Charles University

Faculty of Science

Study programme: Ecology

Mgr. Lenka Procházková

Exploring the diversity of snow algae using polyphasic approach

Studium diverzity sněžných řas s využitím polyfázického přístupu

Doctoral thesis

Supervisor: Doc. RNDr. Linda Nedbalová, Ph.D.

Prague, 2019

Declaration

I hereby declare that I have written this thesis independently, using the listed references; or in cooperation with other paper co-authors. I have submitted neither this thesis, nor any of its parts, to acquire any other academic degree.

Prague, 14th November 2019

.....

Mgr. Lenka Procházková

The doctoral thesis is based on the following papers:

(enclosed as appendices)

I. **Procházková L**, Leya T, Křížková H & Nedbalová L (2019): *Sanguina nivaloides* and *Sanguina aurantia* gen. et spp. nov. (Chlorophyta): the taxonomy, phylogeny, biogeography and ecology of two newly recognised algae causing red and orange snow. *FEMS Microbiology Ecology* 95(6): fiz064.

II. **Procházková L,** Remias D, Holzinger A, Řezanka T & Nedbalová L (2018): Ecophysiological and morphological comparison of two populations of *Chlainomonas* sp. (Chlorophyta) causing red snow on ice-covered lakes in the High Tatras and Austrian Alps. *European Journal of Phycology* 53(2): 230–243.

III. **Procházková L**, Remias D, Řezanka T & Nedbalová L (2019): Ecophysiology of *Chloromonas hindakii*, sp. nov. (Chlorophyceae), causing orange snow blooms at different light conditions. *Microorganisms* 7(10): 434.

IV. **Procházková L,** Remias D, Řezanka T & Nedbalová L (2018): *Chloromonas nivalis* subsp. *tatrae*, subsp. nov. (Chlamydomonadales, Chlorophyta): re–examination of a snow alga from the High Tatra Mountains (Slovakia). *Fottea* 18(1): 1–18.

V. Remias D*, **Procházková L***, Holzinger A & Nedbalová L (2018): Ecology, cytology and phylogeny of the snow alga *Scotiella cryophila* K-1 (Chlamydomonadales, Chlorophyta) from the Austrian Alps. *Phycologia* 57(5): 581–592.

VI. Remias D, **Procházková L**, Nedbalová L & Andersen R A (in press): Two new *Kremastochrysopsis* species, *K. austriaca* sp. nov. and *K. americana* sp. nov. (Chrysophyceae). *Journal of Phycology*, doi: 10.1111/jpy.12937.

VII. Lutz S*, **Procházková L***, Benning LG, Nedbalová L & Remias D (2019): Evaluating amplicon high–throughput sequencing data of microalgae living in melting snow: improvements and limitations. *Fottea* 19(2): 115–131.

VIII. Lukeš M*, **Procházková L***, Shmidt V, Nedbalová L & Kaftan D (2014): Temperature dependence of photosynthesis and thylakoid lipid composition in the red snow alga *Chlamydomonas* cf. *nivalis* (Chlorophyceae). *FEMS Microbiology Ecology* 89(2): 303–315.

IX. Řezanka T, Nedbalová L, **Procházková L** & Sigler K (2014): Lipidomic profiling of snow algae by ESI-MS and silver-LC/APCI-MS. *Phytochemistry* 100: 34–42.

X. Osterrothová K, Culka A, Němečková K, Kaftan D, Nedbalová L, **Procházková L** & Jehlička J (2019): Analyzing carotenoids of snow algae by Raman microspectroscopy and high-performance liquid chromatography. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy* 212: 262–271.

* These authors contributed equally to this work.

Authors' contributions

I. L. Procházková, T. Leya, L. Nedbalová and H. Křížková designed the study and independently participated in field-samplings. L. Procházková, T. Leya and H. Křížková carried out light and electron microscopy. L. Procházková, T. Leya and H. Křížková got sequences of molecular markers. L. Procházková did phylogenetic analyses, created haplotype networks and performed spatial-structure statistical analyses. L. Procházková wrote major part of the manuscript. T. Leya wrote one part on taxonomic treatment and contributed gently in several other parts of the text. L. Procházková, T. Leya and L. Nedbalová participated in the final improvements of the manuscript.

II. L. Procházková, D. Remias and L. Nedbalová prepared the original paper concept. L. Procházková and D. Remias jointly did field sampling, investigation of spatial population density and conducted light microscopy. L. Procházková carried out molecular laboratory work, PAM measurements and scanning and transmission electron microscopy. L. Procházková and D. Remias did drafting and writing the manuscript. D. Remias performed pigment analysis. T. Řezanka was responsible for the fatty acid analysis. Final editing of the manuscript was done by A. Holzinger, T. Řezanka and L. Nedbalová.

III. L. Procházková, D. Remias and L. Nedbalová prepared the original paper concept. L. Procházková, D. Remias and L. Nedbalová participated in field samplings. D. Remias isolated the strain. L. Procházková and D. Remias conducted PAM measurements. L. Procházková did molecular laboratory work, phylogenetic analyses, light and electron microscopy, D. Remias performed pigment analysis. T. Řezanka was responsible for the fatty acid analysis. L. Procházková wrote the manuscript and D. Remias contributed to the respective parts of the text. Final editing of the manuscript was done by T. Řezanka and L. Nedbalová.

IV. L. Procházková, L. Nedbalová and D. Remias prepared the original paper concept. L. Procházková and D. Remias jointly did field-sampling. L. Procházková conducted PAM measurements. L. Procházková did molecular laboratory work, light and electron microscopy, D. Remias performed pigment analysis. T. Řezanka was responsible for the fatty acid analysis. L. Procházková wrote the manuscript and D. Remias contributed to the respective parts of the text. Editing of the final manuscript was done by T. Řezanka and L. Nedbalová.

V. D. Remias and L. Procházková equally contributed to this work. They prepared the original paper concept and jointly did the field-sampling. L. Procházková conducted PAM measurements, molecular laboratory work and statistical analyses. D. Remias did light microscopy. L. Procházková was responsible for scanning electron microscopy. L. Procházková and A. Holzinger performed transmission electron microscopy. D. Remias and L. Procházková jointly wrote the manuscript. Final editing of the manuscript was done by A. Holzinger and L. Nedbalová.

VI. D. Remias and L. Procházková prepared the concept. D. Remias did the field sampling and isolated the strain. L. Procházková performed molecular laboratory work, phylogenetic analyses, scanning and transmission electron microscopy. R. Andersen carried out light microscopy. R. Andersen, D. Remias and L. Procházková wrote jointly the manuscript with the input from L. Nedbalová.

VII. S. Lutz and L. Procházková equally contributed to this work. The study was designed by D. Remias, S. Lutz and L. Procházková. D. Remias and L. Procházková carried out field work and light microscopy observations. S. Lutz completed the generation and analysis of the high-throughput sequencing data. L. Procházková did Sanger sequencing, ITS2 rRNA secondary structure analyses and preparation of sequence structure alignments. S. Lutz and L. Procházková carried out the manual verification of operation taxonomic unit assignments for 18S rDNA and ITS2 rDNA respectively. The manuscript was written by S. Lutz, L. Procházková and D. Remias with input from L. G. Benning and L. Nedbalová.

VIII. M. Lukeš and L. Procházková contributed equally to this work. L. Nedbalová and D. Kaftan prepared the original paper concept. L. Nedbalová isolated the strain. L. Procházková and V. Shmidt did oxygen evolution measurements. L. Procházková sequenced gene coding for D1 protein. D. Kaftan and M. Lukeš did electron transfer rate measurements. M. Lukeš performed lipid and fatty acid analyses. D. Kaftan performed statistical analyses. The manuscript was written by M. Lukeš, L. Procházková and D. Kaftan with input from L. Nedbalová.

IX. L. Nedbalová and T. Řezanka prepared the paper concept. L. Nedbalová collected samples in the field. L. Procházková performed molecular laboratory work. T. Řezanka was responsible for the fatty acid analysis. K. Sigler did pigment analysis. The manuscript was written by T. Řezanka and L. Nedbalová.

X. K. Osterrothová and J. Jehlička prepared the paper concept. L. Nedbalová, L. Procházková, D. Kaftan, A. Culka and J. Jehlička did field sampling. L. Procházková and L. Nedbalová did species identification. K. Osterrothová, A. Culka and J. Jehlička carried out Raman spectroscopy analyses. D. Kaftan performed HPLC analyses. The manuscript was written by K. Osterrothová with significant input of all the co-authors.

On behalf of all the co-authors, I declare the participation of Lenka Procházková in completing the research and writing the papers, as described above.

.....

Linda Nedbalová

Acknowledgements

Firstly, I would like to express my deep thanks to my supervisor **Linda Nedbalová** for giving me much freedom and trust to take the direction of my research I like to, for the financial support, great coordination, reliability and her excellent reviewing activities once a manuscript was nearly ready for submission.

Secondly, I am very pleased to collaborate with **Daniel Remias** (Applied University, Wels, Austria) who introduced me into scientific field work, showed me the best practice in the field work, including the professional time management and safety risk mitigation in the Alps as well as in the Arctic. I have liked a lot all the joint field expeditions, joint paper concepts planning and their fruitful realisations.

Thirdly, I admire **Jasna Vukić**, my colleague from the office at the Department of Ecology. Her never ending motivation to learn new methods and approaches has been always a source of inspiration for me.

Similarly, I am pleased to share the same office with a nice and easy-going colleague such as **Veronika Sacherová**.

It has been a pleasure for me to stay for a while in a laboratory of enthusiastic **Andreas Holzinger** (University of Innsbruck, Austria), usually before or after field samplings in the Alps. I have felt here welcome and motivated to come again.

Moreover, my thanks belong to **Tomáš Hájek** (University of South Bohemia, České Budějovice), for his willingness in a distant field trip assistance and his optimism. Moreover, my thanks belong also to support of **Jaromír Lukavský** (Institute of Botany of the Czech Academy of Sciences, Třeboň) and **Jana Kvíderová** (Centre for Polar Ecology, České Budějovice) during the physiologic experiments at Institute of Botany of the Czech Academy of Sciences in Třeboň. My thanks belong to **David Kaftan** for his assistance during all long-day long measurements of oxygen evolution at the University of South Bohemia, České Budějovice.

Next, I have been happy to discuss and share experience of phylogenetic, taxonomic topics, details of laboratory methods or sample preparation for electron microscopy with other experienced colleagues at the Charles University such as **Kateřina Procházková**, **Martin Pusztai**, **Pavel Škaloud**, **Martina Pichrtová**, **Dora Čertnerová**, **Jan Šťastný**, **Miroslav Hyliš**, **Lenka Flašková** and some others.

The fundamental thanks belong to my mum **Irena Červenková** who helped me a lot by taking care for my daughter Ludmila especially when I was abroad on field expeditions. I am grateful to my beloved husband **Petr Procházka** for his patience, trust and ability to understand all the aspects of the scientific research I have conducted.

Funding of this work was provided by the Czech Science Foundation projects 14-00227S, 17-00027S, 18–02634S.

Abstract

Snow algae cause blooms in slowly melting snowfields in mountain and polar regions. Although they are excellent models for the study of life in extremely cold environments, their taxonomical diversity, geographical distribution and variety of physiological strategies used to cope with their harsh environment are only partially understood.

This work was focused on green algae from the order Chlamydomonadales and in one case on golden algae from the order Hibberdiales. An integrative approach was applied to characterise species, including the sequencing of several molecular markers (18S rDNA, ITS2 rDNA, *rbc*L) to reveal genotypes and infer phylogenetic positions. Light and electron microscopy were conducted to describe the detailed structure of cell wall surfaces and intracellular compartments. Moreover, fatty acid and pigment profiling were carried out to provide new insights into the adjustments of metabolic pathways in these algae. Rapid light curve measurements were used as a proxy of light preferences of photosystem II.

Firstly, one of the main algae responsible for causing the phenomenon of red snow was shown to represent a single, monophyletic lineage, independent from other algae within the Chlamydomonadales (**paper I**). Therefore, the new genus *Sanguina* (S.) was described, with two closely-related species, *S. nivaloides* and *S. aurantia.* Using molecular methods, a cosmopolitan distribution of *S. nivaloides* in polar and alpine regions was demonstrated. Secondly, the physiology of *Sanguina nivaloides* was compared to another red snow forming species (*Chlainomonas* sp.) thriving at ice-covered high alpine lakes, the former exhibited high photophysiological plasticity and had significantly lower polyunsaturated fatty content (**paper II**). Furthermore, a new species *Chloromonas* (*C*.) *hindakii* causing orange snow blooms was described (**paper III**). Multiple populations were collected over a wide altitudinal gradient, and the exploration of light preferences of field samples and a laboratory strain showed a high intraspecific ability to adapt their photosynthesis to different light conditions. Additionally, the old taxon *Scotiella tatrae* was transferred to *C. nivalis* and reduced to a subspecies as *C. nivalis* subsp. *tatrae* (**paper IV**), likely representing a variation of a common cryoflora species with distinct cell wall morphology until now known only from the Tatra Mts (Slovakia/Poland). Next, *Scotiella cryophila* is considered to be the asexual cyst of *C. rosae* var. *psychrophila*; however, field-collected cysts identifiable as *S. cryophila* originating from the Austrian Alps were phylogenetically separated from the authentic strain of *C. rosae* var. *psychrophila* from North America and thus probably represent a new species (**paper V**). Two new species of golden algae were described; *Kremastochrysopsis austriaca* causing yellow snow, and *K. americana* from a pond (**paper VI**). The application of high throughput amplicon sequencing was evaluated for the characterization of snow algal communities. An optimized workflow was proposed for such projects to assist in accurate biodiversity analyses (**paper VII**). A strain of *C. reticulata* isolated from red snow exhibited a range of unusual physiological characteristics: invariant high growth over a broad range of temperatures, high electron transfer rates between the quinon Q_A and Q_B, and the dominance of phosphatidylglycerol in thylakoid membranes in its chloroplasts (**paper VIII**). In the first lipidomic study of snow algae (**paper IX**), *Chloromonas* snow species accumulated various triacylglycerols with fatty acid chains in various stereospecific positions. Raman spectra of carotenoids of various snow algae communities were detected and interpreted (**paper X**).

The presented thesis contributes to our understanding of the taxonomical and physiological diversity of microalgae in mountain and polar snow fields. It shows that the biodiversity of these extreme environments is underestimated, and provides new insights into the biogeography of snow algae. Furthermore, the results reflect the species-specific dynamic nature of responses of metabolic profiles and photosynthesis in the changeable snow environment.

Abstrakt

Sněžné řasy způsobují zabarvení sněhu v pomalu tajících sněhových polích v horských a polárních oblastech. Přestože jsou vynikajícími modely pro studium života v extrémně chladném prostředí, naše dosavadní porozumění taxonomické rozmanitosti, geografického rozšíření a rozmanitosti fyziologických strategií sněžných řas v přizpůsobení se jejich drsnému prostředí jsou pouze částečná.

Tato práce se věnuje zeleným řasám z řádu Chlamydomonadales a v jednom případě zlativkám z řádu Hibberdiales. V práci byl použit integrativní přístup k charakterizaci druhů včetně sekvenování několika molekulárních markerů (18S rDNA, ITS2 rDNA, *rbc*L) za účelem odhalení genotypu a odvození fylogenetické příbuznosti druhů. Pomocí světelné a elektronové mikroskopie byly popsány detailní struktury povrchu buněčné stěny a vnitrobuněčného uspořádání. Kromě toho byly sledovány profily mastných kyselin a pigmentů, aby se získal nový pohled na úpravu metabolických drah těchto řas. Rychlá měření světelných křivek byla použita pro odhad světelných preferencí fotosystému II.

Bylo prokázáno, že jedna z hlavních řas způsobujících fenomén červeného sněhu je monofyletická linie v rámci skupiny Chlamydomonadales (**článek I**), byl popsán nový rod *Sanguina* se dvěma blízce příbuznými druhy *S. nivaloides* a *S. aurantia*. Pomocí molekulárních metod bylo prokázáno celosvětové rozšíření druhu *S. nivaloides* v polárních a alpských oblastech. Za druhé, fyziologie druhu *Sanguina nivaloides* byla porovnána s dalším druhem tvořícím červené zabarvení sněhu (*Chlainomonas* sp.), který se vyskytuje na povrchu ledové pokrývky vysokohorských jezer: prvně jmenovaný druh vykazoval vysokou fotofyziologickou plasticitu a měl výrazně nižší obsah polynenasycených mastných kyselin (**článek II**). Dále byl popsán nový druh *Chloromonas* (*C.*) *hindakii* způsobující oranžově zbarvený sníh (**článek III)**. Populace tohoto druhu byly nalezeny na širokém gradientu nadmořské výšky, byly zkoumány světelné preference terénních cyst a laboratorního kmene, výsledky dokládají vysokou míru přizpůsobení fotosyntézy různým světelným podmínkám v rámci druhu. V další studii byl starý druh *Scotiella tatrae* převeden na *C. nivalis* a redukován na poddruh *C. nivalis* subsp. *tatrae* (**článek IV**), který pravděpodobně představuje variantu běžného druhu kryoflóry s výraznou morfologií buněčné stěny. Poddruh je doposud známý pouze z Vysokých Tater (Slovensko/Polsko). Dále, termín *Scotiella cryophila* je považován pro označení nepohlavní cysty *C. rosae* var. *psychrofila*; práce ukázala, že cysty z rakouských Alp identifikovatelné jako *S. cryophila* byly překvapivě fylogeneticky oddělené od typového kmene *C. rosae* var. *psychrophila* ze Severní Ameriky, a proto pravděpodobně představují nový druh (**článek V**). Byly popsány dva nové druhy zlativek: *Kremastochrysopsis austriaca* tvořící žluté zabarvení sněhu a *K. americana* z tůně (**článek VI**). V další studii jsme vyhodnotili použití ʿhigh-throughputʾ amplikonového sekvenování pro charakterizaci společenstev sněhových řas. Pracovní postup pro takto zaměřené studie jsme optimalizovali s cílem zpřesnit výsledky analýzy biologické rozmanitosti (**článek VII**). Kmen řasy *C. reticulata* izolovaný z červeného sněhu vykazoval řadu neobvyklých fyziologických vlastností: rostl v širokém rozmezí teplot a byla zaznamenána vysoká rychlost přenosu elektronů mezi chinonem Q_A a Q_B, v thylakoidních membránách chloroplastů převažoval fosfatidylglycerol (**článek VIII**). První lipidomická studie sněžných řas ukázala, že sněžné řasy rodu *Chloromonas* akumulovaly rozmanité triacylglyceroly s řetězci mastných kyselin v různých steoreospecifických pozicích (**článek IX**). Ramanova spektroskopie byla použita pro detekci a interpretaci karotenoidů rozmanitých společenstev sněžných řas (**článek X**).

Předkládaná práce přispívá pochopení taxonomické rozmanitosti mikroskopických řas v podmínkách sněhových polí horských a polárních oblastí. Ukazuje se, že biodiverzita těchto extrémních prostředí je nedostatečně prozkoumaná a poskytuje nový pohled na biogeografii sněžných řas. Výsledky dále odráží druhově charakteristickou změnu různých metabolických profilů a fotosyntézy v proměnlivém prostředí sněhu.

Table of content

1 Introduction

1.1 Why to work with snow algae?

Snow algae are a taxonomically and physiologically diverse group of phototrophic microorganisms living in extreme conditions. They are interesting because of their biodiversity, phylogeny and biogeography, and can serve as a model to study cellular adaptations to harsh environments (e.g. light and temperature stress). They also play an important role in melting snow and glacial ecosystems as primary producers. They can be used to describe community interactions with bacteria and fungi, or be screened for biotechnologically promising compounds. Recent studies have analysed the effect of massive blooms on snow albedo and subsequent scenarios of global change. In addition, cryoflora is beautiful, both macroscopically as the major constituent of snow blooms in the field, and especially under the microscope. Last but not least, research on cryoflora allows the researcher to visit remote places full of surprises.

1.2 Geographic and altitudinal distribution of snow algae habitat

Slowly melting snowfields may be found all around the world in mountain and polar regions (Kol 1968). The availability of liquid water between snow and ice crystals is a key factor for the development of a snow algae populations, as tiny freshwater channels provide a solution of nutrients and dissolved gases.

The timing of the development of snow algal blooms is driven by complex factors; however, the main pattern is determined by an interplay of the latitude and altitude of a site. In mid-latitude regions of the Northern hemisphere, e.g. in the border mountains of the Czech Republic and parts of Japan, snow algae are found annually where snow remains at least until the beginning of May (Fukushima 1963, **own observation**). In the first half of May, coloured snow spots in the Czech Mountains are usually small (a few centimetres in diameter; Nedbalová et al. 2008; **paper III**). At these sites, the earliest successful sampling in the course of the season can be conducted, depending on spring precipitation and prevailing air temperature, already in late March or mid-April, similarly as in the Chenango Valley in

the USA (Hoham et al. 2006). Nowadays (taking global warming into account), the best time to collect large blooms (dozens of centimetres to a few square meters) at high alpine sites in central Europe is from the end of May on, e.g. in the Tyrolean Alps (Austria) (Remias et al. 2005; **papers I, II, VII**), and from mid-June on in the High Tatra Mountains (Slovakia, Poland) (**papers II, III, IV**). In the Maritime Antarctic, the suitable time for harvesting snow algae blooms is during the austral summer months of January and February (Davey et al. 2019), while in the Arctic June to August is ideal (Lutz et al. 2014). Cryoflora in the tropical regions have been sampled in summer seasons in New Guinea (Kol & Peterson 1976), Ecuador (Nedbalová & Sklenář 2008) and Mt. Kilimanjaro in Kenya-Tanzania (Vimercati et al. 2019a).

The range of altitude where snow algae are found varies in mid-latitude mountains in Europe, e.g. in the Alps from ~1500 to ~3,600 m. a.s.l. (Kol 1961), and in Krkonoše and Šumava in the Czech Republic from ~700 m to ~1,600 m a.s.l. (Fott et al. 1978, Kociánová et al. 1989, Lukavský 1993, Nedbalová et al. 2008). In the USA altitudes range from ~1,800 up to 4,300 m a.s.l. in the Sierra Nevada, California (Thomas 1972), from ~400 to ~1,400 m a.s.l. in the region of New England and the state of New York (Hoham et al. 1993) and from ~1,800 up to ~3,400 on Niwot Mountain in Colorado and in the state of Washington (Brown et al. 2016). In South America, snow algae have been found up to ~5,300 m a.s.l. in Chile (Vimercati et al. 2019b), and in Japan they have been reported from ~200 m a.s.l. to 3,400 m a.s.l. on the Shiretoko Peninsula and Mt. Fuji, respectively (Fukushima 1963). In polar regions, snow algae may be found in maritime regions directly on the coast (Müller et al. 1998) as well at more elevated inland locations (Lutz et al. 2015a). Similarly, snow algae can occur at 'pristine' locations at polar sites far away from any signs of apparent nutrient inputs (human settlement, animal colonies), e.g. on remote inland glaciers on Svalbard (Leya 2013), as well as close to hiking trails, on mountain pastures and skiing slopes (Hoham et al. 1993, Remias - pers. comm.) and next to penguin colonies (Remias et al. 2013a).

1.3 Melting snow as a challenging habitat

Microorganisms such as algae living in the mountain snowpack are subject to several environmental stressors, including excessive UV and visible light irradiation (Thomas & Duval 1995, Sommaruga & Psenner 1997), daily freeze-thaw cycles (Edwards et al. 2007), limited nutrients (Müller et al. 1998, Hiltbrunner et al. 2005) and a short growing season (a few weeks per year) (Beniston et al. 2003). However, microalgae have adapted to survive a wide range of extreme environments on Earth (Rothschild & Mancinelli 2001).

Solar radiation, namely photosynthetically active radiation (PAR; 400–700 nm), is the energy source for microalgal photosynthesis. Typical light conditions at a high alpine locality in the Tyrolean Alps (2,400 m a.s.l.) in summer were found to include PAR peaks at noon with values between ~2,100 µmol photons m^{-2} s^{-1} (maximum under full sun) and < 1000 µmol photons m^{-2} s^{-1} (minimum under scattered cloudy skies) (Remias et al. 2010a; **paper II**). UV-A radiation (315–400 nm) showed a peak of 48.9 W m^{-2} and UV-B radiation (280–315 nm) of 0.3 W m^{-2}. Under scattered cloudy conditions, the UV radiation declined to a range from 30.2 to 5.2 W m^{-2} and from 105 to 20.3 mW m^{-2}, respectively. At alpine sites in the Tyrolean Alps, in the Rocky Mountains, in the New Zealand Alps and Krkonoše Mts., maximal PAR irradiances up to ~2500 µmol photons m^{-2} s^{-1} have been reported (Sommaruga & Psenner 1997, Gorton et al. 2001, Novis 2002a, Nedbalová et al. 2008).

In contrast, considerably lower ambient UV and PAR are common in polar regions, e.g. around noon on a sunny day in summer at the glacier Longyearbreen (Svalbard), values varied between ~1,000 and 1,100 µmol PAR m^{-2} s^{-1}, UVA was in the range of 20.8–23.1 W m^{-2} and UVB between 0,05–0,06 W m^{-2} (Remias et al. 2012a). The maximum incident PAR flux measured in the Arctic and Antarctic reaches approximately 1,500 µmol m^{-2} s^{-1} (Stibal & Elster 2007; Davey et al. 2019).

Although the snow surface may be exposed to high intensities of incident radiation, cells located a few cm below the snow surface are exposed to much lower light levels, since the penetration of light decreases logarithmically with increasing depth. For instance, 5 cm below the snow surface (at elevation of 3,700 m a.s.l.), PAR reached ~20% of the PAR and UV-A, B value at the snow surface at an alpine site (Gorton & Vogelmann 2003). At noon at

a high alpine site (2435 m a.s.l.), PAR 20 cm below the snow surface equalled only 2% of surface amounts during sunny weather and 1.4% in cloudy conditions (**paper V**). In addition, other factors also play a role in light transmittance in snowpack, such as the sizes of the snow grains, weather conditions and time of the day (especially in the polar region in late summer, when the sun descends close to the horizon).

Nutrients are deposited on snow by wind, precipitation, weathering of surrounding rock, volcanic eruptions and animals. In alpine and polar regions, the electrical conductivities (EC) of melt water are usually very low (EC <10 $\mu S.cm^{-1}$) (Müller et al. 1998, Leya 2004, Lutz et al. 2015a, Remias et al. 2016) suggesting that the snow environment is limiting in nutrients. In contrast, in proximity to a nutrient source, e.g. bird colonies, EC in snow can reach up to ~1,000 $\mu S.cm^{-1}$ (Ling & Seppelt 1998). A Saharan dust event led to a strong enrichment in Fe in snow at a high-altitude site in the Italian Alps (Mauro et al. 2018). Moreover, snowfields at locations near sea level are influenced by sea spray (e.g. EC=100 $\mu S.cm^{-1}$; Leya et al. 2001). At forested localities, leaching of coniferous litter and debris can be an important source of nutrients for the growth of snow microorganisms (Hoham 1976). Overall, NH_4-N is usually the most abundant inorganic nitrogen form (Nedbalová et al. 2008, Remias et al. 2010a, Novis 2002a). Higher nitrate concentrations have been present in samples without an apparent algal presence when compared to samples with flagellates at Whiteface Mts., New York (Hoham et al. 1989), which may indicate an effective utilisation of nutrients.

pH values of meltwater in polar regions range between 6.7-8.1 on Windmill Islands, Antarctica (Ling & Seppelt 1998), 7.4-7.5 in the Antarctic Peninsula (Remias et al. 2013a), 4.4-6.2 and 5.0-5.7 [rarely 7.5-7.9] on the Northwestern Svalbard (Müller et al. 1998, Leya 2004), 6.1-7.2 around Longyearbreen on Svalbard (**own observation**), 4.7-7.0 on a glacier in the North-Eastern Greenland (Lutz et al. 2014), and 4.9-7.9 on glaciers and ice-caps in Iceland (Lutz et al. 2015a). In mid-latitude regions, pH in the Alps ranged from 4.6-6.2 (Remias et al. 2010a, 2016; **paper II, V**) and from 5.2-7.0 in the High Tatras (Kol 1975; **paper III, IV**). Snow algae activity affect pH values in snow (Hoham et al. 1989); e.g. in the Adirondacks of Upstate New York, the pH was more basic in samples containing algae (5.17 vs. 4.98) as a result of carbon dioxide consumption during photosynthesis.

Liquid water availability in snow can be expressed as snow wetness (Novis 2002a) or as a snow-water equivalent (referred to as 'snow/liquid water content' [SWC] or 'snow density'; e.g. Hoham 1975a, Nedbalová et al. 2008; **papers I–V**). Its value is influenced by the occurrence of heavy rains (Novis 2002a), air temperatures during the day, and also depends on the depth of sampling (Hoham & Mullet 1977). Water availability was shown to have an impact on dominant life cycle stages of *Chloromonas* in snow, with sexual stages more common at lower and cysts (or zygotes) more common at higher SWC values (Hoham & Duval 2001). An increase in abundance of *Chlainomonas koliae* (Hardy & Curl) Hoham took place with proceeding melting (Novis 2002a).

Climatic factors, air circulation and local topographical differences play a role in snow accumulation (Niedzwiedz 1992) and thus in the establishment of long-lasting slowly melting snowfields suitable for the development of snow algal populations (Spijkerman et al. 2012). Snow algae maintain populations from year to year in approximately the same localities (Hoham & Duval 2001, Remias et al. 2016), indicating the presence of a local 'seed bank'. Localities of snow algae differ in their ambient irradiance, such as exposed high-light sites above timberline (Gorton et al. 2001), semi-shaded sites in deeper parts of semi-permanent snowdrifts (Remias et al. 2013b) and low-light sites close to or below coniferous tree canopies (Nedbalová et al. 2008). The latter also significantly differs in the higher availability of nutrients leached from the coniferous litter (Hoham 1976, Hoham et al. 2008a). During their seasonal life cycle, one species may be exposed to different irradiance regimes since the exact position of a cell in the snow pack changes over time in relation to several factors, including the ability to move (i.e. flagellate stages), meltwater flow, and the presence of horizontal ice layers and vertical ice fingers (Hoham & Duval 2001, Pomeroy & Brun 2001). Exceptional localities for snow algae include melting snow fields within high alpine ice packs (Novis 2002a), in glaciers (Remias et al. 2016), and in basal ice sheaths isolated from permafrost ground (Remias et al. 2013b). All provide a prolonged period of meltwater availability due to delayed water outflow from the snow. In contrast, snowfields located on rock, soil and permafrost show higher water drain during melting (Wharton & Vinyard 1983), and cells are subject to desiccation or high temperatures after snow melt.

1.4 Snow algae

1.4.1 Defining ʻtrueʼ snow alga

Snow algae are members of several main algal groups (Chlorophyceae, Trebouxiophyceae, Chrysophyceae, Dinophyceae, Cryptophyceae). The term snow alga generally refers to freshwater microalgae found in snow, and traditionally glacial habitats are included as well. However, there is still an ongoing discussion on what it exactly means to be a ʻtrueʼ (autochthonous) snow alga, and which organisms should be considered as ʻxenobiontsʼ (i.e. more or less accidently present in the habitat). Based on experimental testing of several snow algal strains, Hoham (1975b) suggested that physiologically, a true snow alga does not grow at temperatures above 10°C and its growth optimum is at lower temperatures. Similarly, according to Leya (2013) the physiological criterion of being a psychrophile, obligatorily adapted to snow temperatures, is a prerequisite to be regarded as an autochthonous freshwater snow alga. A temperature threshold of 15°C for optimal growth is used to separate psychrophilic (i.e. obligate cryophilic) from psychrotrophic algae (i.e. facultative psychrophilic) (Morita 1975, Helmke & Weyland 1995). The latter are non-obligate cryophiles as they survive elevated temperatures (>20°C) and have a growth optimum above 15 °C.

An ecological approach has been recently proposed and is used in this thesis, namely that an alga which *naturally* grows and reproduces *only* in meltwater between snow or ice crystals is a ʻtrueʼ snow alga (Daniel Remias 2012). This definition has several consequences, such as some alga assumed to be primarily soil alga (i.e. allochtonous algae like several species of *Raphidonema*) are not considered part of the cryoflora in *sensu stricto* though they can cause visible blooms. Under this definition, *not* all algae able to cause snow blooms can be regarded as true snow algae.

1.4.2 Defining facultative snow alga

A facultative snow alga is defined here as a plastid-bearing microorganism reproducing in various habitats including snow, e.g. soil, salt water, ponds, etc. For instance, in maritime Antarctica and maritime Svalbard (Leya 2013) coastal green snow can be found

consisting of actively dividing cells of several species that primarily reproduce in soil, e.g. representatives of the genera *Klebsormidium*, *Ulothrix,* and *Raphidonema*. Some species in these genera are regarded as native soil dwellers that are occasionally transported by wind or water to snow surfaces where they may cause a short-term green snow phenomenon, though the cells show many signs of morphological damage (Stibal & Elster 2005).

1.4.3 Snow blooms

The spectacular snow bloom phenomenon was reported for the first time by Aristotle in the fourth century BC (Aristotle n.d.). While he recognized that something had to contribute to this colouration, red snow was usually mistaken for mineral deposits or pollen until the 19th century, when scientists began to pay more close attention to this unusual event after a red snow sample from Greenland was delivered to Europe (Ross 1819).

Snow blooms can be defined as macroscopically visible mass accumulations of freshwater microalgae in melting mountain or polar snow, and in glacier-based snowfields (**Figure 1**). The snowfield colouration due to algae can be red, pink, orange, yellow, green or grey (Kol 1968). The rarest snow coloration is blue, caused by cyanobacteria (Kol 1955a, b). The factors responsible for snow colourations are a combination of the pigment contents per cell and the cell concentration per snow volume, with blooms generally becoming macroscopically visible once cell counts exceed one thousand 'giant' red cells (Remias et al. 2016) or six thousand tiny vegetative cells per ml of meltwater (Hoham & Duval 2001). Red, pink and orange snow colouration is usually associated with the presence of extra-plastidal secondary carotenoids, mainly astaxanthin (Remias et al. 2005, Leya et al. 2009). A yellow bloom is usually caused by the dominance of cells containing the primary carotenoid fucoxanthin (Petrushkina et al. 2017). Green snow is typical for mass accumulations of cells where chlorophylls dominate and that (still) lack secondary pigments. Grey snow or purple ice associated with algal blooms on glaciers is caused by dark brownish vacuolar phenolic compounds (Ling & Seppelt 1990, Remias et al. 2012b). Grey snow may also be caused by inorganic particles, though snow algae in low abundances might be present (Kol 1966). However, in some cases grey/black snow or pinkish/reddish snow can be

almost macroscopically indistinguishable from snow coloured by inorganic particles (Fukushima 1963, **own observations from the Polish Tatra Mountains and glaciers in the European Alps**). Therefore, light microscopy in the field is always recommended. Moreover, this may also reveal the presence of scattered algal cells in snow patches without any signs of colour at the snow surface (Hanzelová et al. 2018, Brown 2019). Last but not least, yellow snow close to mountain paths or skiing slopes may be the result of animal or human needs.

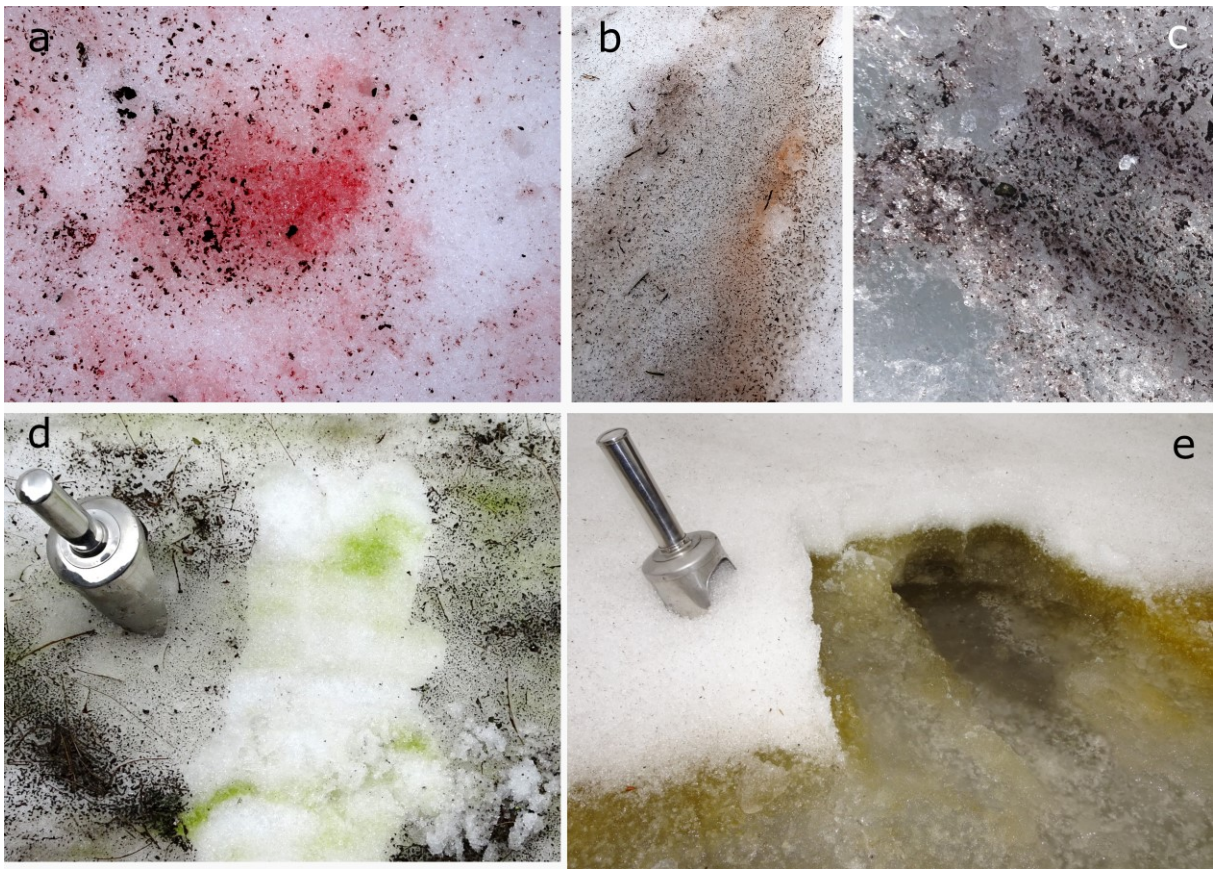

Figure 1. Details of coloured snow or ice caused by microalgae: **(a)** red snow in Tyrolean Alps, Austria, **(b)** orange snow in Krkonoše Mts., Czech Republic, **(c)** grey to purple ice on a glacier, Bernina Range, Switzerland, **(d)** green snow in the High Tatra Mts., Slovakia, **(e)** yellow slush around Longyearbreen Svalbard, Norway.

1.4.4 Taxonomy & phylogeny

Globally, the dominant snow algae are flagellates belonging to genera of green algae such as *Sanguina* (formerly assigned to "*Chlamydomonas"*; **paper I**), *Chlainomonas* (Hoham 1974a, b; Novis 2002a; **paper II**), *Chloromonas* (Hoham & Mullet 1977; **paper III, IV**) and *Smithsonimonas* (Kol 1942).

An independent lineage within Chlamydomonadaceae causing red snow was recognized by Leya (2004). Later, the new genus *Sanguina* was described, with *Sanguina nivaloides* Procházková, Leya & Nedbalová as its type (**paper I**), based on a polyphasic investigation of spherical field cysts (sequencing of three molecular markers, light and electron microscopy of cells). No culturable isolates exist yet for this alga. Green, flagellated isolates from red snow field samples of *Sanguina nivaloides* have often been misinterpreted as having resulted from red spherical cysts or even vice versa; e.g. Novakovskaya et al. (2018) and **paper VIII** thought to have observed such a phenomenon when linking green isolates of *Chloromonas* (*C.*) *reticulata* (Goroschankin) Gobi from snow samples to red cysts in the same sample.

The traditional green algal genus *Chloromonas* includes mesophilic, cold-tolerant and cold-adapted microorganisms. According to Barcytė et al. (2018a), phylogenetic analyses of 18S rDNA and *rbc*L markers showed a paraphyletic origin of *Chloromonas* within Chloromonadinia, with genetically, morphologically and ecologically well-defined clades. Clade 1,2 and 3 in *Chloromonas* were delimitated according to Hoham et al. (2002) Clade 1 (containing true *Chloromonas*) encompasses mesophilic and psychrotolerant organisms (Barcytė et al. (2018b), **paper VIII**). Clade 2 encompass psychrophilic organisms forming snow blooms (clades A, B [**papers III–V**], C, D *sensu* Matsuzaki et al. 2019), whereas clade 3 includes mesophilic algae only. Furthermore, no prominent sexually produced dormant cysts have been described within clade 1 (though asexual resting spores were reported for *C. rosae* var. *psychrophila* Hoham, Bonome, Martin & Leebens-Mack; Hoham et al. 2002). Consistent differences in flagellate morphology (the shape of chloroplasts, presence or absence of stigma and pyrenoid) between the three clades were summarized by Barcytė et al. (2018a). These researchers suggested that the genus *Chloromonas* could be understood as just a part of clade 1 (= core *Chloromonas*), a subclade containing the type species, and corresponding only to *C. reticulata* clade *sensu* Pröschold et al. (2001). According to Barcytė et al. (2018a), other clades or lineages should be carefully revised and new generic names should be proposed.

Less frequently reported flagellates in snow are golden algae (Czosnowski 1948, Hindák 1969). A new species causing yellow blooms in snow was described as *Kremastochrysopsis austriaca* Remias, Procházková & R. A. Andersen (**paper VI**) and showed

no close phylogenetic relation to other snow dwelling chrysophytes such as *Chromulina chionophilia* Stein (Stein 1963, Hoham 1975b), *Hydrurus* sp. (Remias et al. 2013b, **own observation**) or *Ochromonas*-like flagellates (Tanabe et al. 2011). Rarely reported flagellates in snow include euglenoids (Hoham & Blinn 1979), cryptophytes (Javornický & Hindák 1970) and dinophytes (Gerrath & Nicholls 1974).

Other non-flagellated algae are passively transported on snowpack by wind or by meltwater from adjacent soil, e.g. trebouxiophyceaen alga *Raphidonema nivale* Lagerheim (Stibal & Elster 2005) and desmids *Mesotaenium berggrenii* (Wittrock) Lagerheim (Ling & Seppelt 1990) or *Ancylonema nordenskiöldii* Berggern (Lutz et al. 2014). The latter two are known to primarily cause glacier blooms. Reports on cyanobacteria in snow are sparse (Kol 1955a, b).

The taxonomy of snow algae has historically placed strong emphasis on the morphology of characteristic cysts (e.g. '*Scotiella*' in Fritsch 1912, Chodat 1922), since no culturable isolates were known due to the difficulty of inducing vegetative cell production from field-collected material. In the past two decades, methods of molecular phylogenetics have been applied in the course of taxonomic studies (e.g. Muramoto et al. 2008). Also, new cultivation techniques and multigene (multimarker) phylogenetic analyses have demonstrated that field cysts morphologically identified as *C. nivalis* (Chodat) Hoham & Mullet (Hoham & Mullet 1977, 1978) and *C. brevispina* (Fritsch) Hoham, Roemer & Mullet (Hoham & Mullet 1979) in fact consisted of multiple species: one Japanese lineage of *C. nivalis* aplanozygotes was conspecific with *C. miwae* (Fukushima) Muramoto, Nakada, Shitara, Hara & Nozaki (Matsuzaki et al. 2015), and one lineage of *C. brevispina* cysts from Japan was assigned to *C. krienitzii* Matsuzaki & Nozaki which was described based on a newly established strain (Matsuzaki et al. 2015). Most recently, a comparative morphological analysis of field cysts and vegetative strains together with phylogenetic analysis resulted in the species description of *C. muramotoi* Matsuzaki, Nozaki & Kawachi (Matsuzaki et al. 2019). Scanning electron microscopy demonstrated that the cysts of *C. muramotoi* are different from those of *C. miwae*, based on the arrangement of the flanges developing on the cell wall. Although *Chloromonas* dwelling in snow are a relatively well-studied group, there is still an apparently underestimated (and unknown) biodiversity.

1.4.5 Biogeography & dispersal

Snow algae are well known from mountain, alpine and high latitude regions worldwide. They have been reported from Europe (e.g. Kol 1968, Komárek et al. 1973, Lukavský 1993, Nedbalová et al. 2008), Asia (e.g. Fukushima 1963, Yoshimura et al. 2000, Takeuchi et al. 2006a, Matsuzaki et al. 2019), New Zealand (Thomas & Broady 1997, Novis 2002a,b), North America (e.g. Kol 1942, Garric 1965, Hardy 1966, Hoham et al. 1993, Brown et al. 2016) and South America (e.g. Takeuchi & Kohshima 2004, Nedbalová & Sklenář 2008), Australia (Marchant 1982), Africa (Duval et al.1999, Vimercati et al. 2019a), Arctic (Kol & Eurola 1974, Müller et al. 1998, Leya 2004, Spijkerman et al. 2012, Kvíderová 2012, Remias et al. 2013b) and Antarctica (e.g. Fritsch 1912, Ling & Seppelt 1998, Remias et al. 2013a,b).

In the last century, several snow algae have been assumed to have cosmopolitan geographical distribution because the (more or less) uniform morphology of their cysts seems to be consistent, e.g. "*Chlamydomonas nivalis*" (Bauer) Wille (Kol 1968), *C. nivalis* (Hoham & Mullet 1978) and *C. brevispina* (Hoham et al. 1979). The latter two species turned out to be collective taxa (see above). There is not much known yet about the geographic distribution of the newly described snow alga species (e.g. Matsuzaki et al. 2015, 2018, 2019).

In addition, a lack of sampling for sequencing in many regions worldwide makes it difficult to evaluate patterns of geographical distribution. A few snow algal species are regarded as endemic (or regional), e.g. *Scotiella tatrae* Kol was described and reported only from the High Tatra Mountains (Slovakia) so far (Kol 1965, 1968). However, this species has been recently re-examined and reduced to a subspecies, *C. nivalis* subsp. *tatrae* Procházková, Remias, Řezanka & Nedbalová (**paper IV**). *C. polyptera* (Fritsch) Hoham, Mullet & Roemer was reported from the Maritime Antarctic close to coastal animal colonies (Ling 1996, Remias et al. 2013a). By contrast, a disjunct geographic distribution of snow *Chlainomonas* with populations in North America and New Zealand was accompanied by relatively low sequence divergence in *rbc*L sequences (Novis et al. 2008). A molecular investigation of field collected cysts from Europe, North America and South America as well as from both polar regions showed a cosmopolitan distribution of the red snow forming species *Sanguina nivaloides* (**paper I**).

Generally, one may infer that the presence of small sized cells (Gradinger & Nürnberg 1996) that are resistant to excessive visible light, high UV and desiccation (Holzinger et al. 2016) may be favourable for long distance dispersal. Indeed, it was experimentally demonstrated that unicellular algal propagules may remain viable after long-distance transport (Marshall & Chalmers 1997), as was also indicated by metatranscriptomic profiling of Eukaryota in clouds at alpine site (Amato et al. 2019). Some microorganisms (e.g. fungi) have been reported to be viable up to an altitude of 77 km (DasSarma & DasSarma 2018). Moreover, the colonisation of new geographical regions or ecological resources may be an important genetic diversification force in protists, despite their enormous population sizes and cosmopolitan dispersal (Škaloud et al. 2019). That study found that the silica-scaled chrysophyte *Synura sphagnicola* Korshikov - occurring in slightly acidic bogs - underwent rapid diversification during the late Pleistocene, with the most recent haplotypes being ecologically and biogeographically much more differentiated than the older, morphologically distinguishable lineages that diverged in the Miocene. This finding indicates that periods of divergence in eukaryotic microorganisms (at least in some lineages) may be equivalent to the estimated time of speciation in plants and animals. However, a better understanding of global and regional patterns of microbial dispersal and what environmental factors control the development of microbial communities in complex natural systems is needed (Schmidt et al. 2014).

Microalgae may be passively dispersed by prevailing air streams (Brown et al. 1964, Schlichting 1969), ocean currents (Gillespie et al. 2012), birds (e.g. birds migrating between the two polar regions, such as the Arctic tern), insects (e.g. Revill et al. 1967), mammals (Kristiansen 1996), and reptiles (Višić et al. 2019) as well as by humans (e.g. snow algal cysts with a gelatinous matrix were thought to be transported from one ski slope to another, Hoham et al. 1993). Humans as a vector can also be illustrated by the introduction of several Japanese macroalga by into European waters by the translocation of shellfish stocks. Sporophyte stages of these alga may survive in the digestive tracts of bivalves in aquafarms (Schwinghamer et al. 1994). Other examples of human influence include algal dispersal due to ballast water discharges from ships (Smayda 2007).

1.4.6 Biodiversity & sequencing of environmental samples

Currently, there are several molecular markers employed for DNA-based species identification and/or barcoding for principal algal groups; e.g. for green algae the following markers are recommended: *rbc*L, *tuf*a, 18S rDNA, ITS rDNA, and 26S rDNA (Leliaert et al. 2014). Concerning environmental samples, only virtually monospecific field blooms or isolated single cells from snow are suitable for Sanger sequencing (Remias et al. 2013a, Matsuzaki et al. 2015; **papers I–V, IX**). In contrast, mixed communities are ideal targets for state-of-the-art high-throughput (i.e. metagenomic) amplicon sequencing, e.g. using the Illumina system (**paper VII**). Both approaches provide different results, leading to insights into the biodiversity and biogeography of snow algal communities (using culture independent methods). The choice of molecular marker(s) is usually a trade-off reflecting the availability of reference data for certain groups of investigated microorganisms and its resolution sensitivity for this group(s).

One of the first metagenomic studies of snow algal assemblages was based on a single marker. Lutz et al. (2015a) investigated the partial 18S rDNA (V4–V5 hypervariable region of the otherwise conservative marker) of eukaryotic communities at three different Arctic sites. Only four algal phylotypes were detected (*Chloromonas*, *Raphidonema*, two uncultured Chlamydomonadaceae) (Lutz et al. 2015a). Similarly, partial 18S rDNA high throughput sequencing was applied to the so far under-investigated cryoflora at a nival site above 5,000 m a.s.l. in the Chilean Andes (Vimercati et al. 2019b). The authors focused on microbial communities in thin blades of hardened snow known as ʿnieves penitentesʾ, and the most abundant operation taxonomic unit was 100% identical with "*Chlamydomonas nivalis*" CCCryo RS_0015-2010 (the type specimen of *Sanguina nivaloides*, **paper I**), while the second and third most common operational taxonomic units (OTUs) belonged to two still undescribed species of *Chloromonas.*

In **paper VII**, we proposed an updated approach - short reads of 18S rDNA data from Illumina should be accompanied by the generation of new Sanger sequence libraries from local samples to compensate for a lack of reference data in public databases. This approach was used in the first microbial investigation of melting snow in Africa at Mt. Kilimanjaro on the Tanzania-Kenya border (Vimercati et al. 2019a). Kilimanjaro is the most isolated high-

altitude peak on Earth, making this site ideal for testing ideas related to microbial dispersal and biogeography. In that study, the periglacial ecosystems of Kilimanjaro were revealed to harbour algae related to the genera *Chloromonas* and "*Chlamydomonas*" (Vimercati et al. 2019a). Sequences of green algae found on ice and in soil were closely related to those found in similar environments such as European alpine snow (Remias et al. 2010a), Arctic glaciers (Leya et al. 2004) and Antarctic snow (Remias et al. 2013a). Taking a closer look and manually performing a comparison of the selected ~1,720 bp long new 18S rDNA Sanger sequences with available data in public databases, the clone under accession number KX771795 turned out to be almost genetically identical with the red snow forming alga "*Chlamydomonas nivalis*" CCCryo RS_0015-2010 from Svalbard (JQ790560.1 in Remias et al. 2013a; **paper I**). Moreover, the clone KX771778 was closely related to the reddish snow forming species *C. nivalis* Gassan-B from Japan (LC012714.1 in Matsuzaki et al. 2015) and *C. hindakii* Procházková & Remias from the High Tatras (Poland) (**paper III**).

Since several *Chloromonas* species share more than 99 percent identity in the hypervariable V3-V4 region of the 18S rRNA gene (**paper V, paper VII**), that section of this marker is not specific enough for the resolution of closely related *Chloromonas* species. In contrast, the use of a hypervariable marker for metagenomic sequencing (ITS2 rDNA) allowed the possibility to test the intraspecific heterogeneity of the most common species across regions and years (Brown et al. 2016). The two most common snow algae (affiliated with "*Coenochloris* sp." and "*Chlamydomonas* sp.") were shown to have a distinct haplotype diversity distribution locally and regionally in red snowfields in Colorado and Washington, USA. Their results suggested that snow algae are communities of clones within a discrete patch yet are heterogeneous across the landscape. Thus, these communities are likely structured via strong priority effects, intense kin competition and dispersal limitations. The above mentioned North American sequences of "*Coenochloris* sp." and "*Chlamydomonas* sp." have recently been assigned to the two newly described species *Sanguina nivaloides* (**paper I**) and *Sanguina aurantia* Leya, Procházková & Nedbalová (**paper I**).

A bipolar study of red snow used Illumina sequencing and combined both markers, i.e. the conservative (18S rDNA) and the highly variable (ITS2 rDNA) in combination with long reads of 18S rDNA by Sanger sequencing (Segawa et al. 2018). They showed that red-algal

blooms in polar regions comprised mainly cosmopolitan phylotypes (e.g. "*Chlamydomonas*"-snow group B; conspecific with *Sanguina nivaloides* - **paper I**) but also included endemic phototrophs, which were distributed either in the Arctic or Antarctica (Segawa et al. 2018).

One of the weak points of most studies conducting environmental sequencing of snow algal assemblages is the lack of any light microscopy of the harvested samples. This creates an information gap that could be useful for later correct OTU affiliation. Furthermore, it is fruitful to compare differences in the taxonomic composition based on a combined use of a conservative marker (e.g. 18S rDNA) and of a hypervariable marker (e.g. ITS2 rDNA). All these gaps, limitations and possible improvement in the high-throughput amplicon sequencing (HTS) approach were evaluated in detail in **paper VII**. Finally, the biodiversity of the cryoflora of the European Alps was investigated for the first time by these state-of-the-art methods (**paper VII**).

Moreover, detailed single-species studies focused on intraspecific genetic variability, accompanied with evaluations of morphological variability and geographical distribution are still rare in the context of snow algae. Using Sanger sequencing of monospecific field blooms, this was performed for the red snow species ʿ*Chlamydomonas nivalis*ʿ (**paper I**), and the same strategy was applied to multiple populations of the species producing cysts morphologically assignable to *C. nivalis* (**paper III**).

1.4.7 Complex life cycles of chlamydomonadacean algae

Combined field and lab observations of partial life cycles (from vegetative flagellates to zygote) done for field material have provided insights into the life cycle of several snow dwelling *Chloromonas* (e.g. Hoham 1975a, Hoham & Mullet 1977, Hoham et al. 1979, 1983). Unfortunately, however, the life cycle for most snow algae is not known, due to several reasons. In some cases, there is no vegetative strain for the species available, since there are problems inducing the germination of field collected cyst under lab conditions. In other cases, the development of cysts from vegetative strains under laboratory conditions has not yet been observed. For instance, the red snow forming species *Sanguina nivaloides* has only been found in the form of cysts in field so far (**paper I**). Therefore, all diagrams describing

the life cycle of this species are just hypothetical, with no empirical proof in terms of molecular data (Müller et al. 2001, Sattler et al. 2010, Remias 2012). Consequently, the following illustrates the complexity of the snow algal cycle using the example of snow dwelling *Chloromonas*.

The vegetative cells of the genus *Chloromonas* may act as gametes, and sexual reproduction begins when the flagella of two gametes from two opposite mating types (isogamy or anisogamy) come into contact, with four-flagellate plano-zygotes being produced (Hoham & Mullet 1977, Hoham et al. 1979). Later, the four flagella get lost and an immotile, presumably diploid, zygote is formed (Remias et al. 2010a, **own observation**). Combined field and lab observations of partial life cycles have attempted to reconstruct life cycles for *C. brevispina* (partly likely *C. krienitzii*) Hoham et al. 1979, Matsuzaki et al. 2015, **own observation**), *C. hohamii* Ling & Seppelt (Hoham et al. 1983, Ling & Seppelt 1998), *C. nivalis* (Hoham & Mullet 1977), and *C. pichinchae* Wille (Hoham 1975a). However, in laboratory conditions neither sexual reproduction nor the production of cysts have been observed for several *Chloromonas* species from snow even under nitrogen starvation (Matsuzaki et al. 2018, 2019). Other factors possibly inducing sexual mating have been tested as well, such as the spectral composition of light, irradiance level, photoperiod and cell density. It was shown that the response is species specific. Significantly more mating occurred under a prolonged photoperiod (day:night, in hours) of 24:0 than 12:12 for homothallic *C. chenangoensis* Hoham, Berman, Rogers, Felio, Ryba & Miller (Hoham et al. 2009). Also, density-dependent mating was found for the closely related heterothallic *C. tughillensis* Hoham, Bergman, Rogers, Felio, Ryba & Miller (Hoham et al. 1998) with a preference of 1.5 x 10^6 cell ml^{-1} (when compared to 0.5 x 10^6 cell ml^{-1} and 1.0 x 10^6 cell ml^{-1}). In the case of *Chloromonas* sp.-D, a prolonged duration of blue light (430-460 nm peak) was preferred in comparison with the reference light (530-700 nm peak), both with an irradiance level of 95 µmol photons m^{-2} s^{-1} (Hoham et al. 2000). All the three above-mentioned species originated from shaded sites close to canopies.

A key factor in the success of chlamydomonadacean snow algae to colonize melting snow is very likely their seasonal life cycle, as I will illustrate for a snow alga of the genus *Chloromonas* (**Figure 2**) living in mid-latitude mountains (Hoham & Ling 2000, Sattler et al. 2010):

At the beginning of the melting season (e.g. in the Alps at an elevation of about 2,000 m a.s.l. in April), one can hardly see any snow discoloration. Snow algae from the previous seasons are assumed to be resting at the interface between the snow and soil (or rock) as resistant stages (cysts). The snow starts to melt slowly, and the cysts apparently recognize the availability of liquid water and germinate (which is accompanied by mitosis or meiosis). Then, flagellate stages are released and migrate upwards to the sub-surface layers, where they mate (in case of gametes) or become individually immotile. With ongoing melting the cells are transformed into new cysts, and accumulated and exposed at the snow surface. After total snowmelt these resistant stages should survive inactively over the rest of the year in the soil or on bare rock. At this point, they can be subject to airborne long-distance transport by wind.

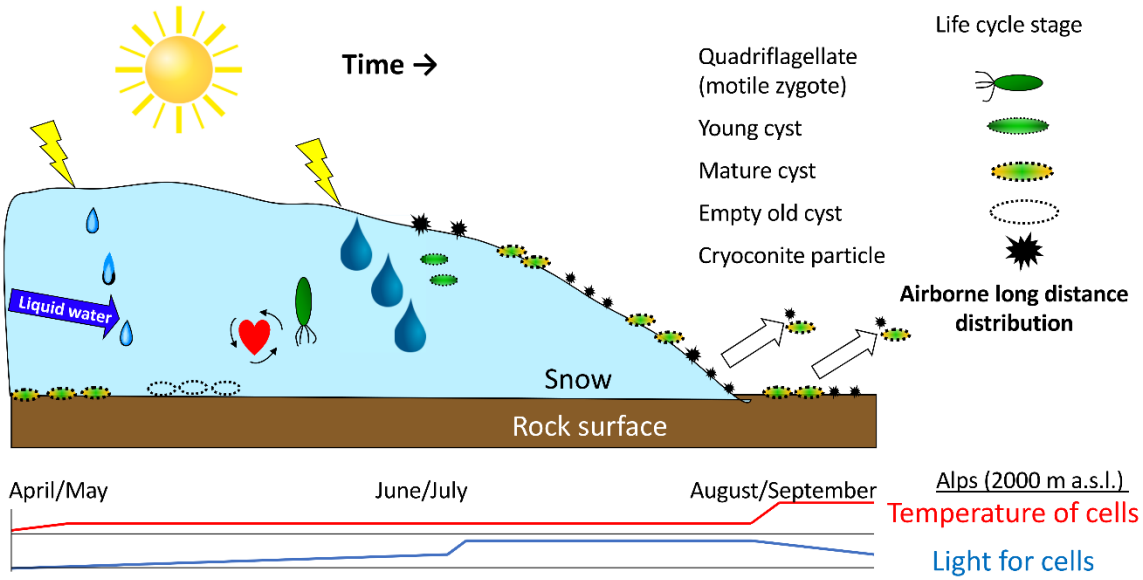

Figure 2. Typical seasonal life cycle of a snow alga (*Chloromonas nivalis*), based on observation over many seasons in European Alps [Figure modified by LP with a permission from Sattler et al. 2010]

The main physiological and cytological differences between the green cells of the flagellate phase and the reddish immotile cells of cyst phase were summarized in Remias (2012) (**Figure 3**): Briefly, cysts are reproductively inactive, accumulate reserve deposits and are resistant to abiotic stresses (e.g. freezing and high irradiation), while flagellate stages can migrate to reach optimal irradiances for photosynthesis and are reproductively active but sensitive to high irradiance and freezing. Reddish snow consisting of cysts is usually found

at open sites, while green snow consists commonly of flagellate stages and is found on the snow surface at shady sites (Fukushima 1963, Kol 1968).

The presence of cysts (or zygotes) as resistant 'resting stages' increases the range of environmental conditions in which a species can survive, ensuring the persistence of a species over time. Nitrogen depletion (particularly NO_3^-) in melting snow and an increase in snow water content seem to coincide with a shift in the life cycle from a vegetative phase to resistant cysts in *Chloromonas* alga (Hoham 1975a, Hoham et al. 1979, 1983). Interestingly, other factors such as species-specific viral infections might also be important for snow algae life cycles. Together with high cell density, it was shown that both nitrogen depletion and viral infections trigger the formation of resting stages at the end of bloom in a marine diatom (Pelusi 2019).

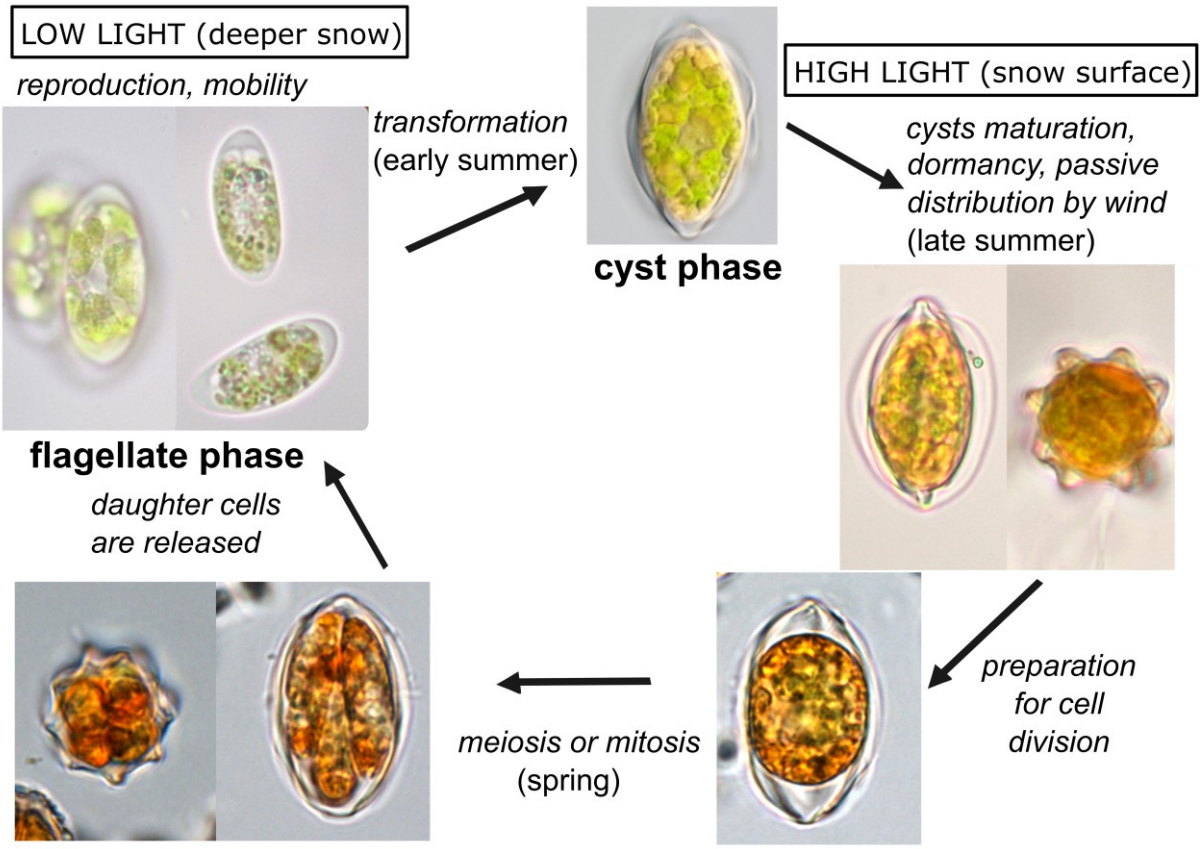

Figure 3. Main ecophysiological differences between two life cycle stages (flagellates, cysts) in the seasonal life cycle of a chlamydomonadacean snow alga. [Figure modified by LP with a permission from Remias (2012), illustrated here on example of *Chloromonas hindakii* (**paper III**)]

The freezing processes combined with prolonged dormancy are important factors resulting in the meiosis (or mitosis) of cyst stages of several snow *Chloromonas* species

during autumn (Hoham et al. 1979). Interestingly, simulation of these conditions in the laboratory has been recently successfully applied in enabling the easier establishment of strains for *Chloromonas* species dwelling in snow (**paper III**).

The assumed presence of haploid vegetative stages and diploid zygotes in the life cycle of chlamydomonadacean snow algae is an example of a heteromorphic life cycle where a prolonged haploid/diploid stage exists, and as consequence, transformation into cysts and the germination of cysts are spatially and temporally separated. This may have several impacts, including the colonisation of new locations by a snow alga being facilitated by high capabilities for uniparental reproduction (i.e. selfing, asexuality or both) (Krueger-Hadfield et al. 2016). This hypothesis is interesting in the context of the uncertainty of dispersal strategies and the lack of knowledge of geographic distribution patterns for many snow algae.

1.4.8 The role of snow algae in ecosystems

Painter et al. (2001) used the range of chlorophyll absorption to quantify snow algal biomass in the snow cover with an airborne imaging spectrometer to estimate snow-algae densities in the Sierra Nevada Mountains of California. A trial scan of their field site found a mean concentration of 1,300 cells ml^{-1} over an area of 0.495 km^2 of snow. They assumed all the algal cells were in the top 10 cm of snow, so the areal biomass concentration was estimated to be 0.033 $g.m^{-2}$. Takeuchi et al. (2006b) investigated the spatial distribution of red snow algae on the Harding Icefield, Alaska using the range of carotenoid absorption retrieved from a satellite image. The averaged mean carbon content estimated from the algal biomass of red snow for the icefield in the image was 1.2 kg km^{-2}.

Williams et al. (2003) integrated daily measurements of gas exchange to show a CO_2 uptake around 2,300 µmol m^{-2} day^{-1} in heavily colonized red snow patches in the Snowy Range of the Rocky Mountains, which indicates that summer snowfields can be productive. Moreover, these authors combined gas-exchange measurements with remote sensing of algal concentrations, estimating an overall annual net primary production for a red snowfield of 5 g m^{-2}. This represents ~2% of the area-specific productivity of arctic heathlands (Shaver 2001, Williams et al. 2003). Similarly, Lutz et al. (2014) measured net

photosynthesis rates in three sample types at the Mittivakkat Glacier the southeast Greenland: red snow (snow algae dominated in abundances), a brown-blackish biofilm (a mixture of snow and glacier algae), and grey ice (glacier algae dominated). All showed positive trends (the highest value was for the biofilm), indicating that snow and glacier algae are important primary colonizers and net primary producers that differ spatially (elevation) and temporally in their distribution in a glacier (Lutz et al. 2014). Hamilton & Havig (2017) measured carbon fixation rates of snow algae communities on supraglacial snowfields at glaciers in the Pacific Northwest. Their geochemical, isotopic, and microcosm data suggest these assemblages were surprisingly not limited by phosphorus or fixed nitrogen availability (Hamilton & Havig 2017), but the addition of inorganic carbon stimulated primary productivity (Hamilton & Havig 2018).

Snow and glacier algae support the microbial community that coexists with them, which includes heterotrophic bacteria (Terashima et al. 2017), fungi (Brown et al. 2015), ciliates (Bidigare et al. 1993), rotifers, nematodes (Hoham 1989), ice-worms (Shain et al. 2001), and springtails (Sattler et al. 2012). These algae may also play an important role in supraglacial and periglacial snow food webs, since they actively sequester iron, manganese, and phosphorus leached from minerals sourced from the local rocks (Hamilton & Havig 2017). Bacteria adhered to the outer smooth cell wall of red cysts of "*Chlamydomonas nivalis"* were visualised by scanning electron microscopy (Weiss 1983). Modern approaches such as metagenomic profiling of the pro- and eukaryotic communities in Japan showed patterns of the co-occurrence of certain microorganismal groups: species belonging to the subphylum Betaproteobacteria were frequently detected in both green and red snow, while members of the phylum Bacteroidetes were also prominent in red snow (Terashima et al. 2017). It is assumed that these bacteria can utilize the available carbon sources in algae-rich environments and may promote algal growth in the field as well as in laboratory conditions (Terashima et al. 2017) with essential nutrients, such as vitamins (Croft et al. 2005). Bacterial abundances and production rates were higher in red snow containing algae than in noncolored snow (Thomas & Duval 1995). However, bacterial abundances can also increase in surface snow during the melting season in the absence of or prior to snow blooms, probably due to the utilization of dissolved organic carbon in snow meltwater (Segawa et al. 2005).

1.4.9 Mechanisms of physiological adaptation

1.4.9.1 Pigments

Three major classes of photosynthetic pigments occur among algae: chlorophylls, carotenoids (carotenes and xanthophylls) and phycobilins. Their individual abundances are characteristic for certain main taxonomic groups (Hoek et al. 1995). For green algae (which dominate in snow blooms), the following pigments are especially important: chl-*a*, chl-*b*, β-carotene, lutein, violaxanthin, neoxanthin, episodically antheraxanthin and zeaxanthin (Bidigare et al. 1993, Remias et al. 2005). Carotenoids participate in several plastidal processes: a) in light harvesting and transfer of the photons to chlorophylls, b) as antioxidative agents protecting the thylakoid membranes from oxidation due to reactive oxygen species and other radicals. Interestingly, cleavage carotenoids products that are volatile cause a smell (Watson 2003), and blooms caused by *Sanguina nivaloides* are often called watermelon snow since the red snowfield smells faintly of fruit due to volatile degradation products of astaxanthin esters.

All carotenoids involved in photosynthesis are classified as primary carotenoids. Under certain scenarios, e.g. when cells are exposed to extreme conditions, certain microalgae have the ability to produce secondary carotenoids, especially astaxanthin, canthaxanthin and echinenone (Leya et al. 2009). These ketocartenoids (i.e. carotenoids containing a carbonyl group) play an essential role in snow algae. Generally, both high light levels as well as nitrogen depletion are known to induce secondary carotenoid synthesis (Sun et al. 2008, Cordero et al. 2010). Other factors that have been shown to play a role are increased concentrations of iron ions (Wang et al. 2013), and temperature, salt stress and combined stresses (Lemoine & Schoefs 2010). It is unknown if endogenous mechanisms in snow algae induce astaxanthin production in the course of their seasonal life cycle. The amount of accumulated astaxanthin in a cell is expressed as its ratio to chlorophyll-a; the highest value so far of 34.1:1 has been reported for *Sanguina nivaloides* cysts on Svalbard (Müller et al. 1998). High amounts of extra-plastidal astaxanthin provide a multifunctional stress defence: astaxanthin shields the photosystem against excessive irradiation including short episodes of enhanced UV-B (Remias et al. 2010b). Enantiomers of astaxanthin differ in their spectral absorption properties: while all-*trans*-astaxanthin has

its maximum absorbance in the visible light range (470 nm), 13Z-*cis*-astaxanthin (and its esters) also has additionally a significant capability of UV protection (around 375 nm) (Remias & Lütz 2007; **paper II**). Moreover, these abundant lipid droplets help in freezing resistance since they occupy large volume of cells and therefore reduce the water content that would otherwise increase the dangers of increased lysis due to crystalline ice formation (Remias et al. 2005). Finally, astaxanthin helps in enhancing the cytosolic resistance to oxidative stress (mediated by excess light, UV-B irradiation) and nutritional stress (Lemoine & Schoefs 2010).

Under nitrogen limitation, cells can redirect their biochemical reduction potential away from proteins to nitrogen-free metabolites such as fatty acids or carotenoids. Since the amount of storable intraplastidal carotenoids is limited by the physiological demand of the photosynthetic apparatus and the solubility of these non-polar compounds, an alternative strategy is activated: the synthesis of non-photosynthetic secondary carotenoids and their export into the cytoplasm and deposition in lipid globules. There, the astaxanthin with its two hydroxyl groups is usually esterified with fatty acids (Remias et al. 2005).

The lack of laboratory cultures for many snow algae also causes gaps in the knowledge about their physiology. Interestingly, the accumulation of secondary carotenoids in some zygotes of *Chloromonas* (e.g. *Cryocystis granulosa* (Kol) Kol ex Komárek & Fott, *Chloromonas nivalis*, *Chloromonas brevispina* in forested snowfields) appears to be related to the aging of cells as well as to ambient irradiance levels (Hoham & Duval 2001, Remias et al. 2010a, **own observation**). Field cysts of *Chlainomonas* sp. sampled later in the season had a higher astaxanthin to chlorophyll ratio when compared to the population collected earlier in the season (**paper II**). Analyses of field samples revealed that the pigment composition of algal communities is variable and dependent on sampling time and location: e.g. it dramatically changed on a glacier in Greenland during a 2 week melting season (Lutz et al. 2014). Similarly, snow algae communities from Iceland collected sooner in the season and at higher elevation glaciers (with later melting periods) were dominated by chlorophylls (31–100% of total pigments), followed by secondary carotenoids (between 0 and 69%) and then by primary carotenoids (up to 8%) (Lutz et al. 2015a). In contrast, snow algal communities collected later in the melting season showed higher secondary carotenoid

contents (up to 69%) (Lutz et al. 2015a). An increase of the astaxanthin to chlorophyll-*a* ratio in relation to cyst maturity was also documented for a population of *Chloromonas nivalis* in the Austrian Alps (Remias et al. 2010a).

Astaxanthin was shown to be the pigment responsible for the red colour in cytoplasmatic lipid bodies in cysts of "*Chlamydomonas nivalis"* (Remias et al. 2005; **paper II**), *Chlainomonas* sp. (**paper II**) and the reddish/orange colour of cysts of *Chloromonas* species in snow (Remias et al. 2013a; **papers III, IV**), though in lower quantities. Interestingly, two closely related species markedly differed in their accumulation of astaxanthin, despite the fact that they dwelled in the same habitat on Svalbard: small orange cysts affiliated with *Sanguina aurantia* exhibited astaxanthin to chlorophyll ratios from 1 to 3 (Müller et al. 1998; **paper I**), while dark red cysts of *Sanguina nivaloides* had ratios from 10 to 34 (Müller et al. 1998; **paper I**). The *Chloromonas* representatives, which accumulate less astaxanthin than *Sanguina*, were suggested to compensate for this with a larger pool of carotenoids of the xanthophyll cycle in order to protect the chloroplast against excessive irradiation (Remias et al. 2010a).

Peak profiles of secondary carotenoids may be used for a rough chemotaxonomy (Remias et al. 2016). While in *Chlainomonas* sp. astaxanthin is mainly esterified with two fatty acids and is thus more non-polar, in *Sanguina nivaloides* and *Chloromonas nivalis* less non-polar monoesters prevail (Bidigare et al. 1993, Remias et al. 2010a, 2016a; **paper II**). Astaxanthin provides chloroplast protection mainly against visible light (Gorton et al. 2001). Under elevated UV-B radiation, impaired (but not inhibited) photosynthesis and an increase of secondary pigments took place in *Sanguina nivaloides*, indicating a trade-off between autotrophic productivity on the one hand and UV-protection on the other. The high amounts of secondary carotenoids attenuated the changes during the exposed conditions. This attenuation explains why violaxanthin was the only xanthophyll cycle pigment found to be typical for low-light-adapted thylakoids (antheraxanthin and zeaxanthin were below detection limits) (Remias et al. 2010b). UV tolerance is relevant for snow alga especially during the atmospheric transport of cysts after snowmelt, when they are exposed to much higher UV doses than at collection sites in Alps or even at high latitude localities.

Adaptation strategies to high light stress other than secondary carotenoids have been demonstrated for Trebouxiophyceae and several species of Chlorophyceae (Leya et al. 2009). *Raphidonema nivale* and *Raphidonema sempervirens* Chodat exhibited an unusually high pool of primary xanthophyll cycle pigments and did not accumulate any secondary carotenoids. The xanthophyll cycle was also shown to be very effective for photoprotection in snow chrysophytes *Ochromonas* (*O.*) *smithii* Fukushima and *O. itoi* Fukushima (Tanabe et al. 2011). In contrast, the transition from non-stressed to stressed (elevated light, nitrogen deficiency) conditions in trophic stages of several snow and permafrost algae of Chlorophyceae decreased the yield of all primary pigments, while all secondary carotenoids and α-tocopherol markedly increased (Leya et al. 2009). An increased accumulation of α-tocopherol during cyst maturation was reported for field samples of *Chloromonas nivalis* (Remias et al. 2010a) and *Chlainomonas* sp. (**paper II**).

1.4.9.2 Phenolic secondary metabolites

Mesotaenium berggrenii, and *Ancylonema nordenskiöldii*, the most prominent algae dwelling at melting bare glacier surfaces, have different protective strategies: secondary carotenoids like astaxanthin has never been detected in their cells, but rather they accumulate high amounts of a polar brownish pigment in vacuoles (Remias et al. 2009, 2012a; **own observation**). These secondary metabolites were revealed to possess an unusual benzotropolone backbone (known from oxidation products of black tea), and the main compound was identified as purpurogallin carboxylic acid-6-O-β-D-glucopyranoside (Remias et al. 2012b). This phenol, together with its congener, exhibit a broad spectral absorption covering the entire UV A and B range, and also a large part of the visible light range (Remias et al. 2012a, b). Moreover, it may act as a chemical defence against grazers (e.g. glacier springtails) due to its tannic attributes, or may represent a 'physiological sink' for surpluses of reductive energy from photosynthesis that cannot be invested, in e.g. growth and cell division, because of abiotic limitations (e.g. temperature, nutrients). Since such phenolic compounds are nitrogen-free, its abundant synthesis by algae is not dependent on eutrophic conditions. Phenolic substances have also been reported from other species of Zygnematales (Streptophyta), i.e. *Spirogyra* (Nishizawa et al. 1985), *Zygnemopsis* (Figueroa et al. 2009),

Zygnema (Pichrtová et al. 2013) and *Zygogonium* (Aigner et al. 2013). Concerning chlamydomonadacean snow algae, total phenolic and free proline contents, as well as antioxidant protection factor, were found to increase in "*Chlamydomonas nivalis"* under UV-A and UV-C light exposure (Duval et al. 2000).

1.4.9.3 Functional profiling of snow microbial communities

One of the first insights into snow ecosystem functioning *in situ* was gained in studying the pro- and eukaryotic green and red snow communities on Feiringsbreen, Svalbard (Lutz et al. 2015b). Green snow represented a carbon and nutrient rich environment, and was dominated by the algae *Microglena* sp. with a metabolic profile characterized by key metabolites involved in growth and proliferation. In contrast, the nutrient poor red snow habitat was colonized by various *Chloromonas* species with a high abundance of storage and reserve metabolites, likely in order to face upcoming severe conditions. Recently, an investigation of snow algal communities in Ryder Bay, Antarctic Peninsula (Davey et al. 2019) showed that green snow was protein-rich, had a high chlorophyll content, and contained many metabolites associated with nitrogen and amino acid metabolism, while red snow communities had a higher carotenoid content and contained more metabolites associated with carbohydrate and fatty acid metabolism. Interestingly, in green snow dominant compounds (fungi were dominant in 18S rDNA reads) such as the amino acid lysin and its metabolite aminoadipic acid were detected (Table S4 in Davey et al. 2019), which is a precursor for penicillin synthesis in fungi that produce α-aminoadipate (Fazius et al. 2012). In red snow (Davey et al. 2019) found glycerol, sugar alcohols and other low molecular weight carbohydrates that are associated with osmotic acclimation such as mannitol and glycerol (Egger & Karsten 2010). The glycerolipid and fatty acid composition was similar between green and red blooms, which is consistent with the classical hypothesis that a high degree of fatty acid saturation is related to membrane stability at low temperatures (Bidigare et al. 1993).

1.4.9.4 Photosynthesis

Snow and glacial ice habitats are quite different than mesophilic ones. A number of physiological adaptations are expected considering the low temperatures and extreme irradiation. Algae adjust both how effectively they absorb photons and how efficiently they turn absorbed light energy into chemical energy via photosynthesis.

Primary productivity is a term used to describe the rate at which plants and other photosynthetic organisms produce organic compounds in an ecosystem. For snow algae, this has been measured by carbon fixation (Fogg 1967, Thomas 1972, Javornický 1973, Mosser et al. 1977, Williams et al. 2003, Hamilton & Havig 2018) and by measurements of changes in oxygen concentrations in light and dark bottles (Lutz et al. 2014).

The relationship between the rate of photosynthesis and light intensity can be described by 'light curves' (LC; P/I curves), which assess not only the present photosynthetic capacity, but the algal activity over a wide range of ambient light intensities (Falkowski & Raven 1997). There are several ways to measure photosynthetic performance. A direct method utilises oxygen evolution monitoring (Remias et al. 2005; **paper VIII**), while an indirect method utilises the measurement of rapid light curves (RLCs) using variable chlorophyll fluorescence (Strasser et al. 2000, Stibal & Elster 2007; **papers II–V**). In the latter, a parameter called the electron transport rate, or ETR, is closely related to the photosynthetic activity measured by oxygen evolution or oxygen dioxide uptake (Beer et al. 1998). Relative ETR (rETR) approximates the rate of electrons pumped through the photosynthetic chain (Ralph & Gademann 2005). Photon energy captured by a chlorophyll-*a* molecule can either drive photosynthesis, be converted to heat, or be emitted as chlorophyll fluorescence. The latter provides the most appropriate means for screening the dynamic processes occurring in the photosystem II (PSII) reaction centres, i.e. the efficiency of light energy utilization, diurnal changes in PSII photochemistry, or electron transfer rates (Ralph & Gademann 2005).

Traditional light response curves and rapid light curves have three distinct sections: an initial light-limited region, a light-saturated region, and with even higher irradiance (supra-saturating) a region where the curve may tend to decline. With traditional light response curves, this decline is usually associated with photoinhibition (Henley 1993);

however, with RLCs this decline could be linked to the dynamic down-regulation of PSII (White & Critchley 1999), not *true* photoinhibition as there is (commonly) insufficient time for photodamage to occur. For quantitatively comparing LCs, several characteristic parameters such as α (the slope of the linear part of the curve), I_k (the light saturation coefficient) and P_{max} (the maximum rate when light becomes saturating; $rETR_{max}$ in RLC) are calculated (Ralph et al. 2002; **papers II–V**). Finally, these photobiologic assays are temperature dependent, and this fact can be used to make conclusion about light preferences of an alga.

RLCs of the snow alga *Chloromonas brevispina* under a spruce canopy in the Krkonoše Mts. (Czech Republic) have indicated that the photochemical activity of cysts is measurable *in situ* (Kvíderová et al. 2005). This is of relevance, since for many snow algae no living strain is available yet. At Svalbard, photosynthetic parameters changed over the course of the snow melting season in a single spot dominated by orange cysts (*Sanguina aurantia*, **paper I**) in relation to weather changes (higher irradiances corresponded with higher rETR, alpha, I_k), and likely also as a consequence of the cell maturation process (**paper II**). These cysts were mostly saturated later in the season, with the saturating depth reaching more than 2 cm on some days. A higher accumulation of secondary screening pigments outside the plastids resulted in a reduced amount of photosynthetic active radiation available for the photosystems (Stibal & Elster 2007). By contrast, purple cysts from the same region (*S. nivaloides*, **paper I**) seem to have never reached saturation (Stibal & Elster 2007). Cysts of this species from the Austrian Alps also never became inhibited up to 2100 µmol photons m^{-2} s^{-1} (Remias et al. 2005; **paper II**). This probably reflects a combination of abundant secondary carotenoid accumulation (Müller et al. 1998; **paper II**) in combination with a photoprotectively advantageous localisation of the plastid in the centre of the cell (Remias et al. 2016; **paper I**).

In the Maritime Antarctic, oxygen evolution measurements of cysts of the green alga *C. polyptera* and zoospores of the chrysophyte *Hydrurus* sp. showed no sign of photoinhibition at higher irradiance levels (~1,300 µmol photons m^{-2} s^{-1}) (Remias et al. 2013a, b). Interestingly, two very closely related taxa, *C. nivalis* from the Tyrolean Alps and *C. nivalis* subsp. *tatrae*, exhibited similar photosynthetic performance (**paper IV**), reflecting the fact that they thrive in similar habitats at open sites, but they differed in relative

astaxanthin accumulation, the latter having three times more (**paper IV**). In contrast, fluorometric measurements demonstrated that the cysts of *Scotiella cryophila* Chodat K-1 (a member of the *Chloromonas* clade based on 18S rDNA sequencing) were adapted to low light conditions. That was exactly the situation deeper in the snow where population was found (20 cm below the surface) (**paper V**). Snow algae live in a changeable environment. With proceeding melting, cells that previously grew in low light conditions deeper in the snow will be exposed at the snow surface. Such cells have to rearrange their photosynthetic apparatus and its antennae, e.g. relocate light harvesting complexes between PSII and PSI, alter the pigment composition, e.g. accumulate secondary carotenoids, or rely on energy dissipation, e.g. via the xanthophyll cycle (Tanabe et al. 2011, Lyon & Mock 2014, Hopes et al. 2017).

The first ecophysiological comparison of populations of the same cryoflora species from two different mountain ranges was done for *Chlainomonas* sp. (High Tatra Mts. in Slovakia, Tyrolean Alps in Austria). The photosynthetic rates of the alga from both sites were consistent in their response to irradiance, with photoinhibition not noticed up to high light intensities of 1,300 μmol photons m^{-2} s^{-1} (**paper II**), corresponding to previous findings for this species based on oxygen evolution measurements (Remias et al. 2016). However, the former population (sampled later in the season) needed only half the irradiance than the latter (collected earlier in the season) to become saturated (I_k) (**paper II**). This can be explained by the advanced snowmelt and associated irradiance stress experienced later in the season (**paper II**). An acclimation to the long-term higher PAR and UVR conditions at certain locations may also be responsible for observed differences between two populations of vegetative flagellates of *Chloromonas* species (Kvíderová 2010).

Recently, a new species causing orange snow, *C. hindakii*, was described (**paper III**) and demonstrated for the first time for a snow alga that the photosynthetic apparatus can adapt to quite different light conditions (**paper III**). This was achieved by comparing RLCs of a laboratory vegetative strain and field cysts collected from shaded, partly shaded and fully exposed snowfields. This showed an intraspecific flexibility of the photophysiology, which apparently allows this species to thrive at quite different light conditions in snow (**paper III**).

1.4.9.5 Lipid compounds

Major metabolic adaptations enabling the cell growth and photosynthesis of snow algae at low temperatures are associated with the maintenance of membrane fluidity. Lipids are the main structural components of biological membranes, the main storage site for energy, and are involved in cell signalling.

Three main classes are recognized: neutral lipids, phospholipids and glycolipids. Neutral lipids in microalgae are represented predominantly by triacylglycerols (TAGs), diacylglycerols, sterols and sterol esters (Leblond & Chapman 2000). Microalgae deposit neutral lipids in organelles referred to as lipid bodies or oil globules, while phospholipids and glycolipids are part of biomembranes. Nutrient availability, temperature and PAR are the key factors influencing fatty acid composition in lipids (Roessler 1990). Very high contents of polyunsaturated fatty acids (PUFAs) were found in both principal life cycle stages of chlamydomonadacean snow algae, namely in cysts of *Chlainomonas* and *Chloromonas* (**papers II–IV**) and vegetative flagellates of *Chloromonas* (**paper III**, **IX**). This indicates the importance of PUFAs in resilience under a range of harmful conditions and the adjustment of the photosynthetic apparatus (Kugler et al. 2019). Desaturation of FAs promotes a looser packing of lipids and a decrease in the temperature of membrane solidification at low temperatures (Morgan-Kiss et al. 2006). Lipid droplet formation leads to striking ultrastructural alterations in the cell during cyst maturation (Remias et al. 2010a; **paper IV**), generally associated with an overall reduction of the plastid and decreased stacking of thylakoid membranes (**paper III, V**). PUFAs formed more than 75% of the total fatty acids in a *Chloromonas brevispina* population from Šumava, Czech Republic; vegetative flagellates dominated the field sample, and only a few cysts were present (Řezanka et al. 2008). On the other hand, Bidigare et al. (1993) reported significantly lower PUFA from green snow of *Chlamydomonas* spp. sampled in Antarctica (28%). However, that result was based on single measurement in the field. There is only limited knowledge about the variability of fatty acid profiles of snow algal strains under varying controlled conditions in the laboratory (Spijkerman et al. 2012).

In snow algal cysts, neutral lipids were composed predominantly of saturated lipids, whereas phospholipids and glycolipids were composed predominantly of PUFAs, suggesting

their key role in membrane fluidity adjustment (**paper II, IV)**. Interestingly, in flagellate stages, PUFAs prevailed even in the neutral lipids of *C. hindakii* (**paper III**). Although cysts and flagellate stages of *C. hindakii* contained the same quantities of PUFAs, differences in the FA profile were found: For the flagellates, the most abundant PUFA was hexadecatetraenoic acid,16:4 (4Z,7Z,11Z,13Z), which was three times higher than for cysts of the same species. Similarly, this FA was important in flagellates of *C. remiasii* Matsuzaki, Nozaki & Kawachi isolated from the Arctic (strain CCCryo 005-99) and kept under nitrogen deficient conditions (Spijkerman et al. 2012) and green field samples of *C. pichinchae* from mountains in the Czech Republic (**paper IX**). Generally, the PUFAs found prevailing in *Chloromonas* snow species are linoleic acid, 18:2 (9Z,12Z), α-linolenic, 18:3 (9Z,12Z,15Z), and stearidonic acid, 18:4 (6Z,9Z,12Z,15Z) (**papers II–IV**), which is consistent with the mean content of individual FAs in 22 strains of *Chloromonas* screened in a study by (Lang et al. 2011).

Concerning the intraspecific variability of populations sampled in different geographical regions, no significant difference in FA profiles was found between field samples of *Chlainomonas* sp. from two mountain ranges in central Europe that were identical in their ITS2 rDNA sequences. In the case of *Sanguina nivaloides*, the monounsaturated oleic acid, 18:1 (9Z), dominated the Svalbard field sample (sample 9/10 1b, Spijkerman et al. 2012), while it was 4.5 times lower in an Austrian population where α-linolenic was the most abundant FA (**paper II**). One may assume that FA profiles are influenced by adaptations to latitudinal differences of ambient conditions, genetic variability between the samples, and/or the time of sampling over the course of the season.

Photosynthetic membranes of cyanobacteria, algae and plants are unique in their richness of glycolipids dominated by galactolipids followed by sulfolipids. Interestingly, an unusually high content of the phospholipid phosphatidylglycerol was found in thylakoid membranes (>80%) of the green alga *C. reticulata* isolated from red snow. This charged lipid may provide structural flexibility that is a prerequisite for electron transfer at the acceptor side of PSII at low temperatures (5°C) (**paper VIII**). In order to understand the cellular physiology, comprehensive identification and precise quantification of lipids is crucial in lipidomic research (**paper IX**).

1.5 Climate change: Are habitats of cryoflora threatened?

Studies of the global deep-sea oxygen ($^{18}\delta O_2$) and carbon ($^{13}\delta C$) isotope records indicate that the Earth has experienced continuous temperature changes. It has drifted from extremes of expansive warmth with ice-free poles, to extremes of cold with massive continental ice-sheets and polar ice caps. During the Cretaceous period (144 to 65 Ma), tropical flora and fauna thrived in polar regions. The Cenozoic era (65 Ma up to present) followed the Cretaceous and marked a significant shift in planetary temperature. The Earth began to cool down about 50 Ma BP (see Figure 9.1 in Marshall 2011). The onset of the Quaternary glacial cycles was at about 2.5 Ma. The planet has experienced more than 40 glacial-interglacial episodes during this period so far. The cycles have increased in magnitude over the past million years (see Figure 4 in Lisiecki & Raymo 2005), indicating that the glaciations have become more severe. Currently we live in an interglacial period that is expected to come to an end perhaps 20,000 years from now.

Insights into the complex interactions between snow and climate were recently published by Thackeray et al. (2019). In the northern hemisphere, the extent of snow cover has experienced a significant decline, at rates of 3.1% and 13.6% in May and June per decade over the past half-century (1967–2018) (Bormann et al. 2018). Increasing surface temperatures are expected to delay the onset of the snow season and promote earlier melting (Brown et al. 2009), with the greatest changes to happen in spring. Snow in the mid-latitudes appears to be most sensitive to climate change (Mudryk et al. 2017) and mid-latitude areas below 1200 m a.s.l. are assumed to suffer complete snow cover loss by the end of 21st century (Marty et al. 2017).

In the last fifty years, 335 ± 144 gigatons of glacier ice have melted away globally, resulting in a global sea-level contribution of 0.92 ± 0.39 millimetres, per year (Zemp et al. 2019). This can be compared to ~120 m of ice layer covering whole area of Austria. One major current contributor to the global sea-level rise was the melting of heavily glacierized regions, with approximately one third originating from Alaska (Zemp et al. 2019). There were also large contributions from regions with less glacierization but strongly negative specific mass changes, such as Western Canada, USA, and Southern Andes. The Himalayas were the only region that exhibited mass gain over the full observation period (Zemp et al. 2019).

This implies that glaciers could almost disappear in certain mountain ranges in this century, while heavily glacierized regions will continue to contribute to sea-level rises beyond the year 2100. Taking a closer look at the European Alps, glaciers are still present in France, Switzerland, Italy and Austria. Under strong warming (implying a high CO_2 emission scenario), these glaciers are projected to almost disappear within one century (Zekollari et al. 2019). Even a scenario of moderate warming does not bring much hope for the ʿpermanent iceʾ of the European Alps, as only about one-third of the present-day glacial volume will remain in 2100 (Zekollari et al. 2019). Even more at risk are equatorial glaciers and mountains with snow (e.g. Mount Kilimanjaro in Africa); these are currently disappearing rapidly and we are likely the last generation able to observe these ʿcold islandsʾ in the tropics (Zawierucha & Shain 2019).

The consequences of dramatic changes of seasonal snow cover and glacier decrease will have a huge impact on supplies of drinking water, and further uses such as hydropower generation, industry and functioning of meltwater-dependent mountain agriculture, especially in cold-arid regions with seasonal water scarcity. This is particularly important in tropical Andes of Peru and Himalaya (Thompson et al. 2011, Nüsser et al. 2019). In addition, further socioeconomical impacts of glacial retreat include the uncertain development of tourism as a major economic support for developing countries, for instance Mountain Kilimanjaro in Tanzania (Mölg et al. 2008).

Recent climate changes are also clearly of relevance for the presence of cryoflora. In addition, however, cryoflora also impacts the results of climate change. Light absorbing impurities on surfaces are affecting the climate through changes in snow albedo. It is calculated as the percentage of incident light (400-700 nm) which is reflected by snow (Thomas & Duval 1995, (Lutz et al. 2014). Snow and glacier algal blooms have been shown to have significant effects on albedo reduction (Lutz et al. 2016, Ganey et al. 2017, Stibal et al. 2017). Other impurities in snow include carbonaceous particles (Warren 1984), mineral dust (Fugazza et al. 2015, Mauro et al. 2018), and other organic matter (Takeuchi & Li 2008). With regard to grain size, water content and presence of any impurities, the albedo of snow is dramatically changing. In the Tioga Pass area, Sierra Nevada, California, fresh snow was reported to have 90±2% while old snow had 58±5.5% (William H Thomas & Duval 1995). At Mittivakkat glacier in south eastern Greenland, the differences in albedo from the clean

(~75±5%) to red snow (~49±8%) and to biofilms (~20±4%; glacier and snow algae) were attributed to an increased secondary cell pigmentation and mineral contents. Cell concentrations and life cycle stages also play a role. Masses of green snow made of vegetative flagellates show decreased solar reflection of even ~30% (Feiringbreen, Svalbard; (Lutz et al. 2015b). Such microbial darkening phenomenon further accelerate snow and ice melting. In four Arctic locations, Lutz et al. (2016) estimated an overall decrease in snow albedo from red snow algal blooms over the course of one melt season of 13%. This indicates that such a bio-albedo effect should be considered in climatic models.

1.6 Perspectives of the snow algal research

Due to global warming, the existence of cryoflora on tropical glaciers (e.g. (Vimercati et al. 2019a) and many mid-latitude mountain ranges (e.g. Nedbalová et al. 2008) are very threatened, and they thus urgently deserve focused research before these habitats will be gone forever.

The acquisition of new snow and glacier algal strains is the first step for further analysis of their detailed cytology, ecophysiology, genomes (molecular taxonomy) and for biotechnology (e.g. Hulatt et al. 2017). To my knowledge, there is no existing laboratory strain of the most prominent snow and glacier algae such as *Sanguina nivaloides* or *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* (Williamson et al. 2019). Acquiring knowledge about detailed environmental changes inducing snow algal cysts to germinate, or the establishment of continuous growth of glacier algae, is necessary. Both problems seem to depend on simulation of the precise abiotic conditions present in the field. I assume that glacier species have intracellular sensors that realize if the situation does not perfectly match that in the field – stopping growth endogenously. A technically accurate simulation of such field conditions in the laboratory is likely needed.

Genomes, transcriptomes, and reverse genetic tools serve as important platforms for advanced studies on algal ecology, evolution, and adaptation (Hopes et al. 2017, Lyon & Mock 2014). Currently, there are only a few polar/snow green algae with a published genome, e.g. the psychrotolerant freshwater green alga *Coccomyxa subellipsoidea* Acton

C-169 (Blanc et al. 2012), and snow alga *Chloromonas brevispina* UTEX SNO96 (J. A. Raymond 2014) and *Kremastochrysopsis austriaca* DR75b (Raymond & Remias accepted). Further genomes of snow algae are currently under work (S. Lutz - pers. comm.). Still, there is much to discover in the function of genes. For instance, there are over 2,300 genes (~30% of all genes) in the *Coccomyxa subellipsoidea* genome with no known homologs in sequenced mesophilic chlorophytes (Blanc et al. 2012). Most of the genes involved in defence, detoxification, and carbohydrate metabolism display higher sequence similarity to bacteria rather than to green algae, suggesting possible acquisition by horizontal gene transfer. Similarly, this origin has also been suggested for ice-binding proteins in the snow alga *Chloromonas brevispina* (Raymond 2014).

The production of knock-down lineages (e.g. Kugler et al. 2019) of snow algal strains for genes of enzymes involved in lipid metabolism (e.g. FAs synthases, elongases, lipases, desaturases) should show a link between certain functions of PUFAs and the ability of an alga to thrive in the extreme conditions of melting snow.

2 Research objectives and methods of this thesis

In the present thesis, I performed both field and laboratory observations and experiments to answer the following questions:

- **What are the phylogenetic relationships of snow algae causing blooms? Do the newly described species show any close phylogenetic relationship to other psychrophilic species? Is there a hidden molecular diversity within the widely distributed species *Chloromonas (C.) nivalis*?**
  The molecular phylogeny was inferred based on the tree construction of 18S rDNA and *rbc*L (papers I, III, V, VI). If almost identical sequences of the closest taxon had already been published before, no new phylogenetic tree was generated (paper II, IV).
- **What are the closest relatives to these species? What is the genetic variability among populations of a single species?**
  The relationship with closely related reference species and among samples of a single species was evaluated by the construction of ITS2 rRNA secondary structures (a search for compensatory base changes was conducted) (papers I–V, VII).
- **What are the pros and cons of using 18S rDNA and ITS2 rDNA for the evaluation of amplicon high-throughput sequencing data (HTS)? Are ITS2 rDNA transcript secondary structures comparison and oligotyping helpful in delineating the cryptic diversity of dominant species?**
  The differences in the algal community structure obtained using the two markers were summarised in non-metric multidimensional scaling ordination graphs. Furthermore, it was checked whether outcomes of the different taxonomic assignment strategies for both markers were cross-correlated with traditional light microscopy observations (paper VII).
- **Is it helpful to complement HTS data with newly generated traditional Sanger sequencing data for a custom-based reference library?**
  Sanger sequencing of local abundant taxa was carried out to obtain new reference sequences, and it was checked whether the taxonomic assignments improved once the additional reference sequences were included into the custom-made reference sequence database (paper VII).
- **Are morphological characteristics of snow algal cells consistent with their phylogeny reconstruction based on molecular data?**
  Cell surface morphology details and intracellular ultrastructure were inspected by using light, scanning and transmission electron microscopy (papers I–VII)
- **Is there any pattern in the geographical distribution of haplotypes of a cosmopolitan species? Is there any significant relationship between the geographical distance and the sequence similarity of ITS2 rDNA?**

The geographic distribution of haplotypes was detected by establishing a haplotype network. To test the existence of spatial patterns, the Mantel test and spatial principal component analyses were carried out (paper I)

- **Does the spatial distribution of the snow algal population on an ice-covered lake correlate with the availability of liquid water in the snow?**
  The spatial distribution of snow water content and the cell concentration in snow on the ice cover of a high alpine lake was investigated along a transect (paper II)
- **Are there any differences in adaptation strategies between populations, between different life cycle stages of one species, or between closely related taxa in terms of pigment and lipid profiles?**
  Pigment (HPLC) and fatty acid (GC-MS) analyses were conducted for: two populations of *Chlainomonas* sp. (Austrian Alps versus High Tatra Mountains), a laboratory strain and a population of field-collected cysts of *C. hindakii*, and a population of *C. nivalis* subsp. *tatrae* from the High Tatra Mountains (papers II, III, IV). Using Raman spectroscopy, data were obtained from monospecific and mixed field snow algae samples (paper X). The lipid composition of field samples of *Chloromonas* flagellates was studied in detail using high-resolution mass spectrometry (ESI-MS), and the analysis of triacylglycerols (silver-LC and NARP-LC/MS) identified regioisomers containing PUFA (paper IX).
- **Do the light preferences reflect the conditions in the original habitat? Do populations from high light conditions become photoinhibited at higher irradiances than those from low light conditions?**
  Using rapid light curves, three populations of *C. hindakii* consisting of cysts (collected above timberline, next to a spruce canopy and 3-5 cm below the snow surface) and a laboratory strain of the same species were compared (paper III). Additionally, cysts of *C. nivalis* subsp. *tatrae* from the snow surface (paper IV) and cysts of *Scotiella cryophila* causing a green bloom at the depth of 20 cm below the snow surface (paper V) were studied.
- **What is the temperature dependence of the growth and photosynthetic activity of a *Chloromonas* strain isolated from snow compared to a mesophilic strain of *Chlamydomonas reinhardtii*?**
  In a wide range of temperatures (5 to 35°C), the autotrophic growth of both strains was assayed by measurements of total chlorophyll content and oxygen evolution rates were determined (paper VIII).
- **What are the possible mechanisms behind the photosynthesis adaptation of the *Chloromonas* strain isolated from snow to low temperature?**
  The temperature dependence on the electron transfer rate from quinon Q_A to quinon Q_B and lipid composition of thylakoid membranes were investigated for *C. reticulata* acclimated for temperatures ranging from 5 to 35°C. Amino acid changes in the sequence of the D1 protein in comparison with other taxa were detected (paper VIII).

3 Outline of the original papers (I–X)

This thesis consists of ten papers (**I–X**). Eight papers (I–VI, IX, X) focused on microalgae causing snow discolouration by massive reproduction, while paper VII is methodologically motivated, using a metagenomic evaluation of whole green algae assemblages present in melting snow. Paper VIII investigated the physiology of an alga isolated from red snow but is likely not responsible for the colouration.

Paper I (Procházková et al. 2019, *FEMS Microbiology Ecology*) focused on spherical cysts commonly found in red snow worldwide and formerly affiliated with the name of *Chlamydomonas nivalis*. The samples originated from alpine habitats in Europe, North America, South America and both polar regions. No culturable isolate of the investigated alga is available. Molecular analyses of the field cysts revealed the presence of a single independent lineage within the Chlamydomonadales (well separated from genera *Chlamydomonas* and *Chloromonas*). The genus *Sanguina* was described, with *Sanguina nivaloides* as its type. It is distinguishable from other red cysts forming alga by the number of cell wall layers, cell size, cell surface morphology and habitat preference. *S. nivaloides* is a diverse species containing 18 haplotypes in the dataset according to ITS2 rDNA. It has a cosmopolitan distribution with an absence of geographical structuring, indicating an effective dispersal strategy with the cysts being transported all around the globe, including trans-equatorially. Additionally, *Sanguina aurantia* was described, with small spherical orange cysts often clustered by means of mucilaginous sheaths, and causing orange blooms in snow in subarctic and Arctic regions.

Paper II (Procházková et al. 2018, *European Journal of Phycology*) investigated a species causing massive red blooms in a special habitat of slush on ice-covered high alpine lakes. Two populations (identical in four molecular markers) in the High Tatras in Slovakia and Austrian Alps consisted mostly of smooth-walled quadriflagellates. They shared similar photosynthetic performances, very high levels of polyunsaturated fatty acids and abundant astaxanthin accumulation, comparable to the red cysts of *Chlamydomonas nivalis* (now *Sanguina nivaloides*). Higher levels of α-tocopherol,13Z-isomer of astaxanthin and secondary pigments were accumulated in the Slovak population in comparison with the Austrian population, probably reflecting harsher environmental conditions for the former in the late melting season. *Chlainomonas* sp. has been misinterpreted several times as *Chlamydomona nivalis*, therefore we compared these two species using a polyphasic approach and showed that the latter species shows high photophysiological plasticity and has significantly lower PUFA content. An annual cycle of lake-to-snow colonization by *Chlainomonas* sp. from slush layers deeper in the ice cover was proposed. Our results point to an ecologically highly specialized cryoflora species, whose geographic distribution is likely to be more widespread than previously assumed.

Paper III (Procházková et al. 2019, *Microorganisms*) focused on the ecophysiology and identity of previously unknown species responsible for orange snow blooms in three mountain ranges in the central Europe. The cysts within the blooms morphologically

resembled those of *Chloromonas nivalis*. Molecular and morphological traits of field and cultured material showed that they represent a new species, *Chloromonas hindakii* sp. nov. For the first time for a snow alga, cyst stages collected in a wide altitudinal gradient and the laboratory strain were compared in terms of the performance of photosystem II (using PAM fluorometry). Results indicated that the physiologic light preferences of each population reflected the conditions in the original habitat. A high content of polyunsaturated fatty acids (about 60% of total lipids) and the accumulation of the carotenoid astaxanthin were observed. These are regarded as adaptations to coping with extreme environmental conditions of snow that include low temperatures, freeze-thaw cycles and variable light intensity. The intraspecific ability of adaptations of the photosynthetic apparatus to different irradiance regimes seems to be crucial for thriving in different snow habitats.

Paper IV (Procházková et al. 2018, *Fottea*) re-examined a snow alga morphologically fitting the original description of *Scotiella tatrae* and causing brownish snow blooms near its type locality in the High Tatra Mountains (Slovakia). The investigated alga turned out to be genetically (sequencing of four markers) very closely related to *Chloromonas nivalis* from the Austrian Alps. Therefore, *S. tatrae* was transferred into the latter taxon and reduced to a subspecies as *C. nivalis* subsp. *tatrae*. Both exhibit a similar photosynthetic performance, thrive in similar habitats at open sites above timberline, but differ in astaxanthin accumulation and the number of aplanozygote cell wall flanges. In a field sample of *C. nivalis* subsp. *tatrae*, polyunsaturated fatty acids formed nearly 50 % of total lipids, dominating in phospholipids and glycolipids. *C. nivalis* subsp. *tatrae* likely represents a variation of a common cryoflora species with distinct morphology.

Paper V (Remias et al. 2018, *Phycologia*) explored the photobiology, cytology and phylogenetic position of cysts causing sub-surficial green snow in the Austrian Alps, Tyrol, and morphologically identifiable as the snow alga *Scotiella cryophila sensu* Chodat, initially described from Switzerland. Cysts of *S. cryophila* K-1 had secondary cell walls with pronounced rib-like surface structures, and contained several small spherical plastids. Cysts performed active photosynthesis at temperature conditions close to the freezing point, and were photoinhibited at irradiances corresponding exactly to habitat conditions occurring at a depth below the snow surface where the population was found. Phylogenetic analyses using several molecular markers indicated that *S. cryophila* K-1 is related to *Chloromonas*, known to contain several snow algae. The taxon formed an independent lineage and was clearly genetically distinct from the type strain of *Chloromonas rosae* var. *psychrophila* from North America that is supposed to have morphologically identical cysts.

Paper VI (Remias et al., accepted, *Journal of Phycology*) described the cellular morphology, ultrastructure and phylogenetic position of two new species of golden algae: *Kremastochrysopsis austriaca* causing yellow snow blooms in the Austrian Alps and its sister species *K. americana* isolated from a pond in USA. When grown in culture, both taxa showed a characteristic hypo-neustonic growth of active flagellates, with older cells forming palmeloid colonies at the bottom of the culture vessel. *K. austriaca* had no close phylogenetic relation to other psychrophilic chrysophytes, e.g. *Chromulina chinophilia*, *Hydrurus* sp., and *Ochromonas*-like flagellates.

Paper VII (Lutz et al. 2019, *Fottea*) evaluated the limits and possible improvements of high-throughput amplicon sequencing analysis applied to green algal assemblages in melting alpine snow. The taxonomical assignment of DNA sequences strongly depended on the quality of the reference databases used. Furthermore, for accurate identification a combination of the manual inspection of automated assignments and oligotyping of the abundant 18S rDNA OTUs and ITS2 rDNA secondary structure analyses were needed. The use of one marker can be misleading because of the low variability (18S rDNA) or scarcity of references (ITS2 rDNA). HTS outputs need to be thoroughly checked when the organisms are poorly represented in databases. An optimized workflow for accurate diversity analyses includes consistent sampling, a two-molecular-marker approach, light-microscopy-based guidance, the generation of appropriate reference sequences and a final manual verification of taxonomic assignments.

Paper VIII (Lukeš et al. 2014, *FEMS Microbiology Ecology*) investigated the temperature dependent response of growth, photosynthetic electron transfer rate and fatty acid composition of a strain of *Chloromonas reticulata* isolated from red snow. The growth and oxygen evolution rate were low at 2 °C yet progressively enhanced to 10 °C and were significantly higher at temperatures from 5 to 15 °C in comparison with the mesophilic control *Chlamydomonas reinhardtii*. Unprecedent high rates of Q_A to Q_B electron transfer were found. The thermodynamics of the process revealed the existence of an increased structural flexibility that we explain by amino acid changes in the D1 protein combined with the physico-chemical characteristics of the thylakoid membrane composed of > 80% negatively charged phosphatidylglycerol.

Paper IX (Řezanka et al. 2014, *Phytochemistry*) described the development of methods enabling the rapid determination of total lipids in algae, and detailed the identification and quantification of a complex mixture of natural triacylgylcerols (TAGs) by silver-LC/APCI-MS and NARP-LC/APCI-MS. Both types of chromatography readily identified TAGs containing 16:3 and 16:4 acids in the molecule, both qualitatively and semiquantitatively. The genus *Chloromonas* was concluded to be a potential biotechnological source of C16 PUFAs. For the first time, lipidomic profiling of field samples of snow algae using high-resolution mass spectrometry (ESI-MS) was performed.

Paper X (Osterrothová et al. 2019, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*) tested the potential of Raman spectroscopy to determine the primary and secondary carotenoid pigments in different species and life-cycle stages of snow algae. The performance of Raman spectrometry to the reference method of biological pigment analysis, high-performance liquid chromatography, was compared. According to the Raman spectrometry, red cysts were rich in astaxanthin. Flagellated green cells commonly contained lutein as a major carotenoid, together with minor amounts of β-carotene and varying amounts of antheraxanthin, violaxanthin and neoxanthin. Aplanozygotes contained mixtures of both primary and secondary carotenoids. However, the ability of Raman spectroscopy to discriminate between structurally slightly differing carotenoid pigments or several carotenoids in an admixture from an unknown biological system remains limited.

4 Summary and conclusions

Snow algae are found in melting snowfields in polar and mountain regions. These habitats are regarded as ephemeral and extreme. Microalgae living in such sites are exposed to several abiotic stresses. This thesis summarizes recent findings on the genetic diversity, ecophysiology and morphology of these organisms.

Phylogeny, morphology and taxonomy

This thesis includes new data on less-known or poorly-described green microalgae, and taxonomic descriptions of novel green and golden algae species (**papers I, III, IV–VI**). *Chlainomonas* sp. was shown here to regularly cause red snow on lake-ice covers in the High Tatras and the Austrian Alps (**paper II**). Still, analyses of multiple molecular markers in further cryoflora species of *Chlainomonas* (i.e. *Chlainomonas koliae*, *Chlainomonas rubra* (Stein & Brooke) Hoham) are needed to determine the exact taxonomic position of each species within the clade (Novis et al. 2008). Three newly described or revised taxa (*C. hindakii*, *Scotiella cryophila*, *C. nivalis* subsp. *tatrae*) belong to a group of morphologically and taxonomically closely related snow phytoflagellates (**papers III–V**), and most of them were formerly associated with the collective taxon *C. nivalis* (Hoham & Mullet 1977). In addition, I showed that it is possible to distinguish cysts of these species morphologically using ultrastructural traits (e.g. the number of cell wall flanges) from cysts of other snow algae (**paper III**).

Moreover, this thesis demonstrates that sequencing of field-collected cysts is a successful strategy for deciphering the phylogenetic position of several species causing prominent blooms (**paper I–V, IX**), such as *Sanguina* and *Chlainomonas* sp., as the establishment of actively growing isolates has not yet been successful for either. However, only virtually monospecific blooms can be used for this purpose (Remias et al. 2013a, **paper I–V, IX**). The selection of ʻcleanʼ snow spots depends on light microscopy in field prior to harvest (**paper I**). An alternative approach utilizes single cell sequencing (Muramoto et al. 2008).

An insight into biogeography of snow algae

Concerning geographical distributions, this thesis shows that either cosmopolitan (**paper I**) or likely local (endemic) taxa (**paper IV**) can dominate melting snow fields. For *Chlainomonas* sp., other similar habitats (frozen alpine lakes, flat snow-covered glaciers) with red blooms need to be investigated to evaluate geographic distributions. Furthermore, molecular reinvestigations of strains from North American sites that were designated *C. rosae* var. *psychrophila* (Hoham et al. 2002) and North American field cysts that are morphologically identical to *Scotiella cryophila* are needed. Re-sampling of the type locality of the latter taxon in western Switzerland (Chodat 1922) could be crucial. While additional surveys have to be performed, it seems probable that the golden alga *Kremastochysopsis austriaca* is distributed in several areas of the Austrian Alps (**paper V**).

Intraspecific genetic variability

This thesis also demonstrates that the red bloom causing alga *Sanguina nivaloides* is a diverse species, containing at least 18 haplotypes of ITS2 rDNA (**paper I**), while a single snow spot is usually made by single clone (**paper VII**), thus corresponding to the assumed evolutionary explanation proposed by (Brown et al. 2016). This finding also helps explain why Sanger sequencing of monospecific spots can be successful when applied to snow algal blooms. Genetic variability below the species level might be elucidated using a microsatellite-based approach (Nagai et al. 2007) developed from single cell genomics (Muramoto et al. 2010).

Improvements and limitations of high-throughput amplicon sequencing when applied to snow algae communities

Long distance dispersal strategies of snow algae have hardly been investigated. New techniques like HTS of several molecular markers will be useful in establishing the biogeography of snow algae (Lutz et al. 2016). However, evaluations of biodiversity at the species level using these current metagenomics methods are susceptible to several technical biases (summarized in **paper VII)**. This can be demonstrated e.g. by light microscopy, which indicated that some high alpine snow samples were dominated by a few

algal species that were not always reflected in the sequencing dataset. Consequently, HTS amplicon data need to be handled with care if applied to habitats or groups of organisms that are (highly) underrepresented in molecular databases (**paper VII**). Further studies may find it helpful to utilize the optimized protocol proposed here (**paper VII**).

Pigment composition: comparisons between populations, closely related taxa, and unrelated species thriving at similar habitats

The intracellular pigment ratio of astaxanthin to chlorophyll significantly differs by one up to two magnitudes between three main taxa causing snow blooms: *Chloromonas* spp. (**paper III, IV**), *Chlainomonas* sp. and *Sanguina nivaloides* (**paper II**). These results are in line with previous reports (Müller et al. 1998, Remias et al. 2005, 2016), and correspond with findings that the latter two species are restricted to exposed habitats in term of irradiance, where a high accumulation of astaxanthin in extraplastidal lipid bodies plays an inevitable role in multifunctional stress defence (Remias et al. 2010b). In contrast, members of the former genus may occupy latitudinal gradients from forested to alpine sites; these cells rely more on heat dissipation in the xanthophyll cycle (Remias et al. 2010a). Closely related taxa (two *Chloromonas*) or even two genetically identical populations (for ITS2 rDNA; two *Chlainomonas* sp.) shared similar physiological properties (in term of RLCs) and ecological requirements (high alpine sites), but differed slightly in the abundance of secondary carotenoids (**paper II, IV**). It is assumed that cell aging (maturation of cysts over the course of the season) also plays a role in the extent of astaxanthin accumulation (Remias et al. 2010a).

Fatty acid and pigment profiles of the two contrasting life cycle stages: flagellates and cysts

This thesis compares for the first time the pigment and fatty acid profiles of two contrasting life cycle stages in one *Chloromonas* species (**paper III**). Cysts were astaxanthin rich, while lutein was the major carotenoid with minor amounts of β-carotene in flagellates (**paper III, X**). However, both stages were almost consistent in their fatty acid profile, with PUFAs dominating (**paper III**). These pigment findings correspond with the proposed main ecophysiological differences between immotile cysts and motile flagellates (Remias

2012). On the other hand, fatty acid desaturation is one of the core adaptations to extreme temperature conditions in melting snow to keep membrane fluidity, independent of algal life cycle stage (Morgan-Kiss et al. 2006).

Temperature dependent growth and photosynthesis

Alternatively, this thesis demonstrates a novel complex of physiological adaptations of a cryotolerant *Chloromonas* species to perform photosynthesis at a wide range of temperatures. This included a unique lipid composition of thylakoid membranes around and within PSII and amino acid changes in the D1 protein structure when compared to a mesophilic counterpart (**paper VII**). Interestingly, the strain is a member of the *Chloromonas* snow algal "clade 1" *sensu* (Hoham et al. 2002), which encompasses psychrotolerant and mesophilic organisms (e.g. *C. rosae var. psychrophila*, Hoham et al. 2008b; *C. svalbardensis* Barcyté & Hodač, *C. arctica* Barcyté & Hodač, Barcytė et al. 2018a,b). By contrast, other *Chloromonas* species investigated in this thesis are members of "clade 2" containing psychrophilic algae (e.g. *C. pichinchae*, Hoham 1975b; *C. tughillensis*, *C. chenangoensis*, Hoham et al. 2008b; *C. hindakii*, **paper III**). Finally, this thesis presents the first lipidomic study of *Chloromonas* snow dwelling species based on field samples (**paper IX**).

Other physiologically interesting compounds in snow algae

For *Sanguina* spp. and *Chlainomonas* sp. and three newly described *Chloromonas* taxa, other physiological aspects such as the occurrence of antifreeze agents, accumulation of soluble carbohydrates (osmolytes), and presence of ice binding proteins (Raymond 2014) have not yet been studied. But saccharose and glycerol as main soluble carbohydrates may occur in the cysts of snow dwelling *Chloromonas,* since these compounds were already found in *C. polyptera* in maritime Antarctic (Remias et al. 2013a) and *C. nivalis* in the Austrian Alps (Remias et al. 2010). Interestingly, the recently described species *K. austriaca* (**paper VI**) appears to secrete a protein that affects the growth of ice (Raymond & Remias accepted). In other ice-associated algae, such proteins mitigate damage

to the cells by ice or help maintain an aqueous environment. This is the first time an ice-active substance has been observed in golden algae (Raymond & Remias accepted).

Mycosporine-like amino acids act as UV screening compounds, but are not expected in the taxa described in this work, since they have not yet been found in either chlamydomonads (Remias et al. 2010a, b) or glacier desmids (Remias et al. 2012b). They were detected, however, in various members of the Trebouxiophyceae (Karsten et al. 2005), and some members of this algal group are involved in green snow (e.g. *Koliella*). One may assume that in the nitrogen limited conditions of melting snowfields/glaciers these compounds are either too expensive to be produced, or they are substituted for by nitrogen-free pigments such as phenols or carotenoids.

Photosynthesis and cellular ʿmemoryʾ of light conditions in the field

Light preferences of snow algae (measured by RLCs) reflected the conditions from which they originated (**paper II–V**). Still, detailed mechanisms of physiologic adaptations among life cycle stages (e.g. "*Scotiella cryophila"* cysts) remain unexplained: drastic cellular changes must take place, e.g. during photoacclimation from low light conditions deep below the snow surface to high-light exposure on the surface – a change happening within a few weeks (**paper V**). Interestingly, this thesis documents the high intraspecific photophysiological plasticity of photosystem II for cyst samples of one *Chloromonas* species collected from high and low light conditions (**paper III**). The response of a cold-adapted alga exposed to different light conditions can be investigated, along with screening which responses are activated (e.g., effect on pigments, proteins) (La Rocca et al. 2015).

Cultivation essays for snow algal strains isolation

Establishing cultures from field cysts has been reported to be generally difficult (Hoham et al. 1979, Matsuzaki et al. 2019). The generation of flagellates from cysts abundantly found in the field is of great interest to scientists considering that this step is tricky, and it has never successfully performed for many species of snow algae so far. This thesis found for *C. hindakii* that this culturing is possible, at least for several taxa within

the genus *Chloromonas*, when carried out under suitable laboratorial conditions simulating the temperature regime of melting snow (**paper III**). Contraction of the protoplast was found to be a very typical advance step (prior to putative meiosis), and the production of 4 daughter cells is a common phenomenon for many *Chloromonas* field cysts as well (**paper IV, V**). However, the key factor enabling daughter cells to be released from the mother cell is likely the regular application of gentle freeze thaw cycles. Additionally, sampling at known locations early in the season or in deep slush layers might help uncover vegetative stages (e.g. *Sanguina nivaloides* or *Chlainomonas* sp.), enabling remaining uncertainties about their life cycles to be resolved using viable cultures/isolates.

Concluding remarks

From the results described above, I conclude that the application of a polyphasic approach to snow algae, including the molecular analysis of multiple DNA regions, and description of the ecology, cytology and physiology are helpful tools in revealing species identities and evaluating cryoflora biodiversity of polar and alpine regions. To sum up, this thesis demonstrates that the biodiversity of cryoflora is still not thoroughly explored. The results presented in this thesis bring new insights to snow algal biogeography. Moreover, I propose a "best practice" when dealing with unculturable algal material in order to explore a species photobiology and physiology and infer phylogeny. This thesis also demonstrates the crucial need for generating reference sequence databases. The proposed optimized protocol for high throughput amplicon sequencing is essential to obtain accurate biodiversity assessments. Although snow algae are a diverse group of phylogenetically unrelated microorganisms, they appear to convergently use a few similar adaptations to thrive in the extreme conditions of melting mountain and polar snow.

While I hope the outcomes and conclusions of this thesis are interesting from the point-of-view of basic research, established snow algal strains might also be subject to screening of metabolite profiles (lipids, pigments, soluble carbohydrates) and used in applied research (Řezanka et al. 2018, Kaftan et al. 2012).

5 References

Aigner S., Remias D., Karsten U. & Holzinger A. (2013). Unusual phenolic compounds contribute to ecophysiological performance in the purple-colored green alga *Zygogonium ericetorum* (Zygnematophyceae, Streptophyta). *J Phycol* **49**: 648–660.

Amato P., Besaury L., Joly M., Penaud B., Deguillaume L. & Delort A. M. (2019). Metatranscriptomic exploration of microbial functioning in clouds. *Sci Rep* **9**: 1–12.

Aristotle. (n.d.). *History of animals*.

Barcytė D., Hodač L., Nedbalová L. & Elster J. (2018a). *Chloromonas svalbardensis* n. sp. with insights into the phylogroup Chloromonadinia (Chlorophyceae). *J Eukaryot Microbiol* **65**: 882–892.

Barcytė D., Hodač L., Nedbalová L. & Elster J. (2018b). *Chloromonas arctica* sp. nov., a psychrotolerant alga from snow in the High Arctic (Chlamydomonadales, Chlorophyta). *Int J Syst Evol Micr* **68**: 851–859.

Beer S., Vilenkin B., Weil A., Veste M., Susel L. & Eshel A. (1998). Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Mar Ecol Prog Ser* **174**: 293–300.

Beniston M., Keller F., Koffi B. & Goyette S. (2003). Estimates of snow accumulation and volume in the Swiss Alps under changing climatic conditions. *Theor Appl Climatol* **76**: 125–140.

Bidigare R. R., Ondrusek M. E., Kennicutt II M. C., Iturriaga R., Harvey H. R., Hoham R. W. & Macko S. A. (1993). Evidence for a photoprotective function for secondary carotenoids of snow algae. *J Phycol* **29**: 427–434.

Blanc G., Agarkova I., Grimwood J., Kuo A., Brueggeman A., Dunigan D. D., Gurnon J., Ladunga I., Lindquist E., Lucas S., Pangilinan J., Pröschold T., Salamov A., Schmutz J., Weeks D., Yamada T., Lomsadze A., Borodovsky M., Claverie J.-M., Grigoriev I. V. & Etten J. L. Van. (2012). The genome of the polar eukaryotic microalga *Coccomyxa subellipsoidea* reveals traits of cold adaptation. *Genome Biol* **13**: R39.

Bormann K. J., Brown R. D., Derksen C. & Painter T. H. (2018). Estimating snow-cover trends from space. *Nat Clim Change* **8**: 924.

Brown R., Larson D. & Bold H. (1964). Airborne algae: Their abundance and heterogeneity. *Science* **143**: 583–585.

Brown R. D. & Mote P. W. (2009). The response of Northern Hemisphere snow cover to a changing climate. *J Climate* **22**: 2124–2145.

Brown S. P. & Jumpponen A. (2019). Microbial ecology of snow reveals taxa-specific biogeographical structure. *Environ Microbiol* **77**: 946–958.

Brown S. P., Olson B. J. S. C., & Jumpponen A. (2015). Fungi and algae co-occur in snow: an issue of shared habitat or algal facilitation of heterotrophs? *Arct Antarct Alp Res* **47**: 729–749.

Brown S. P., Ungerer M. C. & Jumpponen A. (2016). A community of clones: snow algae are diverse communities of spatially structured clones. *Int J Plant Sci* **177**: 432–439.

Chodat R. 1922. Matériaux pour l'histoire des algues de la Suisse. *Bull Soc Bot Genève*, série 2, **13**: 66–114.

Cordero B. F., Obraztsova I., Martín L., Couso I., León R., Vargas M. Á. & Rodríguez H. (2010). Isolation and characterization of a lycopene β-cyclase gene from the astaxanthin-producing green alga *Chlorella zofingiensis* (Chlorophyta). *J Phycol* **46**: 1229–1238.

Croft M. T., Lawrence A. D., Raux-Deery E., Warren M. J. & Smith A. G. (2005). Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. *Nature* **438**: 90–93.

Czosnowski J. (1948). O zakwicie neustonowym *Chrysotilos tatrica* n. sp. na Gubalówce pod Zakopanem. *Poznanski Tow. Przyj. Nauk 11. Prace Kom. Biol.* **4**: 46–55.

DasSarma P. & DasSarma S. (2018). Survival of microbes in Earth's stratosphere. *Current Opinion in Microbiology* **43**: 24–30.

Davey M. P., Norman L., Sterk P., Huete-Ortega M., Bunbury F., Loh B. K. W., Stockton S., Peck L. S., Convey P., Newsham K. K. & Smith A. G. (2019). Snow algae communities in Antarctica – metabolic and taxonomic composition. *New Phytol* **222**: 1242–1255.

Duval B., Duval E. & Hoham R. W. (1999). Snow algae of the Sierra Nevada, Spain, and High Atlas mountains of Morocco. *Int Microbiol* **2**: 39–42.

Duval B., Shetty K. & Thomas W. H. (2000). Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. *J Appl Phycol* **11**: 559–566.

Edwards A. C., Scalenghe R. & Freppaz M. (2007). Changes in the seasonal snow cover of alpine regions and its effect on soil processes: A review. *Quatern Int* **162–163**: 172–181.

Egger A. & Karsten U. (2010). Low molecular weight carbohydrates in red algae – an ecophysiological and biotechnological perspective. In Seckbach J, Chapman D, Weber A (Eds). Cellular origins, life in extreme habitats and astrobiology red algae in the genomics age. (pp. 445–456). Springer, Berlin.

Falkowski P. G. & Raven J. A. (1997). *Aquatic photosynthesis*. Aquatic. Blackwell, Oxford, pp. 375.

Fazius F., Shelest E., Gebhardt P. & Brock M. (2012). The fungal α-aminoadipate pathway for lysine biosynthesis requires two enzymes of the aconitase family for the isomerization of homocitrate to homoisocitrate. *Mol Microbiol* **86**: 1508–1530.

Figueroa F. L., Korbee N., Carrillo P., Medina-Sánchez J. M., Mata M., Bonomi J. & Sánchez-Castillo P. M. (2009). The effects of UV radiation on photosynthesis estimated as chlorophyll fluorescence in *Zygnemopsis decussata* (Chlorophyta) growing in a high mountain lake (Sierra Nevada, Southern Spain). *J Limnol* **68**: 206–216.

Fogg G. E. (1967). Observations on the snow algae of the South Orkney Islands. *Philos T Roy Soc B* **252**: 279–287.

Fott B., Rejmánek M. & Štursa J. (1978). Prvý nález červeného sněhu v Krkonoších [First record of red snow in Krkonoše (Giant Mountains)]. *Opera Corcontica* **15**: 109–112.

Fritsch F. E. (1912). Freshwater algae collected in the South Orkneys by Mr. R. N. Rudmose Brown, B.Sc. of the Scottish National Antarctic Expedition, 1902–04. *Bot J Linn Soc* **40**: 293–338.

Fugazza D., Senese A., Azzoni R. S., Smiraglia C., Cernuschi M., Severi D. & Diolaiuti G. A. (2015). High-resolution mapping of glacier surface features. The UAV survey of the Forni glacier (Stelvio national park, Italy). *Geogr Fis Dinam Quat* **38**: 25–33.

Fukushima H. (1963). Studies on cryophytes in Japan. *J Yokohama Munic Univ, Ser C, Nat Sci* **43**: 1–146.

Ganey G. Q., Loso M. G., Burgess A. B. & Dial R. J. (2017). The role of microbes in snowmelt and radiative forcing on an Alaskan icefield. *Nat Geosci* **10**: 754–759.

Garric R. K. (1965). The cryoflora of the Pacific Northwest. *Am J Bot* **52**: 1–8.

Gerrath J. F. & Nicholls K. H. (1974). A red snow in Ontario caused by the dinoflagellate, *Gymnodinium pascheri*. *Can J Bot* **52**: 683–685.

Gillespie R. G., Baldwin B. G., Waters J. M., Fraser C. I., Nikula R. & Roderick G. K. (2012). Long-distance dispersal: A framework for hypothesis testing. *Trends Ecol Evol* **27**: 47–55.

Gorton H. L., Williams W. E. & Vogelmann T. C. (2001). The light environment and cellular optics of the snow alga *Chlamydomonas nivalis* (Bauer) Wille. *Photochem Photobiol* **73**: 611–620.

Gorton H. L. & Vogelmann T. C. (2003). Ultraviolet radiation and the snow alga *Chlamydomonas nivalis* (Bauer) Wille. *Photochem Photobiol* **77**: 608–615.

Gradinger R. & Nürnberg D. (1996). Snow algal communities on Arctic pack ice floes dominated by *Chlamydomonas nivalis* (Bauer) Wille. *Proc. NIPR Symp. Polar Biol* **9**: 35–43.

Hamilton T. L. & Havig J. (2017). Primary productivity of snow algae communities on stratovolcanoes of the Pacific Northwest. *Geobiology* **15**: 280–295.

Hamilton T. L. & Havig J. R. (2018). Inorganic carbon addition stimulates snow algae primary productivity. *ISME J,* doi:10.1038/s41396-018-0048-6.

Hanzelová M., Vido J., Škvarenina J., Nalevanková P. & Perháčová Z. (2018). Microorganisms in summer snow patches in selected high mountain ranges of Slovakia. *Biologia* **73**: 1177–1186.

Hardy J. T. (1966). *Identification, culture, and physiological ecology of cryophilic algae*. Oregon State University. M.S. Thesis, Oregon State University, Corvallis. 62 pp.

Helmke E. & Weyland H. (1995). Bacteria in sea ice and underlying water of the eastern Weddell Sea in midwinter. *Mar Ecol Prog Ser* **117**: 269–288.

Henley W. J. (1993). Measurement and interpretation of photosynthetic light-response

curves in algae in the context of photoinhibition and diel changes. *J Phycol* **29**: 729–739.

Hiltbrunner E., Schwikowski M. & Körner C. (2005). Inorganic nitrogen storage in alpine snow pack in the Central Alps (Switzerland). *Atmos Environ* **39**: 2249–2259.

Hindák F. (1969). Brownish snow in the High Tatras. *Biologia* **24**: 80–85.

Hoek C., Mann D., Jahns H. M. & Jahns M. (1995). *Algae: an introduction to phycology*. Cambridge university press, 637 p.

Hoham R. W. (1974a). New findings in the life history of the snow alga, *Chlainomonas rubra* (Stein et Brooke) comb. nov. (Chlorophyta, Volvocales). *Syesis* **7**: 239–247.

Hoham R. W. (1974b). *Chlainomonas kolii* (Hardy et Curl) comb. nov. (Chlorophyta, Volvocales), a revision of the snow alga, *Trachelomonas kolii* Hardy et Curl (Euglenophyta, Euglenales). *J Phycol* **10**: 392–396.

Hoham R. W. (1975a). The life history and ecology of the snow alga *Chloromonas pichinchae* (Chlorophyta, Volvocales). *Phycologia* **14**: 213–226.

Hoham R. W. (1975b). Optimum temperatures and temperatures ranges for growth of snow algae. *Arct Alp Res* **7:** 13–24.

Hoham R. W. (1976). The effect of coniferous litter and different snow meltwaters upon the growth of two species of snow algae in axenic culture. *Arct Alp Res* **8**: 377–386.

Hoham R. W. 1989. Snow as a habitat for microorganisms. In C. P. McKay & W. L. Davis (Eds), *Exobiology and future Mars missions, NASA Conf. Publ. 10027* (pp. 32–33). Ames Res. Center, Moffett Field, California.

Hoham R. W., & Mullet J. E. (1977). The life history and ecology of the snow alga *Chloromonas cryophila* sp. nov. (Chlorophyta, Volvocales). *Phycologia* **16**: 53–68.

Hoham R. W. & Mullet J. E. (1978). *Chloromonas nivalis* (Chod.) Hoh. & Mull. comb. nov., and additional comments on the snow alga, *Scotiella*. *Phycologia* **17**: 106–107.

Hoham R. W. & Blinn D. W. (1979). Distribution of cryophilic algae in an arid region, the American Southwest. *Phycologia* **18**: 133–145.

Hoham R. & Ling H. (2000). The effects of chemicals and physical factors on their life cycles and populations. In Seckbach J. (Ed.). *Cellular origin and life in extreme environments, Journey to diverse microbial worlds: Adaptation to exotic Environments* (pp. 131–145). Kluwer Press: The Netherlands.

Hoham R. W., & Duval B. (2001). Microbial ecology of snow and freshwater ice with emphasis on snow algae. In H. G. Jones, J. W. Pomeroy, D. A. Walker, & R. W. Hoham (Eds), *Snow ecology: An interdisciplinary examination of snow-covered ecosystems* (pp. 168–228). Cambridge: Cambridge University Press.

Hoham R. W., Roemer S. C. & Mullet J. E. (1979). The life history and ecology of the snow alga *Chloromonas brevispina* comb. nov. (Chlorophyta, Volvocales). *Phycologia* **18**: 55–70.

Hoham R. W., Mullet J. E. & Roemer S. C. (1983). The life history and ecology of the snow

alga *Chloromonas polyptera* comb. nov. (Chlorophyta, Volvocales). *Phycologia* **61**: 2416–2429.

Hoham R. W., Yatsko C. P., Germain L. & Jones H. G. (1989). Recent discoveries of snow algae in Upstate New York and Quebec Province and preliminary reports on related snow chemistry. In *Proceedings of the 46th Annual Eastern snow conference*). Québec City, Québec, Canada, pp. 196–200.

Hoham R. W., Laursen A. E., Clive S. O. & Duval B. (1993). Snow algae and other microbes in several alpine areas in New England. In M. P. T. Ferick & Pangburn T. (Eds), *Proceedings of the Fiftieth Annual Eastern Snow Conference*. Québec City, Québec, Canada, pp. 165–173.

Hoham R. W, Schlag E. M., Kang J. Y., Hasselwander A. J., Behrstock A. F., Blackburn I. R., Johnson, R. C. & Roemer S. C. (1998). The effects of irradiance levels and spectral composition on mating strategies in the snow alga, *Chloromonas* sp. -D, from the Tughill Plateau, New York State. *Hydrol Process* **12**: 1627–1639.

Hoham R. W, Marcarelli A. M., Rogers H. S., Ragan M. D., Petre B. M., Ungerer M. D., Barnes, J. M. & Francis D. O. (2000). The importance of light and photoperiod in sexual reproduction and geographical distribution in the green snow alga, *Chloromonas* sp.-D (Chlorophyceae, Volvocales). *Hydrol Process* **14**: 3309–3321.

Hoham R. W., Bonome T. A., Martin C. W. & Leebens-Mack J. H. (2002). A combined 18S rDNA and rbcL phylogenetic analysis of *Chloromonas* and *Chlamydomonas* (Chlorophyceae, Volvocales) emphasizing snow and other cold-temperature habitats. *J Phycol* **38**: 1051–1064.

Hoham R. W, Berman J. D., Rogers H. S., Felio J. H., Ryba J. B. & Miller P. R. (2006). Two new species of green snow algae from Upstate New York, *Chloromonas chenangoensis* sp. nov. and *Chloromonas tughillensis* sp. nov. (Volvocales, Chlorophyceae) and the effects of light on their life cycle development. *Phycologia* **45**: 319–330.

Hoham R. W., McCay T. S., Poirier M. B. & Bell T. (2008a). Balsam fir leaf litter extract stimulates growth of the green snow alga *Chloromonas rosae* var. *psychrophila* (Chlorophyta, Volvocales) from Whiteface Mountain, New York. *Nova Hedwigia* **86**: 133–140.

Hoham R. W., Frey F. M., Mohn W. W., Felio J. H., Todd S., Duncan J. E. & Banghart J. B. (2008b). Optimum growth temperatures of three species of green *Chloromonas* snow algae from Upstate New York and the White Mountains, Arizona. *Arct Antarc Alp Res* **40**: 355–363.

Hoham R. W, Frey F. M., Berman J. D., Ryba J. B., Duncan J. E., Forbes A. A., Goodridge, B. M. & Miller P. R. (2009). The effects of irradiance level, photoperiod, and cell density on sexual reproduction in the green snow alga, *Chloromonas chenangoensis* (Chlorophyta, Volvocales), from Upstate New York. *Nova Hedwigia* **89**: 1–16.

Holzinger A., Allen M. C. & Deheyn D. D. (2016). Hyperspectral imaging of snow algae and green algae from aeroterrestrial habitats. *J Photoch Photobio B:* **162**: 412–420.

Hopes A., Thomas D. N. & Mock T. (2017). Polar microalgae: functional genomics, physiology,

and the environment. In Margesin R. (Ed.), *Psychrophiles: from biodiversity to biotechnology: Second Edition*. Springer, Cham, pp. 305–344.

Hulatt C. J., Berecz O., Egeland E. S., Wijffels R. H. & Kiron V. (2017). Polar snow algae as a valuable source of lipids? *Bioresource Technol* **235**: 338–347.

Javornický P. (1973). A field method for measuring the photosynthesis of snow and aerophytic algae. *Algol Stud* 8*/Arch Hydrobiol-Suppl* **14**: 363–371.

Javornický P. & Hindák F. (1970). *Cryptomonas frigoris* spec. nova (Cryptophyceae), the new cyst-forming flagellate from the snow of the High Tatras. *Biologia* **25**: 241–250.

Kaftan D., Lukeš M. & Nedbalová L. (2012). Photosynthetic microorganisms for production of phosphatidylglycerol (PG) and ways to increase the content of PG in the said photosynthetic microorganisms. *CZ patent 303645*.

Karsten U., Friedl T., Schumann R., Hoyer K. & Lembcke S. (2005). Mycosporine-like amino acids and phylogenies in green algae: *Prasiola* and its relatives from the Trebouxiophyceae (Chlorophyta). *J Phycol* **41**: 557–566.

Kociánová M., Štursová H., Štursa J., Vaněk J. & Vávra V. (1989). Nové nálezy červeného sněhu v Krkonoších. [New records of red snow in the Krkonoše Mountains]. *Opera Corcontica* **26**: 151–158.

Kol E. (1942). The snow and ice algae of Alaska. *Smith Msc Coll* **101**: 1–36.

Kol E. (1955a). Színes hó a Bükk-hegységben [Coloured snow in the Bükk-Mountains]. *Botanikai Közlemények* **46**: 61–68.

Kol E. (1955b). Blauer Schnee im Gebiet der Kleinen Küküllő. *Ann Hist-Natur Mus Nat Hung* **6**: 93–96.

Kol E. (1961). Über roten and grünen Schnee der Alpen. *Verh Internat Verein Limnol* **XIV**: 912–917.

Kol E. (1965). Roter Schnee von *Scotiella* in der Hohen Tátra. *Ann Hist-Natur Mus Nat Hung* **57**: 145–148.

Kol E. (1966). Snow algae from the valley of the Morskie Oko lake in the High Tatra. *Ann Hist-Natur Mus Nat Hung Pars Botanica* **58**: 161–168.

Kol E. (1968). *Kryobiologie. Biologie und Limnologie des Schnees und Eises. I. Kryovegetation.* (Die Binnengewässer Einzeldarstellungen aus der Limnologie und ihren Nachbargebieten Volume XXIV). Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, 216 p.

Kol E. (1975). Cryobiological researches in the High Tatra I. *Acta Bot Hung* **21**: 61–75.

Kol E & Eurola S. (1974). Red snow algae from Spitsbergen. *Astarte* **7**: 61–66.

Kol E. & Peterson J. A. (1976). Cryobiology. In U. Hope, G.S., Peterson, J.A., Allison, I. & Radok U. (Eds), *The Equatorial Glaciers of New Guinea.* (pp. 81–91). Rotterdam: Balkema.

Komárek J., Hindák F. & Javornický P. (1973). Ecology of green kryophilic algae from Belanské

Tatry Mountains (Czechoslovakia). *Arch Hydrobiol/Suppl* **41**: 427–449.

Kristiansen J. (1996). Dispersal of freshwater algae - A review. *Hydrobiologia* **336**: 151–157.

Krueger-Hadfield S. A., Kollars N. M., Byers J. E., Greig T. W., Hammann M., Murray D. C., Murren C. J., Strand A. E., Terada R., Weinberger F. & Sotka E. E. (2016). Invasion of novel habitats uncouples haplo-diplontic life cycles. *Mol Ecol* **25**: 3801–3816.

Kugler A., Zorin B., Didi-Cohen S., Sibiryak M., Gorelova O., Ismagulova T., Kokabi K., Kumari P., Lukyanov A., Boussiba S., Solovchenko A. & Khozin-Goldberg I. (2019). Long-chain polyunsaturated fatty acids in the green microalga *Lobosphaera incisa* contribute to tolerance to abiotic stresses. *Plant & Cell Physiol* **60**: 1205–1223.

Kvíderová J. (2010). Characterization of the community of snow algae and their photochemical performance in situ in the Giant Mountains, Czech Republic. *Arct Antarct Alp Res* **42**: 210–218.

Kvíderová J. (2012). Research on cryosestic communities in Svalbard: the snow algae of temporary snowfields in Petuniabukta, Central Svalbard. *Czech Polar Rep* **2**: 8–19.

Kvíderová J., Stibal M., Nedbalová L. & Kaštovská K. (2005). The first record of snow algae vitality *in situ* by variable fluorescence of chlorophyll. *Czech Phycol* **5**: 69–77.

La Rocca N., Sciuto K., Meneghesso A., Moro I., Rascio N. & Morosinotto T. (2015). Photosynthesis in extreme environments: responses to different light regimes in the Antarctic alga *Koliella antarctica*. *Physiol Plantarum* **153**: 654–667.

Lang I., Hodač L., Friedl T. & Feussner I. (2011). Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biol* **11**: 124.

Leblond J. D. & Chapman P. J. (2000). Lipid class distribution of highly unsaturated long chain fatty acids in marine dinoflagellates. *J Phycol* **36**: 1103–1108.

Leliaert F., Verbruggen H., Vanormelingen P., Steen F., López-Bautista J. M., Zuccarello G. C. & De Clerck O. (2014). DNA-based species delimitation in algae. *Eur J Phycol* **49**: 179–196.

Lemoine Y., & Schoefs B. (2010). Secondary ketocarotenoid astaxanthin biosynthesis in algae: A multifunctional response to stress. *Photosynth Res* **106**: 155–177.

Leya T. (2004). *Feldstudien und genetische Untersuchungen zur Kryophilie der Schneealgen Nordwestspitzbergens*. Dissertation. Shaker Verlag, Aachen, 145 p.

Leya T. (2013). Snow algae: adaptation strategies to survive on snow and ice. In Stan-Lotter H., Seckbach J., Oren A. (Eds), *Cellular origin, life in extreme habitats and astrobiology, volume 27, Polyextremophiles: Life under multiple forms of stress* (pp. 401–423). Dordrecht: Springer.

Leya T., Müller T., Ling H. U. & Fuhr G. (2001). Psychrophilic microalgae from north-west Spitsbergen, Svalbard: their taxonomy, ecology and preliminary studies of their cold adaptation using single cell electrorotation. *Nova Hedwigia Beiheft* **123**: 551–570.

Leya T., Müller T., Ling H. U. & Fuhr G. R. (2004). Snow algae from North-Western Spitsbergen (Svalbard). In Wiencke C. (Ed.), *The coastal ecosystem of Kongsfjorden, Svalbard : Synopsis of biological research performed at the Koldewey Station in the years 1991–2003 (Berichte zur Polar- und Meeresforschung 492)* (pp. 46–54). Bremerhaven.

Leya T., Rahn A., Lütz C., & Remias D. (2009). Response of arctic snow and permafrost algae to high light and nitrogen stress by changes in pigment composition and applied aspects for biotechnology. *FEMS Microbiol Ecol* **67**: 432–443.

Ling H. U. (1996). Snow algae of the Windmill Islands Region, Antarctica. *Hydrobiologia* **336**: 99–106.

Ling H. U. & Seppelt R. D. (1990). Snow algae of the Windmill Islands, continental Antarctica. *Mesotaenium berggrenii* (Zygnematales, Chlorophyta) the alga of grey snow. *Antarct Sci* **2**: 143–148.

Ling H. U. & Seppelt R. D. (1998). Snow algae of the Windmill Islands, continental Antarctica. 3. *Chloromonas polyptera* (Volvocales, Chlorophyta). *Polar Biol* **20**: 320–324.

Lisiecki L. E. & Raymo M. E. (2005). A Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records. *Paleoceanography* **20**: 1–17.

Lukavský J. (1993). First record of cryoseston in the Bohemian Forest Mts. (Šumava). *Algol Stud* **69**: 83–89.

Lutz S., Anesio A. M., Jorge Villar S. E. & Benning L. G. (2014). Variations of algal communities cause darkening of a Greenland glacier. *FEMS Microbiol Ecol* **89**: 402–414.

Lutz S., Anesio A. M., Edwards A., & Benning L. G. (2015a). Microbial diversity on Icelandic glaciers and ice caps. *Front Microbiol* **6**: 307.

Lutz S., Anesio A. M., Field K., & Benning L. G. (2015b). Integrated “Omics”, targeted metabolite and single-cell analyses of arctic snow algae functionality and adaptability. *Front Microbiol* **6**: 1–17.

Lutz S., Anesio A. M., Raiswell R., Edwards A., Newton R. J., Gill F. & Benning L. G. (2016). The biogeography of red snow microbiomes and their role in melting arctic glaciers. *Nat Commun* **7**: 11968.

Lyon B. & Mock T. (2014). Polar microalgae: new approaches towards understanding adaptations to an extreme and changing environment. *Biology* **3**: 56–80.

Marchant H. J. (1982). Snow algae from the Australian Snowy Mountains. *Phycologia* **21**: 178–184.

Marshall S. J. (2011). *The Cryosphere*. Princeton University Press, 288 p.

Marshall W. & Chalmers M. (1997). Airborne dispersal of Antarctic terrestrial algae and cyanobacteria. *Ecography* **20**: 585–594.

Marty C., Schlögl S., Bavay M. & Lehning M. (2017). How much can we save? Impact of different emission scenarios on future snow cover in the Alps. *Cryosphere* **11**: 517–

529.

Matsuzaki R., Kawai-Toyooka H., Hara Y. & Nozaki H. (2015). Revisiting the taxonomic significance of aplanozygote morphologies of two cosmopolitan snow species of the genus *Chloromonas* (Volvocales, Chlorophyceae). *Phycologia* **54**: 491–502.

Matsuzaki R., Nozaki H. & Kawachi M. (2018). Taxonomic revision of *Chloromonas nivalis* (Volvocales , Chlorophyceae) strains, with the new description of two snow-inhabiting *Chloromonas* species. *PLoS ONE* **13**: e0193603.

Matsuzaki R., Nozaki H., Takeuchi N., Hara Y. & Kawachi M. (2019). Taxonomic re-examination of "*Chloromonas nivalis* (Volvocales , Chlorophyceae) zygotes" from Japan and description of *C . muramotoi* sp. nov. *PlosONE* **14**: e0210986.

Mauro B. Di, Garzonio R., Rossini M., Filippa G., Pogliotti P., Morra U., Migliavacca M., Baccolo G., Delmonte B., Maggi V., Dumont M., Tuzet F., Lafaysse M., Morin S., Cremonese E. & Colombo R. (2018). Saharan dust events in the European Alps: role on snowmelt and geochemical characterization. *Cryosphere* **13**: 1147–1165.

Mölg T., Hardy D. R., Cullen N. J. & Kaser G. (2008). Tropical glaciers, climate change, and society: Focus on Kilimanjaro (East Africa). In Orlove B., Wiegandt E. & Luckman B. (Eds), *Darkening peaks: Glacier retreat, science, and society* (pp. 168–182). University of California Press: Berkeley, London.

Morgan-Kiss R. M., Priscu J. C., Pocock T., Gudynaite-Savitch L. & Huner N. P. A. (2006). adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiol Mol Biol R* **70**: 222–252.

Morita R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**: 144–167.

Mosser J. L., Mosser A. G. & Brock T. D. 1977. Photosynthesis in the snow: the alga *Chlamydomonas nivalis* (Chlorophyceae). *J Phycol* **13**: 22–27.

Mudryk L. R., Kushner P. J., Derksen C. & Thackeray C. (2017). Snow cover response to temperature in observational and climate model ensembles. *Geophys Res Lett* **44**: 919–926.

Müller T., Bleiß W., Rogaschewski C.-D. M. S. & Fuhr G. (1998). Snow algae from northwest Svalbard : their identification, distribution, pigment and nutrient content. *Polar Biol* **20**: 14–32.

Müller T., Leya T., & Fuhr G. (2001). Persistent snow algal fields in Spitsbergen: Field observations and a hypothesis about the annual cell circulation. *Arct Antarc Alpine Res* **33**: 42–51.

Muramoto K., Kato S., Shitara T., Hara Y. & Nozaki H. (2008). Morphological and genetic variation in the cosmopolitan snow alga *Chloromonas nivalis* (Volvocales, Chlorophyta) from Japanese mountainous area. *Cytologia* **73**: 91–96.

Muramoto K., Nakada T., Shitara T., Hara Y. & Nozaki H. (2010). Re-examination of the snow algal species *Chloromonas miwae* (Fukushima) Muramoto et al., comb. nov. (Volvocales, Chlorophyceae) from Japan, based on molecular phylogeny and cultured material. *Eur J Phycol* **45**: 27–37.

Nagai S., Lian C., Yamaguchi S., Hamaguchi M., Matsuyama Y., Itakura S., Shimada H., Kaga S., Yamauchi H., Sonda Y., Nishikawa T., Kim C. H. & Hogetsu T. (2007). Microsatellite markers reveal population genetic structure of the toxic dinoflagellate *Alexandrium tamarense* (Dinophyceae) in Japanese coastal waters. *J Phycol* **43**: 43–54.

Nedbalová L. & Sklenář P. (2008). New records of snow algae from the Andes of Ecuador. *Arnaldoa* **15**: 17–20.

Nedbalová L., Kociánová M. & Lukavský J. (2008). Ecology of snow algae in the Giant Mts. *Opera Corcontica* **45**: 59–68.

Niedzwiedz T. (1992). Climate of the Tatra Mountains. *Mt Res Dev* **12**: 131–146.

Nishizawa M., Yamagishi T., Nonaka G. I., Nishioka I. & A. Ragan M. (1985). Gallotannins of the freshwater green alga *Spirogyra* sp. *Phytochemistry* **24**: 2411–2413.

Novakovskaya I. V., Patova E. N., Boldina O. N., Patova A. D. & Shadrin D. M. (2018). Molecular phylogenetic analyses, ecology and morphological characteristics of *Chloromonas reticulata* (Goroschankin) Gobi which causes red blooming of snow in the subpolar Urals. *Cryptogamie Algol* **39:** 199–213.

Novis P. M. (2002a). Ecology of the snow alga *Chlainomonas kolii* (Chlamydomonadales, Chlorophyta) in New Zealand. *Phycologia* **41**: 280–292.

Novis P. M. (2002b). New records of snow algae for New Zealand, from Mt Philistine, Arthur's Pass National Park. *New Zeal J Bot* **40**: 297–312.

Novis P. M., Hoham R. W., Beer T. & Dawson M. (2008). Two snow species of the quadriflagellate green alga *Chlainomonas* (Chlorophyta, Volvocales): Ultrastructure and phylogenetic position within the chloromonas clade. *J Phycol* **44**: 1001–1012.

Nüsser M., Dame J., Kraus B., Baghel R. & Schmidt S. (2019). Socio-hydrology of "artificial glaciers" in Ladakh, India: assessing adaptive strategies in a changing cryosphere. *Reg Environ Change* **19**: 1327–1337.

Painter T. H., Duval B., Thomas W. H., Mendez M., Heintzelman S. & Dozier J. (2001). Detection and quantification of snow algae with an airborne imaging spectrometer. *Appl Environ Microb* **67**: 5267–5272.

Pelusi A. (2019). Cues triggering formation and germination of resting stages in marine diatoms. PhD thesis. The Open University, 243 p.

Petrushkina M., Gusev E., Sorokin B., Zotko N., Mamaeva A., Filimonova A., Kulikovskiy M., Maltsev Y., Yampolsky I., Guglya E., Vinokurov V., Namsaraev Z. & Kuzmin D. (2017). Fucoxanthin production by heterokont microalgae. *Algal Res* **24**: 387–393.

Pichrtová M., Remias D., Lewis L. A. & Holzinger A. (2013). Changes in phenolic compounds and cellular ultrastructure of Arctic and Antarctic strains of *Zygnema* (Zygnematophyceae, Streptophyta) after exposure to experimentally enhanced UV to PAR Ratio. *Microb Ecol* **65**: 68–83.

Pomeroy J. W. & Brun E. (2001). Physical properties of snow. In Jones H. G., Pomeroy J. W.,

Walker D. A. (Eds), *Snow ecology: An interdisciplinary examination of snow-covered ecosystems* (pp. 45–126). Cambridge: Cambridge University Press.

Pröschold T., Marin B., Schlösser U. G. & Melkonian M. (2001). Molecular phylogeny and taxonomic revision of (Chlorophyta). I. Emendation of *Chlamydomonas* Ehrenberg and *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and gen. nov. and *Lobochlamys* gen. nov. *Protist* **152**: 265–300.

Ralph P. J. & Gademann R. (2005). Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquat Bot* **82**: 222–237.

Ralph P. J., Gademann R., Larkum A. W. D. & Kühl M. (2002). Spatial heterogeneity in active chlorophyll fluorescence and PSII activity of coral tissues. *Mar Biol* **141**: 639–646.

Raymond J. A. (2014). The ice-binding proteins of a snow alga, *Chloromonas brevispina*: probable acquisition by horizontal gene transfer. *Extremophiles* **18**: 987–994.

Raymond J. & Remias D. (accepted). Ice-binding proteins in a Chrysophycean snow alga: acquisition of an essential gene by HGT. *Front Microbiol*

Remias D. (2012). Cell structure and physiology of alpine snow and ice algae. In Lütz C. (Ed.), *Plants in Alpine Regions. Cell physiology of adaption and survival strategies* (pp. 175–186). Wien: Springer.

Remias D. & Lütz C. (2007). Characterisation of esterified secondary carotenoids and of their isomers in green algae: a HPLC approach. *Algol Stud* **124**: 85–94.

Remias D., Lütz-Meindl U. & Lütz C. (2005). Photosynthesis, pigments and ultrastructure of the alpine snow alga *Chlamydomonas nivalis*. *Eur J Phycol* **40**: 259–268.

Remias D., Holzinger A. & Lütz C. (2009). Physiology, ultrastructure and habitat of the ice alga *Mesotaenium berggrenii* (Zygnemaphyceae, Chlorophyta) from glaciers in the European Alps. *Phycologia* **48**: 302–312.

Remias D., Karsten U., Lütz C. & Leya T. (2010a). Physiological and morphological processes in the Alpine snow alga *Chloromonas nivalis* (Chlorophyceae) during cyst formation. *Protoplasma* **243**: 73–86.

Remias D., Albert A. & Lütz C. (2010b). Effects of realistically simulated, elevated UV irradiation on photosynthesis and pigment composition of the alpine snow alga *Chlamydomonas nivalis* and the arctic soil alga *Tetracystis* sp. (Chlorophyceae). *Photosynthetica* **48**: 269–277.

Remias D., Holzinger A. Aigner S. & Lütz C. (2012a). Ecophysiology and ultrastructure of *Ancylonema nordenskiöldii* (Zygnematales, Streptophyta), causing brown ice on glaciers in Svalbard (high arctic). *Polar Biol* **35**: 899–908.

Remias D., Schwaiger S., Aigner S., Leya T., Stuppner H. & Lütz C. (2012b). Characterization of an UV- and VIS-absorbing, purpurogallin-derived secondary pigment new to algae and highly abundant in *Mesotaenium berggrenii* (Zygnematophyceae, Chlorophyta), an extremophyte living on glaciers. *FEMS Microbiol Ecol* **79**: 638–648.

Remias D., Wastian H., Lütz C. & Leya T. (2013a). Insights into the biology and phylogeny

of *Chloromonas polyptera* (Chlorophyta), an alga causing orange snow in Maritime Antarctica. *Antarct Sci* **25**: 648–656.

Remias D., Jost S., Boenigk J. Wastian J. & Lütz C. (2013b). *Hydrurus*-related golden algae (Chrysophyceae) cause yellow snow in polar summer snowfields. *Phycol Res* **61**: 277–285.

Remias D., Pichrtová M., Pangratz M., Lütz C. & Holzinger A. (2016). Ecophysiology, secondary pigments and ultrastructure of *Chlainomonas* sp. (Chlorophyta) from the European Alps compared with *Chlamydomonas nivalis* forming red snow. *FEMS Microbiol Ecol* **92**: fiw030.

Revill D. L., Stewart K. W. & Schlichting Jr. H. E. (1967). Passive dispersal of viable algae and protozoa by certain craneflies and midges. *Ecology* **48**: 1023–1027.

Řezanka T., Nedbalová L. & Sigler K. (2008). Unusual medium-chain polyunsaturated fatty acids from the snow alga *Chloromonas brevispina*. *Microbiol Res* **163**: 373–379.

Řezanka T., Lukavský J., Cepák V. & Procházková L. (2018). A production strain of the *Bracteacoccus bullatus* alga for the production of oils containing essential unsaturated fatty acids, a method of producing these oils and the use of this strain for the industrial production of these oils. *CZ patent 2017-331* (application number), *307402* (document number).

Roessler P. G. (1990). Environmental control of glycerolipid metabolism in microalgae: commercial Implications and future research directions. *J Phycol* **26**: 393–399.

Ross J. (1819). *A voyage of discovery, made under the order of the Admirality, in his Majesty's ships Isabella and Alexander for the purpose of exploring Baffin's bay, and Inquiring into the probability of a North-West passage.* London: John Murray.

Rothschild L. J. & Mancinelli R. L. (2001). Life in extreme environments. *Nature* **409**: 1092–1101.

Sattler B., Remias D., Lütz C., Dastych H. & Psenner R. (2010). Leben auf Schnee und Eis. In Erschbamer B. & Koch E. M. (Eds), *Glaziale und periglaziale Lebensräume im Raum Obergurgl* (pp. 229–250). Innsbruck University Press.

Sattler B., Post B., Remias D., Lutz C., Lettner H. & Psenner R. (2012). Cold Alpine regions. In Bell E. (Ed.), *Life at extremes. Environments, organisms, and strategies for survival* (pp. 138–154). Wallingford, OX: CABI.

Schlichting H. E. (1969). The importance of airborne algae and protozoa. *Japca J Air Waste Ma* **19**: 946–951.

Schmidt S. K., Nemergut D. R., Darcy J. L. & Lynch R. (2014). Do bacterial and fungal communities assemble differently during primary succession? *Mol Ecol* **23**: 254–258.

Schwinghamer P., Hawryluk M., Powell C. & MacKenzie C. H. (1994). Resuspended hypnozygotes of *Alexandrium fundyense* associated with winter occurrence of PSP in inshore Newfoundland waters. *Aquaculture* **122**: 171–179.

Segawa T., Miyamoto K., Ushida K., Agata K., Okada N. & Kohshima S. (2005). Seasonal

change in bacterial flora and biomass in mountain snow from the Tateyama Mountains, Japan, analyzed by 16S rRNA gene sequencing and real-time PCR. *Appl Environ Microb* **71**: 123–130.

Segawa T., Matsuzaki R., Takeuchi N., Akiyoshi A., Navarro F., Sugiyama S., Yonezawa T. & Mori H. (2018). Bipolar dispersal of red-snow algae. *Nat Commun* **9**: 3094.

Shain D. H., Mason T. A., Farrell A. H. & Michalewicz L. A. (2001). Distribution and behavior of ice worms (*Mesenchytraeus solifugus*) in south-central Alaska. *Can J Zool* **79**: 1813–1821.

Shaver G. R. (2001). NPP Tundra: Toolik Lake, Alaska, 1982 (Oak Ridge National Laboratory Distributed Active Archive Center). Retrieved from www.daac.ornl.gov

Škaloud P., Škaloudová M., Doskočilová P., Kim J. I., Shin W. & Dvořák P. (2019). Speciation in protists: Spatial and ecological divergence processes cause rapid species diversification in a freshwater chrysophyte. *Mol Ecol* **28**: 1084–1095.

Smayda T. J. (2007). Reflections on the ballast water dispersal-harmful algal bloom paradigm. *Harmful Algae* **6**: 601–622.

Sommaruga R. & Psenner R. (1997). Ultraviolet radiation in a high mountain lake of the Austrian Alps: Air and underwater measurements. *Photochem Photobiol* **65**: 957–963.

Spijkerman E., Wacker A., Weithoff G. & Leya T. (2012). Elemental and fatty acid composition of snow algae in Arctic habitats. *Front Microbiol* **3**: 1–15.

Stein J. R. (1963). A *Chromulina* (Chrysophyceae) from snow. *Canad J Bot* **41**: 1367–70.

Stibal M. & Elster J. (2005). Growth and morphology variation as a response to changing environmental factors in two Arctic species of *Raphidonema* (Trebouxiophyceae) from snow and soil. *Polar Biol* **28**: 558–567.

Stibal M. & Elster J. (2007). Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by pulse amplitude modulation fluorometry. *FEMS Microbiol Ecol* **59**: 265–273.

Stibal M., Box J. E., Cameron K. A., Langen P. L., Yallop M. L., Mottram R. H., Khan A. L., Molotch N. P., Chrismas N. A. M., Quaglia F. C., Remias D., Smeeth P. C. J. P., van den Broeke M. R., Ryan J. C., Hubbard A., Tranter M., van As D. & Ahlstrøm A. P. (2017). Algae drive enhanced darkening of bare ice on the Greenland ice sheet. *Geophys Res Lett* **44**: 11463–11471.

Strasser R. J., Srivastava A. & Tsimilli-Michael M. (2000). The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M., Pathre U. & Mohanty P. (Eds) *Probing Photosynthesis: Mechanism, Regulation & Adaptation* (pp. 445–483). Taylor and Francis, London.

Sun N., Wang Y., Li Y. T., Huang J. C. & Chen F. (2008). Sugar-based growth, astaxanthin accumulation and carotenogenic transcription of heterotrophic *Chlorella zofingiensis* (Chlorophyta). *Process Biochem* **43**: 1288–1292.

Takeuchi N. & Kohshima S. (2004). A snow algal community on Tyndall Glacier in the

Southern Patagonia Icefield, Chile. *Arct Antarct Alp Res* **36**: 92–99.

Takeuchi N. & Li Z. (2008). Characteristics of surface dust on Ürümqi Glacier No. 1 in the Tien Shan Mountains, China. *Arct Antarct Alpine Res* **40**: 744–750

Takeuchi N., Uetake J., Fujita K., Aizen V. B. & Nikitin S. D. (2006a). A snow algal community on Akkem Glacier in the Russian Altai A snow algal community on Akkem glacier in the Russian Altai mountains. *Ann Glaciol* **43**: 378–384.

Takeuchi N., Dial R., Kohshima S., Segawa T. & Uetake J. (2006b). Spatial distribution and abundance of red snow algae on the Harding Icefield, Alaska derived from a satellite image. *Geophys Res Lett* **33**: L21502.

Tanabe Y., Shitara T., Kashino Y., Hara Y. & Kudoh S. (2011). Utilizing the effective xanthophyll cycle for blooming of *Ochromonas smithii* and *O. itoi* (Chrysophyceae) on the snow surface. *PLoS ONE* **6**: e14690.

Terashima M., Umezawa K., Mori S., Kojima H. & Fukui M. (2017). Microbial community analysis of colored snow from an alpine snowfield in Northern Japan reveals the prevalence of Betaproteobacteria with snow algae. *Front Microbiol* **8**: 1481.

Thackeray C. W., Derksen C., Fletcher C. G. & Hall A. (2019). Snow and climate: feedbacks, drivers, and indices of change. *Curr Clim Change Rep,* https://doi.org/10.1007/s40641-019-00143-w.

Thomas W. H. (1972). Observations on snow algae in California. *J Phycol* **8**: 1–9.

Thomas W. H. & Duval B. (1995). Sierra Nevada, California, U.S.A., snow algae: snow albedo changes, algal-bacterial interrelationships, and ultraviolet radiation effects. *Arct Alp Res* **27**: 389–399.

Thomas W. H. & Broady P. A. (1997). Distribution of coloured snow and associated algal genera in New Zealand. *New Zeal J Bot* **35**: 113–117.

Thompson L. G., Mosley-Thompson E., Davis M. E. & Brecher H. H. (2011). Tropical glaciers, recorders and indicators of climate change, are disappearing globally. *Ann Glaciol* **52**: 23–34.

Vimercati L., Darcy J. L. & Schmidt S. K. (2019a). The disappearing periglacial ecosystem atop Mt. Kilimanjaro supports both cosmopolitan and endemic microbial communities. *Sci Rep* **9**: 10676.

Vimercati L., Solon A. J., Krinsky A., Arán P., Porazinska D. L., Darcy J. L., Dorador C. & Schmidt S. K. (2019b). Nieves penitentes are a new habitat for snow algae in one of the most extreme high-elevation environments on Earth. *Arct Antarct Alp Res* **51**: 190–200.

Višić H., Filek K., Mucko M., Trotta A., Panagopolou A., Lukač M., Corrente M., Di Bello A., Gračan R. & Bosak S. (2019). Metagenomic characterization of the surface biofilm on Mediterranean loggerhead sea turtles. *Seventh European Phycological Congress, Keynote and Oral Papers*, *Eur J Phycol* **54**: 59.

Wang Y., Liu Z. & Qin S. (2013). Effects of iron on fatty acid and astaxanthin accumulation in mixotrophic *Chromochloris zofingiensis*. *Biotechnol Lett* **35**: 351–357.

Warren S. G. (1984). Impurities in snow: effects on albedo and snowmelt. *Ann Glaciol* **5**: 177–179.

Watson S. B. (2003). Cyanobacterial and eukaryotic algal odour compounds: Signals or by-products? A review of their biological activity. *Phycologia* **42**: 332–350.

Weiss R. L. (1983). Fine structure of the snow alga (*Chlamydomonas nivalis*) and associated bacteria. *J Phycol* **19**: 200–204.

Wharton R. A. & Vinyard W. C. (1983). Distribution of snow and ice algae in Western North America. *Madroño* **30**: 201–209.

White A. J. & Critchley C. (1999). Rapid light curves: A new fluorescence method to assess the state of the photosynthetic apparatus. *Photosynth Res* **59**: 63–72.

Williams W. E., Gorton H. L. & Vogelmann T. C. (2003). Surface gas-exchange processes of snow algae. *P Natl Acad Sci USA* **100**: 562–566.

Williamson C. J., Cameron K. A., Cook J. M., Žárský J. D., Stibal M. & Edwards A. (2019). Glacier algae: A dark past and a darker future. *Front Microbiol* **10**: 524.

Yoshimura Y., Kohshima S., Takeuchi N. Seko K., & Fujita K. (2000). Himalayan ice-core dating with snow algae. *J Glaciol* **46**: 335–340.

Zawierucha K. & Shain D. H. (2019). Disappearing Kilimanjaro snow – Are we the last generation to explore equatorial glacier biodiversity? *Ecol Evol* **9**: 8911–8918.

Zekollari H., Huss M. & Farinotti D. (2019). Modelling the future evolution of glaciers in the European Alps under the EURO-CORDEX RCM ensemble. *The Cryosphere* **13**: 1125–1146.

Zemp M., Huss M., Thibert E., Eckert N., McNabb R., Huber J., Barandun M., Machguth H., Nussbaumer S. U., Gartner-Roer I., Thomson L., Paul F., Maussion F. & Cogley J. G. (2019). Global glacier mass changes and their contributions to sea-level rise from 1961 to 2016. *Nature* **568**: 382–386.