages within the Trebouxiophyceae and Chlorophyceae. However, it is reasonable to assume that the organisms also differ in their biochemical composition. Chemical characters often constitute adaptational mechanisms to the organisms' environment and can contribute to understand the distinct phylogenetic relationships and evolutionary background within these morphological similar species.

The cell wall component ergosterol and pattern of polyols proved to be suitable chemotaxonomic markers. A total of 45 cocoid species were analyzed via different HPLC methods. Concentrations of the respective markers depended on the physiological state of the cultures and varied between 0.25 - 4.5  $\mu\text{g mg}^{-1}$  DW (ergosterol) and 7 - 450  $\mu\text{mol g}^{-1}$  DW (polyols). Ergosterol was detected in aquatic cocoid species, whereas polyols were a common feature of terrestrial species. These results sustain the suggested trichotomy of the Trebouxiophyceae into three lineages: *Chlorella*-, *Trebouxia*- and *Oocystis*-lineage. Furthermore, chemical markers detectable by HPLC can provide a fast, cost-efficient and valuable tool to identify or assign cocoid production strains in biotechnological applications.



## TUFA STROMATOLITES: POLYPHASIC CHARACTERIZATION OF CYANOBACTERIA AND DIATOMS FROM AN UNUSUAL HABITAT

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Tufa stromatolite associated biofilms were investigated within the framework of the DFG-funded research unit FOR 571 'Geobiology of Organo- and Biofilms'. These biogenic substrates host a considerable diversity of cyanobacteria and diatoms. Both groups produce high amounts of exopolymers which are possibly involved in CaCO<sub>3</sub> nucleation and calcification processes. Therefore, we analyzed changes in the taxonomic composition of the cyanobacteria/diatom communities along a gradient of different calcification intensities. For taxa identification a polyphasic approach was used. This approach comprised 1) the cloning of SSU rDNA-markers directly from the environmental material as well as 2) the analysis of isolated unialgal cultures using microscopy and SSU rDNA. As an example of recent intense tufa formation, hardwater creeks from two different areas in Germany were studied: Westerhöfer Bach near Göttingen in Lower Saxony (51°46'N/10°7'E) and Deinschwanger Bach near Neumarkt in Bavaria (49°39'N/11°48'E). For diatoms about 400 sequences of 18S rDNA and for cyanobacteria about 400 sequences of 16S rDNA were analyzed by an OTU-based approach using 98% cut off for diatoms and 97% for cyanobacteria. Full sequences of the OTU-representatives and cultures were phylogenetically analyzed together with available reference sequences. 24 diatom and 40 cyanobacterial lineages were detected. Communities of cyanobacteria and diatoms varied considerably among the 11 studied high-calcified downstream sites and two non-calcified spring sites. Several lineages from both cyanobacteria and diatoms exhibited clear preferences towards microhabitats of intense calcification.



## ALGEN UND PROTOZOEN – STÄRKERE SICHTBARKEIT IN DER GLOBAL BIODIVERSITY INFORMATION FACILITY (GBIF)

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Die Global Biodiversity Information Facility ([www.gbif.org](http://www.gbif.org)) ist ein internationaler Verbund von Staaten und Organisationen, die Biodiversitätsdaten über das Internet verfügbar machen. Biodiversitätsdaten sind Belegdaten, Observationsdaten und Daten zu Lebendkultursammlungen. Zurzeit stellt GBIF-D *Pflanzen, Algen & Protisten* 5,8 Millionen Datensätze für das GBIF-Netzwerk bereit.

GBIF-D ([www.gbif.de](http://www.gbif.de)) sowie GBIF-D *Pflanzen, Algen & Protisten* analysieren Datenlage und Nutzerbedürfnisse für die Einbindung neuer Datenquellen in das GBIF-Netzwerk im Rahmen des BMBF-unterstützten Verbundes (GBIF-D: Kompetenzzentren innovativer Datenmobilisierung, Projekt 01 LI 1001 A-F). Für eine bessere Sichtbarkeit und Nutzbarkeit der Datenbestände wird deshalb ein Datenportal entwickelt, das Ende 2012 online gehen wird. An konkreten Erweiterungen für das Jahr 2012 sind u.a. die Anbindung der Bilddatenbestände von Plankton\*Net, die Sammlungsdatenbestände der Sammlung für Algenkulturen in Göttingen (SAG) sowie die Anbindung protozoologischer Datenbestände geplant. Laufend wird an der verbesserten Datenintegration vorhandener Datenanbieter gearbeitet.

Bereits seit 2005 liefert das *AlgaTerra* Informationssystem ([www.algaterra.org](http://www.algaterra.org)) Daten über Mikroalgen an GBIF. Die verwendete BioCASE-Providersoftware erlaubt die vollständige Anbindung reicher Datenbestände. Im Bereich der Bilddaten nutzt *AlgaTerra* inzwischen moderne Mikroskopie- und Servertechnologien, um qualitativ hochwertige Bilddaten verarbeiten, speichern und bereitstellen zu können. Die konzeptorientierte *AlgaTerra* Datenbank wird im Sommer 2012 auf die *EDIT Platform for Cybertaxonomy* migrieren, die zunehmend für taxonomische und Checklisten-Datenbestände Verwendung findet.

Diese Initiativen zielen auf eine stärkere Nutzbarkeit der vorhandenen Datenbestände durch Phykologie und Protozoologie sowie die Gewinnung weiterer Partner, die Qualitätsdaten an das globale GBIF-Netzwerk liefern, um den Open Access-Ansatz auch im Bereich der Biodiversitätsdaten von Ein- und Wenigzellern voranzubringen.



**BIODIVERSITY AND ECOPHYSIOLOGY OF A COMMON TERRESTRIAL GREEN-ALGAL GENUS *KLEBSORMIDIUM* (STREPTOPHYTA) ISOLATED FROM SOIL OF THREE BIODIVERSITY EXPLORATORIES (GERMANY)**

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The genus *Klebsormidium* belongs to a group of green algae (Streptophyta), which are together with others closely related to the land plants. They are able to form characteristic biofilms and distributed in almost all terrestrial habitats from tundra of the Polar areas, to desert and arid regions of both sites of the equator. *Klebsormidium* is a typical representative of biological crusts, which play an important ecological role in primary production, nitrogen fixation, nutrient cycling, water retention and stabilization of soils. We collected from three large-scale and long-term research sites in Germany (Schorfheide-Chorin, Hainich-Dün, and Schwäbische Alb) soil samples and isolated more than 70 strains of *Klebsormidium*. To detect the genetic diversity of these strains, we sequenced the internal transcribed spacer region of the nuclear ribosomal cistron (ITS-1, 5.8S rDNA, and ITS-2). The molecular phylogeny of the ITS rDNA sequences showed that the strains of *Klebsormidium* belong to the previously discovered clades, which are designated as clades A-G *sensu* Rindi et al. (2011). To discriminate *Klebsormidium* at species level, we used the secondary structures of ITS-2 rDNA sequences as DNA barcode marker and compared these results with the morphology of the described species. Interestingly, the European isolates of *Klebsormidium* showed a high phenotypic plasticity, but only a little genetic diversity. To support these findings, we studied the growth rate by different temperatures and other adaptation mechanisms.



## ALIGNMENT AND PHYLOGENETIC ANALYSES OF THE COMPLETE rRNA OPERON USING THE SUESSIALES AS A TEST CASE

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Phylogenetic analyses in the Dinophyceae using nuclear-encoded rDNA have mostly employed either the SSU rRNA gene or the 5'-terminus of the LSU rDNA. Very few studies were based on both rRNA genes or even on the complete rRNA operon. We used the dinoflagellate order Suessiales as a test case to compare resolution and topologies of phylogenetic trees inferred from complete and partial rRNA operons. The experimental setup consisted of 20 taxa of the Suessiales and a dataset with approximately 5000 aligned characters. This alignment was split, and nine different partitions were analyzed. Each partition contained different genes or gene fragments. By comparison of the tree topologies as well as the bootstraps and posterior probabilities we found some interesting differences. The trees show topology differences for the SSU and for the shortest LSU rDNA partition, and the resolution was improved with increasing alignment length. The results suggest that the complete rRNA operon or at least a combination of SSU and partial LSU rDNA enhance resolution in molecular phylogenetic analyses of the Suessiales.

## NON-PHOTOCHEMICAL QUENCHING IN DIATOMS IS REGULATED BY BLUE LIGHT PHOTORECEPTORS

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The microorganisms of the phytoplankton have to adapt their photosynthetic apparatus to ambient light conditions in order to achieve an optimal photosynthetic performance. Diatoms are an ecologically important group of phytoplankton. However, little is known about their specific light intensity perception mechanism. In the present study, *Phaeodactylum tricornutum* was cultivated semi-continuously in a bioreactor under illumination with blue, red and white light with equal amounts of photosynthetically absorbed radiation ( $Q_{\text{phar}}$ ). For all light qualities, algae were grown under light-limited (LL) and saturating light (SL) conditions. Interestingly, cultures grown under illumination with red light exhibited an extraordinary low non-photochemical quenching (NPQ). This effect was observed in cells under both LL and HL growth conditions. In contrast, the NPQ of white and blue light-grown cultures was clearly higher and light intensity dependent. Analysis of the thylakoid membrane proteome of blue and red light grown cultures revealed that Lhcx1, the key protein for the regulation of NPQ in diatoms, was up-regulated in blue light cultures compared to red light cultures. Accordingly, two putative binding motives for the





blue light receptor (AUREO1a) were found in the promoter region of the Lhcx1 gene. The presented data demonstrates that the capacity of NPQ in diatoms is regulated by blue light-absorbing photoreceptors and not by other sensors like the redox state of the plastoquinone pool. If this would display a general feature of the photoacclimation of diatoms, the light intensity perception of this algal group would be remarkably different to that of higher plants and green algae.

### COMPARATIVE FUNCTIONAL BIODIVERSITY OF SEA ICE ALGAL COMMUNITIES

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Sea ice appears to be a hostile habitat with regards to its abiotic factors. In spite of these harsh conditions sea ice is densely populated by a still underexplored microbial community which represents an ecosystem of global significance. Our aim is to carry out comprehensive molecular and taxonomic analyses of representative sea ice samples from Antarctic and Arctic oceans. In a previous project a seminal dataset on 18S biodiversity and metatranscriptomes of Antarctic sea ice samples was generated. More than 20 18S rDNA libraries have been constructed and 5 metatranscriptomes were sequenced, but a large scale comparison of all data is lacking. The present project aims to compare Antarctic and Arctic sea ice algal communities. This will be achieved by constructing 18S rDNA/rRNA libraries to determine the total (hidden) biodiversity and to reveal the active part of the biodiversity of the sea ice community. Furthermore, the construction of cDNA libraries and sequencing of “Expressed Sequence Tags” (ESTs) from all samples will give an estimate of the abundance and nature of the transcripts present in the samples. By comparing the phylogenies we will correlate the presence and abundance of certain taxonomic groups to the abundance and nature of transcripts. Finally, possible correlations between biodiversity, transcriptional activity and abiotic factors will be analyzed and the newly generated Arctic dataset will be compared to the already established datasets from Antarctic sea ice communities.



**CHARACTERIZATION OF SULFATED EXOPOLYSACCHARIDES EXCRETED BY  
CYANOBACTERIA OF THE GENERA *ARTHROSPIRA*, *GLOEOTHECE* AND  
*PHORMIDIUM***

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Cyanobacteria are photoautotrophic gram-negative prokaryotes. Many of them produce slime layers and release polysaccharides. These exopolysaccharides (EPS) enhance the adhesive capacity of cells to surfaces and are suspected to be reliable for protection against desiccation or antibacterial agents (biofilms). Such EPS are of great interest in technological and industrial applications because of their rheological properties or their capability to remove toxic metals from polluted water or as pharmaceuticals e.g. with antiviral activities [1,2]. *Arthrospira platensis* SAG 21.99 (*A. plat.*) and *Phormidium* sp. SAG 47.90 (*P. sp.*) belong to the order of Oscillatoriales (subsection III of cyanobacteria) and both are multi-cellular, filamentous strains. *A. plat.* has to be equated taxonomically with *Spirulina platensis* (former name) [2] which is marketed as dietary supplement named 'Spirulina'. *Gloeotheca membranacea* SAG 26.84 (*G. memb.*) is an unicellular cyanobacterium and belongs to the subsection I (Chroococcales).

According to our analysis, the EPS of these three species show a complex composition with 7 to 8 different neutral sugars and uronic acids. The EPS are substituted with sulfate and pyruvate groups. Furthermore, proteinaceous compounds are bound to all three EPS. According to Ion Exchange Chromatography all EPS consist of 2 to 3 differently charged main fractions.

Detailed results of sugar composition of total EPS and EPS-subfractions of all three cyanobacteria species are presented.

[1] De Philippis R et al. (1998) FEMS Microbiol Rev 22:151-175.

[2] Rechter S et al. (2006) Antivir Res 72:197-206.



## TWO DIFFERENT EXOPOLYSACCHARIDES FROM CYANOBACTERIA OF THE GENUS *SYNECHOCYSTIS*

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Cyanobacteria can synthesize exopolysaccharides (EPS) in various amounts and of high variability consisting of 2 - 9 different monosaccharides [1]. Up till now only two amino sugars are known to occur in cyanobacterial EPS: N-acetyl-glucosamine and N-acetyl-galactosamine [2].

For the first time we identified N-acetyl-fucosamine (2-acetyl-amido-2,6-dideoxy-D-galactose) as a component of the EPS of *Synechocystis aquatilis* SAG 90.79 (*S. aquatilis*). The monosaccharide composition of these EPS is very different from that of other known cyanobacteria [2]. They contain about 20% of sulfate groups and mainly consist of only four sugars: fucose (42%), arabinose (34%), fucosamine (17%) and glucose (1%). According to NMR analyses *S. aquatilis* seems to build up a polysaccharide with irregular arrangement of the monomer sugar units.

In contrast, the EPS of *Synechocystis pevalekii* SAG 91.79 (*S. pevalekii*) are rather comparable to other cyanobacterial EPS [2] and contain mannose (25%), glucose (19%), galactose (12%), fucose (10%), rhamnose (8%), xylose (8%), glucosamine (5%) and arabinose (3%).

Beside polysaccharides also proteins are mentioned to occur in ethanol-precipitated fractions of purified cyanobacterial cultivation media [3]. About ~39% of protein were detected in the isolated EPS of *S. pevalekii*, but none in the EPS of *S. aquatilis*. Our results show the high heterogeneity of cyanobacterial exometabolites even within one genus.

[1] Bertocchi C et al. (1990) Arch Microbiol 150:558-563.

[2] De Philippis RS et al. (2001) J of Appl Phycol 13:293-299.

[3] Kawaguchi T, Decho AW (2000) Prep Biochem Biotechnol 30 (4):321-330.





## NOMENKLATUR IM GRENZBEREICH VON PHYKOLOGIE UND PROTOZOLOGIE: MARGESCHNEIDERTE CODES ODER EIN UNIVERSELLER BIOCODE?

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Als Phykologen, Protozoologen und Protistologen werden wir bei der Neuentdeckung und Beschreibung von Arten mit der Frage konfrontiert, welchen Nomenklatur-Code wir benutzen wollen. Auf den ersten Blick erscheint dies einfach: alle Organismen mit Chloroplasten werden dem botanischen zugerechnet und alle anderen dem zoologischen Code. Dass diese Zuordnung nicht mehr funktioniert, zeigen uns neuere Phylogenien sowie Taxa aus den Euglenen, Dinoflagellaten, Blaualgen. Bedeutet dies nun, dass wir für jedes Monophylum oder speziell für die Protisten einen eigenen Code benötigen? Oder schaffen wir es, durch Harmonisierung der existierenden Codes einen BioCode für alle Organismen zu etablieren? Welche weiteren Forderungen müsste dieser neue Code erfüllen, damit er unserer modernen taxonomischen Forschung dient?



# DNA - BARCODING WORKSHOP



## ABSTRACTS

In alphabetical order by first author



## ASSESSMENT OF SPECIES DIVERSITY WITHIN THE CHLORELLACEAE USING MORPHOLOGICAL AND MOLECULAR DATA (BARCODES)

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The family Chlorellaceae was morphologically defined as comprising solitary or seldom in colonies living bristle-less green algae which reproduce mainly by autospores. Molecular ribosomal SSU / ITS analyses changed the scope of this family in many ways. Beside the common spherical phenotype, several taxa with considerably different morphology, which were formerly classified into other families of coccoid green algae cluster within this family. Now taxa with bristles, mucilage envelopes, colonial morphology or reproduction by oogamy are to be included into the Chlorellaceae as well. This high morphological diversity combined with a close phylogenetic relationship provides a good opportunity to evaluate ribosomal regions as molecular barcodes for the Chlorellaceae. Here we present on the example of selected genera the delineation of species based on different molecular signatures, e.g. the compensatory base changes (CBC) concept or the 5.8S rRNA – ITS 2 region.

### NOTES:





## ASSESSING DISTRIBUTION PATTERNS IN TERRESTRIAL ALGAE FROM VARIOUS HABITATS: A DNA-BARCODING APPROACH

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Terrestrial algae tolerate broad ranges of and rapid changes in environmental conditions. Their ability to form resting stages makes them easily transported even over long distances. Thus it may be reasonable that terrestrial algae may occur worldwide everywhere where environmental conditions are favorable. This is further supported by ongoing research of our group which focuses on algae from soils and other terrestrial habitats from distant geographical areas and diverse habitats. Ecuador tropical mountain rain forest, dry steppe vegetation and hypersaline Solonchak soils of Ukraine, temperate soils of Germany, and isolates from Arctica and Antarctica were studied. Despite their differences, the studied sites may even be rather similar at certain seasons or substrates and thus comparable to test for distribution patterns. As DNA barcodes, we employed the ITS2 rDNA for green algae, the plastid-encoded psbA-rbcL spacer for Xanthophyceae and segments of 18S rDNA for diatoms. As an example, for green algae of the tropical mountain forest new lineages were found, but at the same time barcodes identical with those from temperate regions as well. A similar situation seems to prevail also for terrestrial diatoms sampled in the tropical mountain forest. For *Chlorella*-like isolates and Tribonemataceae (Xanthophyceae) from Antarctic soils no phylogenetic clades corresponding to a limited geographical region were found, but the presence of populations distinct from those of other regions were indicated by the highly variable barcode sequences.

### NOTES:



## PITFALLS OF DNA BARCODING METHODS: THE CRYPTOPHYCEAE AS A TEST CASE

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DNA barcoding – the identification of species by means of short variable DNA regions – has become common in animals and fungi. In embryophyte plants, macroalgae, but also in protistan lineages, efforts are going on to establish DNA barcoding systems using different types of barcode markers. The accuracy of a DNA barcoding system highly depends in density of taxon sampling, but also in how well a group is systematically characterized. Identification of species will fail if taxa are poly- or paraphyletic. Since barcode markers are usually short, identification engines rely on searches constraint to the group in question. Different from embryophytes or animals, however, many protistan lineages are systematically not well characterized and an *a priori* assignment to constrain the search to a group may be difficult to impossible (e.g. in environmental sequencing or picoplankton), which poses additional challenges on protistan barcoding projects. The performance of identification with the blast suite e.g. highly depends in taxon sampling, barcode marker, chosen blast settings, but also proves to be highly vulnerable to sequencing errors.

The establishment of DNA barcoding systems for an identification of microscopic organisms should be done more careful than currently practiced for animals.

### NOTES:



## ITS-2 AS UNIVERSAL DNA BARCODE MARKER FOR PROTISTS

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DNA sequences are a powerful tool in systematics and molecular phylogeny of protists and have given new insights into the evolution of this group of organism. However, it has not yet proven as rewarding for taxonomic categorization. DNA Barcoding might close this gap. The goal of the International Barcoding Initiative is to find a single, universal, short DNA fragment, which is easy to sequence and leads to a clear species identification. The mitochondrial cytochrome oxidase subunit I (coxI) was proposed by the barcoding initiative and is mostly used by zoologists. However, for certain groups like higher plants and several groups of microalgae coxI is too conserved to separate organism at the species level. In our study we used the second Internal Transcribed Spacer (ITS-2) of the nuclear ribosomal gene cistron. This locus has suggested a high degree of predictability across eukaryotes, is easy to sequence, and its secondary structure can be used for comparison at species and generic level. The main objection to the ITS-2 usage as barcode marker was the difficulty in aligning these sequences and the prediction of the secondary structure. However, with the help of the new computer programmes and the easy recognition of two hallmarks in the secondary structure, these problems are resolved. ITS-2 also gives additional information about the species concept. For example, compensatory base changes (CBC) in the 30 bp highly conserved region of Helix III of ITS-2 correlate with the extent of sexual compatibility. A difference of even one CBC in this region predicts a total failure of crossing. The question is whether use of addition of highly conserved region of ITS-2 can be used as DNA Barcode marker for describing species of protists.

### NOTES:



## ASSESSING THE HYPERVARIABLE D1-D2 REGION OF THE LSU rDNA FOR CILIATE BARCODING

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The barcode of life for identifying species is the front line in discovery, monitoring and research. It is specifically useful for micro-organisms, which for technical and biological reasons are usually difficult to identify for all but a few experts. Barcoding reduces ambiguities due to morphological identifications and can unmask morphologically similar species. This is why the Consortium for the Barcode of Life (CBOL: <http://www.barcodeoflife.org/>) has initiated the Protistan Working Group (ProWG). In the framework of this initiative, we have tested two hypervariable gene regions, the V4 region of the small subunit (SSU) ribosomal DNA (SSU rDNA) as well as the D1-D2 region of the large subunit (LSU) rDNA as potential DNA barcode markers in ciliates. Ciliates have the advantage of a relatively solid morphospecies concept as a basis for DNA barcoding, even though cryptic species are also known. We analyzed 15 species (54 different strains) of the *Paramecium aurelia*-complex, as well as nine other *Paramecium* species (15 strains in total). We found a large overlap of genetic distances in the V4 region between intra- and interspecies, suggesting that this gene fragment is unsuitable for DNA barcoding. By contrast, for the D1-D2 region, the variation within species is in a significant number of cases lower than divergence among (sibling) species, thus showing the characteristic “barcode gap”. Because the preservation and deposition of a voucher-specimen is a prerequisite in DNA barcoding and PCR-amplification of a barcode gene relies on “destructive” sampling, we also suggest a way to accomplish this goal.

### NOTES:



## DIATOM DNA BARCODING REVISITED

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DNA barcoding is a tool that uses a short, standard portion of DNA to identify organisms based on a shared database of DNA sequences. In diatoms, a consensus on an appropriate DNA barcode has not been reached, and several markers are still in discussion (e.g. nuclear 18S rRNA, 28S rRNA, 5.8S rRNA + ITS2, mitochondrial *cox1*, plastid *rbcL*). Because conventional morphological identification of diatoms demands i. a. specialised in-depth knowledge, DNA barcoding is especially interesting in regard to high throughput methodology (e.g. NGS) used in the analysis of environmental samples. Diatoms are present in all types of water bodies and their species diversity is influenced greatly by environmental conditions. This means that diatom occurrence and abundances are suitable indicators of water quality.

Therefore we have established standard laboratory procedures for DNA barcoding in diatoms to develop a standardised identification tool to serve routine water quality assessments. We selected the short V4 segment (about 390 bp) of the SSU (18S) rRNA gene which is applicable for the identification of diatom taxa, and elaborated a routine protocol including standard primers for this group of microalgae. The SSU of about 200 taxa, representing limnic diatom diversity, were analysed to ensure the universality of the primer binding sites and the discriminatory power of the proposed barcode region. This DNA barcode is also currently practically applied to investigate the water quality in the river system Neisse/Oder (Czech Republic and Germany). The results are cross referenced to the findings of morphological analysis of the same samples.

### NOTES:





## ABSTRACTS

## TALKS AND POSTERS

In alphabetical order by first author



## MORPHOLOGIC DATA UNRAVEL THE PARAPHYLY OF THE TINTINNID GENUS *FAVELLA* IN SSU rRNA TREES (CILIOPHORA, SPIROTRICHEA, TINTINNINA)

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Phylogenies of the small subunit ribosomal RNA (SSU rRNA) gene reveal a paraphyly of the genus *Favella* with two distinctly separate clusters: one branched rather early in the tintinnid evolution just after the genus *Eutintinnus*, the other groups with the more highly developed genera *Rhabdonella* and *Metacylis*. Recently, the SSU rRNA gene sequence and ciliary pattern of *Favella ehrenbergii*, the type of the genus, were provided. The somatic ciliature comprises a right, left, and lateral ciliary field as well as ventral and dorsal kineties. As common, the ventral kinety is monokinetidal and abuts on the right field. The lorica wall has a monolaminar texture with alveoli and a smooth surface. *Favella arcuata*, which clusters with *Rhabdonella* and *Metacylis* in the SSU rRNA genealogies, differs from *F. ehrenbergii* in (i) a ventral kinety composed of a monokinetidal anterior and a dikinetidal posterior portion separate from the right field by an unciliated stripe and (ii) a lorica wall with pores and surface ridges. These lorica features are shared by *Rhabdonella* and *Metacylis angulata*. The differences in the morphologies and gene sequences justify not only the establishment of a new genus, but also its separation from *Favella* on familial level. Supported by the Austrian Science Foundation (FWF, Project P20461).

## RNAi-REGULATED CHROMATIN-MODIFICATIONS ENABLE MUTUAL EXCLUSIVE TRANSCRIPTION

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Small RNAs (siRNAs) are involved in post-transcriptional gene-silencing (PTGS) and transcriptional gene-silencing (TGS). In the latter case, the regulation of transcriptional activity becomes realized by covalent modifications of the chromatin. Here, we investigated the post translational histone modifications which enable exclusive transcription of subtelomeric surface antigen genes. Our results indicate that active and silent antigens show a different pattern of almost all analyzed histone modifications and *in vitro* run on experiments indicate these modifications to be responsible for transcriptional activity. We could demonstrate that transcriptional activation of surface antigen genes is correlated with accumulation of H3K4me3 and H3K9ac in the 5'-region of the genes. Consequently, transcriptional silent genes reveal these post-translational histone modifications predominantly in their 3'-regions showing a mirror image of active genes. We therefore conclude that transcriptional control of surface antigen genes is controlled by the initiation of transcriptional





activity. Surprisingly, also high levels of H3K27me3 can be detected in some areas of the genes which might be responsible for the general dynamics of these loci because the switching behaviour of surface antigen expression requires that the chromatin state can anytime become altered. As this variability, as well as serotype stability is controlled by small RNAs, expression and inheritance of serotypes are epigenetically controlled.

### **NUCLEARIA SP. AUS DEM ZÜRICHSEE MIT LOS SYMBIONTOS LIVE IN CONCERT**

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Bei der Erforschung von Lebensgemeinschaften, die Protisten und Bakterien eingehen, werden immer mehr enge und persistente Assoziationen zwischen eu- und prokaryotischen Zellen aufgedeckt. Solche Assoziationen werden ganz allgemein als Symbiosen bezeichnet, die von Mutualismus bis hin zu Parasitismus reichen. In dieser Studie wurden eine nackte filose Amöbe und deren unterschiedliche Bakteriensymbionten in Kultur untersucht. Der Einzeller wurde aus dem Zürichsee isoliert und gehört der Gattung *Nuclearia* an. Die meisten beschriebenen Arten dieser Gattung können zwei Erscheinungsformen annehmen (kugelig oder amöboid). Unser Isolat weist eine phylogenetische Identität von 99-100% mit *Nuclearia thermophila* auf (Yoshida et al. 2008, jenes Isolat stammt aus 30°C warmen Wasser des Yunoko Sees – Japan). Allerdings unterscheiden sich *Nuclearia thermophila* und *Nuclearia* sp. (Zürichsee) in vielen Charakteristika. Bemerkenswert ist, dass bei der von Yoshida et al. (2008) entdeckten Amöbe keine Symbionten beobachtet wurden, wohingegen *Nuclearia* sp. (Zürichsee) eine Schleimhülle mit schön angeordneten und ausgerichteten Stäbchenbakterien aufweist. Mit Hilfe von CARD-FISH und elektronenmikroskopischen Untersuchungen wurden zudem auch im Zellinnern der Amöbe Bakterien (*Gammaproteobakterien*) nachgewiesen. Die Symbiose der *Nuclearia* sp. mit den Endosymbionten wurde mit Wachstumsexperimenten, kombiniert mit der Quantifizierung der zellinternen Endosymbionten untersucht. Bei der mikroskopischen Beobachtung der Bakterien in der Schleimhülle wurde offensichtlich, dass es sich um mehrere, morphologisch unterschiedliche Ektosymbionten handelt. Unser Isolat geht also gleichzeitig mit mehreren Bakterienarten Symbiosen ein. Die Resultate legen nahe, dass die Beziehung mit den Endosymbionten eine obligate Symbiose ist, wohingegen die Interaktion mit den Ektosymbionten eine fakultative Symbiose mit unterschiedlichen Arten darstellt. Welche Rolle die Bakteriensymbionten beim Abbau toxischer filamentöser Cyanobakterien einnehmen (Hauptnahrungsquelle der Amöbe), ist ebenso Teil dieser Studie.



## A MARINE ENVIRONMENTAL PYROSEQUENCING VIEW OF TRADITIONALLY TERRESTRIAL AND FRESHWATER COLPODEAN CILIATES

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Our knowledge of any clade of organisms is affected by where and how sampling has occurred, and by the methodologies that have been implemented in analyzing the samples. Colpodean ciliates are prime an example. Culture-dependent investigations that have focused on this clade have targeted terrestrial and freshwater environments. These studies have concluded that the almost 200 described species are primarily found in these environments, with only three closely related marine species known. Here we show that intensive culture-independent investigations using pyro-sequencing have uncovered additional marine colpodean lineages. Rather than being primarily terrestrial and freshwater, colpodean ciliates may be comprised of numerous new marine species that await culturing and morphological description.

## **OBERTRUMIA AUREA (CILIOPHORA) AND ITS TOXIC FOOD, THE FILAMENTOUS CYANOBACTERIUM *PLANKTOTHRIX RUBESCENS***

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*Planktothrix rubescens*, a harmful filamentous cyanobacteria, is the most dominant primary producer in Lake Zurich. This cyanobacterium stores several secondary metabolites such as microcystins (MC) which serve as grazer defense and are considered to be toxic for eukaryotes. Nevertheless, a few organisms are described to feed on *P. rubescens*, mainly protists. The ciliate *Obertrumia aurea* (Nassophorea) belongs to these organisms and was first observed in Lake Zurich in 1941 by E. Thomas. Since then, there was no new report of *O. aurea* in Lake Zurich until 2009 when we observed and isolated this ciliate.

We identified the species by live observations and protargol staining. In growth experiments we could show that the ciliate abundance is correlated to food concentration and that the highest growth rates are reached at concentrations found in the lake during *P. rubescens* blooms. However, in the pelagic zone we never observed *O. aurea* in the dense *P. rubescens* layer during summer or in other depths during the mixis of the lake (winter / spring). *O. aurea* could only be detected in samples taken from the litoral zone. It still remains an open question why *O. aurea* is not more abundant in the lake, although there is plenty of *P. rubescens* available. We suppose that top-down control by predators and parasites limits the ciliate population in the open water column. In addition, grazing experiments were done by offering cyanobacterial species that produce other metabolites than MC. We found that not all filamentous cyanobacteria are suitable as a food source for *O. aurea* and that the ciliate might need time to adapt to some cyanobacterial species.



## ENVIRONMENTAL SELECTION OF PROTISTAN PLANKTON COMMUNITIES IN HYPERHALINE ANOXIC DEEP-SEA BASINS, EASTERN MEDITERRANEAN SEA

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Deep hypersaline anoxic basins in the Eastern Mediterranean Sea are some of the most polyextreme habitats on Earth due to the combination of nearly saturated salt concentration, absence of light, anoxia, and high hydrostatic pressure. The geographical proximity and unique chemical characteristics of these basins make them ideal for testing hypotheses on influencing effects of environmental selection and distance on the shaping of protistan communities. T-RFLP analyses were performed on water samples from the brines and seawater/brine interfaces of five basins: Discovery, Urania, Thetis, Tyro and Medee. Analyses of similarities between the ten individual T-RF profiles based on both, peak abundance and peak incidence were used to assess the beta-diversity across biogeographic barriers (between brines of different isolated DHABs) and along environmental gradients (interface samples versus brines). While a significant distance effect on the separation of the samples from these 5 basins was not detected, environmental gradients from the brines across the interface into the “regular” deep-sea water appear to act as biogeographic barriers that likely lead to environmental selection in the DHAB protistan plankton communities. Environmental segregation appears to be responsible for observed dissimilarities among the individual predominant populations, with sodium, magnesium, sulfate and oxygen emerging as the dominant driving environmental factors.



## MYXOMYCETES (AMOEBOZOA) PHYLOGENIES

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The myxomycetes plasmodial slime-moulds are distinctive amoebae that form macroscopic fruiting bodies, very common on litter or decaying logs in forests. These protists form a monophyletic taxon in the phylum Amoebozoa. Myxomycetes (also called Myxogastria) include more species (ca. 900 recognized) than all other Amoebozoa. They are present in nearly every terrestrial environment but also, as amoebae or flagellates, in aquatic environments where they cannot form fruiting bodies. They have a complex life cycle culminating in the formation of mainly macroscopic fruiting bodies highly variable in shape and colour. We summarize here the major phylogenetic results about Conosa, "Mycetozoa" and Myxomycetes of the past ten years and present novel findings. At a deeper level, we find strong support for the monophyly of Myxogastria and Dictyostelia, while Protostelia appear to be polyphyletic. In Myxomycetes, there are two major divisions according to spore color, i.e. a dark-spored clade (comprising the orders Echinosteliales, Physarales and Stemonitales), and a bright-spored clade (Trichiales and Liceales). Possible underlying evolutionary scenarios will be discussed.



**REDISCOVERY OF *PARAMECIUM CHLORELLIGERUM* KAHL, 1935, A SECOND ZOOCHLORELLAE-BEARING *PARAMECIUM* SPECIES BELONGING TO THE *P. NEPHRIDIATUM* CLADE**

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There is a common believe that the genus *Paramecium* contains only one green (by symbiotic algae) species, viz., *P. bursaria* Focke. However, Kahl (1935) described a second green species, *P. chlorelligerum*, which escaped most *Paramecium* students because he mentioned it only in the 1935 addendum to his four previous monographs. We rediscovered *P. chlorelligerum* in a moorland pond of southern Germany (Kreutz and Foissner 2006), where it occurred together with *P. bursaria* and many microaerophilic ciliates, such as *Loxodes striatus* and *Euplotes diadalos*, and a great abundance of the sulphur bacterium *Beggiatoa alba*. We investigated *P. chlorelligerum* with classical (in vivo and in silver preparations) and molecular (18S rDNA) methods. This showed that it belongs to the *P. nephridiatum* clade, while *P. bursaria* belongs to the *P. putrinum* clade, demonstrating that a green algal symbiosis developed independently in two clades. Morphologically, *P. chlorelligerum* and *P. bursaria* differ by the following features: (i) with vs. without an ellipsoidal swimming state, (ii) caudal cilia on average 29  $\mu\text{m}$  vs. 18  $\mu\text{m}$  long, (iii) micronucleus on average 5.7  $\times$  2.4  $\mu\text{m}$  vs. 14  $\times$  7  $\mu\text{m}$  in size; (iv) contractile vacuoles with collecting vesicles vs. radial collecting canals, (v) contractile vacuoles each with a single vs. two to three excretory pores, (vi) symbiotic algae on average 7.7  $\times$  6.2  $\mu\text{m}$  vs. 5.5  $\times$  5.1  $\mu\text{m}$  in size.

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## IDENTIFICATION, FUNCTIONAL ROLES AND ECOSYSTEM SERVICES OF PROTOZOA IN SOIL

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Protozoa are the major consumers of bacterial production in soil, forming the base of the heterotrophic eukaryotic food web that channels the energy flow via bacteria to higher trophic levels in soil (i.e. the bacterial energy channel). Despite small sizes of protozoa in soil (5-200  $\mu\text{m}$ ), their high abundance and fast turnover make them one of the key regulators of bacterial biomass and nutrient cycling.

Even though they occupy important functional roles, we still have only a vague idea on the identity of the dominant protist taxa in soil. One major reason for the general ignorance of protists in environmental studies is methodological difficulties in quantifying small protists in the opaque soil environment, their uneven distribution and the lack of taxonomic expertise. However, recent developments in high-throughput sequencing and in the cultivation of so-called uncultivable protists now allow closing the methodological gap on this functionally important trophic link in the soil food web.

Within the EU-project EcoFINDERS we aim at designing DNA-based barcodes for dominant protozoan taxa in soil. Protozoan diversity will be compared between five long-term observatories across Europe using high-throughput sequencing.

Cultivation of amoebae from Dutch and Sardinian grassland soils to improve phylogenetic information of this rarely studied assembly of organisms indicates an enormous diversity. Based on culture isolates, we constructed phylogenetic trees based on two genes (18s rDNA and cytochrome oxidase 1) to decipher deep-relationships among protozoa and to identify genetic barcodes targeting individual taxa for pyrosequencing, which we are currently conducting.

Ecological studies investigating protozoan grazing of bacteria has been shown to be a major structuring force for bacterial diversity in the plant rhizosphere. Therefore we suggest that protozoa may provide an important ecosystem service by removing pathogenic microorganisms from soils. Laboratory experiments with selected dominant protozoan taxa will be performed to investigate the effects of protozoan predation on different pathogenic and beneficial soil microorganisms.

Preliminary results indicate that bacterial spores are resistant to predation i.e. germinate and grow inside food vacuoles, while vegetative bacteria are killed by protozoan predation. This will be tested along with the dependency of morphology and feeding behaviour of protozoa on bacterial predation and related to soil ecosystem services.



## MOLEKULARE MARKERGENE ZUR UNTERSUCHUNG BIOGEOGRAPHISCHER MUSTER VON DIATOMEEN AM BEISPIEL VON *PINNULARIA VIRIDIS* (BACILLARIOPHYCEAE)

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Diatomeen (Bacillariophyta) sind eine weit verbreitete Gruppe heterokonten Algen und spielen eine wichtige Rolle in biogeochemischen Zyklen. Ihr wichtigstes Charakteristikum ist die aus Siliziumdioxid ( $\text{SiO}_2$ ) bestehende Zellwand, anhand derer sie identifiziert werden können. Da die Schalenmorphologie von Diatomeen jedoch innerhalb einer Art stark variieren kann, bringt die Identifizierung anhand morphologischer Details Schwierigkeiten mit sich. Eine alternative Möglichkeit zur Identifizierung von Diatomeen bieten molekulare Methoden, wie DNA-Barcoding. In dieser Studie wurden verschiedene Barcodemarker (18S rDNA, 28S rDNA, ITS, *cox*, *rbcl*) getestet. Dabei erwiesen sich das *rbcl*-Gen und die D2-D3-Region der 28S rDNA als am praktikabelsten. Anhand dieser beiden Barcodemarker wurde die genetische Variation der Diatomeenart *Pinnularia viridis* in drei Gewässern untersucht. Im Vergleich dazu wurde auch die morphologische Variation dieser Art analysiert. Die Ergebnisse dieser Untersuchungen zeigen, dass es sich bei den untersuchten Zellen von *P. viridis* sowohl um eine Morphospezies als auch um eine genetische Einheit handelt.

## ANALYSIS OF ENDOGENOUS REGULATORY SHORT RNAs INVOLVED IN THE MUTUAL EXCLUSIVE GENE EXPRESSION

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Antigenic variation is a powerful mechanism enabling hiding or adhesion phenotypes in eukaryotic protists. Increasing evidence indicates such variable surface antigen coats to be regulated by RNA interference. Therefore, small regulatory RNAs are necessary to silence all but one gene of the antigen gene family. Characterising this mutual exclusive mechanism in the ciliate *Paramecium*, northern blots unexpectedly show two classes of siRNAs (~20nt and ~22nt) and at least one of them (~20nt) is dependent of RdRP3, a RNA dependent RNA-Polymerase. Furthermore, biochemical treatment with phosphatase and periodate indicate that both classes of siRNAs are 5'-monophosphorylated and surprisingly show a covalent modification at the 3'-end. In agreement with the silencing phenotype of Hen1, a RNA-Methyltransferase, we conclude that siRNAs involved in the regulation of surface antigens carry a 2'-O-Methylation at their 3'-end which distinguishes them from other small RNAs associated with post-transcriptional silencing. Further experiments suggest that these small RNAs are responsible for epigenetic control of surface antigen expression by placing permanent marks at the genes.



## SHEDDING LIGHT ON VAMPIRES: THE PHYLOGENY OF VAMPYRELLID AMOEBAE REVISITED

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With the advent of molecular phylogenetic techniques the polyphyly of naked filose amoebae has been proven. They are interspersed in several supergroups of eukaryotes and most of them already found their place within the tree of life. Although the 'vampire amoebae' have attracted interest since the middle of the 19th century, the phylogenetic position and even the monophyly of this traditional group are still uncertain. In this study clonal co-cultures of eight algivorous vampyrellid amoebae and the respective food algae were established. Culture material was characterized morphologically and a molecular phylogeny was inferred using SSU rDNA sequence comparisons. We found that the limnetic, algivorous vampyrellid amoebae investigated in this study belong to a major clade within the Endomyxa CAVALIER-SMITH, 2002 (Cercozoa), grouping together with a few soil-dwelling taxa. They split into two robust clades, one containing species of the genus *Vampyrella* CIENKOWSKI, 1865, the other containing the genus *Leptophrys* HERTWIG & LESSER, 1874, together with terrestrial members. Supported by morphological data these clades are designated as the two families Vampyrellidae ZOPF, 1885, and Leptophryidae fam. nov. Furthermore the order Vampyrellida WEST, 1901 was revised and now corresponds to the major vampyrellid clade within the Endomyxa, comprising the Vampyrellidae and Leptophryidae as well as several environmental sequences. In the light of the presented phylogenetic analyses morphological and ecological aspects, the feeding strategy and nutritional specialization within the vampyrellid amoebae are discussed.





**EXPANDING CHARACTER SAMPLING FOR THE MOLECULAR PHYLOGENY OF  
EUPLOTID CILIATES (PROTOZOA, CILIOPHORA) USING THREE MARKERS, WITH A  
FOCUS ON THE FAMILY URONYCHIIDAE**

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Although euplotida ciliates are widely used as model organisms in multiple fields of biology, details of their phylogenetic relationships remain unresolved despite a rich history of investigation with small subunit (SSU) rDNA sequences and other characters. Here, six genera in *Diophrys*-like complex and three other euplotid genera are sampled for SSU-rDNA, ITS1-5.8S-ITS2 and LSU-rDNA, and their phylogenies were inferred with unconstrained and constrained analyses. In general, the concatenated analyses infer more reliable, less ambiguous phylogenies with higher node support values. The following conclusions can be made: 1) four well-supported clades are consistently detected in the family Uronychiidae, forming into two subgroups, which challenge the traditional arrangement based on morphological similarities; 2) the subfamily Diophryinae is paraphyletic; 3) the monophyly of *Paradiophrys* and the establishment of *Apodiophrys* and *Diophryopsis* is fully supported by concatenated data; 4) *Apodiophrys* and *Paradiophrys* form independent lineages, at the subfamily level, from other *Diophrys*-like genera; and 5) the highly specialized *Pseudodiophrys* nests within *Diophrys*.



## CILIATE COMMUNITY COMPOSITION IN TWO LAKES OF DIFFERENT TURBIDITY RESULTING FROM GLACIER RETREAT

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The rapid glacier retreat in many parts of the world has resulted in large changes in water transparency with some lakes turning more turbid or turning transparent when the connection to the glacier is lost. To understand how those changes affect planktonic ciliates, we investigated the community structure in one turbid and one transparent alpine lake both originating from the same glacier. In the turbid lake, we found a 3-fold higher ciliate abundance and a 6-fold higher number of species compared to the transparent one (17.1 vs. 5.9 ind. ml<sup>-1</sup> and 31 vs. 5 species). In these lakes, we detected predominantly euplanktonic species such as the prostomatids *Balanion planctonicum* and three *Urotricha* spp. (91% of the total abundance in the turbid and up to 99% in the transparent lake) and haptorids such as *Askenasia* cf. *chlorelligera*. However, particle-associated ciliates were exclusively found in the turbid lake. To understand the species-specific vertical distribution of the ciliates in these lakes and possible factors that may influence them, optical characteristics, abiotic parameters, and biotic factors such as bacteria, phyto- and zooplankton were also assessed. In both lakes, the ciliates vertical distribution is mainly influenced by food availability whereas in the highly transparent lake also predation and ultraviolet radiation seem to influence the pattern found. Our results suggest that changes in lake turbidity caused by glacier retreat affect the composition and vertical distribution of the planktonic ciliate community.

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## THE SEC6 PROTEIN IS REQUIRED FOR FUNCTION OF THE CONTRACTILE VACUOLE IN *CHLAMYDOMONAS REINHARDTII*

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Contractile vacuoles (CVs) are key players of osmoregulation in many protists. To investigate the mechanism of CV function in *Chlamydomonas*, we isolated novel osmoregulatory mutants. 4 isolated mutant cell lines carried the same 33,641 b deletion rendering the cell lines unable to grow under strong hypotonic conditions. One mutant cell line (Osmo75) was analyzed in detail. Mutant cells contained a variable CV morphology (multiple small CVs (major phenotype), enlarged 1 or 2 CVs or no light microscopically visible CVs at all). These findings indicate that the mutant is impaired in homotypic vacuolar and exocytotic membrane fusion. Furthermore the mutants displayed a long flagella phenotype. One of the affected genes is the only SEC6 homologue in *Chlamydomonas* (CreSEC6). The SEC6 protein is a component of the exocyst complex required for efficient exocytosis. Transformation of the Osmo75 mutant with CreSEC6GFP construct rescued the mutant completely (osmoregulation and flagellar length). Rescued strains overexpressed CreSEC6 and displayed a modified CV activity. CVs were significantly larger, whereas the CV contraction interval remained unchanged leading to increased water efflux rates. These results indicate that the CreSEC6 is essential for CV function and required for homotypic vesicle fusion during diastole and water expulsion during systole. In addition CreSEC6 is not only necessary for CV function, but possibly influencing the CV cycle in an indirect way and flagellar length control in *Chlamydomonas*.



## PHYLOGENETIC RELATIONSHIPS OF THE HIMATISMENIDA AND TAXONOMY OF AMOEOBOZOA

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The order Himatismenida Page, 1987 (Amoebozoa) comprises discoid lobose amoebae in which part of the plasma membrane surface is covered with a flexible layer of variously structured organic material secreted by the cell. Recent molecular phylogenetic studies of lobose amoebae led to establishment of the current classification of the order Himatismenida that now comprises two suborders: Parvamoebina Cavalier-Smith et Smirnov, 2011 with the family Parvamoebidae (genus *Parvamoeba*) and Tectiferina Cavalier-Smith et Smirnov, 2011 with the families Cochliopodiidae (genera *Cochliopodium* and *Ovalopodium*) and Goceviidae (genera *Gocevia* and *Paragocevia*). A separate order Pellitida Smirnov et Cavalier-Smith, 2011 has also been established recently to accommodate one of the former members of the Himatismenida. In this contribution we will perform an overview of the diversity of the Himatismenida and similar amoebae and analyse their phylogeny based on morphological characters and sequences of nuclear small-subunit ribosomal RNA, actin and mitochondrial cytochrome oxidase 1. We conclude that (1) *Parvamoeba rugata*, the type species of *Parvamoeba* demonstrates some features of the himatismenid locomotive morphotype, while a new species of *Parvamoeba* that we isolated and studied recently, is not distinguishable from the typical cochliopodiids morphologically. Therefore, an inclusion of *Parvamoeba* in the order Himatismenida suggested earlier by actin gene sequence analysis is also confirmed by morphological characters and corresponds to the genuine phylogenetic relationships. (2) Based on ultrastructure and molecular data, the genera *Gocevia* and *Endostelium* should be included in the order Pellitida, an isolated, deeply-branching clade of Amoebozoa, not related to the Himatismenida. Therefore, the himatismenid morphotype has evolved several times independently within lobose amoebae. (3) The ability to “liberate” part of the plasma membrane surface from the external cell coat has evolved at least five times within naked lobose amoebae and is not correlated to the cell coat thickness or structure. (4) While the evolutionary relationships of the Himatismenida revealed with SSU rRNA and COI are more or less congruent, at least at the levels of families and order, actin demonstrates a different evolutionary pattern, complicated by the presence of multiple paralogs.

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## WAS BEDEUTET DAS GBIF-NETZWERK FÜR DIE BIODIVERSITÄTSFORSCHUNG IN DER ALGENKUNDE UND PROTOZOLOGIE?

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Die Global Biodiversity Information Facility ermöglicht den freien Zugang zu Biodiversitätsdaten im Internet. Im Januar 2012 sind bereits über 322 Millionen Datensätze publiziert. Die Vision dieser Initiative ist der ungehinderte Zugang zum Weltwissen der Biodiversität für jede Organismengruppe und jede Nutzungsmöglichkeit. Mit wachsender Datenfülle werden diese Daten zunehmend nutzbar für wissenschaftliche Auswertungen zur Verbreitung der Arten, zum Niche Modelling, für Fragen des Klimawandels und des Naturschutzes.

Im Vergleich zur Situation bei Wirbeltieren und höheren Pflanzen sind nur relativ wenige Fundinformationen und referenzierte Bilddaten für Ein- und Wenigzeller öffentlich im Internet zugänglich. In der Gewässerforschung dominieren bisher Biodiversitätsdaten zu Fischen und submersen Makrophyten, während für Algen und Protozoa noch erheblicher Nachholbedarf in der Datenmobilisierung besteht. Speziell im Bereich mikroskopischer Ein- und Wenigzeller wird die zunehmende Verarbeitung und Publikation von referenzierten Bilddaten zu einem An Schub für bessere Artenkenntnis und feinere Auflösung bei ökologischen Untersuchungen führen können. Um die Ziele der verbesserten Nutzung von Observations- und Bilddaten zu erreichen, mobilisiert GBIF-D *Pflanzen, Algen & Protisten* Biodiversitätsdaten und berät Datenhalter, die ihre Daten mit der Scientific Community teilen wollen (BMBF-Projekt 01 LI 1001 A).

Über die nutzerorientierte Portalfunktion hinaus werden von der Biodiversitätsinformatik innerhalb des GBIF-Netzwerkes Standards und Services entwickelt (TDWG-Standards, BioCASE-Providersoftware, Datenqualitätschecks), um Biodiversitätsdaten zu publizieren, auszutauschen, zu annotieren und in die virtuelle Umgebung der Cybertaxonomy einzubinden. Diese Entwicklungen sind teils für Datenhalter, teils für Nutzer aus Forschung, Monitoring und Behörden nützlich.



## INTERACTIVE EFFECTS OF DISSOLVED NUTRIENTS AND PREY ON POTENTIALLY HARMFUL MIXOTROPHIC DINOFLAGELLATES

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Only recently, many potentially harmful bloom-forming dinoflagellates have been identified to be mixotrophic, allowing them to combine photosynthesis and phagotrophy. However, even though their feeding behavior may be of crucial importance for their bloom dynamics, not much is known about grazing preferences and rates or the relevance of phagotrophy under different environmental conditions for many species. In the present study feeding preferences and grazing rates were determined for different mixotrophic dinoflagellates, as well as interactive effects of dissolved phosphate and prey concentrations for two of them. Phosphate increased both of the dinoflagellates' biovolumes when prey was absent or low, while increasing prey concentrations increased their biovolume when phosphate was limiting. At high phosphate concentrations, however, both of the dinoflagellates' biovolumes decreased with increasing prey concentrations, indicating that they were not able to take advantage of their prey anymore and control their abundances, when these were the better competitors at high nutrient concentrations. Grazing rates decreased with increasing phosphate concentrations for one of the dinoflagellates at low prey concentrations, while grazing rates were independent of prey or nutrients for the other one. Our results indicate that different bloom-forming mixotrophic dinoflagellates may have different impacts on their prey and thus food web dynamics depending on environmental conditions, but that phagotrophy is a relevant survival mechanism especially under nutrient limitation, thus potentially prolonging the bloom duration when nutrients become depleted.

## CRYPTIC DIVERSITY WITHIN THE CHOANOFLAGELLATE MORPHOSPECIES COMPLEX *CODOSIGA BOTRYTIS* – PHYLOGENY AND MORPHOLOGY OF ANCIENT AND MODERN ISOLATES

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Choanoflagellates are closely related to metazoans and fungi according to recent phylogenetic studies; therefore the systematics of these organisms is of particular interest. The choanoflagellate morphospecies *Codosiga botrytis* is the first described choanoflagellate, and is one of the most frequently reported choanoflagellate species. In this study we present phylogenetic and morphological data on eight different strains of *Codosiga botrytis*. Among these there are five ancient strains; these cultures have been established from up to 43,000 years old cysts from Siberian permafrost. We found that based on the variable V4 region of the small subunit



(SSU) of the rDNA, all the investigated freshwater isolates of *Codosiga botrytis*, together with *Sphaeroeca volvox*, form a cluster at the base of all other choanoflagellate species. Moreover, the morphospecies described classically as *Codosiga botrytis* contains at least four different genotypes separated by considerably high genetic distance. All these 'cryptic species' have identical general morphology and cell structure. Strains have a similar life cycle with several different life forms and large morphological plasticity. For the first time we were able to establish cultures from cryo-conserved cysts of choanoflagellates. The ancient strains did not differ significantly in partial SSU rDNA from the modern ones. Besides, no biogeographically pattern could be established. This fact and the low genetic distances of some strains from remote locations support the distribution of dormant stages via air.

### ONTOGENESIS OF *LEPTOPHARYNX COSTATUS COSTATUS* (CILIOPHORA, MICROTHORACIDA) AND ITS PHYLOGENETIC SIGNIFICANCE

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We studied the ontogenesis of a German population of *Leptopharynx costatus costatus*, using standard methods. The ontogenesis is complex due to the preoral kineties and the postoral complex. Fission is homothetogenic (transverse), occurring in freely motile (non-encysted) condition. The parental oral apparatus is reorganized, except of the adoral membranelles. The first sign of division is an elongation of the micronucleus. Stomatogenesis is mixokinetal: the opisthe membranelles 1 and 2 are formed by the oral primordium, while membranelle 3 is produced by the posterior portion of somatic kinety 1. The nascent kinetosomes are generated by the anterior portion of the oral primordium. Kineties 2–8 divide ordinarily; kinety 6 has two anterior kinetids in line with kinety 7. Kinety 9 consists of a dikinetidal anterior portion ("preoral kinety 4") and a monokinetidal posterior portion. Kinety 10 consists of three portions: the group C basal bodies; some dikinetids posterior of the adoral membranelles; and a long, monokinetidal posterior portion. Three of the four preoral kineties originate de novo, while one is produced by the postoral complex, thus being a somatic kinety. The postoral complex consists of two rows of basal bodies: the posterior, ciliated monokinetidal portion of somatic kinety 9 and the middle, non-ciliated dikinetidal portion of somatic kinety 10. The new contractile vacuole appears in early dividers posterior of the adoral membranelles, while the opisthe obtains the parental contractile vacuole.

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## UNRAVELLING THE rDNA OPERON OF EUGLENZOAN FLAGELLATES

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In most eukaryotes, the genes for the small subunit (18S or SSU) and large subunit (5.8S plus 28S or LSU) are present as tandemly repeated linear cistrons in multiple copies located in the chromosomes. Usually, the coding regions for the SSU and LSU rRNA are divided by two internal transcribed spacers, with ITS1 separating 18S from 5.8S and ITS2 parting 5.8S from 28S rDNA.

The unusual ribosomal operon of *Euglena gracilis* (Euglenozoa, Euglenida) is organized as an 11,056 bp circle with 800 - 4,000 extrachromosomal copies comprising the most highly fragmented LSU rDNA known to date. An intergenic spacer region (IGS) dividing 5'-SSU from 3'-LSU rDNA has been reported and a read-around transcription hypothesized. This rDNA organization is known for the derived phototrophic *Euglena gracilis*, the rDNA of the Kinetoplastida is arranged in tandem repeats and the rDNA organization of the Diplonemida is unknown. An analysis of the rDNA operons could clarify the evolution of and sister group relationships within the Euglenozoa, which still remain disputed.

Like *Euglena gracilis*, the examined phagotrophic euglenid and diplonemid species possess a highly fragmented LSU rRNA gene intermitted by additional ITSs. The deciphered rDNA operon of *Rhynchopus* sp. (Euglenozoa, Diplonemida) appears to be circular and the absence in Kinetoplastida raises the question of sister taxa relationships. Whether the presence of the circle constitutes an apomorphy of the Euglenozoa needs further examination. Additionally, assembling a concatenated SSU and LSU rDNA dataset could shed new light on the phylogeny of phagotrophic euglenids and contribute to resolve sister group relationships within the Euglenozoa.





**MICRODOMAIN FORMING STOMATIN PROTEIN SUPERFAMILY IN THE CILIATED  
PROTOZOAN, *PARAMECIUM TETRAURELIA* – MOLECULAR STRUCTURE,  
LOCALIZATION AND FUNCTION**

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The SPFH protein superfamily (stomatatin-prohibitin-flotillin/reggie) is assumed to occur universally in eukaryotes, but from protozoa no information is available. In *Paramecium* we found only stomatins as three subfamilies, *PtSto1* to *PtSto3*, with a total of 20 paralogs. According to cDNA analysis all are expressed. For further analysis we produced family-specific antibodies for fluorescence labeling, gene silencing, and functional tests. With all subfamily members we find a patchy localization at or near the cell surface and especially around the oral cavity, and as intracellular dots (compartments). Silencing of *PtSto1* reduces mechanosensitivity (ciliary reversal upon touching an obstacle). Thus, *PtSto1* may be relevant for positioning of mechanosensitive channels in the plasmalemma. *PtSto2* is also associated with the contractile vacuole complex (more than *PtSto1*), with food vacuoles, and less consistently with the cytoproct and structures along postoral fibers (vesicle delivery to nascent phagosomes). Silencing of *PtSto2* members increases pulsation speed of the contractile vacuole complex and reduces phagocytotic activity of *Paramecium* cells. Whereas for the largely deviating *PtSto3* subfamily subgroup-specific localization and function remain largely open (except some surface association), *PtSto1* and *PtSto2* members are involved in specific superficial and intracellular microdomain-based cell functions – with coincidence of localization and gene silencing effects.



**CALCIUM SIGNALING IN CLOSELY RELATED PROTOZOAN GROUPS (ALVEOLATA):  
NON-PARASITIC CILIATES (*PARAMECIUM*, *TETRAHYMENA*) VS. PARASITIC  
APICOMPLEXA (*PLASMODIUM*, *TOXOPLASMA*)**

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The importance of Ca<sup>2+</sup>-signaling for many subcellular processes is well established in higher eukaryotes, whereas information about protozoa is restricted. Recent genome analyses have stimulated such work also with Alveolates, such as ciliates (*Paramecium*, *Tetrahymena*) and their pathogenic close relatives, the Apicomplexa (*Plasmodium*, *Toxoplasma*). Here we compare Ca<sup>2+</sup> signaling in the two closely related groups. Acidic Ca<sup>2+</sup> stores have been characterized in detail in Apicomplexa, but hardly in ciliates. Two pore channels engaged in Ca<sup>2+</sup>-release from acidic stores in higher eukaryotes have not been stingly characterized in either group. Both groups are endowed with plasma membrane- and Endoplasmic Reticulum-type Ca<sup>2+</sup>-ATPases (PMCA, SERCA), respectively. Only recently was it possible to identify in *Paramecium* a number of homologs of ryanodine and inositol 1,3,4-trisphosphate receptors (RyR, IP<sub>3</sub>R) and to localize them to widely different organelles participating in vesicle trafficking. For Apicomplexa, physiological experiments suggest the presence of related channels. In *Paramecium*, IP<sub>3</sub>Rs are constitutively active in the contractile vacuole complex; RyR-related channels in alveolar sacs are activated during exocytosis stimulation, whereas in the parasites the homologous structure (inner membrane complex) may no longer function as a Ca<sup>2+</sup> store. Scrutinized comparison of the two closely related protozoan phyla may stimulate further work and elucidate adaptation to parasitic life.



## THE SYSTEMATICS OF "ZOOCHLORELLA" REVISITED EMPLOYING AN INTEGRATIVE APPROACH

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Symbiosis of green algae with protozoa and invertebrates has been studied for more than one hundred years. Endosymbiotic green algae are widely distributed in ciliates (e.g. *Paramecium*, *Stentor*, *Climacostomum*, *Coleps*, *Euplotes*), heliozoa (e.g. *Acanthocystis*) and invertebrates (e.g. *Hydra*, *Spongilla*), and have traditionally been identified as named or unnamed species of *Chlorella* Beij. or *Zoochlorella* K. Brandt or referred to as *Chlorella*-like algae or zoochlorellae.

We studied 18 strains of endosymbionts isolated from various hosts and geographical localities using an integrative approach (SSU and ITS rRNA gene sequences including their secondary structures, morphology, physiology, and virus sensitivity). Phylogenetic analyses have revealed them to be polyphyletic. The strains examined belong to five independent clades within the Trebouxiophyceae (*Choricystis*-, *Elliptochloris*-, *Auxenochlorella*- and *Chlorella*-clades) and Chlorophyceae (*Scenedesmus*-clade). The most studied host organism, *Paramecium bursaria*, harbors endosymbionts representing at least five different genera. On the basis of our results, we propose a taxonomic revision of endosymbiotic "*Chlorella*"-like green algae. *Zoochlorella conductrix* K. Brandt is transferred to *Micractinium* Fresen. and *Z. parasitica* K. Brandt to *Choricystis* (Skuja) Fott. It was shown that *Choricystis minor* (Skuja) Fott, the generitype, is a later heterotypic synonym of *C. parasitica* (K. Brandt) comb. nov. A new species, *Chlorella heliozoae*, is proposed to accommodate the endosymbiont of *Acanthocystis turfacea*.



## OXYMONADEN IN DER GÄRKAMMER NIEDERER TERMITEN

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Niedere Termiten beherbergen in ihrem Enddarm eine Fülle symbiontischer Flagellaten aus der Gruppe der Parabasaliden und Oxymonaden. Im Gegensatz zu den Parabasaliden besitzen die Oxymonaden weder einen Golgi-Apparat noch Hydrogenosomen. Nur wenig ist über ihre anaeroben Stoffwechselwege bekannt. Die besiedelten Habitate sind unterschiedlich. Die Gattungen *Monocercomonoides*, *Polymastix* und *Dinenympha* leben frei im Gärkammermilieu, sowie auch die umstrittene Gattung *Opisthomitus*. *Streblomastix* und *Pyrsonympha* können sich zeitweise an die Gärkammerwand anheften. Andere Gattungen, die zeit lebens einen anterioren Fortsatz (Rostellum) besitzen, sind dauerhaft an der Darmwand festgeheftet. Dies stellt eine physiologische Herausforderung dar, denn durch eindiffundierenden Sauerstoff ist die Sauerstoffkonzentration in der Peripherie der Gärkammer relativ hoch. Neben den angehefteten Oxymonaden wird die Gärkammerwand dicht von einem prokaryotischen Biofilm besiedelt. In diesen tauchen kleine Oxymonadenarten regelrecht hinein oder legen sich sogar amöboid eng an die peritrophische Membran des Darms an. Andere, größere Oxymonaden haben einen eigenen Besatz mit Bakterien. Es gibt Hinweise für einen Sauerstoffabbau durch Termitenbakterien, so dass die eng umgebenden Prokaryoten die anaeroben Oxymonaden möglicherweise schützen. Große Oxymonaden wie *Pyrsonympha* ragen weit ins Lumen des Darms hinein und befinden sich dadurch vermutlich mit dem größten Teil des Körpers in der gefahreren anoxischen Zone. Viele Oxymonaden besitzen Endobakterien, die ebenfalls symbiontische Funktionen haben können.



## **MOLEKULARE UND MORPHOLOGISCHE ANALYSE RÄUMLICHER UND ZEITLICHER BIODIVERSITÄTSMUSTER VON CILIATEN IN PFLANZENKLÄRANLAGEN**

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Ciliaten spielen eine Schlüsselrolle bei Abbauprozessen in Kläranlagen. Während einschlägige Untersuchungen zur Biodiversität herkömmlicher Kläranlagen bereits vorliegen, wurden ähnliche Studien an Pflanzenkläranlagen (PKA) noch nicht durchgeführt. Hierzu wurden die Ciliaten morphologisch und mit molekularen Markern charakterisiert. Des Weiteren wurde untersucht, in wie weit geographische und saisonale Einflüsse das Artenspektrum (und Abundanz?) der Ciliaten verändern. Es wurden zudem spezifische DNA Sonden entwickelt, die als diagnostische Werkzeuge zum Nachweis einzelner Arten genutzt werden können. Drei PKA in Deutschland zeigten tendenziell ein ähnliches Taxonspektrum mit saisonalen Änderungen in der Zusammensetzung der Ciliatengemeinschaft. Im Vergleich der geographischen Regionen (Deutschland und Thailand) gab es Übereinstimmungen in wenigen dominanten Taxa, aber nur geringe Übereinstimmungen, wenn alle detektierten Taxa betrachtet werden. Die in Thailand getesteten Anlagen wiesen zudem eine weit höhere Ciliatendiversität auf als die deutschen. Zudem konnte eine signifikante Differenzierung zwischen zwei benachbarten sich nur im Makrophytenbestand unterscheidenden PKA in Thailand aufgedeckt werden. Diese Ergebnisse stellen einen wichtigen Beitrag zur Frage geographischer Verbreitung eukaryotischer Mikroorganismen dar, indem sowohl kleinräumige (ökologische) als auch großräumige (historisch bedingte) Verbreitungsmuster nachgewiesen wurden.

## **ENDOCYTOBIOSIS IN DINOFLAGELLATES**

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Dinoflagellates are important primary producers or consumers of prokaryotes and Eukaryotes in aquatic environments. While Dinoflagellates became model organisms of primary, secondary and even tertiary endosymbiosis with phototrophic protists, showing all different stages of reduction of the endocytobiont endocytobioses with prokaryotes were detected accidentally. This changed with the investigation of harmful algal blooms, dominated by dinoflagellates. During the last years it became obvious that endocytobioses with prokaryotes can be found frequently in different dinoflagellate species and with a variety of bacterial taxa. A few examples of endocytobioses with Dinoflagellates and Prokaryotes are given.



## EVOLUTION OF THE SURFACE ANTIGEN MULTIGENE-FAMILY IN *PARAMECIUM TETRAURELIA*

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We describe the multigene family of variable surface antigens by a bioinformatical approach including gene identification and chromosomal mapping. Approx. 80 intact candidate genes were identified exhibiting the characteristics of the gene family: 7-8 kb length, highly conserved cysteine periodicity and intact C-terminal GPI-anchoring signals. Mapping the genes to the genome indicates that surface antigen genes have subtelomeric localization with direction of the open reading frame towards the telomere. As the *Paramecium* genome evolved by three successive genome duplications, one would assume that highly similar surface antigen genes represent ohnologs. Surprisingly, our data indicates that this is not the case. Moreover, several antigen genes have intrachromosomal duplicates and absence of any introns suggest them for retrocopies. Transcriptional analysis of these isogenes shows that two parameter are crucial for (co-)transcription: homology and localization on the same chromosome. An isogene becomes therefore cotranscribed if localized on the chromosome of the actually expressed antigen gene. The summary of this data allows a model of surface antigen evolution based on gene duplication. Moreover, the analysis of expression patterns of the individual isogenes in combination with their subchromosomal localization brings arguments for subnuclear expression centers forward.



## DO CILIATES SUFFER FROM SUNBURN?

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It is well known for many groups of organisms that too long exposure to sunlight might cause a severe sunburn. Especially the short wavelengths of the light spectrum in the ultraviolet B and A range (280-400 nm) potentially damage DNA and, for example, in protists they consequently lead to reduced motility, division and growth rates. To test how planktonic freshwater ciliates can handle stress caused by ultraviolet radiation (UVR), we carried out a series of experiments including heterotrophic and Chlorella-bearing species. Principally, we exposed the ciliates to different light regimes in three approaches: exposure to the full sunlight spectrum, to photosynthetically active radiation (PAR) only by cutting off the UVR with a specific foil (Mylar) and dark (=control). Additionally, for the mixotrophic *Pelagodileptus trachelioides* and the heterotrophic *Balanion planctonicum* we wanted to identify the wavelengths responsible for potential damage and applied a series of special long-pass cut-off filters (Schott). Overall, we found that the ciliates were adapted to the incident light intensities measured in their original habitat, however, responses were species-specific. Moreover, when the irradiation time to UVR was experimentally extended over natural conditions, we found either 100% mortality or changes in shape and behavior. Even though some ciliates such as *Stokesia vernalis* or *Vorticella chlorellata* showed no morphological abnormalities directly after the full sunlight irradiation, they were not able to survive the next days. Our results indicate that some ciliate species definitely suffer from sunburn, i.e., DNA damage when exposed to increased UVR.



## NOVEL ACTIVE KINETOPLASTIDS ASSOCIATED WITH HYPERSALINE ANOXIC BASINS IN THE EASTERN MEDITERRANEAN DEEP-SEA

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The Eastern Mediterranean Sea is home to deep (3500 m) hypersaline anoxic basins (DHABs), which are among the harshest and most polyextreme environments known on our planet. A combination of saturated salt concentration, high density and pressure, absence of light and lack of oxygen characterize these DHABs. A steep gradient of physico-chemical parameters forms a sharp halocline that separates the high-density brines from the normalsaline and normoxic deep-sea water. Using taxon-specific PCR-primers, we detected numerous molecular signatures of kinetoplastid flagellates in these deep-sea habitats. Some of the amplified SSU rRNA gene sequences (small subunit ribosomal) were only distantly related to cultured representatives suggesting the existence of hitherto undetected kinetoplastids, which might be restricted to the Mediterranean DHABs. They comprise a significant fraction of the protistan communities in the brines and haloclines of different DHABs. Fluorescence *in situ* hybridization results identified an environmental bodonid-related sequence clade. The respective organism represents approximately 10% of the total protistan community in the Discovery DHAB's halocline. The composition of kinetoplastid groups is different in the individual basins under study, which we attribute to environmental selection and the isolated island-character of the DHABs.

## '*CANDIDATUS ANCILLULA TRICHONYMPHAE*', A NOVEL LINEAGE OF ENDOSYMBIOTIC ACTINOBACTERIA IN TERMITE GUT FLAGELLATES

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Termite gut flagellates are colonized by host-specific lineages of ectosymbiotic and endosymbiotic bacteria. Previous studies have shown that flagellates of the genus *Trichonympha* may harbor more than one type of symbiont. Using a comprehensive approach combining cloning with fluorescence *in situ* hybridization and electron microscopy, we investigated the phylogeny and subcellular locations of the





symbionts in a variety of *Trichonympha* species from different termites. 'Endomicrobia' were restricted to *Trichonympha* Cluster I, which comprises flagellates from the termite families Rhinotermitidae and Termopsidae. Instead, *Trichonympha* species of Cluster II, which are present in flagellates of the termite genus *Incisitermes* (family Kalotermitidae), showed a high abundance of endosymbiotic Actinobacteria from a novel lineage restricted to termite guts. These endosymbionts, for which we suggest the name '*Candidatus* Ancillula trichonymphae', formed a monophyletic group and – in contrast to 'Endomicrobia' – preferentially colonized the anterior cell pole of the flagellate host. Ectosymbiotic *Desulfovibrio* populations associated with the cytoplasmic protrusions occurred in both *Trichonympha* clusters, but not in all species.

### AMOEBA GENOMICS – FREE-LIVING VERSUS PARASITIC TRAITS

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The Amoebozoa are considered the sister group of the animals plus fungi and include, among others, so diverse organisms as the free-living and social dictyostelids, the amphizoic acanthamoebae and the parasitic entamoebae. In the past years, several amoebozoan genome projects have been initiated and allow first comparisons and evolutionary conclusions.

Although all available data support the monophyly of the Amoebozoa, the genomic divergence between *Dictyostelium* and *Entamoeba* is higher than the one between animals and fungi. The haploid nuclear genome of the free-living *D. discoideum* is with approximately 34 Mb considerably larger than the one of the endoparasitic *E. histolytica* with around 21 Mb. Until now, only very few gene families have been found that are indeed characteristic for the Amoebozoa. However, genomes completed so far have a high (A+T) content (>75%) and a relatively high percentage of horizontal gene transfer and transposal elements. Interestingly, particularly genes involved in anaerobic metabolism of *E. histolytica* seem to be of prokaryotic origin. *E. moshkovskii*, the only free-living representative of the genus *Entamoeba* has a lower (A+T) content than all other entamoebae. On the other hand, genes involved in motility and signaling of the social amoebae *Dictyostelium* spp. show high similarities to metazoan genes. And finally also unique traits have been found – *Dictyostelium* as well as *Acanthamoeba* have essential tRNAs in their mitochondrial genomes, which is not known from any other organism so far.

This presentation intends to give an overview of the state of knowledge on amoebae genomes, thereby comparing free-living and parasitic representatives of the amoebae.



## MONOGRAPH OF THE HYPOTRICHA AND NOMENCLATOR CILIOPHORUM, TWO USEFUL WORKS FOR ALL WHO DEAL WITH CILIATES

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The Hypotricha are a core-group of the spirotrichous ciliates. About 1000 nominal species have been described and it is estimated that 700 to 800 of them are valid. Four volumes are available (Oxytrichidae; Urostyloidea; Amphisiellidae and Trachelostylidae; Gonostomatidae and Kahliellidae) and volume 5 (Uroleptidae) will be published in 2012/2013. The final volume is in preparation and deals, inter alia, with the “spiralled” genera, for example, *Hypotrichidium*, *Strongylidium*, *Spiretella*, *Chaetospira*. A key to all taxa revised in volumes 1–6 will be a major part of volume 6. When the monograph is complete, the hypotrichs will be the sole very large group of ciliates which is revised in detail. It is difficult to estimate the share of hypotrichs on the total number of ciliate species because no detailed conspectus about this group of protists is available. To overcome this problem, the Nomenclator Ciliophorum is prepared. This catalogue will contain all ciliate names ever published: basionyms of species and infrasubspecific taxa, combinations, genera, and suprageneric taxa. Furthermore, important taxonomic papers are listed for each taxon, so that the nomenclator will be useful not only for taxonomists, but also for ecologists, molecular biologists, and parasitologists, who will gain from such an index, for example, in that they can find the updated name (combination) of a certain organism easily.

The Monograph (vol. 6) and the Hypotricha part of the Nomenclator are supported by the Austrian Science Fund (FWF), project P23415-B17.

## PLASTID LSU rDNA (23S) VARIATION OF *SYMBIODINIUM* (DINOFLAGELLATA) WITHIN *PHYLLODESMIUM* (GASTROPODA)

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The genus *Phyllodesmium* (Gastropoda, Aeolidida) is well known to host symbiotic photosynthetic dinoflagellates of the genus *Symbiodinium* (Alveolata, Dinzoa).

Although these dinoflagellates mostly occur as freeliving cells in fresh and marine water, some are known to exist as endosymbionts (zooxanthellae) in reef-building corals and other invertebrates, for example in the coral feeding sea slug *Phyllodesmium*. During the digestion of the corals *Symbiodinium* cells remain intact embedded in the cerata tissue of the slug. Even those incorporated *Symbiodinium* maintain fully functional plastids to perform photosynthesis that can be measured by PAM (pulse amplitude modulated fluorometry).



Although distribution of *Symbiodinium* is well studied in corals, very little is known about diversity within the genus *Symbiodinium*. Due to extremely restricted morphological and ultrastructural autapomorphies the genus *Symbiodinium* is divided into clades A to I revealed by molecular data. So far *Symbiodinium* clades have not been isolated and molecularly identified in other Aeolidida than *Pteraeolidia ianthina*. In this study we amplified the plastid LSU rDNA (23S) of different *Symbiodinium* clades from the aeolidian *Phyllodesmium* and performed phylogenetic analyses.

### EXPLORING THE ECOLOGICAL FUNCTION OF THE PROTISTAN RARE BIOSPHERE

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Recent interrogations of nucleic acids isolated from environmental samples using next generation sequencing strategies have revealed new dimensions in protistan diversity. Based on these studies, the number of protistan taxa in for example a liter of ocean water increases previous estimates several fold. Such highly diverse and complex protistan communities are formed by a few dozen abundant taxa plus a large collection of rare taxa (the rare biosphere) that escapes traditional molecular methods like clone library construction and Sanger sequencing. The existence of a rare protistan biosphere is believed to have enormous consequences for the function(ing) of any ecosystem. We used 454 amplicon sequencing to test one of the hypothesis suggested for the function of the rare protistan biosphere: namely, the rare protistan biosphere as a seedbank of taxa with an enormous genomic potential to buffer environmental changes. We were able to support this hypothesis through the discovery of nearly all taxon groups in samples from a pre-alpine lake (Piburger See, 913 m a.s.l., Austria), collected during a unique sampling event, which have previously been detected in varying spatio-temporal patterns throughout an annual cycle using traditional light microscopy.



## QUANTITATIVE ESTIMATION OF NEXT-GENERATION-SEQUENCING - SEQUENCING QUALITY AND DETECTION LIMITS -

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Next-Generation-Sequencing (NGS) delivers thousands of sequence reads in a single sequencing run. This huge data set should enable detailed studies of community structures and detection of even rare organisms within the community. Sequence reads can differ in length and quality. Several steps of quality filtering are included in every sequence analysis to exclude low quality sequences and increase the reliability of estimates. We wanted to get an idea of the accuracy of the sequence reads and the limits of detection of even rare taxa obtained by a NGS run. We compared the quality of NGS analysis of different known and already sequenced taxa offered in different concentrations.

We analyzed the possibility to detect underrepresented organisms in a mix of different species. Equal amounts of 13 different species were nearly detectable if mixing the individual PCR products. However, a mixture of equal DNA amounts followed by PCR showed results below the PCR approaches. Consequences for routine NGS studies will be discussed.



## IDENTIFICATION, FUNCTIONAL ROLES AND ECOSYSTEM SERVICES OF PROTOZOA IN SOIL

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Protozoa are the major consumers of bacterial production in soil, forming the base of the heterotrophic eukaryotic food web that channels the energy flow via bacteria to higher trophic levels in soil (i.e. the bacterial energy channel). Despite small sizes of protozoa in soil (5-200  $\mu\text{m}$ ), their high abundance and fast turnover make them one of the key regulators of bacterial biomass and nutrient cycling.

Even though they occupy important functional roles, we still have only a vague idea on the identity of the dominant protist taxa in soil. One major reason for the general ignorance of protists in environmental studies is methodological difficulties in quantifying small protists in the opaque soil environment, their uneven distribution and the lack of taxonomic expertise. However, recent developments in high-throughput sequencing and in the cultivation of so-called uncultivable protists now allow closing the methodological gap on this functionally important trophic link in the soil food web.

Within the EU-project EcoFINDERS we aim at designing DNA-based barcodes for dominant protozoan taxa in soil. Protozoan diversity will be compared between five long-term observatories across Europe using high-throughput sequencing.

Cultivation of amoebae from Dutch and Sardinian grassland soils to improve phylogenetic information of this rarely studied assembly of organisms indicates an enormous diversity. Based on culture isolates, we constructed phylogenetic trees based on two genes (18s rDNA and cytochrome oxidase 1) to decipher deep-relationships among protozoa and to identify genetic barcodes targeting individual taxa for pyrosequencing, which we are currently conducting.

Ecological studies investigating protozoan grazing of bacteria has been shown to be a major structuring force for bacterial diversity in the plant rhizosphere. Therefore we suggest that protozoa may provide an important ecosystem service by removing pathogenic microorganisms from soils. Laboratory experiments with selected dominant protozoan taxa will be performed to investigate the effects of protozoan predation on different pathogenic and beneficial soil microorganisms.

Preliminary results indicate that bacterial spores are resistant to predation i.e. germinate and grow inside food vacuoles, while vegetative bacteria are killed by protozoan predation. This will be tested along with the dependency of morphology and feeding behaviour of protozoa on bacterial predation and related to soil ecosystem services.



## MAGNIFLASH

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Der 2007 entwickelte Makrokonverter mit lichtoptischer Blitzleitung namens „*MagniFlash*“ schlägt mit einem außergewöhnlichen Linsensystem die Brücke zwischen Makrofotografie und Mikroskopie. Auf das Objektiv einer Spiegelreflex-Kamera aufgeschraubt fängt er mit flexiblen Lichtleitern den integrierten Kamerablitz ab und leitet das Licht in die Brennebene der stark vergrößernden Linsenanordnung (20 oder 40 dpt). Mit einem Abbildungsmaßstab von 2:1 erreicht das System Vergrößerungen wie stationäre Stereolupen, kann im Gegensatz dazu jedoch auf jeder Exkursion dabei sein. Die Mobilität ist vor allem dann ein großer Vorteil, wenn die Objekte fragil, vergänglich oder geschützt sind. Durch die extrem geringen Belichtungszeiten kann sogar spielend aus der Hand fotografiert werden. *MagniFlash* ist somit für den mobilen Einsatz prädestiniert und erleichtert dem Anwender z.B. durch magnetische Linsenfassungen die Handhabung im Gelände. Die Aufnahmen mit *MagniFlash* von Details höherer Pflanzen, Moosen, Flechten, Insekten, Kristallen oder auch von technischen Gegenständen überzeugen durch eine große Schärfentiefe und sind oftmals sehr ästhetisch. Auch für Makroalgen, aeroterrestrische Algen oder aber Schleimpilze hat sich *MagniFlash* bestens bewährt. Das Poster stellt neben der Funktionsweise und den Vorzügen der Erfindung einige Ergebnisse aus den Bereichen Phykologie und Protistologie vor. Besucher des Posters können sich zudem gerne selbst an diversen Testobjekten von der Funktionalität des *MagniFlash*-Systems überzeugen.

## DNA BARCODING IN FORAMINIFERA

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Foraminifera are a major group of protozoans that successfully colonizes aquatic and terrestrial habitats. They are characterized by a granular pseudopodial network called granuloreticulopodia and most of them feature a test (shell) that is composed of an organic, agglutinated or calcareous wall and has one or multiple chambers. Some species have developed symbiotic relationships with various groups of algal protists and bacteria. The identification of foraminiferal species is mainly based on the morphology of their tests, which leads to difficulties when dealing with morphologically variable species and impedes the detection of sibling species.

DNA barcoding refers to a technique that characterizes species using DNA fragments specific to each species. DNA barcode sequences are very short relative to the entire genome and they can be obtained reasonably quickly and cheaply.

Our Forambarcoding website proposes a complementary identification system based on DNA barcodes. The record of each species in the database comprises its general



description, photos, collection data, DNA sequences, and references to related publications. The database is manually curated and differs from other foraminiferal databases by including only species, for which both molecular and morphological data are available. Our objective is to provide a complete, high quality and freely accessible resource of information about modern foraminiferal species.

### **STUDIES ON THE PHYLOGENY AND ECOLOGY OF NON-ACANTHOECID CHOANOFAGELLATES (CRASPEDIDA)**

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Choanoflagellates are small (3-10  $\mu\text{m}$ ) heterotrophic flagellates which are globally distributed in marine and freshwater. They have a single flagellum surrounded by a collar ('chóanos' = collar) with microvilli. Choanoflagellates are of great evolutionary interest because of their close relationship to Metazoa and of great ecological importance due to their ubiquitous distribution, their small size and significant impact on the food web as filter feeders on suspended bacteria.

Currently choanoflagellates are classified into three families - Salpingoecidae (non-loricates), Acanthoecidae (nudiform loricates) and Stephanoecidae (tectiform loricates). Molecular data, mainly based on SSU rDNA, shows that on the one hand the phylogeny of loricate species is well defined and monophyletic families exist. On the other hand the former families of Salpingoecidae and Codosigidae, based on morphologic characters only, were abandoned as they were clearly not monophyletic.

At the moment there are two hypothetical concepts regarding the phylogeny of the order of Craspedida, containing at present only one family, the Salpingoecidae. 1- Based on their ecological characteristics, a division into marine and limnic clusters and 2- similar to the Acanthoecida a developmental clustering due to their morphological characteristics ("cup-shaped" and "flask-shaped" thecae). Here, we will present our first results regarding the salinity tolerance of different, up to now unsequenced limnic and marine isolates of craspedid choanoflagellates and their phylogenetic position.



## LIFE CYCLE DIVERSITY AND FUNCTIONAL ULTRASTRUCTURE OF THE CHOANOFAGELLATE GENUS *CODOSIGA*

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47b

*Codosiga botrytis* is the first described choanoflagellate species, characterised by a multicellular trophozoite cluster situated on a stalk. It is one of the most commonly reported freshwater choanoflagellate morphospecies. In the present study we investigated 10 *Codosiga* strains collected from different parts of the world, to analyse their life cycle, ultrastructure and phylogenetic affiliations. The *Codosiga* strains are extremely polymorphic with a complex life containing about 30-40 different life stages. These include swimmers, stalked forms, mucilage forms, filopodial forms, budding forms, splayed tentacle forms, interbridged forms, gliding amoebae and cysts. Several forms are very similar or morphologically identical with other freshwater choanoflagellate genera. The ultrastructure of the strains is very similar, and is in agreement with the basic choanoflagellate architecture. Only slight differences could be revealed in the morphology and position of the Golgi apparatus, in the structure of the nucleus, and in the cyst structure; these are not sufficient for the morphological distinction of the phylogenetically far cryptic strains. The formation and work of several functionally important cellular structures and processes could be revealed, like the role of the sheath during the feeding process, the formation of main stalks and substalks, the complex division process, the mucilage production and its role in colony formation, the structure of the interbridges, as well as the process of cyst formation and the production of surface structures on the cysts.





## BENEFICIAL EFFECTS OF NAKED AMOEBAE (*ACANTHAMOEBA CASTELLANII*) ON PLANT GROWTH, NITROGEN UPTAKE AND CARBON ASSIMILATION VARIES WITH MAIZE (*ZEA MAYS*) CULTIVAR

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Nitrogen (N) limits plant growth in most terrestrial ecosystems. After mineralization, plant residues form a major source of N for plants growth. Plants invest significant amounts of photosynthates into the rhizosphere that enhance mineralization capacity of N by plant species specific microbial communities. Protozoa foster plant N uptake by re-mobilizing N from bacterial biomass ('microbial loop' in soil).

To evaluate whether cultivars of plant species differentially affect the functioning of the 'microbial loop', a microcosm experiment was carried out with three cultivars (cv) of maize (*Zea mays*; cv MB862, cv Bangui and cv PR39T45) and the naked amoebae *Acanthamoeba castellanii*. Plant biomass and stable isotopes (<sup>15</sup>N) were used to determine plant growth and N uptake from plant residues in soil. Photosynthesis was assessed by measurements of net CO<sub>2</sub> assimilation rates.

Growth, N uptake and CO<sub>2</sub> assimilation in the presence of naked amoebae varied among the investigated maize cultivars. Generally, MB862 was not affected by the presence of naked amoebae, whereas total shoot <sup>15</sup>N uptake was increased by 30% in Pr39T45. Bangui responded the strongest to naked amoebae with shoot biomass and total mass of <sup>15</sup>N in roots being increased by 40%. Further, Bangui almost doubled its net CO<sub>2</sub> assimilation rate in presence of naked amoebae.

The results suggest that plant-protozoa interactions strongly depend on plant cultivar. Therefore, cultivar specific plant-microbial interactions in the rhizosphere need to be considered to improve breeding strategies aiming to enhance N uptake of crops.



## ALGEN UND PROTOZOEN – STÄRKERE SICHTBARKEIT IN DER GLOBAL BIODIVERSITY INFORMATION FACILITY (GBIF)

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Die Global Biodiversity Information Facility ([www.gbif.org](http://www.gbif.org)) ist ein internationaler Verbund von Staaten und Organisationen, die Biodiversitätsdaten über das Internet verfügbar machen. Biodiversitätsdaten sind Belegdaten, Observationsdaten und Daten zu Lebendkultursammlungen. Zurzeit stellt GBIF-D *Pflanzen, Algen & Protisten* 5,8 Millionen Datensätze für das GBIF-Netzwerk bereit.

GBIF-D ([www.gbif.de](http://www.gbif.de)) sowie GBIF-D *Pflanzen, Algen & Protisten* analysieren Datenlage und Nutzerbedürfnisse für die Einbindung neuer Datenquellen in das GBIF-Netzwerk im Rahmen des BMBF-unterstützten Verbundes (GBIF-D: Kompetenzzentren innovativer Datenmobilisierung, Projekt 01 LI 1001 A-F). Für eine bessere Sichtbarkeit und Nutzbarkeit der Datenbestände wird deshalb ein Datenportal entwickelt, das Ende 2012 online gehen wird. An konkreten Erweiterungen für das Jahr 2012 sind u.a. die Anbindung der Bilddatenbestände von Plankton\*Net, die Sammlungsdatenbestände der Sammlung für Algenkulturen in Göttingen (SAG) sowie die Anbindung protozoologischer Datenbestände geplant. Laufend wird an der verbesserten Datenintegration vorhandener Datenanbieter gearbeitet.

Bereits seit 2005 liefert das *AlgaTerra* Informationssystem ([www.algaterra.org](http://www.algaterra.org)) Daten über Mikroalgen an GBIF. Die verwendete BioCASE-Providersoftware erlaubt die vollständige Anbindung reicher Datenbestände. Im Bereich der Bilddaten nutzt *AlgaTerra* inzwischen moderne Mikroskopie- und Servertechnologien, um qualitativ hochwertige Bilddaten verarbeiten, speichern und bereitstellen zu können. Die konzeptorientierte *AlgaTerra* Datenbank wird im Sommer 2012 auf die *EDIT Platform for Cybertaxonomy* migrieren, die zunehmend für taxonomische und Checklisten-Datenbestände Verwendung findet.

Diese Initiativen zielen auf eine stärkere Nutzbarkeit der vorhandenen Datenbestände durch Phykologie und Protozoologie sowie die Gewinnung weiterer Partner, die Qualitätsdaten an das globale GBIF-Netzwerk liefern, um den Open Access-Ansatz auch im Bereich der Biodiversitätsdaten von Ein- und Wenigzellern voranzubringen.



## COMPARISON OF DNA ISOLATION METHODS FOR DIVERSITY STUDIES

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A plethora of different DNA isolation methods, utilized to study diversity of environmental samples, exists. Many of those protocols are specifically adjusted to the properties of aquatic or soil samples. The demands on DNA isolation methods are isolating preferably total community DNA in high quality, suitable for PCR and further molecular analyses as well as being reasonable and time-saving.

In this study we tried to establish a standardized DNA isolation method for comparative molecular analyses of aquatic and terrestrial habitats. Therefore we compared the efficiency of different lysis instruments and furthermore of commercial kits and an ISO standard using an optimized lysis protocol particularly with regard to quantity and quality of the extracted DNA.

## MOBICOS (MOBILE AQUATIC MESOCOSMS): EINE NEUE PLATTFORM FÜR INTERDISZIPLINÄRE WASSERFORSCHUNG

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Die Durchführung kontrollierbarer, manipulierbarer und replizierbarer Experimente stellt die empirische Gewässerforschung vor eine große Herausforderung. Während im Labor in der Regel nur stark vereinfachte Abbilder der Natur erzeugt werden können, sind die experimentellen Möglichkeiten in Freilandstudien häufig sehr eingeschränkt. Freilandmesokosmen können diese Problematik teilweise lösen und eine Brücke zwischen den verschiedenen Versuchsansätzen schlagen. In Fließwasserlaboren werden verschiedene Versuchseinrichtungen mit unverändertem oder spezifisch manipuliertem Freilandwasser gespeist, ohne dabei den Freilandbezug zu stark einzubüßen. Durch die containerbasierte Bauweise erweitert die hier vorgestellte Versuchsplattform MOBICOS die Grundidee von Fließwasserlaboren um ein hohes Maß an Mobilität und Flexibilität.



## FEEDING OF PROTISTS ON TOXIC FILAMENTOUS CYANOBACTERIA

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Mass occurrences of harmful filamentous cyanobacteria are of increasing importance in freshwaters due to ongoing global warming and changing nutrient stoichiometry. Many cyanobacteria produce and store intracellular secondary metabolites, which may affect growth, survival and life history traits of potential consumers. Best known are the microcystins (MCs), cyclic heptapeptides, which are efficient inhibitors of eukaryotic protein phosphatases. Acute toxicity of purified MCs was determined repeatedly against various freshwater metazoans. However, protists and in particular ciliates have been described as efficient predators of toxic filamentous cyanobacteria. In addition to a literature review, we present micrographs and first quantitative data about several protists ('flagellates', 'amoebae' & ciliate species) feeding on the harmful cyanobacterium *Planktothrix rubescens* in Lake Zurich. We document various complex behaviour patterns, how protists can ingest large filaments or feed on fragments of cyanobacterial cells. Further, some isolated protistan species of Lake Zurich could be successfully cultivated on different filamentous cyanobacterial species, storing other secondary metabolites than MCs. Thus, protists must have developed strategies to cope with MCs but also with various other cyanobacterial toxins. It is our aim to understand how these protists, being eukaryotes, are able to survive their toxic diet either by own detoxifying mechanisms or in symbiosis with endo- or ectosymbiotic bacteria. This knowledge is needed for a better understanding of natural degradation processes of MCs.

## A NEW SPECIES OF THE CILIATE *TETRAHYMENA* (CILIOPHORA, OLIGOHYMENOPHOREA) ISOLATED FROM GROUNDWATER SAMPLES FROM CAPE TOWN, SOUTH AFRICA

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Species belonging to the genus *Tetrahymena* are relatively easy to distinguish from other hymenostomes because they show clear-cut generic characteristics. Problems arise when trying to differentiate them at the intrageneric level, since they are



essentially identical in morphology. These difficulties to identify species within the genus *Tetrahymena* are aggravated by the existence of cryptic and sibling species, as well as the phenotypic plasticity associated to the polymorphic life cycles exhibited by some species. Since the study of morphology do often not allow discriminating between *Tetrahymena* species, different identification approaches have been used and combined over the years, including ecology, biochemistry and/or genetics. In this context, DNA barcoding has increased its popularity for species identification, particularly because it is thought that it can be used without needing taxonomic expertise. Among the known DNA barcodes, the mitochondrial cytochrome-c oxidase subunit I (*cox1*) gene has been recently proven by different authors to be a valuable taxonomic tool to successfully assign *Tetrahymena* isolates to the species level. A new species of *Tetrahymena* was isolated from samples from a groundwater well in Cape Town, South Africa. We have combined alpha-taxonomy and molecular data to characterize this new species, since this approach has recently emerged as an effective tool due to its ability to conquer the challenges of classifying organisms as *Tetrahymena* prone to simple or convergent morphologies.

### INDIVIDUALITÄT BEI BAKTERIEN UNTER GRAZINGEINFLUSS

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Individuelle Unterschiede in physiologischen Prozessen von Organismen und deren phänotypischer Erscheinungsbilder könnten intrinsische Dynamiken von Populationen (Auftreten von chaotischem Verhalten, stabilen Grenzzyklen, gedämpfte Oszillationen) maßgeblich beeinflussen. Sie könnten sowohl bei Pro- als auch Eukaryoten selbst unter homogenen Umweltbedingungen auftreten.

Individuelle Unterschiede innerhalb einer Population können eine schnelle Reaktion auf veränderte biotische oder abiotische Einflüsse ermöglichen und so das Überleben einer Population zu sichern.

In der vorliegenden Studie wurde mit einem Zwei-Arten-System, bestehend aus einer Bakterienart (*Bacillus subtilis*) und einer Protozoenart (*Tetrahymena pyriformis*), unter Laborbedingungen untersucht, in welchem Maß der Einfluss von Fraß durch Protozoen die individuellen Unterschiede in der respiratorischen Aktivität von Bakterien veränderte, wobei sowohl Versuche im Batch als auch in einem Chemostatsystem vorgenommen wurden.



## FUNCTIONAL ANALYSIS OF ENDOSYMBIOTIC GENES: TRACING THE ROLE OF R-BODIES IN THE KILLER TRAIT

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R-bodies are coiled proteinaceous ribbons produced by *Paramecium* endosymbionts belonging to the genus *Caedibacter*. These intracellular bacteria confer upon their hosts a phenomenon called the killer trait. It is the ability to kill symbiont-free competitors called sensitives. The R-body is the crucial element of this process, but despite many efforts, the actual role of R-bodies in killing sensitive paramecia is still not satisfactory clarified. The open question is whether the R-body acts as transmitter for a yet unidentified toxin or whether it directly kills sensitive paramecia having intrinsic cytotoxic effects. In the present study, this problem is addressed by heterologous expression of *Caedibacter taeniospiralis* R-body in *Escherichia coli* followed by a detailed analysis of its potential intrinsic toxic effect on feeding sensitive *Paramecium tetraurelia*. Using this approach, we can exclude any eventual effects of additional, unidentified factors produced by *C. taeniospiralis* and thus observe the impact of the recombinant R-body itself. No cytotoxic effects of recombinant R-bodies were detected following this approach, strengthening the hypothesis that R-bodies act as releasing system for an unidentified *C. taeniospiralis* toxin.

## INFLUENCE OF HYDROSTATIC PRESSURE ON SOME DEEP-SEA HETEROTROPHIC FLAGELLATES

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The importance of the 'microbial loop' is well described for marine pelagic systems. Very little is known, however, about heterotrophic flagellates from the largest part of the biosphere, the abyssal. Our clone library studies revealed a large diversity of flagellate genotype present in the deep sea. In order to investigate whether the marine heterotrophic flagellates can potentially act as important *bacterivorous* in the deep sea, we isolated flagellates of different phyla from the abyssal and studied their ecology. We used an experimental system that allowed the study of growth rates and survival rates at different hydrostatic pressures and temperatures. Experiments carried out on various heterotrophic flagellates subjected to various pressures showed that certain flagellates can survive and grow under deep sea pressure. Survival rates were strain specific. Generally, deep sea isolates were more tolerant with regard to hydrostatic pressure and were in some cases barophilic. As a



conclusion, heterotrophic flagellates - mostly ignored in deep-sea studies - possibly act as major bacteria predators in deep sea.

## EXPLORING THE FUNCTION OF LOROXANTHIN IN LIGHT-HARVESTING COMPLEX II OF GREEN ALGAE

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The carotenoid loroxanthin (19-hydroxy-lutein) is present in many green algal species. In *Chlamydomonas reinhardtii*, loroxanthin partially replaces lutein in the light-harvesting complexes of photosystem II (LHCII), but the biological significance of this exchange is still unknown. Here, we observed an increase of the loroxanthin/lutein ratio in *C. reinhardtii* and other green algae at higher temperatures suggesting that loroxanthin may contribute to thermal stabilization of LHCII. We pursued this idea by *in vitro* reconstitution of recombinant LHCII from *C. reinhardtii* with mixtures of chlorophyll a, chlorophyll b, neoxanthin, and either lutein or loroxanthin. Interestingly, the resulting complexes were virtually identical in terms of pigment stoichiometries, absorbance properties, fluorescence excitation spectra, circular dichroism spectra and thermal stability. A major difference, however, was a 15-20 % higher fluorescence emission of loroxanthin-containing LHCII. Notably, *in vitro* reconstitutions of recombinant LHCII from the vascular plant *Pisum sativum* showed similar effects although loroxanthin is not present in higher plants. In agreement with the *in vitro* results, native LHCII from *C. reinhardtii* with different loroxanthin/lutein ratios showed a higher fluorescence quantum yield for preparations with increased loroxanthin content. Thus, we suggest that the modulation of the loroxanthin content may allow green algae to adjust the light-harvesting efficiency of LHCII to the capacities of the electron transport chain and Calvin cycle under varying environmental conditions.



## MORPHOLOGY, PHYLOGENETIC RELATIONSHIPS, AND pH RESPONSE OF TWO AS YET UNDESCRIBED *OXYTRICHA* SPECIES (CILIOPHORA, HYPOTRICHA)

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We investigated the morphology, phylogeny, and pH response of two undescribed *Oxytricha* species isolated from two acid mining lakes (pH ~2.6) located in Lusatia (Germany) and Langau (Austria). The Langau species, tentatively named *Oxytricha acidotolerans* by Weisse et al. (2011, Ecosphere), is about 60–80 × 25–40 μm in life, has an ordinary 18-cirri pattern, about 24 adoral membranelles, 3 μm long dorsal bristles, three caudal cirri, two macronuclear nodules, two micronuclei, and six dorsal kineties. The species from Lusatia is highly variable in the main characters (body size, shape, infraciliature, nuclear apparatus) making a useable description almost impossible. Both species differ by 3% in their SSU rDNA and occur in a cluster containing, inter alia, other oxytrichids like *Onychodromopsis flexilis*, *Cyrtohymena citrina*, and *Paraurostyla weissei*. However, *Oxytricha granulifera*, the type species of *Oxytricha*, is distinctly separated, demonstrating the immature taxonomy of this group. Our ecological hypothesis was that the shape of the pH reaction norm would not differ between our closely related species. Ciliate growth rates measured in the laboratory were calculated from changes in cell numbers vs. time. Results revealed a broad pH niche for *O. acidotolerans*, with positive growth rates over the entire pH range investigated, peaking at moderately acidic conditions (pH 5.2). Cyst formation was positively and linearly related to pH. *Oxytricha* sp. from Lusatia was more sensitive to pH and did not survive at circum-neutral pH. Our experimental results characterize *O. acidotolerans* as an acidotolerant species, while *Oxytricha* sp. is acidophilic. We reject our hypothesis that similar habitats would harbour ciliate species with virtually identical pH reaction norm.

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**FACULTATIVE ANAEROBIC CHOANOFAGELLATES FROM THE BALTIC SEA  
REDOXCLINE *CODOSIGA BALTICA* N. SP. AND *CODOSIGA MINIMA* N. SP.**

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We isolated two strains of choanoflagellates from suboxic water layers of the temporarily anoxic Gotland Deep of the central Baltic Sea, and maintained them under oxic conditions using bacteria as a food. These naked choanoflagellates produce stalked colonies and thus belong to the genus *Codosiga* James-Clark 1866. Both strains differ morphologically from each other by shape of the cell, mitochondrial peculiarities, and from other *Codosiga* species by their small size and the environment where they have been isolated. Gene sequence analyses based on 18S and 28S rRNA genes shows that our isolates form a monophyletic clade of *Codosiga* spp, but are well separated from *Codosiga gracilis*. *Codosiga baltica* is more closely related to *C. gracilis* (*p*-distance 4.8%) than *C. minima* (*p*-distance to *C. gracilis* 11.6%). Here, we describe *Codosiga minima* n. sp. and *Codosiga baltica* n. sp. based on morphology and molecular phylogeny. The 18S rRNA sequences of *C. baltica* and *C. minima* branch off together with clonal sequences from other hypoxic environments indicating a preference for oxygen depleted or anoxic habitats of both species.

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