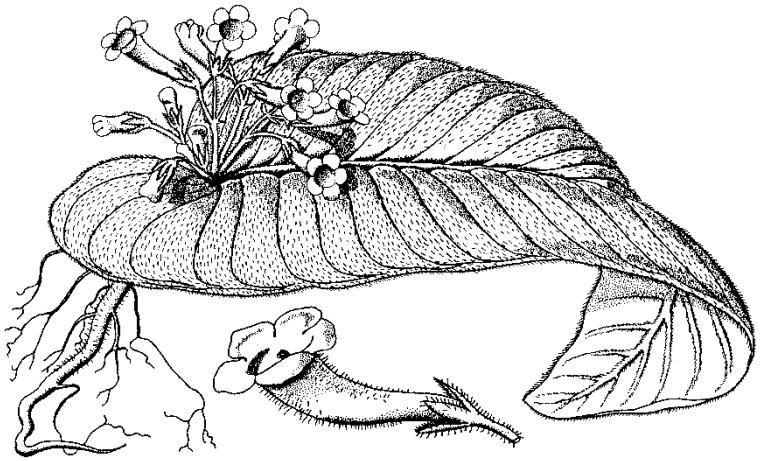
FRITSCHIANA

85



Veröffentlichungen aus dem
Institut für Pflanzenwissenschaften
der Karl-Franzens-Universität Graz

Silke WERTH & Walter OBERMAYER (editors)

Lichen Genomics Workshop II
Institute of Plant Sciences, University of Graz, Austria
2–5 November 2017
(Program and Abstracts)

Graz, 31. Oktober 2017

Hofrat Prof. Dr. Karl FRITSCH

(∗ 24.2.1864 in Wien, † 17.1.1934 in Graz)

Karl FRITSCH studierte nach einem Jahr in Innsbruck an der Universität Wien Botanik und wurde dort 1886 zum Dr.phil. promoviert; 1890 habilitierte er sich. Nach Anstellungen in Wien wurde FRITSCH 1900 als Professor für Systematische Botanik an die Universität Graz berufen, wo er aus bescheidenen Anfängen ein Institut aufbaute. 1910 wurde er Direktor des Botanischen Gartens, 1916 wurde das neu errichtete Institutsgebäude bezogen. Aus der sehr breiten wissenschaftlichen Tätigkeit sind vor allem drei Schwerpunkte hervorzuheben: Floristisch-systematische Studien, besonders zur Flora von Österreich, monographische Arbeiten (besonders über *Gesneriaceae*) und Arbeiten zur systematischen Stellung und Gliederung der Monocotylen. An Kryptogamen interessierten ihn besonders Pilze und Myxomyceten.

Nachrufe: KNOLL F. 1934: Karl Fritsch. - Berichte der Deutschen Botanischen Gesellschaft 51: (157)–(184) [mit Schriftenverzeichnis]. — KUBART B. 1935: Karl Fritsch. - Mitteilungen des Naturwissenschaftlichen Vereins für Steiermark 71: 5–15 [mit Porträt]. — TEPPNER H. 1997: Faszination versunkener Pflanzenwelten. Constantin von Ettingshausen - ein Forscherportrait. - Mitteilungen Geologie und Paläontologie am Landesmuseum Joanneum 55: 133–136. — Im übrigen vgl. STAFLEU F.A. & COWAN R.S. 1976, Taxonomic Literature 1: 892 und BARNHART J.H. 1965: Biographical Notes upon Botanists 2: 12.

Graz, November 1997 Herwig TEPPNER

Die Serie FRITSCHIANA wurde als Publikationsorgan für die zahlreichen Aktivitäten im Zusammenhang mit der botanischen Sammlung des Institutes für Pflanzenwissenschaften, Bereich Systematische Botanik und Geobotanik (vormals Institut für Botanik), der Karl-Franzens-Universität Graz (GZU) gegründet. Vor allem Schedae-Hefte der von den Mitarbeitern herausgegebenen Exsiccatenwerke sollten hier erscheinen, aber auch Exkursionsberichte sowie Listen und Indices besonders wertvoller Bestände in GZU. Das Spektrum wurde mittlerweile auf floristische und kleinere taxonomische Arbeiten (zwischenzeitlich auch auf das Samentauschverzeichnis des Botanischen Gartens) ausgeweitet. Die Schedae-Hefte des von Prof. Dr. Josef POELT begründeten, inzwischen abgeschlossenen Exsiccatenwerkes Plantae Graecenses sind die Vorläufer dieser Schriftenreihe.

Gesamtredaktion:

Dr. Christian SCHEUER, Mag. Dr. Walter OBERMAYER
Karl-Franzens-Universität Graz, Institut für Pflanzenwissenschaften
Bereich Systematische Botanik und Geobotanik, Holteigasse 6
8010 Graz, Österreich/Austria

ISSN 1024-0306
Key title = Abbreviated title: Fritschiana (Graz)

Umschlagsbild: *Carolofritschia diandra* ENGL. (= *Acanthonema strigosum* HOOK.f.); nach einer Zeichnung in HUTCHINSON, J. & HEPPER, F.N. 1963, Flora of West Tropical Africa, Ed. 2, Vol. II: 382.

FRITSCHIANA

Veröffentlichungen aus dem
Institut für Pflanzenwissenschaften
(Bereich Systematische Botanik und Geobotanik)
der Karl-Franzens-Universität Graz

85

Silke WERTH & Walter OBERMAYER (editors)

Lichen Genomics Workshop II
Institute of Plant Sciences, University of Graz, Austria
2–5 November 2017
(Program and Abstracts)

pp. 1–50

Graz, 31. Oktober 2017

Lichen Genomics Workshop II
Institute of Plant Sciences, University of Graz, Austria
2–5 November 2017

Organizing committee:

Silke WERTH*, Philipp RESL*, Christoph HAHN**,
Fernando FERNÁNDEZ MENDOZA*,
Martin GRUBE*

*Institute of Plant Sciences, **Institute of Zoology
NAWI Graz, University of Graz, Austria

ISSN 1024-0306
Key title = Abbreviated title: Fritschiana (Graz)

Date of publication: 31 October 2017
Printed by: Druckservice, Wirtschaftsabteilung der Karl-Franzens-Universität,
Attemsgasse 8/I, 8010 Graz, Austria

Contents

Program, Lichen Genomics Workshop II

Thursday (2 November 2017)

Thu. 09:00–9:45. Registration, SR 32.31 ('seminar room'), Holteigasse 6, 3rd floor.

Thu. 09:45–10:00. Introduction (Martin **GRUBE**), HS 32.01, Holteigasse 6, 1st floor.

Genomics I (genome assembly and annotation)

[chair: Martin Grube]
(HS 32.01, Holteigasse 6, 1st floor)

Thu. 10:00–10:50. KEYNOTE LECTURE. Christoph **HAHN**: Genome assembly and annotation.

Thu. 10:50–11:15. Claudio **AMETRANO**, Felix GREWE, Kerry KNUDSEN, Helge Thorsten LUMBSCH, Lucia MUGGIA, Steven D. LEAVITT: First insights into the genomes of *Lichenothelia* and *Saxomyxces* (Dothideomycetes).

Thu. 11:15–11:40. Ólafur S. **ANDRÉSSON**, Andrey N. GAGUNASHVILI, Sheeba S. BASIL: Distinctive characters of *Nostoc* genomes in cyanolichens.

Techniques and prospects for lichen genomics I

[chair: Martin Grube]
(HS 32.01, Holteigasse 6, 1st floor)

Thu. 11:40–12:00. Elfie **STOCKER-WÖRGÖTTER**: 'Case studies' and experimental approaches for culturing lichen fungi to understand developmental strategies of lichen thalli under various ecological conditions.

Thu. 12:00–12:05. Information from the organizer.

Thu. 12:05–14:00. Lunch break

Practical computer session I

[instructors: Fernando Fernandez Mendoza, Christoph Hahn, Philipp Resl]
(SR 31.01, Schubertstraße 51)

Thu. 14:00–17:00. Introduction to **Linux.**

Thu. 17:10–17:30. [optional] Tour of the lichen herbarium GZU, guided by Walter Obermayer and Astrid Scharfetter (meeting point: in front of HS 32.01, Holteigasse 6).

Welcome reception & poster session

(Foyer of Green Houses, Botanical Garden)

Thu. 18:00–20:00. Reception by a representative of the City of Graz, followed by the poster session.

Friday (3 November 2017)

Fri. 8:15–9:00 [optional] Tour of the Botanical Garden / green houses.

Genomes and functions, transcriptome studies

[chair: Silke Werth]
(HS 32.01, Holteigasse 6, 1st floor)

Fri. 09:00–09:50. KEYNOTE LECTURE. Philipp **RESL**. Linking genomes and functions.

Fri. 09:50–10:10. Antoine **SIMON**, Toby SPRIBILLE, Bernard GOFFINET, Li-Song WANG, Trevor GOWARD, Tatiana PYSTINA, Natalia SEMENOVA, John P. McCUTCHEON, Emmanuël SÉRUSIAUX: Multi-gene phylogeny of the lichen genus *Dendriscosticta* and transcriptome sequencing of photomorph pairs.

Fri. 10:10–10:35. Denis **WARSHAN**, Katharina PAWLOWSKI, Ulla RASMUSSEN: Cyanobacteria in symbiosis with boreal forest feathermosses: from genome evolution and gene regulation to impact on the ecosystem.

Fri. 10:35–10:55. Coffee break

Population genetics

[chair: Manuela Dal Forno]
(HS 32.01, Holteigasse 6, 1st floor)

Fri. 10:55–11:20. Arnar **PÁLSSON**, Marcos A. ANTEZANA: Transcriptional co-option, transcriptional decay and the principles of regulatory evolution.

Fri. 11:20–11:45. Ulrike **RUPRECHT**, Robert R. JUNKER: Climate niche expansion due to generalization in species associations in lichens.

Fri. 11:45–11:55. Christoph **SCHEIDEGGER**: The lichen individual: Insights from fungal microsatellites and outlooks into genomics.

Fri. 11:55–12:05. Inga **JÜRIADO**, Ulla KAASALAINEN, Maarit JÜLHÄ, Jouko RIKKINEN: Genetic diversity of *Nostoc* symbionts in *Peltigera* species (Ascomycota) of hemiboreal habitats in Estonia.

Fri. 12:05–12:15. Pamela **RODRIGUEZ-FLAKUS**: The origin and evolution of intercontinental disjunctions of lichen-forming fungi in fresh-water habitats.

Fri. 12:15–12:20. Information from the organizer.

Fri. 12:20–14:00. Lunch break

Excursion to Riegersburg (+ 'Buschenschank' visit)

(Meeting point: Holteigasse 6)

Fri. 14:00. Departure by bus from Holteigasse 6 to Riegersburg (drive ~45 minutes).

Fri. 18:00–21:00. 'Buschenschank Bernhart' near Riegersburg.

Saturday (4 November 2017)

Microbiome and mycobiome

[chair: Matthias Kaltenböck]
(HS 32.01, Holteigasse 6, 1st floor)

Sat. 09:00–9:50. KEYNOTE LECTURE. Tomislav **CERNAVA**, Martin GRUBE, Gabriele BERG: Recent insights into functioning and persistence of the lichen microbiota.

Sat. 09:50–10:00. Manuela **DAL FORNO**, Masoumeh SIKAROODI, Robert LÜCKING, James D. LAWREY, Patrick GILLEVET, Martin GRUBE: First insights into the microbiota associated with different thallus morphologies in the *Dictyonema* clade.

Sat. 10:00–10:25. Elisa **BANCHI**, David STANKOVIC, Fernando FERNÁNDEZ MENDOZA, Alberto PALAVICINI, Lucia MUGGIA: ITS2 metabarcoding analysis complements data of lichen mycobiome diversity.

Sat. 10:25–10:45. Coffee break

Sat. 10:45–11:05. Martin **GRUBE**. Associated microbes in analyses of lichen symbioses

Techniques and prospects for lichen genomics II

[chair: Matthias Kaltenböck]
(HS 32.01, Holteigasse 6, 1st floor)

Sat. 11:05–11:25. Silke **WERTH**. How does the lichen symbiosis work? Journey into the unknown.

Sat. 11:25–11:35. Jasmin **ALMER,** Silke WERTH: Isolation and disruption of lichen photobionts for gene expression studies.

Sat. 11:35–11:55. Gregor **PICHLER**, Fabio CANDOTTO CARNIEL, Erwann ARC, Wolfgang STÖGGL, Ilse KRANNER: GC-MS-based metabolite profiling as a new tool to study lichens and mycobiont-photobiont symbiotic interactions.

Sat. 11:55–14:00 Lunch break

Practical computer session II

[instructors: Fernando Fernandez Mendoza, Christoph Hahn, Philipp Resl]
(SR 31.01, Schubertstraße 51)

Sat. 14:00–18:00. Read quality control, genome assembly and annotation

Sunday (5 November 2017)

Population genetics, population genomics and phylogenomics

[chair: Fernando Fernandez Mendoza]
(HS 32.01, Holteigasse 6, 1st floor)

Sun. 09:00–09:50. KEYNOTE LECTURE. Fernando **FERNÁNDEZ MENDOZA**: Lichen population genomics and phylogenomics.

Sun. 09:50–10:15. Jessica **ALLEN**, Sean K. McKENZIE, Robin S. SLEITH, Elizabeth ALTER: Isolation by distance and low recombination characterize the population structure of the rock gnome lichen (*Cetradonia linearis*).

Sun. 10:15–10:40. Pradeep K. **DIVAKAR**: Comparative mitochondrial genome analyses of the symbiotic and parasitic fungi.

Sun. 10:40–11:00. Coffee break

Phylogenetics and systematics

[chair: Fernando Fernandez Mendoza]
(HS 32.01, Holteigasse 6, 1st floor)

Sun. 11:00–11:10. Edit **FARKAS**: *Cladonia magyarica*, a lichen species described from Hungary.

Sun. 11:10–11:20. Alice **DA CRUZ LIMA GERLACH**, Rosa Mara BORGES DA SILVEIRA, Philippe CLERC: Systematics of the lichen genus *Usnea* (Parmeliaceae) with emphasis on southern Brazil.

Genomics II (practical considerations)

[chair: Fernando Fernandez Mendoza]
(HS 32.01, Holteigasse 6, 1st floor)

Sun. 11:20–11:30. Silke **WERTH**: Update of ongoing genome projects.

Sun. 11:30–12:10. Fernando **FERNANDEZ MENDOZA**: Budgeting for genomic studies: what sequencing coverage is needed?

*Sun. 12:10–13:00. Lunch break (**catered lunch**, foyer of green houses)*

Practical computer session III

[instructors: Fernando Fernandez Mendoza, Christoph Hahn, Philipp Resl]
(SR 31.01, Schubertstraße 51)

Sun. 13:00–15:00. Phylogenomics.

Sun. 15:00. Closing of workshop.

> Please leave your name tags behind <

Isolation by distance and low recombination characterize the population structure of the rock gnome lichen (*Cetradonia linearis*)

Jessica L. ALLEN, Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland. - jessica.allen@wsl.ch

Sean K. MCKENZIE, Rockefeller University, 1230 York Avenue, New York, NY, U.S.A. - smckenzie@rockefeller.edu

Robin S. SLEITH, The New York Botanical Garden, 2900 Southern Boulevard, Bronx, NY, U.S.A. - rsleith@nybg.org

Elizabeth ALTER, York College, Biology Department, 94-20 Guy R. Brewer Boulevard, Jamaica, NY, U.S.A. - ealter@york.cuny.edu

ALLEN J.L., MCKENZIE S.K., SLEITH R.S. & ALTER E. 2017: Isolation by distance and low recombination characterize the population structure of the rock gnome lichen (*Cetradonia linearis*). Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 5. - ISSN 1024-0306.

Lichenized fungi are evolutionarily diverse and ecologically important, but little is known about the processes driving diversification and genetic differentiation in these lineages. Though few studies have examined population genetic patterns in lichens, geographic distributions are often assumed to be wholly shaped by ecological requirements rather than dispersal limitations. Furthermore, while their reproductive structures are observable, the lack of information about recombination mechanisms and rates can make inferences about reproductive strategies difficult. Here we investigate the population genomics of *Cetradonia linearis*, a rare and narrowly endemic lichen in the southern Appalachians of eastern North America, to test the relative contributions of environmental factors and geographic distance in shaping genetic structure, and to characterize and investigate the mating system. Analysis of genome-wide SNP data indicated strong evidence for both low rates of recombination and isolation by distance, but no evidence for isolation by environment. The species is putatively unisexual as only one mating-type locus was found across individuals. Hindcast species distribution models and the spatial distribution of genetic diversity suggest that *Cetradonia linearis* had a larger range during the last glacial maximum, especially in the southern portion of its current extent. These results contribute to our understanding of intrinsic and extrinsic factors shaping genetic diversity in *Cetradonia linearis*, and more broadly in rare fungi.

Rapid isolation and disruption of lichen photobiont cells

Jasmin ALMER, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - jasmin.almer@edu.uni-graz.at

Silke WERTH, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - silke.werth@uni-graz.at

ALMER J. & WERTH S. 2017: Rapid isolation and disruption of lichen photobiont cells. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 6–7. - ISSN 1024-0306

Cultivation-free, rapid isolation of photobiont cells from lichen thalli can be used to obtain axenic cultures, which are a pre-requirement for photobiont identification, and could be helpful for other analyses requiring a high concentration of more or less pure photobiont cells. Rapid photobiont isolation protocols based on grinding and subsequent low speed centrifugation were already developed in the 1970s (GREEN & SMITH 1974). To obtain total (ribo-)nucleic acids of the photobionts from axenic cultures or lichen thalli, e.g. for quantitative Real-Time PCR studies, a complete disruption of photobiont cell walls and lysis of cells is required. However it remains unclear if cells are disrupted sufficiently in existing protocols for (ribo-)nucleic acid isolation.

The dual purpose of this study was to determine i) the best protocol for rapid photobiont isolation from lichen thalli and ii) the optimal method to disrupt photobiont cells. The following species served as study organisms: The cyanolichen *Peltigera membranacea* and the tripartite lichens *Peltigera leucophlebia, Peltigera britannica*, *Lobaria pulmonaria,* and *Sticta canariensis*.

For the rapid isolation of photobionts, freshly collected thalli were ground with mortar and pestle in distilled water. The resulting suspension was filtered through a sieve with a mesh size of 0.5 mm to block the passage of bigger fragments like hyphae. The filtered solution was then centrifuged, with revolutions per minute and duration differing from species to species. To obtain axenic cultures, the resulting liquid containing a high concentration of photobiont cells was used for serial dilution with sterile ddH_2O. Small subsamples of the dilutions were cultivated on Bold's Basal Medium (BBM) agar with ampicillin. After several weeks of incubation, photobiont colonies were harvested and flash frozen in liquid nitrogen.

Disruption of photobiont cells was performed from frozen cells which were either lyophilised or not, and either ground with a tissue lyser bead mill to disrupt the cell walls, or with mortar and pestle. The degree of disruption of algal cell walls was examined by checking ground samples for the presence of intact algal cells with a light microscope. We compared how well photobiont cells were disrupted with or without a prior lyophilisation step, and with or without the addition of sand during grinding. Lyophilisation dehydrates the algal cells, removing the protective layer of water and making the disruption of the cells easier.

The rapid isolation of photobionts from the lichens was successful for the tripartite lichens, even though the centrifugation steps did not separate the algae completely

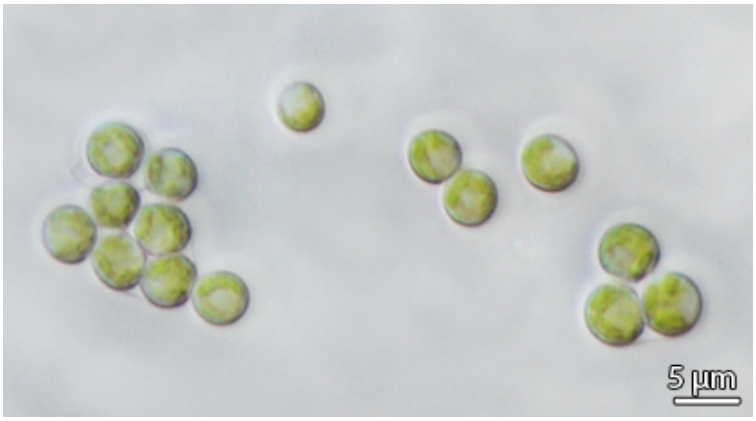
from small hyphal fragments. Highly diluted rapid preparations of *Coccomyxa* and *Symbiochloris* algae grew fairly well into pure colonies. Cyanobacteria, on the other hand, were difficult to rapidly isolate from the lichens, as during centrifugation, cyanobacterial cells pelleted along with hyphal fragments. We did not obtain axenic cyanobacterial cultures from the cells isolated with the rapid isolation technique.

In lyophilised preparations of *Coccomyxa* and *Symbiochloris*, the grinding process led to a complete disruption of the algal cells – even though both algae are known to contain sturdy layers of sporopollenins in their cell walls. Without lyophilisation, a larger number of undisrupted algal cells remained. Also the usage of a bead mill was necessary, as cell walls remained intact when the algae were ground manually with mortar and pestle. Adding a small quantity of sterile sand to the samples seemed to block the grinding process. However, examination with the light microscope was difficult as the sand particles covered the algae causing the state of the algae to be hardly determinable.

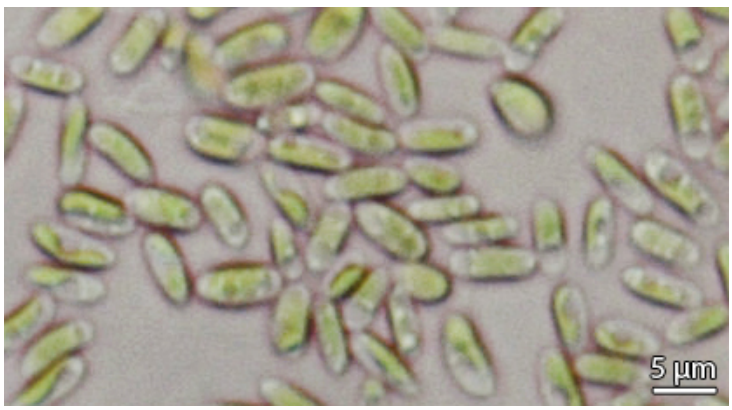
Overall, green algae were successfully isolated in high concentration from rapid photobiont isolations, and lyophilisation and mechanical grinding in a bead mill enabled complete disruption of algal cells, making them accessible for various subsequent analyses of (ribo-) nucleic acids.

References

GREEN T.G.A. & SMITH D.C. 1974: Lichen physiology. XIV. Differences between lichen algae in symbiosis and in isolation. – New Phytologist 73: 753–766.



Symbiochloris sp. from *Sticta canariensis*



Coccomyxa sp. from *Peltigera leucophlebia*

First insights into the genomes of *Lichenothelia* and *Saxomyces* (Dothideomycetes)

Claudio G. AMETRANO, Department of Life Sciences, University of Trieste, via Giorgieri 10, 34127 Trieste, Italy. - claudiogennaro.ametrano@phd.units.it

Felix GREWE, Science & Education, The Field Museum, 1400 S Lake Shore Drive, Chicago, IL 60605, U.S.A. - fgrewe@fieldmuseum.org

Kerry KNUDSEN, Department of Ecology, Czech University of Life Science, Kamycka 129, 165 21 Prague 6, Suchdol, Czech Republic. - kerry.knudsen@ucr.edu

Helge Thorsten LUMBSCH, Science & Education, The Field Museum, 1400 S Lake Shore Drive, Chicago, IL 60605, U.S.A. - tlumbsch@fieldmuseum.org

Lucia MUGGIA Department of Life Sciences, University of Trieste, via Giorgieri 10, 34127 Trieste, Italy. - lmuggia@units.it

Steven D. LEAVITT, Department of Biology and M.L. Bean Life Science Museum, Brigham Young University, 4102 Life Science Building, Provo, UT 84602, U.S.A. - steve_leavitt@byu.edu

AMETRANO C.G., GREWE F., KNUDSEN K., LUMBSCH H.T., MUGGIA L. & LEAVITT S.D. 2017: First insights into the genomes of *Lichenothelia* and *Saxomyces* (Dothideomycetes). Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 8. - ISSN 1024-0306.

Natural rocks and anthropogenic substrates are usually colonized by a group of microcolonial rock-inhabiting fungi (RIF). Due to their polyextremotolerance, RIF can colonize the harshest environments on earth; it was even shown that they can tolerate long-term exposure in outer space. *Lichenothelia* and *Saxomyces* are two genera of RIF with an unclear phylogenetic position within the class Dothideomycetes, although their evolutionary relationships were partly clarified by recent studies. Two *Lichenothelia* and two *Saxomyces* species were sequenced on an Illumina platform to generate high-throughput data for draft genome assemblies. These genomes were mined for orthologous single copy genes to study the phylogenetic placement of RIFs within the class Dothideomycetes. In addition, evolutionary rates and the presence of positive or negative selection patterns will be tested among fungi with different lifestyles (lichens, plant and human pathogens, saprotrophs, RIF). Comparative genomic techniques will be then used to assess features related to extremotolerance in the genomes of these fungi.

Distinctive characters of *Nostoc* genomes in cyanolichens

Ólafur S. Andrésson, Life and Environmental Sciences, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland. - osa@hi.is

Andrey N. Gagunashvili, UCL GOS Institute of Child Health, University College London, Gower Street, WC1E 6BT London, UK. - a.gagunashvili@ucl.ac.uk

Sheeba S. Basil, Life and Environmental Sciences, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland. - ssm5@hi.is

Andrésson Ó.S., Gagunashvili A.N. & Basil S.S. 2017: Distinctive characters of *Nostoc* genomes in cyanolichens. Abstract. - In: Werth S. & Obermayer W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 9. - ISSN 1024-0306.

Cyanobacteria of the genus *Nostoc* can form symbioses and close associations with a number of different organisms, including lichens, bryophytes and biological soil crusts, often predominant in cold terrestrial habitats. Only a restricted group of *Nostoc* strains have the ability to form symbioses. In order to elucidate what characterizes symbiotic *Nostoc* we have sequenced and annotated the genomes of five lichen-derived strains and compared them to other symbiotic and non-symbiotic *Nostoc* genomes.

Nostoc cyanobacteria from *Peltigera membranacea* and *Lobaria pulmonaria* were isolated and cultured on BG11 medium without fixed nitrogen, DNA was extracted and sequenced on the Illumina platform, and the reads were assembled and annotated with reference to well annotated bacterial genomes.

Comparison of six symbiotic vs. eight non-symbiotic *Nostoc* and *Anabaena* genomes allowed identification of several unique gene clusters. One of them had been identified and experimentally verified in the cycad symbiont *Nostoc punctiforme* ATCC 29133. Three of the lichen *Nostoc* strains harbored an alternative nitrogenase system based on vanadium in addition to the canonical molybdenum based system. The frequency of the vanadium system increases in colder habitats rather than with lower molybdenum availability, indicating greater efficiency at lower temperatures. Values for nitrogen isotope discrimination indicate that in some cases the contribution of the vanadium system may be similar to that of the canonical molybdenum system, despite lower expression and lower efficiency.

Annotation and analysis of the mycobiont genome of *Peltigera membranacea* has not yet revealed clear indications of principal determinants of symbiosis, although transcriptome analysis has uncovered several genes with differential expression in aposymbiotic vs. symbiotic tissues. No firm difference could be found in the cytosine DNA methylation of the tissues, despite a nearly 15% level of cytosine methylation genome wide. Repeat elements comprise ~5% of the genome and are heavily methylated, whereas coding regions have about 2.5% of the Cs methylated.

Acknowledgements: This research was supported by the Icelandic Research Fund and the University of Iceland.

ITS2 metabarcoding analysis complements data of lichen mycobiome diversity

Elisa BANCHI, Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy. - elisa.banchi@phd.units.it

David STANKOVIC, Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy, and National Institute of Biology, Marine Biology Station, Fornače 41, 6330 Piran, Slovenia. - david.stankovic@guest.arnes.si

Fernando FERNÁNDEZ MENDOZA, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - fernando.fernandez-mendoza@uni-graz.at

Alberto PALLAVICINI, Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy. - pallavic@units.it

Lucia MUGGIA, Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy, and Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - lucia_muggia@hotmail.com

BANCHI E., STANKOVIC D., FERNANDEZ MENDOZA F., PALLAVICINI A. & MUGGIA L. 2017: ITS2 metabarcoding analysis complements data of lichen mycobiome diversity. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 10–11. - ISSN 1024-0306.

The classical view of lichens as mutualistic, symbiotic associations between one fungus, the mycobiont, and a population of algae, the photobiont, has been revised in a more integrative and comprehensive context in which lichens act as microhabitats where multiple fungi, algae and bacteria coexist and likely contribute to the functions of the symbiotic systems (ARNOLD et al. 2009, MUGGIA & GRUBE 2010, U'REN et al. 2012).

Recently, the diversity of lichen associated fungi, addressed as lichen mycobiomes, has been assessed by both culture-based methods and high throughput amplicon sequencing (HTS) techniques (MUGGIA et al. 2016, FERNÁNDEZ-MENDOZA et al. 2017).

In this study, we analyzed thalli collected from the Koralpe Massif (Austria). We assessed by HTS of the ITS2 spacer the taxonomic composition and diversity of a well-characterized, alpine rock lichen community which included both thalli symptomatically infected by lichenicolous fungi and asymptomatic thalli. The results were compared with those previously obtained using ITS1 as barcode (FERNÁNDEZ MENDOZA et al. 2017). Taxa belonging to the order Chaetothyriales were the major components of the observed lichen mycobiomes. We predicted sequences representative of morphologically characterized lichenicolous fungi and assessed their asymptomatic presence in lichen thalli. We showed how the estimation of species diversity widely differed when using ITS1 or ITS2, which particularly affected the detection of Basidiomycota. The complementary analysis of both ITS1 and ITS2 barcodes is therefore required to reliably estimate lichen mycobiome diversity.

References

ARNOLD A.E., MIĄDLIKOWSKA J., HIGGINS K.L., SARVATE S.D., GUGGER P., WAY A., HOFSTETTER V., KAUFF F. & LUTZONI F. 2009: A phylogenetic estimation of trophic transition networks for ascomycetous fungi: Are lichens cradles of symbiotrophic fungal diversification? - Systematic Biology 58: 283–297.

FERNÁNDEZ MENDOZA F., FLEISCHACKER A., KOPUN T., GRUBE M. & MUGGIA L. 2017: ITS1 metabarcoding highlights low specificity of lichen mycobiomes at a local scale. - Molecular Ecology 26(18): 4811–4830. DOI: 10.1111/mec.14244.

MUGGIA L., FLEISCHACKER A., KOPUN T. & GRUBE M. 2016: Extremotolerant fungi from alpine rock lichens and their phylogenetic relationships. - Fungal Diversity 76: 119–142.

MUGGIA L., GRUBE M. 2010: Fungal composition of lichen thalli assessed by single strand conformation polymorphism. - The Lichenologist 42: 461–473.

U'REN J.M., LUTZONI F., MIĄDLIKOWSKA J., LAETSCH A.D. & ARNOLD A.E. 2012: Host and geographic structure of endophytic and endolichenic fungi at a continental scale. - American Journal of Botany 99: 898–914.

Recent insights into functioning and persistence of the lichen microbiota

Tomislav CERNAVA, Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12/I, 8010 Graz, Austria. - tomislav.cernava@tugraz.at

Martin GRUBE, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - martin.grube@uni-graz.at

Gabriele BERG, Institute of Environmental Biotechnology, Graz University of Technology, Petersgasse 12/I, 8010 Graz, Austria. - gabriele.berg@tugraz.at

CERNAVA T., GRUBE M. & BERG G. 2017: Recent insights into functioning and persistence of the lichen microbiota. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 12–13. - ISSN 1024-0306.

Over the past decade, the lichen microbiota was increasingly in the focus of various molecular studies. While in the first years, primarily the structure, composition, and colonization patterns of the colonizing microorganisms were deciphered, later also their functioning was addressed. We found potential implications of the microbiome in (i) nutrient supply, especially nitrogen, phosphorous and sulfur, (ii) resistance against biotic stress factors, (iii) resistance against abiotic factors, (iv) support of photosynthesis by provision of vitamin B12, (v) fungal and algal growth support by provision of hormones, (vi) detoxification of metabolites, and (vii) degradation of older parts of the lichen thallus (GRUBE et al. 2015). Furthermore, several multi-omics approaches allowed to assign more specifically functional roles to distinct bacterial lineages that are highly abundant in the lichen holobiont. The predominant order of *Rhizobiales* was identified as a commonly shared structural element in different lichen species (HODKINSON & LUTZONI 2009, ERLACHER et al. 2015). Lichen-associated Rhizobiales were later assigned to the general group of 'feeders' in the holobiont, while other distinct bacterial lineages were termed as 'protectors', referring to their potential for biotic and abiotic stress reduction (CERNAVA et al. 2017). These groups are also found in the core microbiome that is passed from one lichen generation to the next via propagules (ASCHENBRENNER et al. 2014).

Ongoing studies demonstrated that the lichen microbiota can efficiently adapt to unfavorable environmental conditions, including arsenic pollution. Under such conditions, the associated microorganisms were shown to still maintain their structure and support their host in detoxification reactions. The analysis of herbarium specimens of *Lobaria pulmonaria* that are several decades old revealed a seemingly viable bacterial community on the outer cortex (CERNAVA et al. 2016). Various molecular, as well as cultivation-dependent methods were employed since then to shed light on the integrity of this unexpected community and gradually disclose its secret. The insights gained in this continuously expanding approach to understand stable and long-lasting coexistence of micro- and macro-organisms, will eventually provide novel solutions for biotechnological applications in the future.

Acknowledgements. This work was supported by a grant of the Austrian Science Fund (FWF) to GB and MG (FWF Project I882).

References

ASCHENBRENNER I.A., CARDINALE M., BERG G. & GRUBE M. 2014: Microbial cargo: do bacteria on symbiotic propagules reinforce the microbiome of lichens? - Environmental Microbiology 16(12): 3743–3752.

CERNAVA T., BERG G. & GRUBE M. 2016: High life expectancy of bacteria on lichens. - Microbial Ecology 72(3): 510–513.

CERNAVA T., ERLACHER A., ASCHENBRENNER I.A., KRUG L., LASSEK C., RIEDEL K., GRUBE M. & BERG G. 2017: Deciphering functional diversification within the lichen microbiota by meta-omics. - Microbiome 5(1): 82.

ERLACHER A., CERNAVA T., CARDINALE M., SOH J., SENSEN C.W., GRUBE M. & BERG G. 2015: Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. - Frontiers in Microbiology 6: 53.

GRUBE M. [and 10 additional authors] 2015: Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. - The ISME journal 9(2): 412.

HODKINSON B.P. & LUTZONI F. 2009: A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. - Symbiosis 49(3): 163–180.

Systematics of the lichen genus *Usnea* (Parmeliaceae) with emphasis on southern Brazil

Alice DA CRUZ LIMA GERLACH, Conservatoire et Jardin Botaniques de la Ville de Genève, Switzerland, and Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Laboratório de Micologia, Avenida Bento Gonçalves, 9500 Campus do Vale, Porto Alegre, Rio Grande do Sul, Brazil. - alice_gerlach @yahoo.com.br

Rosa Mara BORGES DA SILVEIRA, Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Laboratório de Micologia, Avenida Bento Gonçalves, 9500 Campus do Vale, Porto Alegre, Rio Grande do Sul, Brazil. - rosa.silveira@ufrgs.br.

Philippe CLERC, Conservatoire et Jardin Botaniques de la Ville de Genève, 1 ch. de l'Impératrice, 1292 Chambésy/GE, Switzerland. - Philippe.Clerc@ville-ge.ch.

DA CRUZ LIMA GERLACH A., BORGES DA SILVEIRA R.M. & CLERC P. 2017: Systematics of the lichen genus *Usnea* (Parmeliaceae) with emphasis on southern Brazil. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 14–15. - ISSN 1024-0306.

The PhD research of the first author focused on the systematics of *Usnea* from Southern Brazil using an integrative approach. At first morphospecies were identified, using morphology, anatomical and chemistry features. Subsequently a dataset of DNA sequences was generated and phylogenetic inferences using maximum likelihood and Bayesian approaches were performed. Special focus was given to fertile species of *Usnea* using three nuclear markers, ITS and two protein-coding ones, Mcm7 and RPB1. In this framework, detailed taxonomic treatments were provided for seventeen corticolous shrubby species, including five recently described ones. Molecular data are available for most of these taxa.

The second main goal was to explore species boundaries, and to evaluate morphologically circumscribed species and chemotypes in the light of molecular data. Since previous studies showed that the cosmopolitan *Usnea cornuta* is polyphyletic, this aggregate emerged as an optimal choice to perform species delimitation analyses using the multi-species coalescent model implemented on the program STACEY on Beast 2. This recently developed program is one of the few approaches that do not require a priori assignment of individuals to putative species. In order to accomplish this task, we generated a dataset of 156 DNA sequences of 82 specimens of the *Usnea cornuta* aggregate from a broad geographical area (Brazil, Neotropical Andes, Costa Rica, Europe and USA). STACEY provided powerful insights to recognize lineages within this difficult and cosmopolitan group, particularly within clades of recent and rapid diversifications. The *Usnea cornuta* aggregate presents high genetic diversity and split into nine supported clades correlated with chemistry, but each of them remained not clearly morphologically characterized. Our study highlights the importance of understanding patterns of evolution in *Usnea* and it may provide additional insights how evolution occurs in Parmeliaceae, since *Usnea* is a hyper-diverse genus with high speciation rates.

Furthermore the presence of terpenes and other chemical structural families were investigated by thin layer chromatography (TLC) using an alternative spray solvent, anisaldehyde–sulfuric-acid. Further TLC analysis demonstrates the presence of

sugars (e.g. arabitol, saccharose, mannitol) and steroids besides the terpenes already reported for the species studied (*Usnea malmei* and *U. moreliana*). The chemistry profile is useful to recognize these species. However, the terpenes and steroids found remained unidentified and more studies are needed to evaluate their identity as well as their taxonomical significance on the genus *Usnea*.

Acknowledgements: This study was supported by the Swiss Government Excellence Scholarship and CNPq (Brazil). Laboratory work was supported by the IAPT Research Grant in Plant Systematics to AG and was carried out at CJBG. We thank the Conservatoire et Jardin Botaniques de la Ville de Genève et Universidad Complutense de Madrid.

First insights into the microbiota associated with different thallus morphologies in the *Dictyonema* clade

Manuela DAL FORNO, Smithsonian Institution, National Museum of Natural History, Department of Botany MRC 166, 10th Street & Constitution Avenue, NW, Washington, DC 20560 U.S.A. - manudalforno@hotmail.com

Masoumeh SIKAROODI, George Mason University, Department of Biology MSN 4D4, 10900 University Boulevard, Manassas, VA 20110 U.S.A. - msikaroo@gmu.edu

Robert LÜCKING, Botanical Garden and Botanical Museum Berlin, Königin-Luise-Straße 6–8, 14195 Berlin, Germany. - r.luecking@bgbm.org

James D. LAWREY, George Mason University, Department of Biology MSN 3E1, 4400 University Drive, Fairfax, VA 22030 U.S.A. - jlawrey@gmu.edu

Patrick GILLEVET, George Mason University, Department of Biology MSN 4D4, 10900 University Boulevard, Manassas, VA 20110 U.S.A. - pgilleve@gmu.edu

Martin GRUBE, Institute of Plant Sciences, University of Graz, Holteigasse 6, A-8010 Graz, Austria. - martin.grube@uni-graz.at

DAL FORNO M., SIKAROODI M., LÜCKING R., LAWREY J., GILLEVET P. & GRUBE M. 2017: First insights into the microbiota associated with different thallus morphologies in the *Dictyonema* clade. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 16–17. - ISSN 1024-0306.

Dictyonema sensu lato is the most speciose lichen clade in Basidiomycota, with 136 currently described species (LÜCKING et al. 2017, DAL FORNO et al. 2017). The known photobiont of all these lichen species belong to *Rhizonema*, a genus of cyanobacteria so far only found in symbiosis with lichens and liverworts (LÜCKING et al. 2009, Cornejo & SCHEIDEGGER 2015). While most of the diversity of *Dictyonema* s.l. is found in tropical montane regions, several species may also grow in subtropical and temperate regions. The group also shows a wide range of morphologies: the basal clade *Cyphellostereum* and the paraphyletic genus *Dictyonema* are filamentous, while the other three genera, *Acantholichen, Corella* and *Cora*, are squamulose to mostly foliose (DAL FORNO et al. 2013). We aimed to investigate whether the inhabiting microbial communities of these lichens were related in any way to these different morphologies, given that they all present different substrates to which the bacteria could interact. We sequenced the 16S rDNA region (covering the variable regions one and two) with multi-tag pyrosequencing (MTPS) for 695 samples from 18 countries representing all major clades within *Dictyonema* s.l. We found that reads matching the photobiont *Rhizonema* were predominant in all samples. However, after excluding these cyanobacterial reads, the most abundant bacteria belonged to the Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi phyla. Our preliminary results show that there is a non-random composition pattern among the morphological categories with bacterial communities from filamentous species clustering separately from those of foliose species.

Acknowledgements: Authors thank the National Science Foundation for financial support through a Division of Environmental Biology grant (DEB 0841405, PI: J. Lawrey; CoPIs: R. Lücking, P. Gillevet)

and a Postdoctoral Research Fellowship in Biology (PRFB 1609022, PI: M. Dal Forno). Authors are thankful to all collaborators who have contributed valuable *Dictyonema* s.l. collections over the years.

References

CORNEJO C. & SCHEIDEGGER C. 2016: Cyanobacterial gardens: the liverwort *Frullania asagrayana* acts as a reservoir of lichen photobionts. - Environmental Microbiology Reports 8(3): 352–357.

DAL FORNO M., BUNGARTZ F., YÁNEZ-AYABACA A., LÜCKING R. & LAWREY J.D. 2017: High levels of endemism among Galapagos basidiolichens. - Fungal Diversity 85: 45–73.

DAL FORNO M., LAWREY J.D., SIKAROODI M., BHATTARAI S., GILLEVET P.M., SULZBACHER M. & LÜCKING R. 2013: Starting from scratch: evolution of the lichen thallus in the basidiolichen *Dictyonema* (Agaricales: Hygrophoraceae). - Fungal Biology 117: 584–598.

LÜCKING R., DAL FORNO M. [and 13 additional authors] 2017: Turbo-taxonomy to assemble a mega-diverse lichen genus: seventy new species of *Cora* (Basidiomycota: Agaricales: Hygrophoraceae), honouring David Leslie Hawksworth's seventieth birthday. - Fungal Diversity 84: 139–207.

LÜCKING R., LAWREY J.D., SIKAROODI M., GILLEVET P.M., CHAVES J.L., SIPMAN H.J.M. & BUNGARTZ F. 2009: Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognized lineage of filamentous cyanobacteria. - American Journal of Botany 96: 1409–1418.

Pleomorphy in lichens possessing thalloconidia: molecular data do not support the separation of *Umbilicaria decussata* and *U. polaris* as separate species

Evgeny A. DAVYDOV, Altai State University, Lenin Avenue 61, Barnaul, 656049, Russia. - eadavydov@yandex.ru

Sergio PEREZ-ORTEGA, Real Jardín Botánico (CSIC), Claudio Moyano 1, E-28014 Madrid, Spain. - sperezortega@rjb.csic.es

Christian PRINTZEN, Senckenberg Research Institute and Natural History Museum Frankfurt, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany. - christian.printzen@ senckenberg.de

DAVYDOV, E.A., PEREZ-ORTEGA, S. & PRINTZEN, C. 2017: Pleomorphy in lichens possessing thalloconidia: molecular data do not support the separation of *Umbilicaria decussata* and *U. polaris* as separate species. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 18–19. - ISSN 1024-0306.

Many fungal groups display pleomorphism, asexual and sexual reproduction associated with often entirely different looking anamorphic and teleomorphic morphs or life-stages. The availability of molecular genetic data has greatly facilitated the clarification of teleomorph-anamorph relationships (SHENOY et al. 2007). However, lichenized fungi are still very poorly studied in this respect. Heteromorphic life-cycles have been described only for a few microlichens, e.g. *Chaenotheca furfuracea* (HONEGGER 1985) or *Micarea adnata* (COPPINS 1983).

Members of the mostly arctic-alpine lichen family Umbilicariaceae developed a variety of sexual, asexual and vegetative diaspores, which raises questions about the status of many taxa. Do reproductively differing but phenotypically similar individuals belong to different species, i.e. represent ‘species pairs’, or does the same species give rise to sexual and asexual morphs, analogous to teleomorphic and anamorphic states of some non-lichenized fungi?

The aim of the work was to test the species status of the asexual *Umbilicaria decussata* and the sexual *U. polaris*, which may provide further evidence of pleomorphy in lichens with thalloconidia. Molecular phylogeny seems to be the most appropriate tool to test the monophyly of taxa, which may be interpreted in terms of evolutionary relationships.

DNA was isolated from 25 specimens of *Umbilicaria decussata* and 14 of *U. polaris* collected worldwide. Six genetic markers (ITS/5.8S, RPB1, RPB2, MCM7, mtSSU, and mtLSU) were used, 204 sequences altogether. Single loci and the combined datasets we analysed using ML and Bayesian inference. *Umbilicaria polyphylla* was used as outgroup. Haplotype networks were reconstructed for each locus using statistical parsimony. Multispecies coalescent analysis was further used to analyse the multilocus datases. Analyses of molecular variance (AMOVA) were performed in addition.

In all single-marker analyses, none of the two species appeared monophyletic. Moreover, all but one haplotype network showed haplotypes shared between both morphospecies. Even the information from six gene loci does not fully resolve the tree. This is, however, not expected if we consider datasets infraspecific, because gene genealogies from conspecific individuals usually show discordance due to recombination.

Our results suggests, that despite *Umbilicaria decussata* and *U. polaris* are well separated morphologically, they should be treated as one species – *U. decussata*, represented by two morphotypes according to the principal (asexual vs. sexual) reproductive stage (old thalli of *U. decussata* may produce apothecia in addition to thalloconidia). Four different genetic lineages are present in our dataset, but they are not phenotypically diagnosable. The presence and absence of thalloconidia and apothecia is not fully congruent with these four groups but a pattern emerges.

A preliminary conclusion from our results would be that the species *Umbilicaria decussata* represents a recent pleomorphic species in the process of splitting up into at least four different lineages with ancestral polymorphisms still present.

Acknowledgements: The investigation was supported by the Russian Foundation of Basic Research (grants no. 11-04-90704, 14-04-00067 and 15-04-05971). The work in Senckenberg Forschungsinstitut und Naturmuseum was supported by the German Science Foundation (DFG grant PR 567/17-1).

References

COPPINS B.J. 1983: A taxonomic study of the lichen genus *Micarea* in Europe. - Bulletin of the British Museum (Natural History), Botany 11: 17–214.

HONEGGER R. 1985: The hyphomycetous anamorph of *Coniocybe furfuracea*. - The Lichenologist 17: 273–279.

SHENOY B.D., JEEWON R. & HYDE K.D. 2007: Impact of DNA sequence-data on the taxonomy of anamorphic fungi. - Fungal Diversity 26: 1–54.

Comparative mitochondrial genome analyses of the symbiotic and parasitic fungi

Pradeep K. DIVAKAR, Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid 28040, Spain. - pdivakar@farm.ucm.es

DIVAKAR P. 2017: Comparative mitochondrial genome analyses of the symbiotic and parasitic fungi. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 20. - ISSN 1024-0306.

Lichens are a symbiotic assemblage of multiple organisms including algae, fungi and bacteria. They form an ecologically obligate, stable mutualism between an exhabitant fungal partner (the mycobiont) and an inhabitant population of unicellular or filamentous algal or cyanobacterial cells (the photobiont).

Nevertheless, a controlled parasitism phenomenon of lichenization or a parasitic lifestyle of lichenized fungi has also been postulated. Here we aim to compare the complete mitochondrial genomes of the selected taxa of obligate mutualistic and obligate parasitic fungi. Only reference genomes or well-curated mitochondrial genomes of the selected species of obligate mutualistic and obligate parasitic fungi belonging to different major clades of fungi were analyzed. The genome sizes of mutualistic fungi were significantly larger than those of parasitic fungi and this was positively correlated with the number of introns.

Is *Solenopsora cesatii* an indicator of relictual and refugial character of mid-altitudinal forest rock assemblages in the Carpathians?

Zuzana FAČKOVCOVÁ, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia. - zuzana.fackovcova@savba.sk

Marek SLOVÁK, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia. - marek.slovak@savba.sk

Jaromír KUČERA, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia. - jaromir.kucera@savba.sk

Anna GUTTOVÁ, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Dúbravská cesta 9, 845 23 Bratislava, Slovakia. - anna.guttova@savba.sk

FAČKOVCOVÁ Z., SLOVÁK M., KUČERA J. & GUTTOVÁ A. 2017: Is *Solenopsora cesatii* an indicator of relictual and refugial character of mid-altitudinal forest rock assemblages in the Carpathians? Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 21. - ISSN 1024-0306.

High mountain areas, representing island-like ecosystems, are generally characterised by a high level of biological diversity and are considered one of the evolutionary hotspots on the globe. The spatio-temporal isolation effectively hampers dispersal and gene flow among populations, and thus acts as an accumulator of genetic diversity and population divergence. In Europe, besides the southern peninsular mountain systems and Alps, the more northern Carpathians harbour rich biodiversity with endemism richness mainly confined to the alpine but also to the mid-altitudinal zone (500–1500 m a.s.l.). The mid-altitudinal biota could survive glacial periods in extra-Mediterranean refugia, especially in gorges, forest cliffs or similar habitats present in the Carpathians. The lichen *Solenopsora cesatii* occurs predominantly in mid-altitudes of high European mountain ranges (e.g. southern foothills of the Alps, the Pyrenees, foothills of the Dinarides and the Carpathians) and its distribution is apparently linked to typical relic calcareous biotopes. Mid-altitudinal forest biotopes were proven to be potential glacial refugia for numerous moisture demanding temperate organisms: snails, small mammals, trees, and herbs. The question arises, whether the Carpathian localities with the presence of *Solenopsora cesatii* represent remnants of an originally compact and larger distribution range strongly influenced by Pleistocene glaciations, or whether they are the result of recent dispersal events. To test the above mentioned phylogeographic hypotheses, we would like to apply the new high-throughput sequencing methods which can provide a substantial amount of robust data with a sufficiently high resolution.

Acknowledgements: The work is supported by the projects APVV-15-0210 'Distribution potential of different fungal trophic groups in Europe' and VEGA 2/0032/17 'Unraveling processes responsible for the contemporary geographic range of symbiotic organisms with Mediterranean distribution'.

Cladonia magyarica, a lichen species described from Hungary

Edit FARKAS, Institute of Ecology and Botany, MTA Centre for Ecological Research, Hungarian Academy of Sciences, Vácrátót, Hungary, farkas.edit@okologia.mta.hu

FARKAS E. 2017: *Cladonia magyarica*, a lichen species described from Hungary. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 22. - ISSN 1024-0306.

Cladonia magyarica is an aesthetic species among lichens. It was collected in Hungary and described by Vainio in 1927 (see FARKAS & LŐKÖS 1994, PIŠÚT 1961). It is characterised by richly squamulose podetia, a primary thallus, and its lichen secondary metabolites (atranorin in the cortex and fumarprotocetraric acid in the medulla). For a long time it was only known from the Carpathian Basin (and regarded as endemic species), mostly from lowland steppe areas, but its morphological varieties were discovered in other habitats as well (GALLÉ 1968). During the last decades from time to time new records have been announced from various parts of the world from North America to Asia (see LITTERSKI & AHTI 2004, ZRAIK et al. 2016). Due to the isolated situation of its type locality, *Cladonia magyarica* became protected by law [23/2005. (VIII. 31.) KvVM] in Hungary among the first lichen species ever (FARKAS & LŐKÖS 2006). Contrary to the new records questioning the endemic position of the species, arguments are still necessary 'pro et contra', since the today generally accepted molecular genetic evidence is missing. Only a limited number of molecular gentic sequences has been isolated so far (see PINO-BODAS et al. 2013) and an investigation covering all populations, habitats and geographic areas (and especially type specimens) is still in urgent need.

Acknowledgements: Molecular genetic study of this species is supported by the project NKFI K 124341 financed by the National Research Development and Innovation Fund from September 2017.

References

FARKAS E.E. & LŐKÖS L.S. 1994: Distribution of the lichens *Cladonia magyarica* Vain. and *Solorinella asteriscus* Anzi in Europe. - Acta Botanica Fennica 150: 21–30.

FARKAS E. & LŐKÖS L. 2006: Törvényesen védett zuzmófajok Magyarországon (Protected lichen species in Hungary). - Kitaibelia 11(1): 47.

GALLÉ L. (1968): The xerothermic lichen species *Cladonia magyarica* Vain. (A *Cladonia magyarica* Vain. xerotherm zuzmófajról). – Móra Ferenc Múzeum Évkönyve 1968: 237–268.

LITTERSKI B. & AHTI T. 2004: World distribution of selected European *Cladonia* species. - Symbolae Botanicae Upsalienses 34(1): 205–236.

PINO-BODAS R., MARTIN M.P., BURGAZ A.R. & LUMBSCH H.T. 2013: Species delimitation in *Cladonia* (Ascomycota): a challenge to the DNA barcoding philosophy. – Journal of Molecular Ecology Resources 13(6): 1058–1068.

PIŠÚT I. 1961: Lichenologische Bemerkungen 2. - Preslia 33: 369–374.

ZRAIK M., BOOTH J.T. & PIERCEY-NORMORE M.D. (2016): Reports of *Cladonia magyarica* and *C. humilis* in Manitoba. – Evansia 33(3):136–144.

Introduction to lichen phylogenomics and population genomics

Fernando FERNÁNDEZ-MENDOZA, Institut für Pflanzenwissenschaften, Karl-Franzens-Universität Graz. Holteigasse 6, 8010 Graz, Austria. - fernando.fernandez-mendoza@uni-graz.at

FERNÁNDEZ-MENDOZA F. 2017: Introduction to lichen phylogenomics and population genomics. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 23–25. - ISSN 1024-0306.

The introduction of high throughput DNA sequencing technologies (HTS) in the late 2000s caused a revolution in molecular genetics and genome biology. Soon, the simplification of laboratory protocols, and the progressive reduction in per-base sequencing costs allowed a wider community to incorporate HTS methods to address ecological, evolutionary and systematic questions in non-model organisms.

In this context a series of new terms created to emphasize the adoption of the new type of data: **phylogenomics** and **population genomics.** While both disciplines comprise evolutionary surveys in which many –nuclear– loci are used, their scope is to understand genome-wide processes from their early origin. The term **phylogenomics** was coined to refer to evolutionary methods used in gene annotation (EISEN 1998), but slowly evolved to include purely evolutionary surveys. Similarly, **population genomics** was first used in the context of human disease genetics (GULCHER & STEFANSSON 1998) but was rapidly adopted for data-rich population surveys.

Lichens, being semi-open symbiotic systems, provide an incomparable model to study evolutionary and ecological processes associated with the symbiotic lifestyle. Their biotic complexity, while exciting, limits their usability of 'in vivo' sequencing experiments (GRESHAKE et al. 2016). Processing the **metagenome** or **metatranscriptome** of a full lichen thallus is difficult. Even if the photobiont layer is excised, bacteria and fungi make up an important fraction of the sequenced libraries, and interfere in assembly and variant calling. While not necessary, investing in obtaining clonal **axenic cultures** of at least one of the symbionts is a crucial step to generate high quality genomic references. Still, lichen fungi, especially those with well-delimited haploid thalli, are perfect organisms for genomic surveys. First they are haploid, which allows the assembly of a phased genome and not a consensus of two or more sets of chromosomes. Second, their genomes are quite small typically ranging between 30 and 40Mb. And third, depending on the taxon studied, the genomes tend to be very tidy and have few repetitive elements.

The type of data provided by HTS technologies is quite homogeneous: either long reads with a high base calling error (i.e. PacBio, Oxford Nanopore) or short reads with high quality base calls (Illumina, etc.) and homogeneous spacing (i.e. paired end and mate pair libraries). Meanwhile, the type of library preparation, and the philosophy used in processing the reads may result in very different data structures, which strongly influence both downstream analyses and the type of questions to be addressed. A good example is the SNP datatype. SNPs are among the earliest genome-wide data developed and are widely used in microarrays. Extracted from genomic HTS data they provide a simple tool to study non-model animal and plant

species overcoming the limitations imposed by their large diploid/polyploid genomes. However, SNPs are not just substitutions, but strongly stringent biallelic markers expected to be heterozygous. SNPs are simple to obtain from multiple pipelines, but their downstream use in haploid organisms is not straightforward, and potentially bias population genetic and phylogenetic inferences, especially coupled with methods based on SNP frequency spectra. SNPS are widely used in population surveys of diploid non-model organisms coupled with reduced representation libraries (*RadSeq/ GBS*, the different modalities of target enrichment including RNASeq, or poolSeq) that provide a good compromise between sampling size and genomic coverage.

Reduced representation libraries have also been used to study lichen population genomics to achieve wider sampling and reduced costs. However, metagenomic shotgun sequencing of lichens at a low coverage can provide a reasonably sized, phased population dataset with a broad genomic coverage, at least for the mycobiont.

The first phylogenomic surveys in lichen fungi focused in testing the usability of different alignment (read mapping and orthology based) and phylogenetic reconstruction methods in set of mycobiont genomes. LEAVITT et al. (2016) found that standard phylogenetic methods on genomic datasets did not provide different results as those obtained in sanger-based surveys. Similarly, population genetic surveys using GBS libraries of Icelandic *Peltigera membranacea* (WERTH et al. in prep.) did not provide an improved estimate of population structure compared to an SSR dataset, finding also limitations in the ability to identify signals of local adaptation. On the other hand, the Poolseq survey carried out by DAL GRANDE et al. (2017) did identify genome-wide patterns but encountered difficulties interpreting the patterns of local adaptation with protein function, probably due to the interference of population structure and selective sweeps.

Finally, the study of lichen photobionts from a genomic perspective has complications on its own. Cyanobionts have smaller genomes, but the diversity and genome characteristics of chlorobionts, and the paucity of reference genomes make their study more challenging. The problem of having multiple strains/species of chlorobiont is an additional complication. I believe that using a pool sequencing philosophy will soon revolutionize the interpretation of ecological and evolutionary patterns of symbiont association, as amplicon sequencing changed the study of the lichen microbiome.

To sum up, including a genome-wide scope in an evolutionary survey should not only be seen as a way to provide an enriched answer to the same long-standing questions; it completely changes the questions to ask. A great example is provided in the first sentence of the seminal study of LARSON et al. (2014) on species boundaries: "...Species are often viewed as cohesive entities, …as diagnosably distinct or exclusive groups of individuals. In fact, cohesion, diagnosability and exclusivity are properties of individual genes or genome regions and not of whole organisms...". New questions also require new methods, and will lead to even newer questions. I'm eager to see next generation lichenology happening.

References

DAL GRANDE F., SHARMA R., MEISER A. [and 7 additional authors] 2017: Adaptive differentiation coincides with local bioclimatic conditions along an elevational cline in populations of a lichen-forming fungus. - BMC Evolutionary Biology 17: 93.

EISEN J.A. 1998: Phylogenomics: Improving Functional Predictions for Uncharacterized Genes by Evolutionary Analysis. - Genome Research 8: 163–167.

GRESHAKE B., ZEHR S., DAL GRANDE F, MEISER A., SCHMITT I. & EBERSBERGER I. 2016: Potential and pitfalls of eukaryotic metagenome skimming: A test case for lichens. - Molecular Ecology Resources 16: 511–523.

GULCHER J. & STEFANSSON K. 1998: Population genomics: Laying the groundwork for genetic disease modeling and targeting. - Clinical Chemistry and Laboratory Medicine 36(8): 523–527.

LARSON E.L., WHITE T. A., ROSS C.L. & HARRISON R.G. 2014: Gene flow and the maintenance of species boundaries. - Molecular Ecology 23: 1668–1678.

LEAVITT S.D., GREWE F., WIDHELM T., MUGGIA L., WRAY B. & LUMBSCH H.T. 2016: Resolving evolutionary relationships in lichen-forming fungi using diverse phylogenomic datasets and analytical approaches. - Scientific Reports 6, article number 22262.

Cophylogeny between *Tremella* spp. and their lichen hosts: always similar coevolving patterns?

Sandra FREIRE-RALLO, Universität Rey Juan Carlos, Calle Tulipán, s/n, 28933 Móstoles, Madrid, Spain. - s.freirerallo@icloud.com

Mats WEDIN, Swedish Museum of Natural History, Department of Cryptogamic Botany, P.O. Box 50007, SE-104 05 Stockholm, Sweden. - mats.wedin@nrm.se

Ana M. MILLANES, Departamento de Biología y Geología, Física y Química Inorgánica (ESCET), Universidad Rey Juan Carlos, Calle Tulipán, s/n, E-28933 Móstoles, Madrid, Spain. - ana.millanes@urjc.es

FREIRE-RALLO S., WEDIN M. & MILLANES A.M. 2017: Cophylogeny between *Tremella* spp. and their lichen hosts: always similar coevolving patterns? Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 26. - ISSN 1024-0306.

Lichen-inhabiting fungi form fascinating coevolving systems with their hosts. As organisms that live ecologically linked, they provide great possibilities for investigating patterns underlying generation of diversity. Coevolution does not necessarily imply co-speciation, but is one of the mechanisms promoting rapid diversification, and co-speciation is one of the strongest evidences of coevolution. In a co-speciation scenario, the phylogenies of the groups involved mirror each other, but it is in reality rare to find perfectly congruent linked phylogenies. Most frequently these are not fully congruent and other mechanisms (host switch or lineage sorting, among others) explain their joint evolutionary story.

Highly host-specific lichen-inhabiting fungi, however, have been suggested to co-speciate with their hosts. The Tremellales (Basidiomycota, Fungi) include lichenicolous species that are very host-specific, but previous studies between *Biatoropsis* and their *Usnea* and *Protousnea* hosts did not suggest that any co-speciation occured in this system. Here, we extend these studies to another putative lichenicolous tremellalean species complex to assess if, at least in some cases, speciation can be explained by parallel reciprocal evolution between the associated bionts.

Associated microbes in analyses of lichen symbioses

Martin GRUBE, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - martin.grube@uni-graz.at

GRUBE M. 2017: Associated microbes in analyses of lichen symbioses. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 26b. - ISSN 1024-0306.

As self-supporting and long-living symbiotic structures, lichens provide a habitat for many other organisms beside the traditionally considered lichen symbionts – the myco- and the photobionts. In fact, lichens form complex symbiotic systems, which include multiple photobiont lineages, other fungi, and complex communities of bacteria. Omics analyses and amplicon sequencing of lichen symbioses are different approaches to capture this diversity. Amplicon sequencing is a common approach to characterize taxonomic diversity in lichen symbioses, but reconciling gene function assignments with this diversity by omics analyses are still limited by database constraints.

An overview of current knowledge about the diversity of lichen-associated microbial communities and their functional involvement in the symbiotic system will be provided. Despite remaining questions, these data indicate that lichens can be understood as complex systems extending the interaction of myco – and photobionts.

Genome assembly and annotation

Christoph HAHN, Institute of Zoology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria. - christoph.hahn@uni-graz.at

HAHN C. 2017: Genome assembly and annotation. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 27. - ISSN 1024-0306.

In recent years, the emergence of so-called Next Generation Sequencing (NGS) technologies has brought about a paradigm shift in modern biological research. Driven by ever plummeting sequencing costs, the assembly and annotation of genomes, initially the forte of large international consortia, have become a democratized, bottom-up enterprise, now feasible for small research groups, and indeed, individual researchers. Draft genomes for non-model organisms can routinely be constructed in days, even with limited funds. However, handling the exceedingly large amounts of NGS data that are readily accessible to us today is not straightforward and requires specific sets of skills: experience with Linux environments, especially High-Performance Computing (HPC) infrastructure, and the command of simple scripting languages, to name but a few, are often essential for data processing and management. The field of genome assembly in particular, is moving exceptionally fast, with new computational tools and algorithms constantly being developed, assessed and improved.

In this lecture, I will give an introduction to the algorithms and the principle challenges of genome assembly, including an overview of the most recent NGS technologies and their respective data characteristics (sequence length, error rate, cost), with a particular emphasis on their relevance for genome assembly. Based on case studies conducted across a variety of organisms I will introduce the audience to core software tools and approaches essential for successfully completing the assembly and annotation of small to medium sized genomes of non-model organisms.

Shades of olive in the cyanolichen Placynthium

Alica HINDÁKOVÁ, Institute of Botany, Plant Science and Biodiversity Centre SAS, Bratislava, Slovakia. - alica.hindakova@savba.sk

Mats WEDIN, Department of Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden. - mats.wedin@nrm.se

Martin WESTBERG, Museum of Evolution, Uppsala University, Norbyvägen 16, SE-752 36, Uppsala, Sweden. - martin.westberg@em.uu.se

Alica KOŠUTHOVÁ, Department of Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden; Institute of Botany, Plant Science and Biodiversity Centre SAS, Bratislava, Slovakia. - alica.kosuthova@nrm.se

HINDÁKOVÁ A., WEDIN M., WESTBERG M. & KOŠUTHOVÁ A. 2017: Shades of olive in the cyanolichen *Placynthium*. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 28–29. - ISSN 1024-0306.

The role of cyanobacteria as photobionts in the thallus of lichens is well known, but efforts to gain the correct classification of the photobionts still remain (GOMEZ et al. 2016). Moreover, cyanobacteria as epiphytic microorganisms may reliably protect the lichens against the UV radiation (VINCENT 2007), or reduce other environmental stress.

The subject of the study is the *Placynthium nigrum* group with different colour shades and under different environmental conditions. Morphological studies and mtSSU DNA-region has been used as barcode for *Placynthium* samples. The aim is to find the factor responsible for the colour of the lichen. Because there are no secondary metabolites in the thallus, we have tested the colour of the mycobiont hyphae and of the photobiont. The results have not been significant. However, we have observed chroococcal cyanobacteria (e.g. *Gloeocapsa*, *Gloeothece, Gloeocapsopsis, Asterocapsa*) (KOMÁREK & ANAGNOSTIDIS 1998), which were sitting on the thallus of *Placynthium* species and are characterized by cells grouped in coloured mucilaginous colonies rich in photoprotective pigments as gloeocapsin (the surface is blue, red or orange) or scytonemin (brown to black). The ability of colonies with coloured mucilage aggregated into macroscopic mats or layers to 'colour' the substrate is known from studies focusing on the eplitihic cyanobacteria which form the so called 'Tintenstriche' (PENTECOST 1982, HINDÁK 2008). Is there a chance that varying colour of this mucilage might explain the variation in colour of the lichen?

Acknowledgements: Alica HINDÁKOVÁ was supported by the grant agency APVV-15-0210. Alica KOŠUTHOVÁ was funded by the Štefan Schwarz Fund SAS and Swedish Taxonomy Initiative.

References

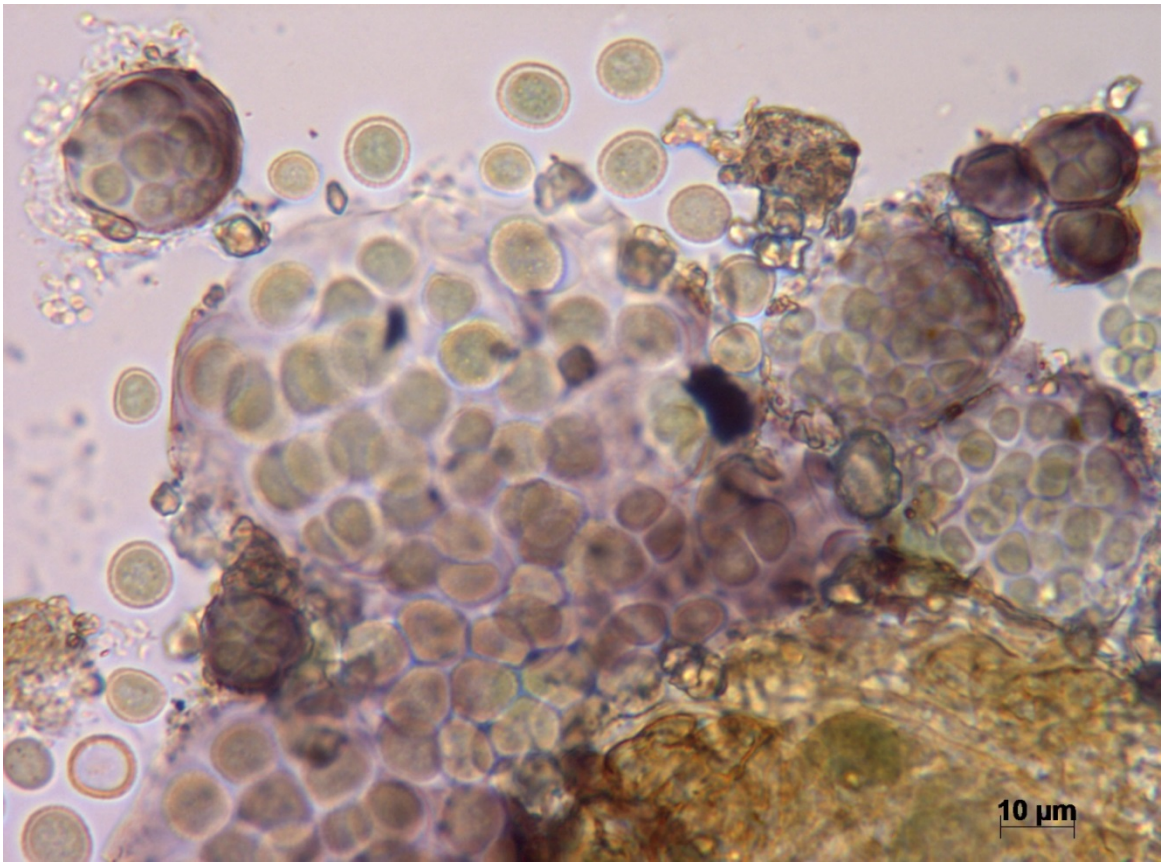
GOMEZ E.B., JOHANSEN J.R., KAŠTOVSKÝ J., BOHUNICKÁ M. & ČAPKOVÁ K. 2016: *Macrochaete* gen. nov. (Nostocales, Cyanobacteria), a taxon morphologically and molecularly distinct from *Calothrix*. - Journal of Phycology 52: 638–55.

HINDÁK F. 2008: Colour Atlas of Cyanophytes. - Bratislava: Veda.

KOMÁREK J. & ANAGNOSTIDIS K. 1998: Cyanoprokaryota 1. Chroococcales. – In: ETTL H., GÄRTNER G., HEYNIG H. & MOLLENHAUER D. (editors). Süßwasserflora von Mitteleuropa 19/1, p. 548. Jena-Stuttgart-Lübeck-Ulm: Gustav Fischer.

PENTECOST A. 1982: A quantitative study of calcareous and Tintenstriche algae from the Malha district, Northern England. - British Phycological Journal 17: 443–456.

VINCENT W.F. 2007: Cold Tolerance in Cyanobacteria and Life in the Cryosphere. - In: SECKBACH J. (editor). Algae and Cyanobacteria in Extreme Environments. Cellular Origin, Life in Extreme Habitats and Astrobiology, pp. 287–301. Dordrecht: Springer.



Gloeocapsa (greenish cells and violet sheets) is sitting on *Placynthium* (orange-brown coloured thallus). Photo taken by Alica HINDÁKOVÁ.

Genetic diversity of *Nostoc* symbionts in *Peltigera* species (Ascomycota) of hemiboreal habitats in Estonia

Inga JÜRIADO, Institute of Ecology and Earth Sciences, University of Tartu, Lai Street 38/40, 51005 Tartu, Estonia. - inga.juriado@ut.ee

Ulla KAASALAINEN, Finnish Museum of Natural History, University of Helsinki, P.O. Box 7, 00014, Helsinki, Finland. - ulla.kaasalainen@helsinki.fi

Maarit JYLHÄ, Department of Biosciences, University of Helsinki, P.O. Box 65, 00014, Helsinki, Finland. - maarit.jylha@helsinki.fi

Jouko RIKKINEN, Finnish Museum of Natural History, University of Helsinki, P.O. Box 7, Helsinki, Finland; Department of Biosciences, University of Helsinki, P.O. Box 65, 00014, Helsinki, Finland. - jouko.rikkinen@helsinki.fi

JÜRIADO I., KAASALAINEN U., JYLHÄ M. & RIKKINEN J. 2017: Genetic diversity of *Nostoc* symbionts in *Peltigera* species (Ascomycota) of hemiboreal habitats in Estonia. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 30. - ISSN 1024-0306.

We studied the genetic diversity of cyanobacterial symbionts in the genus *Peltigera* in Estonia. The study sites were distributed all over Estonia and represented different habitats, including three wooded habitat types (oligotrophic forests, eutrophic forests, and park stands) and three grassland types (alvars, dunes, and roadsides). Three substrate types were distinguished: ground, tree trunks, and rock. A total of 271 specimens of *Peltigera* were collected in more than a hundred study sites. The phylogenetic analyses of the 252 fungal ITS sequences obtained from the *Peltigera* specimens grouped them into 31 OTUs (JÜRIADO et al. 2017). The genotypic identities of the lichen cyanobionts were determined on the basis of tRNALeu (UAA) intron sequences. More than 30 different cyanobiont genotypes were identified, all belonging to a previously known group of *Peltigera*-type *Nostoc* genotypes. Some *Peltigera* taxa (e.g. *P. malacea*, *P. extenuata*) associate with a single or a few closely related *Nostoc* genotypes, others (e.g. *P. leucophlebia*, *P. rufescens*, *P. didactyla*) were found to associate with a spectrum of different *Nostoc* genotypes. However, this selectivity does not reflect the distinct phylogenetic relationships within the genus *Peltigera*; instead it correlates with the mycobiont habitat preference. Habitat-specific segregation of *Peltigera* taxa and symbiotic *Nostoc* genotypes vary along gradients of humidity, from mesotrophic forests to oligotrophic forests and from basic soil alvar grasslands to other xerophytic habitats with acidic soil.

Acknowledgements: Financial support was received from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreements No 659070 and No 705777.

References

JÜRIADO I., KAASALAINEN U. & RIKKINEN J. 2017: Specialist taxa restricted to threatened habitats contribute significantly to the regional diversity of *Peltigera* (Lecanoromycetes, Ascomycota) in Estonia. – Fungal Ecology 30: 76–87.

Transcriptional co-option, transcriptional decay and the principles of regulatory evolution

Arnar PÁLSSON, Institute of Biology, University of Iceland, Sæmundargata 2, 101 Reykjavík, Iceland. - apalsson@hi.is

Marcos ANTEZANA, Institute of Biology, University of Iceland, Sæmundargata 2, 101 Reykjavík, Iceland. - marcos.antezana@gmail.com

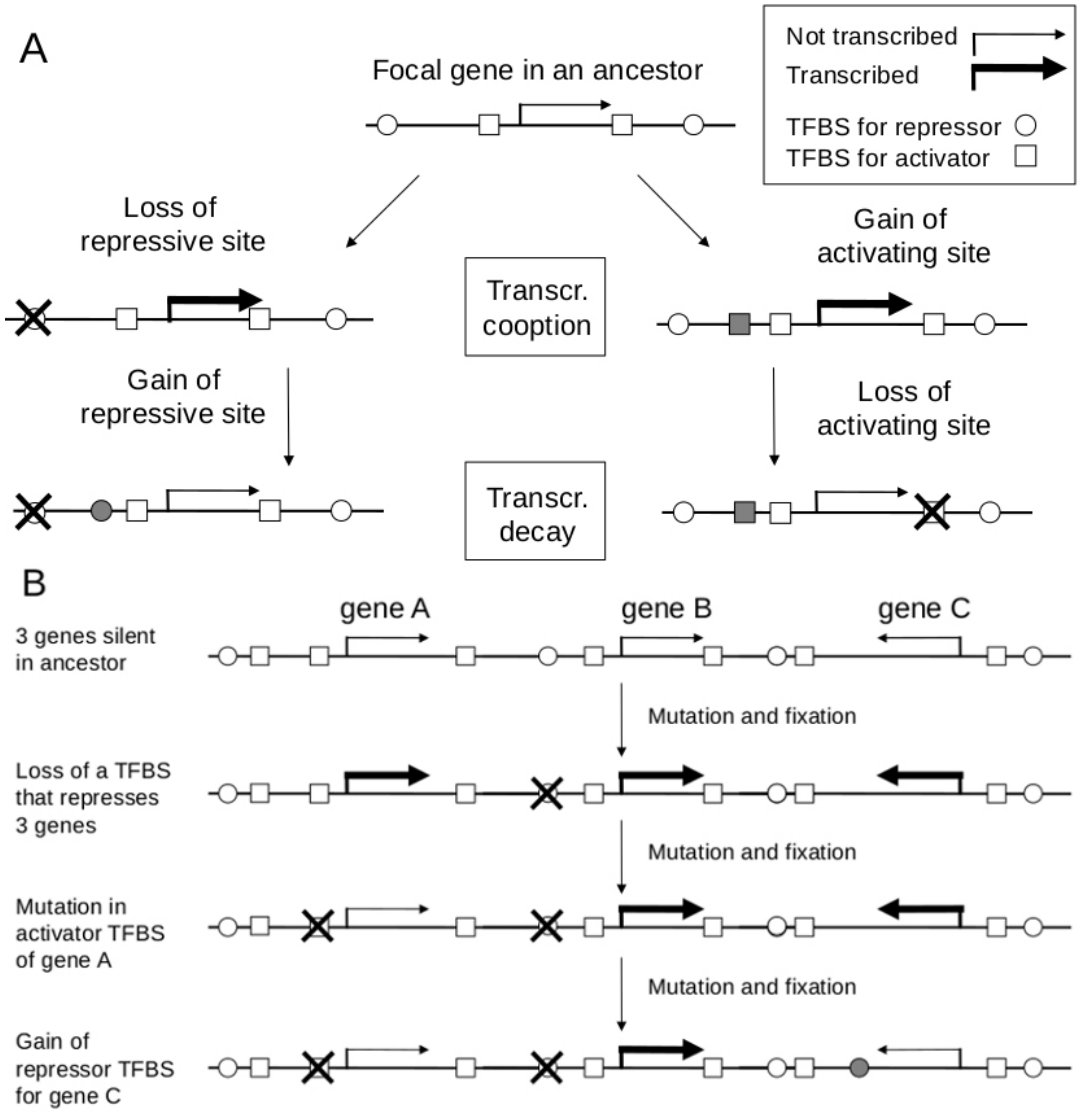
PALSSON A. & ANTEZANA M. 2017: Transcriptional co-option, transcriptional decay and the principles of regulatory evolution. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 31–33. - ISSN 1024-0306.

Regulatory evolution is important for adaptive evolution and the emergence of novelties, in part because regulatory mutations tend to be less pleiotropic than changes in exons. The functional properties of regulatory elements, e.g. short, degenerate motifs, for multiple activators and repressors, dictate the evolution of regulatory DNA (WRAY et al. 2003). Stabilizing selection on transcription may explain the evolutionary turnover of transcription factor binding sites (TFBS) in enhancers (LUDWIG et al. 2000). Genes have been recruited for multiple functions during evolution, by a process called transcriptional co-option (TC) (TRUE & CARROLL 2002). In TC a mutation in a gene (here called focal gene), not previously expressed in a specific tissue or cell population during development, turns the gene on in that tissue and the responsible allele is fixed in the population. As new TFBS arise easily via mutation, it was proposed that any transcription factor can co-opt (influence the transcription of) any gene in the genome (PRUD'HOMME et al 2007). Here we define the opposite scenario, evolution by transcriptional decay (TD). In TD, a mutation reducing strongly the transcription of a focal gene in tissue or developmentally specific fashion is fixed by positive selection. An example of TC would be the recruitment of various crystallins to the vertebrate lens (PIATIGORSKY 2006), and of TD the loss of *Pitx1* expression in pelvic structures in stickleback (Shapiro et al. 2004). Here we will use transcriptional decay and co-option as a prism to explore the principles of regulatory evolution.

TC or TD can arise by mutations in either *trans*-factors (TF) or *cis*-elements (figure part A). While we focus on mutations in *cis*, changes in the structure or tissue specific concentration of a TF can affect expression of a focal gene. Such changes are expected to be pleiotropic, with changes in expression of targets of that TF (if changes are in TF level, then the effects will be more circumscribed). Changes at the level of *cis*-elements will be less pleiotropic. Disruption of TFBS can for example either increase or decrease expression of the focal gene, depending on whether the TF that binds that TFBS represses or activates transcription. The complementary argument applies for mutations that generate TFBS; TC can occur by gain of an activator binding site affecting the focal gene. And in TD a gain of a binding site for repressor near the focal gene.

Regulatory mutations can be pleiotropic. In one scenario, a mutation alters the expression of two or more genes in the same chromosome region. If the fitness increase of the increased expression of the focal gene outweighs the fitness reduction of its chromosome neighbors (figure, part B), then TC can occur. The

stronger the selection, the more serious the regulatory side effects can be. After fixation of such a mutation, we anticipate rounds of refinement where the deleterious expression of nearby genes will be alleviated, e.g. by loss of activator sites or gain of repressor sites (figure, part B). Other regulatory changes (miRNA binding sites, RNA degradation etc.) may play a role, and the complementary case when reduced expression of two or more genes is favored will adhere to the same principles. Similar mechanistic and evolutionary logic will also apply if adaptive expression increase of a focal gene in a tissue, leads to increased expression of the gene in other tissues.



The central principles discussed here are, i) mutations disrupting TFBS can lead to beneficial changes in gene expression, ii) *cis*-regulatory mutations can be pleiotropic, affecting multiple genes and potentially tissues, iii) the likelihood of fixation of such pleiotropic mutations depends on the strength of selection, iv) natural selection is likely to alleviate such side-effects by favoring modifiers (e.g. in *cis* or *trans*). Finally, these principles are likely to apply more generally, to gain and decay of associations between trans-regulators and regulatory motifs in DNA, RNA and proteins. For instance regulatory systems like those controlling mRNA splicing, export, stability and localization, translation, protein maturation and modifications and other cellular regulatory cascades.

Acknowledgements: We thank the organizers of the Lichen Genomics Workshop for the invitation and Baldur KRISTJANSSON for comments.

References

LUDWIG M.Z., BERGMAN C., PATEL N.H. & KREITMAN M. 2000: Evidence for stabilizing selection in a eukaryotic enhancer element. - Nature 403: 564–567.

PIATIGORSKY J. 2006: Evolutionary genetics: seeing the light: the role of inherited developmental cascades in the origins of vertebrate lenses and their crystallins. - Heredity 96: 275–277.

PRUD'HOMME B., GOMPEL N. & CARROLL S.B. 2007: Emerging principles of regulatory evolution. - Proceedings of the National Academy of Sciences of the United States of America 15: 8605–8612.

SHAPIRO M.D. [and 7 additional authors] 2004: Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. - Nature 428: 717–723.

TRUE J.R. & CARROLL S.B. 2002: Gene co-option in physiological and morphological evolution. - Annual Review of Cellular and Developmental Biology 18: 53–80.

WRAY G.A., HAHN M.W., ABOUHEIF E., BALHOFF J.P., PIZER M., ROCKMAN M.V. & ROMANO L.A. 2003: The evolution of transcriptional regulation in eukaryotes. - Molecular Biology and Evolution 20: 1377–1419.

GC-MS-based metabolite profiling as a new tool to study lichens and mycobiont-photobiont symbiotic interactions

Gregor PICHLER, Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria. - gregor.pichler@student.uibk.ac.at

Fabio CANDOTTO CARNIEL, Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria and Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Italy. - fabio.candotto@gmail.com

Erwann ARC, Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria. - erwann.arc@gmail.com

Wolfgang STÖGGL, Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria. - wolfgang.stoeggl@uibk.ac.at

Ilse KRANNER, Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020 Innsbruck, Austria. - ilse.kranner@uibk.ac.at

PICHLER G., CANDOTTO CARNIEL F., ARC E., STÖGGL W. & KRANNER I. 2017: GC-MS-based metabolite profiling as a new tool to study lichens and mycobiont-photobiont symbiotic interactions. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 34–35. - ISSN 1024-0306.

Metabolomics is highly relevant to contemporary scientific research and is also gaining importance in lichenology. Here we report on a gas chromatography - mass spectrometry (GC-MS) based metabolite profiling method. In this assay, extracted metabolites are first submitted to a two-step chemical derivatization using methoximation to stabilize sugars in an open ring conformation and trimethylsilylation to produce thermally stable compounds. The resulting derivatives are then injected into a gas chromatograph coupled to a triple quadrupole mass spectrometer. As a result, each analyte can be associated to a retention time and a specific spectrum with defined mass-to-charge ratios (m/z). The comparison of these informations with different databases (custom build, commercial and publicly available mass spectral libraries) allows the identification of metabolites such as amino-acids, common sugars, organic acids (including TCA cycle intermediate), polyols and phosphorylated sugars. However, the precise identification of compounds not found in GC-MS based libraries may require elaborated extraction and isolation techniques combined with further analyses by high resolution MS and Nuclear Magnetic Resonance Spectroscopy (NMR). As an example, the 14 most abundant low-molecular-weight carbohydrates found in *Flavoparmelia caperata* thalli are arabitol, erythritol, fructose, galactose, glycerol, mannitol, myo-inositol, ribitol, sorbitol, sucrose, trehalose, volemitol, xylitol and xylose. The ability to detect trace amounts of compounds may also enable the exploration of hitherto unknown biochemical processes. Metabolite profiling is particularly valuable to resolve open questions in lichenology, such as the molecular crosstalk and the chemical communication between photobiont and mycobiont upon lichenization, and may help discovering new biochemical pathways (EISENREICH et al 2011). For example, the roles of polyols and other carbohydrates in lichen symbionts is still not fully understood. Importantly, metabolites of lichens and their isolated components are sources of bioactive compounds with potential medicinal properties

(BOUSTIE & GRUBE 2005), and research into lichen metabolites may open new avenues for biotechnological applications.

Acknowledgements. We gratefully acknowledge financial support by a DIAnet Outgoing fellowship (code FP1369874001) to FCC and we thank Daniela Cornelia SPIELMANN for helpful comments.

References

BOUSTIE J. & GRUBE M. 2005: Lichens - a promising source of bioactive secondary metabolites. - Plant Genetic Resources 3: 273–287.

EISENREICH W., KNISPEL N. & BECK A. 2011: Advanced methods for the study of the chemistry and the metabolism of lichens. - Phytochemistry Reviews 10: 445–456.

From genome to function – functional annotations in our favorite non-model organisms

Philipp RESL, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - philipp.resl@uni-graz.at

RESL P. 2017: From genome to function - functional annotations in non-model organisms. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 36–37. - ISSN 1024-0306.

High-throughput sequencing technologies have led to an immense increase of nucleic acid sequence data over the last decades. It is now easier than ever to sequence complete genomes or transcriptomes even for non-model organisms. However the ever-growing amount of data also creates many challenges when it comes to data analyses. One such challenge is how to assign functions to the many genes in newly sequenced genomes to understand links between genotype and phenotype.

Traditionally, functional assignments focus on single (or few) genes and involve laborious laboratory experiments the results of which are interpreted within the metabolic context of single, usually well-studied model organisms. Information on these functionally well-characterized genes forms the core of curated databases of protein functions such as Uni-ProtKB/Swiss-Prot (THE UNIPROT CONSORTIUM 2017). However, despite such joint international efforts, the functional characterization of genes cannot keep up with the speed at which new nucleic acid data are produced, and experimental evidence of gene function still largely stems from model organisms.

Often, putative functions of large numbers of genes are therefore assigned computationally, by comparing unknown genes to databases of well-characterized genes. Comparing whole-sequence similarity (BLAST best-hit approaches) or the similarity of functional domains or sequence motifs (usually based on Hidden Markov Models) are two possible approaches. A number of specialized databases such as SignalP (to identify signal peptides; NIELSEN 2017), CAZy (LOMBARD et al. 2014; a database for carbohydrate active enzymes) or KEGG (KANEHISA et al. 2017; the encyclopedia of genes and genomes) among many others make it possible to put numbers of genes into a functional context. However, in non-model organisms a large number of genes or metabolic pathways may be underrepresented in the commonly utilized databases and thus remain uncharacterized with most analyses.

In this talk, I will present functional annotation results of several lichen-forming fungal genomes with several functional annotation approaches and highlight some of the challenges associated with each of them. I will also introduce the Gene Ontology (GO; ASHBURNER et al. 2000) initiative, which aims to unify the vocabulary of gene product annotations.

References

ASHBURNER M. [and 19 additional authors] 2000: Gene ontology: tool for the unification of biology. - Nature Genetics 25(1): 25–9.

KANEHISA M., FURUMICHI M., TANABE M., SATO Y. & MORISHIMA K. 2017: KEGG: new perspectives on genomes, pathways, diseases and drugs. - Nucleic Acids Research 45: D353–D361.

LOMBARD V., GOLACONDA RAMULU H., DRULA E., COUTINHO P.M. & HENRISSAT B. 2014: The Carbohydrate-active enzymes database (CAZy) in 2013. - Nucleic Acids Research 42: D490–D495. [http://www.cazy.org]

NIELSEN H. 2017: Predicting Secretory Proteins with SignalP. - In: KIHARA D. (editor): Protein Function Prediction, pp. 59–73. Humana Press.

THE UNIPROT CONSORTIUM 2017: UniProt: the universal protein knowledgebase. - Nucleic Acids Research 45(D1): D158–D169.

The origin and evolution of intercontinental disjunctions of lichen-forming fungi in fresh-water habitats

Pamela RODRIGUEZ-FLAKUS, Laboratory of Molecular Analyses, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland. - prodriguez@botany.pl

RODRIGUEZ-FLAKUS P. 2017: The origin and evolution of intercontinental disjunctions of lichen-forming fungi in fresh-water habitats. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 38. - ISSN 1024-0306.

Fresh-water environments and its biodiversity are among the most important for human well-being. During the last years, several worldwide policies have been applied to protect those ecosystems and their services including a good ecological status of their biological elements. The lichen symbioses, subject of this study, are an integral part of those valuable habitats. Several fresh-water lichens are bipolar species showing wide-ranging disjunct distribution patterns.

The main goal of this project is to resolve the origin of the intercontinental disjunctions of those microorganisms, and the underlying historical mechanisms for the evolution of both fungal and algal symbionts, using restriction site-associated DNA sequencing (RAD-seq). These methods have recently been most widely used for the population genetics analyses of other organisms, providing the best combination of read-length, low cost, and highly informative output when compared to traditional sequencing approaches. Fresh-water lichen-forming fungi are an iconic model of mutualistic symbiosis for study from an evolutionary or biogeographical point of view. However, knowledge of origin, evolution or phylogeography of these organisms based on modern molecular approaches is still very basic.

Acknowledgements: The author is partly supported by the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland.

Climate niche expansion due to generalization in species associations in lichens

Ulrike RUPRECHT, University of Salzburg, Department of Ecology & Evolution, Hellbrunnerstraße 34, 5020 Salzburg, Austria. - ulrike.ruprecht@sbg.ac.at

Robert R. JUNKER, University of Salzburg, Department of Ecology & Evolution, Hellbrunnerstraße 34, 5020 Salzburg, Austria. - robert.junker@sbg.ac.at

RUPRECHT U. & JUNKER R.R. 2017: Climate niche expansion due to generalization in species associations in lichens. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 39–40. - ISSN 1024-0306.

Saxicolous crustose lichens are the most successful and often the only vegetation forming organism in extreme habitats in polar and high elevated areas (RUPRECHT et al. 2016). They form symbiotic associations of a fungus (mycobiont) and a photosynthetic partner (green algae and/or cyanobacteria; photobiont). Both partners have species-specific and independent distribution ranges exemplifying that both partners are associated with more than one specialized partner.

We investigated the role of climatic factors driving lichen diversity, distribution and interaction patterns between mycobiont and photobiont along a latitudinal gradient in southern Patagonia (South America) spanning a broad range of climatic conditions.

In total, we identified 49 OTUs of mycobionts that were associated with 16 OTUs of photobionts. Network statistics (BLÜTHGEN et al. 2006) revealed that mycobionts were – on average – more generalized in their associations than the photobionts that were often associated with few mycobionts only. However, the rather specialized photobionts were complemented by two strongly generalized and cosmopolitan species (*Trebouxia*_S02, *Tr*_I01) which cover almost 50% of the accessions.

On the one hand, *N*-dimensional hypervolume analyses using the R package *dynRB* (JUNKER et al. 2016) show clearly that two cryptic species of the mycobiont clade *Lecidea lapicida* (*LP*_Lcd16 and *LP*_Lcd19) are predominantly associated with the same photobiont but occupy strongly diverging climatic niches: *LP*_Lcd16 is only associated with one photobiont and restricted to a small climatic niche. In contrast, the generalist *LP*_Lcd19 expanded its climate niche strongly by associations with three different photobionts.

Our work shows that a strong degree of specialisation is associated with a restricted ecological niche, whereas more generalized associations between myco- and photobionts enable the fungi to colonize a broader range of climatic conditions.

The asymmetry in specialization in myco- and photobionts sheds new light on the ecology and evolution of the associations between the symbiotic partners in lichens.

Acknowledgements: This study was financially supported by the Austrian Science Fund (FWF): P26638.

References

BLÜTHGEN N., MENZEL F. & BLÜTHGEN N. 2006: Measuring specialization in species interaction networks. - BMC Ecology 6: 9.

JUNKER R.R., KUPPLER J., BATHKE A.C., SCHREYER M.L. & TRUTSCHNIG W. 2016: Dynamic range boxes - a robust nonparametric approach to quantify size and overlap of *n*-dimensional hypervolumes. - Methods in Ecology and Evolution 7: 1503–1513.

RUPRECHT U., SØCHTING U. & TÜRK R. 2016: *Porpidia navarina*, a new endemic species from Isla Navarino (southern Tierra del Fuego, Chile). - Herzogia 29: 596–609.

The lichen individual: Insights from fungal microsatellites and outlooks into genomics

Christoph SCHEIDEGGER, Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland, christoph.scheidegger@wsl.ch

Jessica ALLEN, Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland, jessica.allen@wsl.ch

Olga NADYEINA, Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland, olga.nadyeina@wsl.ch

SCHEIDEGGER C., ALLEN J.L. & NADYEINA O. 2017: The lichen individual: Insights from fungal microsatellites and outlooks into genomics. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 41. - ISSN 1024-0306.

The fundamental sampling unit for population genetics studies is the individual. While identifying an individual when working with animals and many plants is simple, it is usually not straightforward with fungi. Lichenized fungi present some particular challenges. Although lichens are readily macroscopically observable in their habitats year-round, unlike most fungi, in many cases it can be difficult to determine where one individual stops and another starts. Some examples of scenarios with ambiguous individuals include corticolous crustose species that do not form zones of inhibition, communities of terricolous *Cladonia*, and chimeric foliose thalli. In this presentation we will highlight examples of ambiguous individuals discovered through microsatellite assays on foliose and crustose species. We will discuss the implications of finding multiple haplotypes in a single sample for population genetic and genomic studies. We will conclude by presenting some practical approaches to identifying and handling the presence of multiple genetic individuals in genomics samples.

Natural products from lichens

Tânia Keiko SHISHIDO, Institute of Biotechnology, University of Helsinki, Viikinkaari 5D, FIN-00014, Helsinki, Finland. - tania.shishido@helsinki.fi

Jouko RIKKINEN, Faculty of Biological and Environmental Sciences, Finnish Museum of Natural History Luomus and Department of Biosciences, University of Helsinki, POBox 7 and POBox 65, FIN-00014, Helsinki, Finland. - jouko.rikkinen@helsinki.fi

Petri AUVINEN, Institute of Biotechnology, University of Helsinki, Viikinkaari 5D, FIN-00014, Helsinki, Finland. - petri.auvinen@helsinki.fi

SHISHIDO T.K., RIKKINEN J. & AUVINEN P. 2017: Natural products from lichens. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 42. - ISSN 1024-0306.

Lichens have been traditionally used as a medicine in human communities around the world (KOSANIĆ et al. 2011, WANG et al. 2014). Diverse microorganism may be found living in lichen-association, such as fungi, algae, cyanobacteria and bacteria. Lichen or isolated lichen-associated strains were found to have antimicrobial, anticancer and antioxidant activities, among others (BOUSTIE et al. 2011, BASILE et al. 2015). The present research project aims to search new antimicrobial drug leads produced in lichen symbiosis. The use of meta-omics approaches, such as meta-genomics, metatranscriptomics and peptidogenomics, allows the study of uncultivated organisms. In this study, a meta-omics research pipeline will be used to search the biosynthetic genes and the compounds produced by microorganism in a lichen association. In addition, the molecular networking of fragments will aid to detect the potential unknown natural products produced in the lichens. The diversity of the bacterial community and their role in the lichen symbiosis will be investigated through the analysis of the metagenomics and metatranscriptomics data. *Cladonia* spp., *Cetraria islandica*, *Platismatia glauca*, *Peltigera* sp., *Umbilicaria* spp., and other lichens were collected from Espoo and Helsinki forest and city areas and will be further analyzed. The expected results include the discovery of new drug leads with antimicrobial activities, the creation of a molecular network that will indicate unique compounds to be further characterized, and the increase in knowledge of the role of the bacterial community in lichens.

Acknowledgements: This work is supported by the grant from Academy of Finland (308266) to TKS.

References

BASILE A., RIGANO D., LOPPI S. [and 9 additional authors] 2015: Antiproliferative, antibacterial and antifungal activity of the lichen *Xanthoria parietina* and its secondary metabolite parietin. - International Journal of Molecular Sciences 16(4): 7861–75.

BOUSTIE J., TOMASI S. & GRUBE M. 2011: Bioactive lichen metabolites: alpine habitats as an untapped source. - Phytochemistry Reviews 10(3): 287–307.

KOSANIĆ M.M., RANKOVIĆ B.R. & STANOJKOVIĆ T.P. 2012: Antioxidant, antimicrobial and anticancer activities of three *Parmelia* species. - Journal of the Science of Food and Agriculture 92(9): 1909–16.

WANG Y., WANG J., CHEONG Y.H. & HUR J.-S. 2014. Three new non-reducing polyketide synthase genes from the lichen-forming fungus *Usnea longissima*. - Mycobiology 42(1): 34–40.

Multi-gene phylogeny of the lichen genus *Dendriscosticta* and transcriptome sequencing of photomorph pairs

Antoine SIMON, Evolution and Conservation Biology, InBIOS research center, University of Liège, Quartier Vallée 1, Chemin de la Vallée 4, B-4000 Liège, Belgium. - asimon@doct.ulg.ac.be

Toby SPRIBILLE, Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A. - spribill@ualberta.ca

Bernard GOFFINET, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269-3043, U.S.A. - bernard.goffinet@uconn.edu

Li-Song WANG, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming 650-204, China. - wanglisong@mail.kib.ac.cn

Trevor GOWARD, UBC Herbarium, Beaty Museum, University of British Columbia, Vancouver, BC V6T1Z4, Canada. - trevor.goward@botany.ubc.ca

Tatiana PYSTINA, Institute of Biology, Komi Science Center UrD RAS, Kommunisticheskaya street 28, Syktyvkar, Russia. - pystina@ib.komisc.ru

Natalia SEMENOVA, Institute of Biology, Komi Science Center UrD RAS, Kommunisticheskaya street 28, Syktyvkar, Russia. - semenova@ib.komisc.ru

John P. MCCUTCHEON, Division of Biological Sciences, University of Montana, Missoula, MT 59812, U.S.A. - john.mccutcheon@mso.umt.edu

Emmanuël SÉRUSIAUX, Evolution and Conservation Biology, InBIOS research center, University of Liège, Quartier Vallée 1, Chemin de la Vallée 4, B-4000 Liège, Belgium. - e.serusiaux@ulg.ac.be

SIMON A., SPRIBILLE T., GOFFINET B., WANG L.-S., GOWARD T., PYSTINA T., SEMENOVA N., MC CUTCHEON J.P. & SÉRUSIAUX E. 2017: Multi-gene phylogeny of the lichen genus *Dendriscosticta* and transcriptome sequencing of photomorph pairs. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 43–44. - ISSN 1024-0306.

A lichen is generally defined as the product of a symbiotic association between a fungus and at least one photosynthetic organism (a green alga and/or a cyanobacterium). Some species of Lobariaceae (Peltigerales, Lecanoromycetes) are remarkable among lichen-forming fungi in that they can form thalli with two different kinds of synthetic partners (i.e., with a green alga or a cyanobacterium). These different lichen bodies, usually referred to as photomorphs, can be morphologically similar, or display extremely different phenotypes as observed in the genus *Dendriscosticta*.

This study looks at evolutionary dynamics in symbiotic systems, using dissimilar lichen photomorphs, that constitute optimal models for addressing fundamental questions in the biology of symbiosis. The main objectives are to:

- identify genes that show differential expression in photomorphs of *Dendriscosticta*, and ultimately characterize the genes that play a predominant role in the symbiosis;
- reconstruct the phylogenetic relationships of members of the genus *Dendriscosticta*, using the most comprehensive sampling to date.

As for the transcriptomic aspect of the study, fresh specimens were collected during field trips in British Columbia (Canada). *Dendriscosticta* specimens were found in eight different sites. RNA libraries were successfully prepared for a total of twelve thalli, including the two morphologically dissimilar photomorphs, while being representative of the geographical distribution in British Columbia. Whole transcriptome sequencing (RNA-seq) was carried out for each prepared library. Each library yielded approximately 30 Gb of high-quality data (~ 45 million reads per library). Illumina reads were pooled and de novo assembled. Coding regions were identified by determining open reading frames (ORFs) from the assembly. Ultimately, counts of differential expression were obtained after the alignment of reads onto the captured ORFs. Our preliminary results show that hierarchical clustering of differentially expressed genes clearly discriminates between photomorphs, but also between species within the *Dendriscosticta wrightii* aggregate (*D*. aff. *wrightii* versus *D*. *borealis*).

In addition to the aforementioned transcriptomic component, a multigene phylogeny has been inferred to provide the first comprehensive evolutionary tree for the genus *Dendriscosticta*. To date, four fungal molecular markers have been sequenced for about 80 specimens of *Dendriscosticta*, including specimens from European Russia, India, North America, Taiwan, and Yunnan. Our results reveal a remarkably high and unsuspected biodiversity in this genus, and helps clarify the taxonomic status of the *Dendriscosticta wrightii* aggregate.

'Case studies' and experimental approaches for culturing lichen fungi to understand developmental strategies of lichen thalli under various ecological conditions

Elfie STOCKER-WÖRGÖTTER, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - elfriede.stocker@uni-graz.at

STOCKER-WÖRGÖTTER E. 2017: 'Case studies' and experimental approaches for culturing lichen fungi to understand developmental strategies of lichen thalli under various ecological conditions. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 45. - ISSN 1024-0306.

The major branch of genomics still focusses on sequencing the genomes of various organisms, later on the knowledge of full genomes has created new possibilities and questions for the field of functional genomics, which is mainly concerned with patterns of gene expression during various conditions.

Lichens colonize many substrata within diverse habitats world-wide, depending on the species and its adaptation. The ability of lichens to colonize diverse habitats could be connected with competition among algal symbionts that associate with the lichen-forming fungus in the thallus. Most lichen fungi cannot survive without an algal partner, rendering lichen fungi obligate symbiotic organisms, where dispersal mechanisms could result in the same or different combinations of symbionts at each dispersal event.

During my presentation I will try to give an overview about culture methods and techniques we performed during the past 20 years. I will select and highlight 'case studies' I did which could be of use for young lichenologists and lichenologists in general to achieve axenic and genetically uniform lichen fungi/mycelia for genomic studies which certainly appears to be still a challenge, considering that lichen thalli are composed of two/three/four symbiotic partners living together with several further epi- and/or endo-lichenic organisms with mainly unknown functions.

Moreover it is hypothesized that some of the secondary metabolic capabilities of lichen fungi may be similarly influenced by selection pressures exerted by other organisms, because relatively little is known of the chemical interactions between different lichen associated organisms common within functioning lichen ecosystems.

Finally, questions like 'what determines and guides thallus morphogenesis in symbiotic fungi/lichen fungi', will be discussed regarding the results of the most successful culture experiments.

Cyanobacteria in symbiosis with boreal forest feathermosses: from genome evolution and gene regulation to impact on the ecosystem

Denis WARSHAN, Life and Environmental Sciences, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland. - warshanded@gmail.com.

Katharina PAWLOWSKI, Department of Ecology, Environment and Plant Sciences, Stockholm University, Svante Arrheniusvägen 20A, Stockholm 114 18, Sweden. - katharina.pawlowsk@su.se

Ulla RASMUSSEN, Department of Ecology, Environment and Plant Sciences, Stockholm University, Svante Arrheniusvägen 20A, Stockholm 114 18, Sweden. - ulla.rasmussen@su.se.

WARSHAN D., PAWLOWSKI K. & RASMUSSEN U. 2017: Cyanobacteria in symbiosis with boreal forest feathermosses: from genome evolution and gene regulation to impact on the ecosystem. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 46–47. - ISSN 1024-0306.

Among dinitrogen (N_2)-fixing some cyanobacteria can establish symbiosis with a broad range of host plants from all plant lineages including bryophytes, ferns, gymnosperms, and angiosperms (RIKKINEN 2017). In the boreal forests, the symbiosis between epiphytic cyanobacteria and the feathermosses *Hylocomium splendens* and *Pleurozium schreberi* is ecologically important (DELUCA et al. 2001, 2008). The main input of biological nitrogen to the boreal forests is through these cyanobacteria, and thus, they greatly contribute to the productivity of this ecosystem (DELUCA et al. 2002, 2008). Despite the ecological relevance of the feathermoss symbiosis, our knowledge about the establishment and maintenance of cyanobacterial-plant partnerships in general is limited, and particularly our understanding of the feathermoss symbiosis is rudimentary.

The first aim of this work was to gain insight into the genomic rearrangements that enabled cyanobacteria to form a symbiosis with feathermosses, and their genomic diversity and similarities with other plant-symbiotic cyanobacteria partnerships. Genomic comparison of the feathermoss isolates with the genomes of free-living cyanobacteria highlighted that functions such as chemotaxis and motility, the transport and metabolism of organic sulfur, and the uptake of phosphate and amino acids were enriched in the genome of plant-symbiotic cyanobacteria.

The second aim was to identify cyanobacterial molecular pathways involved in forming the feathermoss symbiosis and the regulatory rewiring needed to maintain it. Global transcriptional and post-transcriptional regulation in cyanobacteria during the early phase of establishment of the feathermoss symbiosis, and after colonization of the moss were investigated. The results revealed that the putative symbiotic gene repertoire includes pathways never before associated with cyanobacteria-plant symbioses, such as nitric-oxide sensing and regulation, and the transport and metabolism of aliphatic sulfonate.

The third aim was to explore the role of the cyanobacterial community in contributing to the temporal variability of N_2-fixation activity. Results from a field-study

showed that temporal variation in N_2-fixation rates could be explained to a high degree by changes in cyanobacterial community composition and activity. In particular, the cyanobacteria belonging to the genus *Stigonema* - although not dominating the community - appeared to be the main contributors to the N_2-fixation activities. Based on this result, it is suggested that this genus is responsible for the main input of nitrogen in the boreal forest ecosystems.

The last aim was to understand how the relationship between cyanobacterial community composition and N_2-fixation activity will be affected by climatic changes such as increased temperature (11^{o}C compared to 19^{o}C) and CO_2 level (500 ppm compared to 1000 ppm). Laboratory experiments highlighted that 30 weeks of combined elevation of temperature and CO_2 resulted in increased N_2-fixation activity and moss growth rates. The observed increases were suggested to be allocated to reduced cyanobacterial diversity and changes in community composition, resulting in the dominance of cyanobacteria adapted to the future abiotic condition.

Acknowledgements: DOE EMSL-JGI Collaborative Science, Carl Tryggers Foundation and J. Craig Venter Institute.

References

RIKKINEN J. 2017: Cyanobacteria in terrestrial symbiotic systems. - In: HALLENBECK P.C. (editor). Modern topics in the phototrophic prokaryotes. Environmental and Applied Aspects. pp. 243–294. Springer International Publishing Ag. Switzerland.

DELUCA T.H., ZACKRISSON O., NILSSON M.C. & SELLSTEDT A. 2002: Quantifying nitrogen-fixation in feather moss carpets of boreal forests. - Nature 419: 917–920.

DELUCA T.H., ZACKRISSON O., GUNDALE M.J. & NILSSON M.C. 2008: Ecosystem feedbacks and nitrogen fixation in boreal forests. - Science 320: 1181–1181.

How does the lichen symbiosis work? Journey into the unknown

Silke WERTH, Institute of Plant Sciences, University of Graz, Holteigasse 6, 8010 Graz, Austria. - silke.werth@uni-graz.at

WERTH S. 2017: How does the lichen symbiosis work? Journey into the unknown. Abstract. - In: WERTH S. & OBERMAYER W. (editors). Lichen Genomics Workshop II. Institute of Plant Sciences, University of Graz, Austria. 2–5 November 2017. - Fritschiana (Graz) 85: 48–49. - ISSN 1024-0306.

Lichens are… ?

…The symbiotic phenotype of fungi nutritionally dependent on photosynthetic algae or cyanobacteria. The lichen thallus, the structure created by the mutualistic interaction, hosts a diverse community of bacteria and fungi whose functions remain largely unclear.

To understand how the lichen symbiosis works, we have to bring together expertise from different areas of research: morphology, physiology, omics approaches, molecular biology, and others. One topic of interest is the ultrastructure of mycobiont-photobiont contact sites, and different types of contact have been described, depending on identity of the photobiont (reviewed in HONEGGER 1985, 1991). Early physiological studies demonstrated that in symbiosis, cyanobacterial photobionts export glucose while green algal photobionts export polyols to the mycobiont (reviewed in SMITH 1980). This transport involves a vast fraction of the carbohydrates fixed by the photoautotrophic partner. The export of carbohydrates from photobiont cells is terminated within hours after isolation, and then, lichen photobionts instead invest into storage products (GREEN & SMITH 1974).

Even though lichens were the first mutualistic system for which transfer of carbohydrates from a photoautotrophic partner to a heterotrophic partner was investigated in much detail, it is largely unknown to date how the lichen symbiosis works at the molecular level. For example, which genes are involved in transfer of carbohydrates? How important are epigenetic marks for the functioning of the symbiosis?

By making meaningful use of –omics approaches in combination with molecular biology techniques in well-designed experiments, chances are that we shall be getting closer to understanding this important biological phenomenon in the near future.

Acknowledgements: I thank my colleagues from the University of Graz and worldwide for inspiring discussions. This contribution draws strongly on the large body of early physiological work by Sir David Cecil SMITH and coworkers, and on the work on ultrastructure of the mycobiont-photobiont interface by Rosmarie HONEGGER.

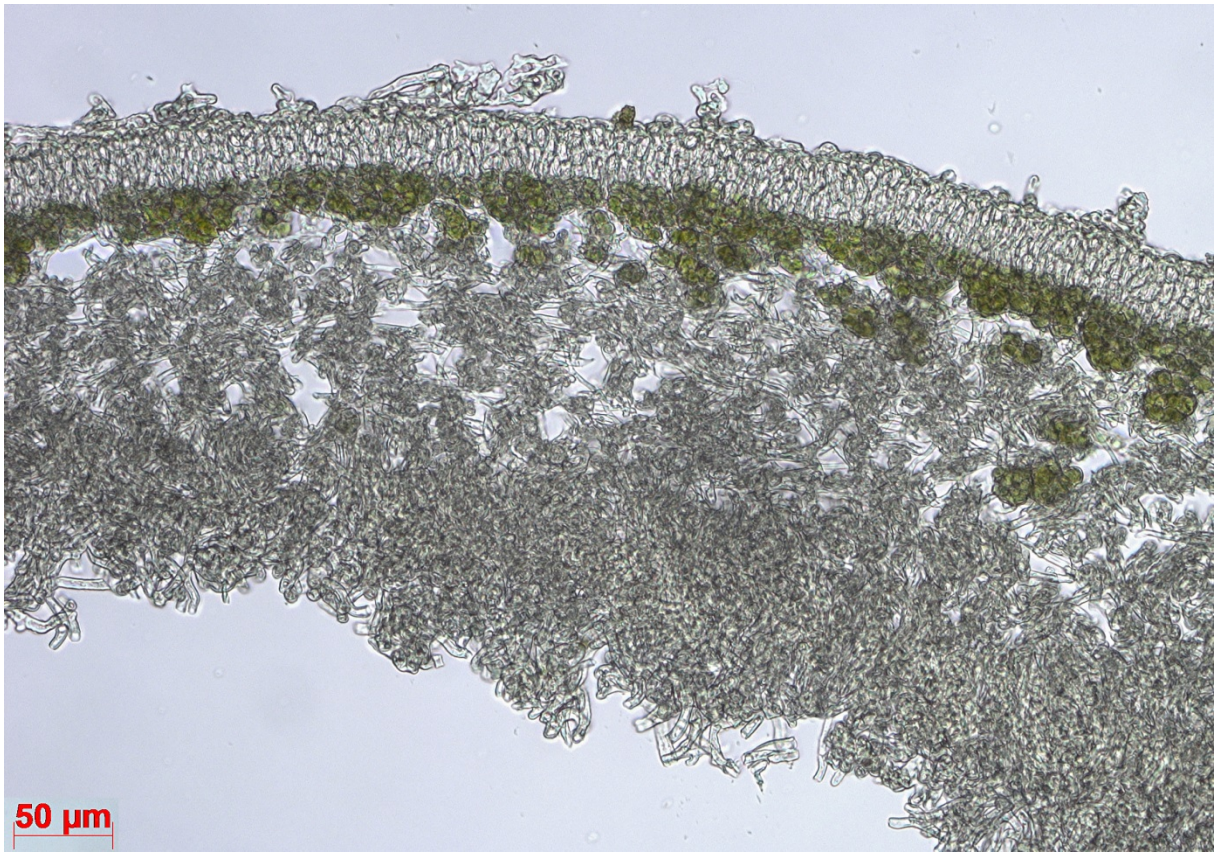
References

GREEN T.G.A & Smith D.C. 1974: Lichen physiology. XIV. Differences between lichen algae in symbiosis and in isolation. – New Phytologist 73: 753–766.

HONEGGER R. 1985: Fine structure of different types of symbiotic relationships in lichens. - In: BROWN D.H. (editor). Lichen physiology and cell biology, pp. 287-302. New York and London. Plenum Press.

HONEGGER R. 1991: Functional aspects of the lichen symbiosis. - Annual Review of Plant Physiology and Molecular Biology 42: 553–578.

SMITH D.C. 1980: Mechanisms of nutrient movement between the lichen symbionts. - In: COOK C.B., PAPPAS P.W. & RUDOLPH E.D. (editors). Cellular interactions in symbiosis and parasitism, pp. 197–228. Columbus: Ohio State University Press.



Peltigera membranacea: Cross section of the thallus. - Photo taken by Walter OBERMAYER

Peltigera leucophlebia: Wet thallus with cephalodia. - Photo taken by Walter OBERMAYER

Bisher erschienen - hitherto published

A complete table of contents (including pdf-files) is available under
https://botanik.uni-graz.at/de/fritschiana/
and under http://www.landesmuseum.at/datenbanken/digilit/?serienr=7405

REDURON Jean-Pierre, ROTTENSTEINER Walter Karl & SCHEUER Christian 2016: Beiträge zur Flora von Istrien V: The Istrian Apiaceae: a new determination key, distribution maps, and a list of specimens housed in the herbarium GZU. - Fritschiana (Graz) 81: 1–80.

SCHEUER Christian 2016: Dupla Graecensia Fungorum (2016, Nos 351–450). - Fritschiana (Graz) 82: 1–36.

OBERMAYER Walter 2016: Dupla Graecensia Lichenum (2016, numbers 1021–1100). - Fritschiana (Graz) 83: 1–23.

HAFELLNER Josef 2016: Lichenicolous Biota (Nos 231–250). - Fritschiana (Graz) 83: 25–40.

HAFELLNER Josef 2016: Additional records of *Gyalideopsis mexicana* (lichenized Ascomycota). - Fritschiana (Graz) 83: 41–45.

HAFELLNER Josef & SPRIBILLE Toby 2016: *Tingiopsidium* - the correct name for *Vestergrenopsis* as currently delimited (Peltigerales, Koerberiaceae). - Fritschiana (Graz) 83: 47–50.

DRESCHER Anton & SCHARFETTER Astrid 2017. Vascular Plants issued by the Herbarium GZU, nos. 1–50. - Fritschiana (Graz) 84: 1–24.

DRESCHER Anton 2017: Alphabetischer Gesamtindex der Dupla plantarum vascularium (1997, 2001, 2002, 2003). - Fritschiana (Graz) 84: 25–30.

DRESCHER Anton 2017: Alphabetischer Gesamtindex der Dupla Salicum (1997, 2001, 2002, 2003). - Fritschiana (Graz) 84: 31–33.

ISSN 1024-0306