Polar Biol (2017) 40:1169–1184
DOI 10.1007/s00300-016-2045-4

REVIEW

Chilling out: the evolution and diversification of psychrophilic algae with a focus on Chlamydomonadales

Marina Cvetkovska[1] · Norman P. A. Hüner[1] · David Roy Smith[1]

Received: 20 February 2016 / Revised: 20 July 2016 / Accepted: 10 October 2016 / Published online: 21 October 2016

Abstract The Earth is a cold place. Most of it exists at or below the freezing point of water. Although seemingly inhospitable, such extreme environments can harbour a variety of organisms, including psychrophiles, which can withstand intense cold and by definition cannot survive at more moderate temperatures. Eukaryotic algae often dominate and form the base of the food web in cold environments. Consequently, they are ideal systems for investigating the evolution, physiology, and biochemistry of photosynthesis under frigid conditions, which has implications for the origins of life, exobiology, and climate change. Here, we explore the evolution and diversification of photosynthetic eukaryotes in permanently cold climates. We highlight the known diversity of psychrophilic algae and the unique qualities that allow them to thrive in severe ecosystems where life exists at the edge. We focus on psychrophilic green algae from the Chlamydomonadales, discussing recent discoveries and directions for future research, and argue that they are among the best available models for studying psychrophily and life at the edge in photosynthetic eukaryotes.

Keywords Algae · *Chlamydomonas reinhardtii* · *Chlamydomonas nivalis* · Cold adaptation · ICE-L · Psychrophily · UWO241

Introduction

Almost 80 % of the Earth's biosphere is permanently below 5 °C, including most of the oceans, the polar, and alpine regions (Feller and Gerday 2003). These seemingly inhospitable places are some of the least studied but most important ecosystems on the planet. They contain a huge diversity of prokaryotic and eukaryotic organisms, many of which are permanently adapted to the cold (psychrophiles) (Margesin et al. 2007). The environmental conditions in such habitats severely limit the spread of terrestrial plants, and therefore, primary production in perpetually cold environments is largely dependent on microbes. Eukaryotic algae and cyanobacteria are the dominant photosynthetic primary producers in cold habitats, thriving in a surprising variety of niches—from perennially ice-covered lakes to the volatile environments of sea ice and snowfields (Morgan-Kiss et al. 2006).

Psychrophiles can be excellent systems for studying adaptation to extreme environments and the diversification of life (Siddiqui et al. 2013). They can also provide clues to possible life on other planets (exobiology) (Gao et al. 2013)—a topic that has gained recent widespread attention with the discovery of liquid water of Mars. Photosynthetic species that flourish in the cold are responsible for a significant proportion of the inorganic carbon fixed each year and thus are crucial for mediating climate change (Lyon and Mock 2014). And the fact that psychrophilic green algae accumulate higher levels of lipids as compared to their mesophilic counterparts makes them attractive targets for commercial biofuel production (Mou et al. 2012).

Here, we review the evolution of psychrophily in plastid-bearing protists. We first explore the definition of psychrophily in eukaryotes and then highlight the diversity and abundance of psychrophilic algae across the eukaryotic

---

Electronic supplementary material The online version of this article (doi:10.1007/s00300-016-2045-4) contains supplementary material, which is available to authorized users.

✉ Norman P. A. Hüner
nhuner@uwo.ca

[1] Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada

domain, documenting some of the driving forces for their survival at frigid temperatures. We pay particular attention to psychrophilic green algae from the Chlamydomonadales, describing some of the best-studied cold-adapted algae to date (*Chlamydomonas* sp. UWO241, *Chlamydomonas nivalis*, *Chlamydomonas* sp. ICE-L). These species are closely related to the model algae, *Chlamydomonas reinhardtii* and *Dunaliella salina*, and are poised to become leading systems for investigating cold adaptation. Finally, we outline directions for future research, such as the exceptional potential of comparative genomic analyses of psychrophilic Chlamydomonadales with *C. reinhardtii*.

“A rose by any other name”: defining psychrophily

Organisms that thrive in cold climates and falter in warmer ones are broadly called psychrophiles. The precise meaning of this term, however, is debated and can differ between disciplines, often leading to confusion (Table 1). For instance, many organisms found in permanently cold environments are psychrotrophs, meaning that they can survive at low temperatures, but their maximal growth temperature is >20 °C. One of the most common definition of psychrophily (Morita 1975) applies to microorganisms that survive and reproduce when temperatures are <15 °C and have an upper survival limit of ≤20 °C, as determined by growth rate. This definition has drawbacks. It does not apply to most cold-adapted eukaryotes, and the upper temperature limit corresponds to that from a typical laboratory setting rather than to a natural environment. Moreover, using growth rate to determine the optimum temperature does not take into account the physiological state of the organism. For instance, although the growth rate of cold-adapted bacteria can increase with the temperature, other key cellular processes, such as protein synthesis and membrane permeability, can be negatively affected (Morgan-Kiss et al. 2006).

Given their remarkable diversity, psychrophilic eukaryotic algae—the focus of this review—are not easily grouped under a single definition of psychrophily. The term photopsychrophile, for example, is regularly used to distinguish psychrophilic photoautotrophs from heterotrophs, but many cold-adapted algae are mixotrophic (Laybourne-Parry and Pearce 2007). Nevertheless, measuring the temperature that provides the highest rate of growth and the upper temperature limit for survival are still the most common approaches for the verification of psychrophily in algae. However, these simplified approaches do not always account for the following:

1. The upper survival temperature can depend on exposure time, which is known to range from one day for unicellular species to three months for multicellular ones (Wiencke et al. 1994). This is especially true in seaweeds, where a single living cell among mostly dead tissue can resume growth under favourable conditions (Wiencke et al. 1994).

Table 1 Available definitions of cold-adapted life

Term	Definition	Organisms	References
Psychrophile or obligate psychrophile	Microorganisms that have optimal growth temperatures of <15 °C and upper cardinal temperatures of <20 °C	Bacteria, Archaea, Yeast, Fungi, Microalgae, Macroalgae	Morita (1975)
Psychrotroph, psychrotolerant, or facultative psychrophile	Organisms that can grow at low temperatures, but have optimal and maximal growth temperatures >15°C and >20 °C, respectively	Bacteria, Archaea, Yeast, Fungi, Microalgae, Macroalgae	Morita (1975)
Stenotherm	Organisms that can only grow and survive within a narrow (10 °C) temperature range	Macroalgae, Invertebrates, Fish	Bligh and Johnson (1973)
Eurytherm	Organisms that can grow at a wide range of temperatures	Macroalgae, Invertebrates, Fish	Bligh and Johnson (1973)
Psychroactive	Organisms capable of growth ≤−1 °C	Bacteria, Archaea	Panikov and Sizova (2006)
Cryophile	Organisms capable of growth at temperatures ranging from −20° to 10 °C	Bacteria, Plants, Invertebrates, Fish	Morita (1975)
Eutectophile	Organisms living at the interphase between water and ice	Bacteria	Deming (2002)

2. Measuring the upper survival temperature is further complicated by temperature-dependent changes in development and reproduction. For example, the psychrophilic fungus *Myriosclerotinia borealis* shifts from vegetative hyphae at low, permissive temperatures to the formation of resting bodies (sclerotia) at high, nonpermissive temperatures; hyphal growth then resumes following exposure to low temperatures (Newsted et al. 1985; Newsted and Hüner 1987). This parallels the germination of a resting cyst after a period of dormancy in some unicellular psychrophilic algae (Daugbjerg et al. 2000; Flaim et al. 2010; Remias et al. 2010b). Complicating matters further, different stages in the algal life cycle can have different temperature requirements, a phenomenon widely documented in macroalgae. For instance, in Antarctic seaweeds, the maturation of reproductive structures only occurs in the austral summer when water temperatures reach 5 °C, and zoospores often have higher upper survival temperatures than sporophytes (Gómez et al. 2009).
3. Maximum growth rates and temperature survival limits are also sensitive to growth media, particularly its salt concentration, as shown for the green alga *Chlamydomonas* sp. ARC (Eddie et al. 2008) and the diatom *Thalassiosira antarctica* (Aletsee and Jahnke 1992).
4. Finally, the growth rate and survival of psychrophilic algae in their natural environment can be reliant on complex interactions with other cold-adapted organisms. For example, various chlamydomonadalean snow algae, including *Chlamydomonas nivalis*, are commonly found in large assemblages of bacteria and fungi, which can form symbiotic or parasitic relationships with the algae (Brown et al. 2015). Similarly, the intensity and duration of large algal blooms dominated by the Antarctic haptophyte *Phaeocystis antarctica* are often governed by the surrounding bacterial communities (West et al. 2008; Delmont et al. 2014), possibly through their requirements for exogenous supply of essential nutrients and vitamins (Croft et al. 2005; Koch et al. 2011).

Given all of the above factors, the definition of psychrophily for eukaryotic algae needs to be based on clearly defined temperature survival limits as well as features relating to metabolism, physiology, and reproduction. For the purpose of this study, we will broadly define psychrophilic eukaryotic algae as naturally existing plastid-bearing protists that are metabolically active and able to reproduce at temperatures permanently close to the freezing point of water and that cannot tolerate more moderate (mesophilic) temperatures (≥20 °C).

Cold and light: potential imbalances in cellular energy budget

In contrast to heterotrophs, a photoautotrophic lifestyle requires the integration of photophysical, photochemical and biochemical processes involved in the conversion of light energy into stable storage forms of carbon for growth and metabolism. As such, access to light energy is a major factor driving the evolution of photosynthetic organisms (Rothschild 2008) and one that has to be considered when discussing the evolution of photopsychrophiles. Sunlight is abundant, its availability is reliable, and it is present in inexhaustible quantities when measured on biological time scales, but its utilization as the ultimate energy source for photoautotrophic life at low temperatures poses unique challenges and dangers.

The processes that convert light into stable carbon forms vary in both their rate constants and their temperature sensitivity. The biophysical processes of light absorption and energy trapping (energy sources) occur on a picosecond to microsecond (10^{-12} to 10^{-6} s) time scale and are temperature insensitive. In contrast, biochemical reactions such as the enzyme-catalysed energy conversion and storage (energy sinks) occur on a time scale that can range from millisecond (10^{-3} s) to hours and days and vary as a function of temperature, with the rates of reaction decreasing significantly in the cold due to thermodynamic constraints (Hüner and Grodzinski 2011). The combination of such extreme disparities in temperature sensitivities coupled with rate constants that differ by 10 orders of magnitude represents a formidable challenge for all photosynthetic organisms, and especially so for photopsychrophiles. When temperatures are low, the rate at which energy is absorbed through the temperature-insensitive energy sources exceeds the rates at which energy is utilized at the temperature-sensitive energy sinks, which leads to imbalances in the cellular energy budget. Such an imbalance is defined as excitation pressure (Hüner et al. 1998), and chronic exposure to excess excitation energy (EEE) can lead to overreduction of the chloroplast electron transport chain (cETC), photoinhibition and photo-oxidative damage due to the generation of harmful reactive oxygen species (ROS), and ultimately cell death (Karpinski et al. 1999; Hüner and Grodzinski 2011; Hüner et al. 2013). Consequently, an essential component in the evolution of photopsychrophile must be the capacity to maintain photostasis (Melis 1998)—a balance between energy input as light and energy utilization through metabolism and growth, under persistent and extreme cold temperatures.

Several mechanisms have evolved to allow the photosynthetic apparatus to balance the flow of energy to attain photostasis. Some multicellular organisms, such as terrestrial freeze-tolerant plants, respond to low temperatures by stimulating sink capacity through the upregulation of photosynthetic CO_2 assimilation and carbon export that results in an increase in biomass accumulation and seed yield (Bravo et al. 2007; Dahal et al. 2014). Unicellular green algae can achieve photostasis by balancing the energy absorbed by the photosystems II (PSII) and I (PSI) of the thylakoid membranes of the chloroplast and thus readjust the redox poise of the cETC and ensure optimal rates of photosynthetic electron transport. This readjustment can occur in the short term (seconds to minutes) via the process of state transitions (Rochaix 2014), which involves the phosphorylation and redistribution of the mobile PSII light-harvesting complex (LHCII) from PSII to PSI in response to overreduction of the cETC. In the long term (minutes to days), the changes in absorption capacities of PSII and PSI can be achieved through transcriptional and translational regulation of their components (Kirchhoff 2013). In addition to the linear electron flow (LEF) from PSII to PSI, overreduction can also induce cyclic electron flow (CEF) where electrons are passed from PSI back to the cETC. This process has been implicated in the prevention of damage due to EEE (Shikanai 2007; Tikkanen and Aro 2014; Chaux et al. 2015). Finally, EEE can be dissipated as heat through non-photochemical quenching (NPQ) via the pigments in the xanthophyll cycle. NPQ is considered to act as a "safety valve" that protects the cETC from photodamage (Demmig-Adams and Adams 1996; Derks et al. 2015; Dietz 2015). Our knowledge on how and to what extent these processes contribute to photosynthetic efficiencies in modern-day photopsychrophiles is limited, but it appears that the dominant photoprotective mechanisms vary depending on the lifestyle of the individual species (reviewed in Lyon and Mock 2014). We suggest that the evolution of psychrophily in photosynthetic organisms must be tightly associated with their capacity to adapt to not only low temperatures, but their light environment as well.

Staying cool: the evolution of psychrophily

The evolution of psychrophily is believed to be a gradual, stepwise process (Wiencke et al. 1994), beginning with slight increases in cold tolerance, allowing a mesophilic species to survive in a seasonally fluctuating and harsh environment. Growth rate and reproduction then slowly adjust to lower and lower temperatures, followed by a reduction in the upper temperature survival limit. Eventually, the species can no longer survive at moderate temperatures, becoming a true psychrophile. This scenario fits with the geological history of the polar regions (Zachos et al. 2001). Antarctic glaciation peaked ~14 Mya, whereas glaciation of the Arctic is thought to have happened no earlier than 3.5 Mya. This might explain why most known psychrophilic algae come from the Antarctic (Online resource 1). But even after existing for millions of years in a cold climate, many Antarctic algae survive—and flourish—well above ambient polar temperatures.

Some have hypothesized that extremophiles evolve from species that thrive in a range of different environments ("generalists") because of their phenotypic plasticity (West-Eberhard 2005). Such plasticity can result from cryptic genetic variation, which is expressed only under certain environmental conditions and has as a phenotypic effect (Schlichting 2008). However, if conditions stabilize over time (e.g. persistent cold temperatures), "generalists" can evolve through natural selection into "specialists", such as psychrophilic algae. The unicellular diatom *Asterionellopsis glacialis* is a good example of a generalist with cryptic genetic variation: it is globally distributed and can tolerate a variety of environmental conditions, including the extreme cold (Kaczmarska et al. 2014).

Many of the traits needed for survival at cold temperatures (drought resistance, high light and high UV tolerance, shade adaptation, low nutrient stress) are also needed in a variety of other environments, such as deserts, tropical and subtropical oceans, and caves (Quesada and Vincent 2012). For example, a reoccurring theme among plastid-bearing extremophiles is mixotrophy—the ability to make use of both inorganic (via photoautotrophism) and organic (via chemoheterotrophism) carbon sources. Although metabolically costly to maintain, mixotrophy can provide obvious benefits for algae living in the low-nutrient and low-light environments such as tropical oceans and polar snowfields (Thingstad et al. 1996; Raven 1997; Zubkov and Tarran 2008). In fact, for some psychrophiles, such as the prasinophyte *Pyramimonas gelidicola* and the cryptophyte *Gemingera cryophila*, mixotrophy contributes significantly to the overall carbon budget, regardless of the season (Laybourne-Parry and Pearce 2007; McKie-Krisberg et al. 2015). We can speculate that organisms that evolved under different stressful conditions could already carry some of the adaptations needed for survival at cold temperatures prior to their radiation into such habitats. This mechanism has been proposed for the radiation of angiosperms in freezing environments (Zanne et al. 2014) and for the spread of several cold-adapted cyanobacterial lineages into polar and alpine regions (Christmas et al. 2015).

The appearance of psychrophilic algae may be linked to major glaciation events (Vincent et al. 2004). The Earth's biosphere likely experienced several intervals of extremely low temperature, coinciding with the emergence and diversification of life. Geological data suggest that during these cooling periods, glaciation extended to the tropics in what is called "snowball Earth", where the entire planet was covered in ice, or "slushball Earth" where a band of open water persisted around the tropics in an otherwise frozen landscape (Hyde et al. 2000). Ubiquitous snow and ice would make ideal conditions for the emergence of psychrophiles, but opponents to this view point out that global freezing would result in mass extinctions. Some have argued that only prokaryotes could survive such conditions; however, the fossil record implies that many eukaryotes prevailed as well (Hoffman and Schrag 2002). Undoubtedly, a "snowball Earth" would have strong evolutionary consequences, especially for photosynthetic eukaryotes exposed to severe shading under the ice, in addition to cold temperatures (Vincent et al. 2004). In such harsh conditions, life could persist in local open water environments, under relatively thin ice (McKay 2000), or in microbial mats, as it is often seen in some present-day polar algae (Vincent et al. 2004). Given that they are composed of concentrated populations of diverse taxa, microbial mats are considered to be ideal environments for evolutionary processes, including mutualism, symbiosis, and even eukaryogenesis (Nisbet and Fowler 1999). During the coldest periods, when near-surface liquid water was rare or non-existent, psychrophiles could enter a dormant life cycle, such as a resting cyst, and survive beneath the ice. This strategy is often encountered in modern psychrophilic algae. For example, the alpine snow alga *Chloromonas nivalis* enters a diploid, non-motile zygotic life stage under freezing winter conditions. During the warmer summer season in meltwater pools, these zygotes undergo meiosis and produce motile haploid offspring that can reproduce asexually (Remias et al. 2010b).

The arrival of warmer weather globally in a "snowball Earth" scenario would have caused the extinction of many obligate psychrophiles, although those with some thermal tolerance could persist, contributing to the post-glaciation diversification of life as observed in the fossil record (Hoffman and Schrag 2002). While this theory does not support the persistence of psychrophily as an ancestral trait, it does provide evidence that organisms with greater phenotypic plasticity in terms of thermal adaptations can not only survive, but also thrive and evolve under harsh temperature conditions, subsequently giving rise to some of the psychrophiles we see today in polar and alpine environments (Vincent et al. 2004).

Counting in the cold: unravelling the diversity of psychrophilic algae

Eukaryotic algae are plastid-bearing protists with complex (and sometimes convoluted) evolutionary histories, as reflected by their phylogeny, which spans four of the five eukaryotic "supergroups" (Keeling 2013) and includes at least 42,000 species (Guiry and Guiry 2016). Indeed, algae are ubiquitous in ocean and freshwater ecosystems and commonly occur in extreme environments, such as deserts, and even exist as symbionts or parasites. Thus, it is not surprising that many algae are found in permanently cold environments, but even so, most species still grow optimally in warm habitats (e.g. psychrotrophs) (Quartino et al. 2005; Komárek and Nedbalová 2007; Kvíderová 2011; Li et al. 2016).

The study of psychrophilic algae is in its infancy, and there are only about 90 confirmed psychrophilic species. Although new algal species are continuously being identified in cold environments, data on their optimal and upper survival temperatures and physiology are usually lacking. Nevertheless, detailed growth statistics are slowly accumulating for a small but eclectic list of plastid-bearing psychrophiles (Fig. 1; Online resource 1). Albeit short, this list includes unicellular and multicellular species and spans major eukaryotic groups, such as green algae, rhodophytes, stramenopiles, and dinoflagellates, reinforcing the idea that psychrophily has evolved (and been lost) multiple times independently throughout eukaryotic evolution. Determining the number of times that psychrophilic algae have arisen is not straightforward. First, there is little information on the precise number of psychrophiles within individual groups, and the phylogenetic relationships among psychrophilic species are typically poorly resolved. Second, it can be hard to determine whether the shift to cold tolerance was gained independently in multiple lineages or whether it represents the ancestral state. That said, given the small number of psychrophilic species in any given family, it is unlikely that the condition is ancestral.

Much of our knowledge of psychrophiles comes from subtidal macroalgae, such as the rhodophyte *Palmaria decipiens*, which dominates Antarctic shallow and deep benthic environments and supports a complex community of epiphytes, grazers, and decomposers (Becker et al. 2011). Substantial data also exist for various unicells, many of which are dominant in polar and alpine snowfields, sea ice, cold-water lakes, and surface transitory ponds (Morgan-Kiss et al. 2006). The diatom *Fragilariopsis cylindrus*, for instance, can be found in Arctic and Antarctic seawater and sea ice and regularly forms large ice-edge blooms (Mock and Hoch 2005). Similarly, the green alga *Chlamydomonas* sp. ICE-L is prominent in Antarctic coastal marine environments, and because of its high lipid content, it is being studied for potential biofuel production (Mou et al. 2012).

Genomic technologies have transformed the field of algal research. Alongside the pioneering genome sequencing studies of the red alga *Cyanidioschyzon merolae* (Matsuzaki et al. 2004) and the green alga *C.*

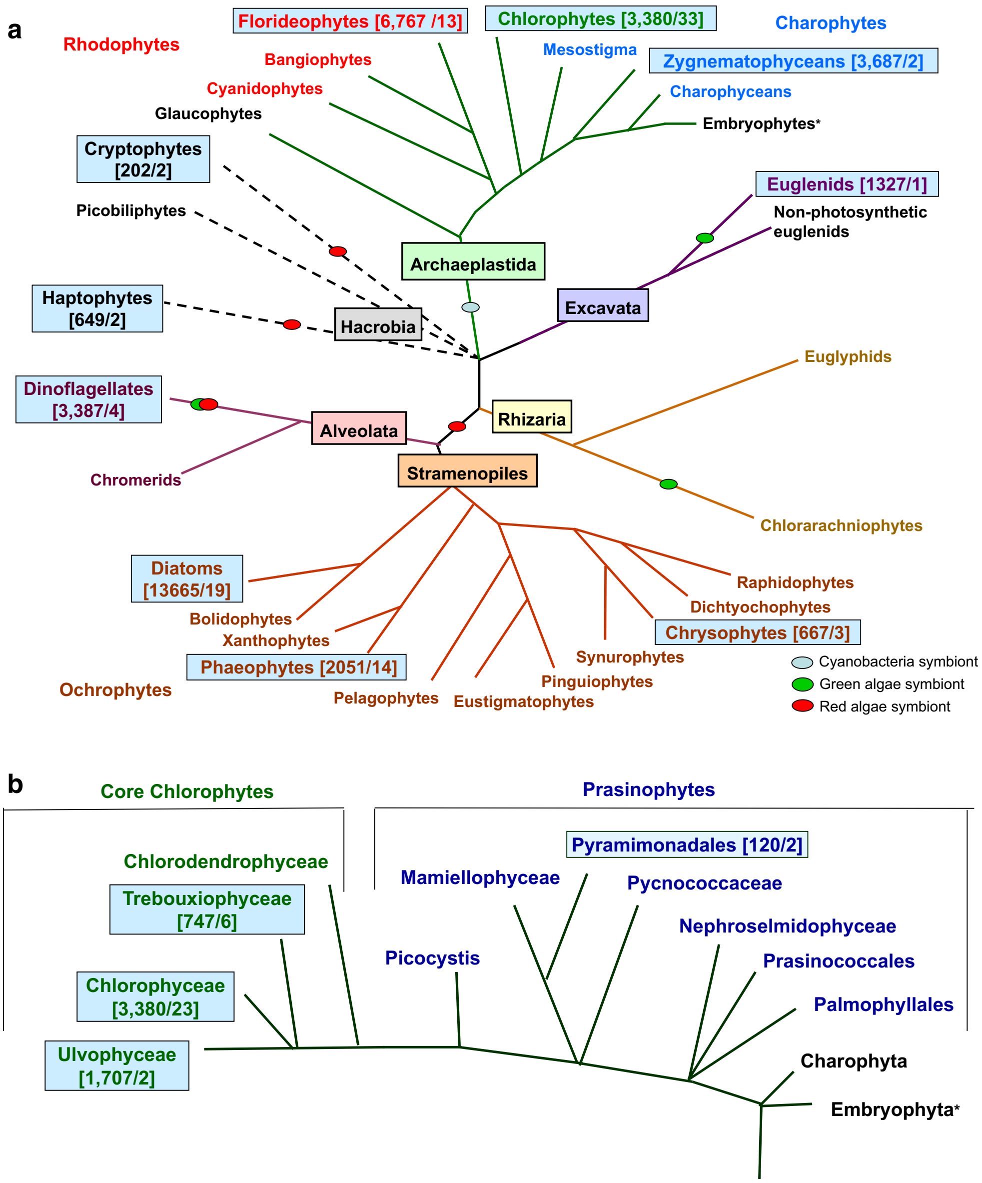

Fig. 1 **a** Tree of eukaryotes highlighting lineages harbouring known psychrophilic algae (Adapted from Burki 2014). Only major eukaryotic groups containing plastid-bearing photosynthetic organisms are shown. The branching pattern does not necessarily represent relationships between lineages. The dotted lines represent uncertain relationships and conflicting positions within the tree. **b** Overview of the phylogeny of the chlorophytes, showing the core chlorophytes and major prasinophytes (adapted from Leliaert et al. 2012). In both **a** and **b**, the lineages containing psychrophiles are boxed in [total number of species/known number of psychrophilic species] *the embryophyte lineage contains several species that are adapted to permanently cold environments, such as the Antarctic plants *Deschampsia antarctica* (Gramineae) and *Colobanthus quitensis* (Caryophyllene). These species, while permanently adapted to the cold, do not fit the definition of psychrophiles presented in this work and thus are not included in our discussion

reinhardtii (Merchant et al. 2007), many other model and non-model algae have had their genomes sequenced, improving our understanding of photosynthesis (*Chlorella variabilis*, Blanc et al. 2010), multicellularity (*Volvox carterii*, Prochnik et al. 2010; *Gonium pectorale*, Hanschen et al. 2016), endosymbiosis (*Guillardia theta* and *Bigelowiella natans*, Curtis et al. 2012), carbohydrate and silica metabolism (*Thalassiosira pseudonana*; Armbrust et al. 2004), and salt tolerance (*Dunaliella salina*, early-release genome, Joint Genome Institute). Although there is not yet a complete genome sequence from a psychrophilic alga, various research teams are in the process of developing one. The United States Department of Energy Joint Genome Institute has generated draft genome assemblies of the psychrophilic diatom *F. cylindrus* and haptophyte *P. antarctica*, and draft transcriptome sequences exist for other cold-adapted algae, many of which were sequenced as part of the Marine Eukaryotic Transcriptome Sequencing Project (Keeling et al. 2014).

Researchers are also using environmental sequencing to characterize algae from cold environments (Bielewicz et al. 2011; Dolhi et al. 2015; Li et al. 2016). Again, the challenge facing this kind of work is that detailed growth rates and metabolic data are often lacking, thus preventing species from being formally categorized as psychrophiles. In other cases, significant amounts of sequencing and growth rate data are available from polar algae that, although living in extremely cold environments, do not meet the strict definition of psychrophily, as is the case for the psychrotolerant green alga *Coccomyxa subellipsoidea*, which has had its entire genome sequenced (Blanc et al. 2012). Nevertheless, these and other studies are giving insights into the evolution of psychrophily.

Chlamydomonadalean algae: an icebox of model psychrophiles

Although plastid-bearing psychrophiles are broadly distributed across the eukaryotic tree of life (Fig. 1a), certain lineages appear to have an abundance of cold-adapted taxa. For instance, the Chlorophyta harbours more than a third of all confirmed photopsychrophiles, almost all of which (23 species) belong to the order Chlamydomonadales (Fig. 1b; Online resource 1).

With more than 1700 described species (Guiry and Guiry 2016), the order Chlamydomonadales has fascinated evolutionary biologists for decades. Its members span the gamut of complexity, from unicells (e.g. *C. reinhardtii*) to undifferentiated colonies (e.g. *G. pectorale*) to multicellular individuals (e.g. *V. carteri*), providing a unique platform for studying major transitions in evolution (Prochnik et al. 2010; Hanschen et al. 2016). The group also harbours non-photosynthetic species (e.g. *Polytomella parva*) (Figueroa-Martinez et al. 2015) and a large number of extremophiles, most notably the halophile *D. salina* and the acidophiles *Dunaliella acidophila* and *Chlamydomonas acidophila* (Varshney et al. 2015). Chlamydomonadalean algae are generally easy to collect, cultivate, and manipulate, and many species are available from public culture collections. Most importantly, there is a large arsenal of genomic and molecular tools that are freely available, including complete genome and transcriptome sequences as well as a host of well-described mutants (Umen and Olson 2012).

Cold-adapted Chlamydomonadales are no less diverse and represent some of the best-studied photopsychrophiles to date. These organisms can be found in a number of polar and alpine environments, such as ice-covered lakes, sea ice, transitory meltwater ponds, and snowfields. They often employ common strategies for coping in permanently cold environments, including the presence of high levels of polyunsaturated fatty acids in their lipid membranes, cold-adapted enzymes, and cryoprotectant compounds. These features can be found in a variety of other photosynthetic and non-photosynthetic organisms living in the cold and have been reviewed in detail elsewhere (Morgan-Kiss et al. 2006; Dolhi et al. 2013; Siddiqui et al. 2013; Lyon and Mock 2014). Certain psychrophilic species, however, are highly specialized and harbour unique features that help them survive in their extreme environments, especially regarding traits related to their photosynthetic machinery. In the following sections, we highlight a few best-known chlamydomonadalean psychrophiles and their shared and unique qualities (Fig. 2).

Chlamydomonas sp. UWO241: the evolution of a lean, green photopsychrophilic machine

The McMurdo Dry Valleys of Antarctica are home to many perennially ice-covered lakes, including Lake Bonney, which is fast becoming one of the leading natural laboratories for studying psychrophily. The frozen surface of Lake Bonney minimizes sediment input and wind-driven mixing, resulting in a uniquely hermetic and stratified environment, largely sheltered from human impact. The organisms living in Lake Bonney, in addition to perpetual cold, experience prolonged periods of darkness, low annual levels of photosynthetically active radiation (PAR), reduced concentrations of inorganic nutrients, and sustained physical and chemical gradients (Morgan-Kiss et al. 2006). Relatively few species can withstand such conditions, and as such, Lake Bonney is characterized by extremely simplified food webs composed of microorganisms. The key eukaryotic communities are strongly stratified in the water column and dominated by chlorophytes, cryptomonads, stramenopiles, and haptophytes. These organisms possess diverse metabolic capabilities (photoautotrophy, mixotrophy, heterotrophy, and/or parasitism), which likely contributes to their survival in this harsh environment (Bielewicz et al. 2011; Li et al. 2016). The unicellular, biflagellate green alga *Chlamydomonas* sp. UWO241 (hereafter UWO241) is one of these organisms.

UWO241 was first isolated more than two decades ago from the frigid, dark waters of Lake Bonney, 17 metres below the surface. It was initially identified as *Chlamydomonas subcaudata* based on cell morphology (Neale and Priscu 1995) and later as *Chlamydomonas raudensis* based on gene sequencing (Pocock et al. 2004), but recent studies have shown that UWO241 is a unique lineage within the Moewusinia clade of the Chlamydomonadales (Fig. 2;

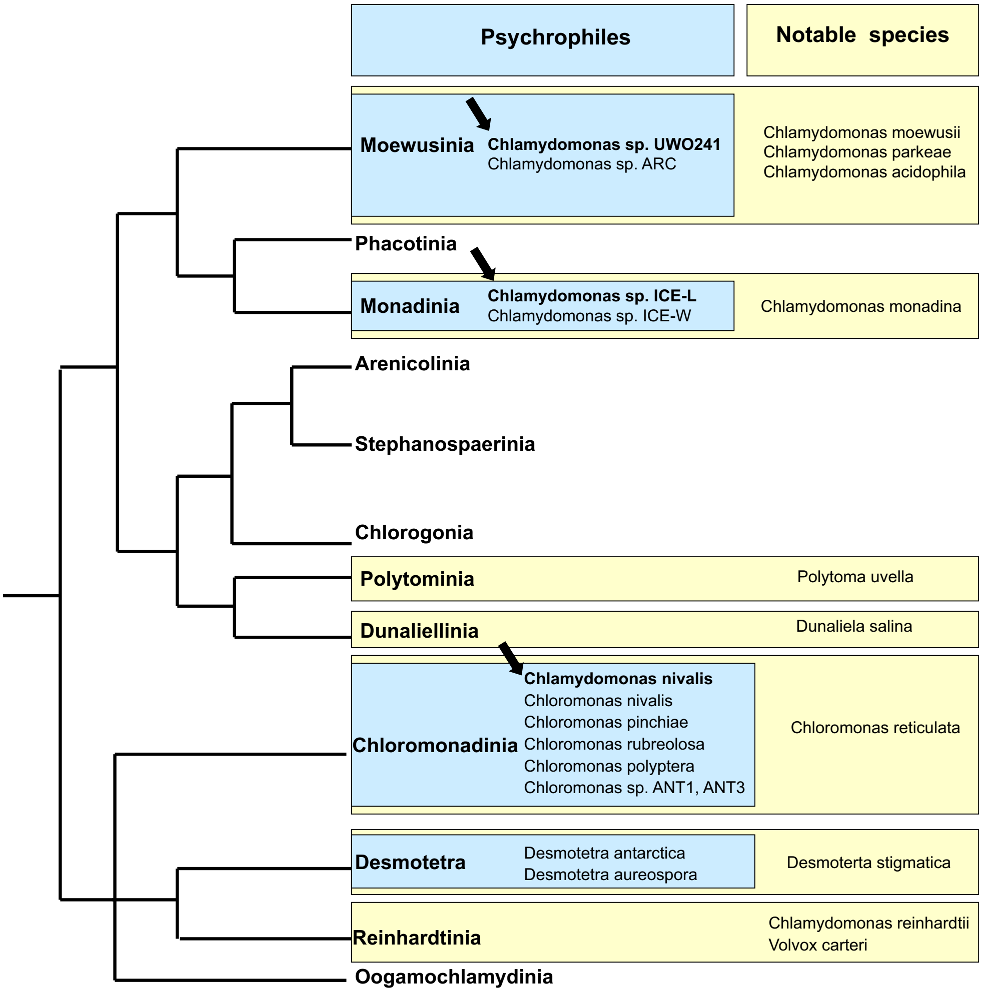

Fig. 2 The position of the known psychrophiles in the Chlamydomonadales phylogenetic tree in relation to important model species (adapted from Nakada et al. 2008; Possmayer et al. 2016). Note that only species with known phylogenetic relationships are included. The three best-studied psychrophilic species described in this review (*Chlamydomonas* sp. UWO241; *Chlamydomonas* sp. ICE-L; *Chlamydomonas nivalis*) are highlighted with a black arrow

Possmayer et al. 2016). In addition to withstanding constantly cold temperatures (~5 °C year round), UWO241 is exposed to perpetual shading (5 µmol photons m^{-2} s^{-1} during midday in summer) and seasonal extremes in photoperiod (e.g. 24-h light during the peak summer), which are biased towards the blue-green spectrum (450–550 nm). Lake Bonney is also phosphorus limited and has a high level of dissolved oxygen (200 % saturation) and high salinity (0.7 M). Consequently, UWO241 has evolved a number of unique cellular and physiological features to cope with the extreme conditions of Lake Bonney, as discussed previously (Morgan-Kiss et al. 2006; Dolhi et al. 2013) (Online resource 2).

The origin of UWO241 in Lake Bonney is unknown, but it might be a descendant from a recent marine ancestor: UWO241 is closely related to the marine alga *Chlamydomonas parkeae* (Possmayer et al. 2016), and Lake Bonney has experienced marine incursions (Laybourne-Parry and Pearce 2007). Alternatively, wind could have carried UWO241 to Lake Bonney during the peak summer months when the lake's edges are often melted. In fact, UWO241 DNA was recovered from a mixed culture grown from a cyanobacterial mat embedded in Lake Bonney surface ice (Raymond and Morgan-Kiss 2013). Wherever it originally came from, UWO241 has undoubtedly undergone significant changes since arriving in Lake Bonney.

At first glance, UWO241 looks similar in structure to its close chlamydomonadalean relatives: it is a 10–15-µm, biflagellated unicell with a single chloroplast, a basally located pyrenoid, and a small eyespot (Fig. 3). Closer inspection, however, reveals a green alga unlike any other—one that has been shaped by the extraordinary conditions of Lake Bonney (Fig. 4). For example, UWO has an unconventional photosynthetic apparatus, tailored to work best at ~8 °C, where it exhibits rates of photosynthesis comparable to those of *C. reinhardtii* at 25–35 °C.

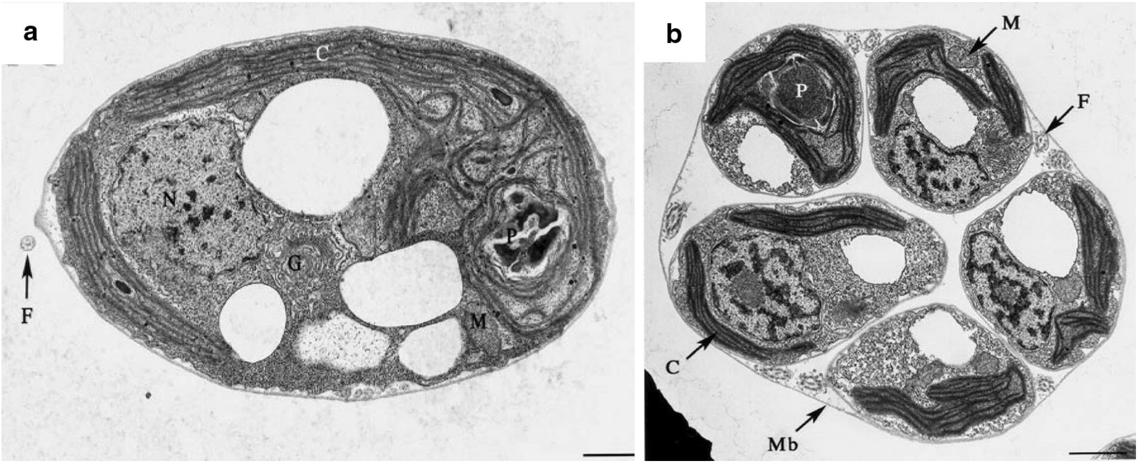

Fig. 3 Electron micrographs of UWO241 grown under laboratory-controlled conditions (8°C/20 μmol photons m^{-2} s^{-1}) showing a single cell (**a**) and a non-motile palmelloid (**b**). *P* pyrenoid, *C* chloroplast, *N* nucleus, *G*, Golgi apparatus, *M* mitochondrion, *F* flagellum. *Scale bars* 1.0 μm (Pocock et al. 2004)

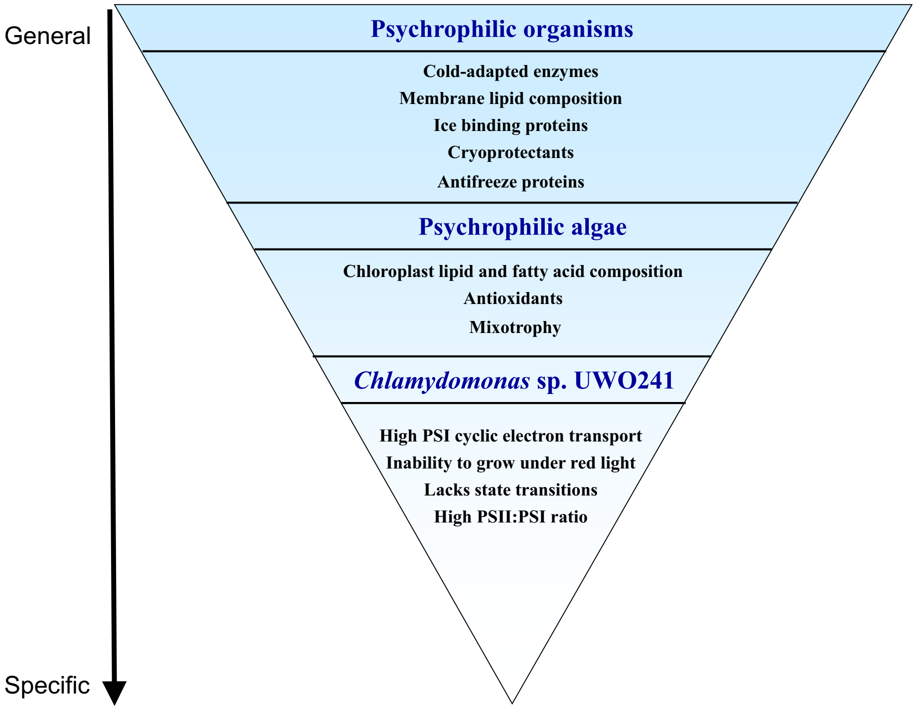

Fig. 4 A hierarchy of characteristics found in psychrophilic organisms. General features are found in most psychrophiles from different taxa and can be considered universal adaptations to permanently cold temperatures. These include changes in the membrane composition to increase its fluidity, the presence of cold-adapted proteins, ice-binding proteins, antifreeze proteins, and cryoprotectants. In addition to these features, psychrophilic algae exhibit adaptations related to their photosynthetic lifestyle, such as chloroplast membrane composition, increased levels of antioxidants, and a mixotrophic lifestyle. Note that some of these survival strategies are also found in organisms living in different harsh environments (i.e. low nutrient stress, Zubkov and Tarran 2008). Finally, it can be expected that individual species will develop a set of unique characteristics due to their specific habitat. UWO241, for instance, has adapted to the environment in Lake Bonney which resulted in the appearance of unique characteristics, such as the lack of state transitions, increased PSI cyclic electron flow, and the inability to grow under red light. We currently do not know whether these characteristics are caused by cold temperatures, the specific light environment that this organism has adapted to, high salinity, or by a combination of multiple factors

Moreover, because it can rapidly repair photosystem II reaction centres, UWO241 can avoid photoinhibition at low temperatures. UWO241 can also shift from predominantly motile single cells at low temperatures to non-motile palmelloid colonies upon exposure to higher temperatures (Pocock et al. 2007).

Among the many unconventional features of UWO241 is its inability to undergo photosynthetic state transitions (i.e. state 1–state 2 transitions), which balance the energy distribution between photosystems I and II (PSI and PSII) (Morgan-Kiss et al. 2002a). Algae regulate the light-harvesting capacities of the two photosystems to optimize rates of photosynthetic electron flow. This regulation, which is mediated by the cytochrome *b6/f* complex and triggered by changes in the redox status of the photosynthetic electron transport chain, results in the reversible phosphorylation of light-harvesting complex II (LHCII) polypeptides and their physical shuttling from PSII to PSI (Rochaix 2014).

UWO241 is unique among all natural photosynthetic organisms studied to date, in that it is permanently locked in State 1 and is unable to reversibly shift to State 2 (Morgan-Kiss et al. 2006). The basis for this unorthodox form of photosynthesis is unknown, but likely linked to the photosynthetic machinery of UWO241. The cytochrome f protein (the largest subunit of the cytochrome *b6/f* complex) from UWO241 has subtle yet important differences in its primary amino acid sequence as compared to its *C. reinhardtii* counterpart, particularly in the small domain, which can impact the enzymes' molecular structure and physical properties. Nevertheless, complementation of the UWO241 gene for cytochrome f (*petA*) in a *C. reinhardtii* background did not prevent state transitions in this model organism (Gudynaite-Savitch et al. 2006). UWO241 does, however, phosphorylate a novel PsbP-like protein associated with the PSI cytochrome *b6/f* supercomplex rather than LHCII polypeptides—a strategy that appears to regulate cyclic electron flow around PSI. It is therefore possible that instead of state transitions, UWO241 achieves optimal rates of photosynthesis via PSI cyclic electron transport (Szyszka-Mroz et al. 2015). It is not yet known whether the lack of state transitions and the induction of PSI electron flow in UWO241 is an adaptation to permanently low temperatures or a trait that has developed in response to other stimuli, such as the precarious availability of sunlight and/or high salt concentrations.

In addition to coping with low temperatures, UWO241 has to survive the austral winter. Polar algae have developed different strategies for withstanding sustained darkness, such as mixotrophy, cyst formation, breakdown of carbohydrate storage molecules, and/or changes to their photosynthetic machinery (Lyon and Mock 2014). The psychrophilic seaweed *Gigartina skottsbergii* (Wiencke 1990), for instance, grows whenever light conditions are favourable. Conversely, UWO241 and other polar algae have a seasonal growth pattern, likely regulated by photoperiod (Morgan-Kiss et al. 2016). The population size of UWO241 in Lake Bonney plummets in early austral winter and then starts to increase in late winter, reaching its maximum level in austral spring, thus allowing the alga to enter the short summer season with an actively growing population (Morgan-Kiss et al. 2006).

A recent study transplanted UWO241 laboratory cultures to the water column of Lake Bonney (Morgan-Kiss et al. 2016) and revealed that during the shift to darkness there was a reduction in the expression of photosynthetic genes and a decline in the fixation of inorganic carbon. This suggests that in the dark UWO241 undergoes a cascade of physiological and molecular alterations to the photosynthetic apparatus, which can quickly be reversed when light becomes available again. For instance, the light-harvesting antenna in UWO241 could shift from an energy dissipation-protective mechanism in winter to an efficient light energy transfer mechanism in summer (Morgan-Kiss et al. 2016). This is in line with data from other photopsychrophiles showing that in darkness the photosynthetic apparatus is kept in a downregulated but functional form (Baldisserotto et al. 2005), which can be rapidly reactivated by sunlight. Such a strategy would presumably be advantageous in polar environments where the growing season is short. Alternatively, UWO241 could undergo a dormant stage as is seen in other polar Chlamydomonadales. For example, the snow alga *Chloromonas nivalis* produces zygotes late into the growing season, which have a deep red colour due to carotenoid accumulation and thick cell walls resistant to harsh conditions (Remias et al. 2010b). Unfortunately, few studies have examined the survival strategies of polar algae to prolonged darkness, and more work is needed to understand this intriguing topic.

Chlamydomonas nivalis: evolution under a bright spotlight

Alpine and polar snowfields are harsh environments characterized by low temperatures, extremely high light levels (up to 5000 μmol photons m^{-2} s^{-1}), intense UV radiation, and frequent freeze–thaw cycles. Yet, there are rich and metabolically active microbial communities within snowfields (Brown et al. 2015). "Coloured snow" is the macroscopic expression of massive algal blooms composed of hundreds of chlorophytes, mainly from the genera *Chlamydomonas* and *Chloromonas* (Hoham and Duval 2001). One of the best-known snow algae is *Chlamydomonas nivalis*, which causes the well-documented phenomenon of "watermelon snow"—red, sweet-smelling patches of snow on exposed snowfields at high altitudes. The cells that make up "watermelon snow" are typically non-motile, red spheroids with a thick cell wall. Although these red cells were originally described as a separate species, it was recently recognized that they are the diploid zygotic stage of the green alga *Chlamydomonas nivalis*, which has a complex life cycle. During warmer summer season, these zygotes undergo meiosis and produce green, motile, and haploid offspring that can multiply asexually. The green motile stage is short-lived, susceptible to freezing damage (Bidigare et al. 1993), and produces a new zygote late in the growing season, which can withstand winter freezing (Müller et al. 1998). This switch from an active, mobile stage to a dormant stage is a common strategy for surviving unfavourable conditions and has been described for other psychrophilic species, including *Chloromonas nivalis* (Remias et al. 2010b), as well as for prasinophytes (Daugbjerg et al. 2000) and dinoflagellates (Flaim et al. 2010).

Originally thought to be a cosmopolitan psychrophile found in polar and alpine snowfields worldwide, *Chlamydomonas nivalis* and related *Chloromonas* species form a closely knit collective of taxa, which are part of the larger Chloromonadinia clade of the Chlamydomonadales (Fig. 2). Recent molecular sequencing studies have shown that isolates of these algae from different geographical locations represent closely related but distinct lineages (Matsuzaki et al. 2015), comprised of both psychrophiles and psychrotrophs (Lukeš et al. 2014). The presence of cold-adapted and cold-tolerant species within the same small group provides an excellent system for studying the emergence of psychrophily, both evolutionarily and physiologically.

A conspicuous feature of *Chlamydomonas nivalis* and its sister species is the abundance of the red secondary pigment astaxanthin (Remias et al. 2005). This keto-carotenoid and its esterified derivatives are present at low levels during the green motile life stages, but accumulate at high levels in cytoplasmic lipid bodies as the cells mature into resting spores (Řezanka et al. 2013; Remias et al. 2016). The physiological role of astaxanthin in these algae is multifold: it prevents photoinhibition and UV damage by protecting the chloroplast from excessive light, likely acts as a powerful antioxidant, and is a metabolic sink for the non-dividing yet metabolically active spores (Remias et al. 2010a, 2016). Although it provides protection from high light, astaxanthin is not unique to snow algae and can be found in high concentrations in other species. For instance, the pathways involved in astaxanthin synthesis have been well defined in the cosmopolitan chlamydomonadalean *Haematococcus pluvialis* (Lemoine and Schoefs 2010), which has been isolated from various habitats worldwide, including the Arctic (Klochkova et al. 2013). In *H. pluvialis*, astaxanthin accumulation is regulated by ROS and the chloroplast redox state, but it is not yet known whether a similar regulation mechanism exists in snow algae.

Chlamydomonas sp. ICE-L: at the forefront of molecular studies on psychrophiles

Since it was first isolated from floating marine ice near the Antarctic coast (Liu et al. 2006a), *Chlamydomonas* sp. ICE-L has been a leading system for studying the molecular biology of psychrophiles. Research on this alga has led to novel methods for isolating protoplasts (Liu et al. 2006b), preparing cDNA libraries (Wu et al. 2010), expressing cold-adapted proteins (Liu et al. 2011), and validating housekeeping genes for real-time PCR (Liu et al. 2012; Mou et al. 2015). *C.* sp ICE-L has also become an attractive target for biofuel production (Mou et al. 2012) and is a promising candidate for space research (Gao et al. 2013) because it can withstand hyper-gravitational stress.

C. sp ICE-W belongs to the Monadina clade of the Chlamydomonadales (Fig. 2), and closely related organisms have been detected in other Antarctic locations such as ice-covered lakes (Li et al. 2016) and cyanobacterial mats collected from freshwater systems (Jungblut et al. 2012). Researchers are currently trying to understand how this psychrophile can survive the harsh conditions in floating ice. For instance, Wang et al. (2015) showed that in *C.* sp ICE-L primary nitrogen metabolism is tightly regulated by light exposure, suggesting that this is a strategy for surviving continuous light in summer and sustained darkness in winter. Similarly, Mou et al. (2013) suggested that *C.* sp ICE-L protects its photosynthetic apparatus during periods of high light by dissipating light energy via a highly inducible non-photochemical quenching (NPQ) mechanism.

C. sp. ICE-L can also synthesize large quantities of polyunsaturated fatty acids (PUFAs), which increase the fluidity of its cell and chloroplast membranes. High levels of PUFAs have been found in a wide diversity of polar algae (Becker et al. 2011; Lyon and Mock 2014), including UWO241 (Morgan-Kiss et al. 2002b). The importance of PUFAs in photosynthetic and non-photosynthetic psychrophiles has been previously recognized (Siddiqui et al. 2013), but researchers are only just beginning to locate the genes for PUFA synthesis in cold-adapted algae. Transcriptomic analyses uncovered five fatty acid desaturases (FADs) in *C.* sp. ICE-L (Zhang et al. 2011; An et al. 2013a). Three of these transcripts (all omega-3 FADs) were found to be of vital importance for low-temperature acclimation because their expression increased with decreasing temperatures and coincided with the accumulation of lipids and fatty acids. These three FADs were also induced under hypersaline conditions (An et al. 2013b), demonstrating that increased levels of PUFAs are vital not only under temperature stress, but also under salinity stress. FAD genes have also been found in UWO241 (Dolhi et al. 2013) and are particularly abundant in the genome of the psychrotroph *C. subellipsoidea* (Blanc et al. 2012). A global transcriptomic study of *C.* sp. ICE-L showed that FAD genes, as well as genes encoding molecular chaperones and membrane transport proteins, have high levels of sequence identity to genes present in psychrophilic Antarctic bacteria (Liu et al. 2016). Thus, *C.* sp. ICE-L might have acquired bacterial FADs via horizontal gene transfer. Eukaryotic gene acquisition from prokaryotes is believed to be a driving force for evolution in extreme environments (Schönknecht et al. 2014) and is the possibility of the mechanism through which UWO241 and other psychrophiles acquired genes for ice-binding proteins (Raymond and Kim 2012; Raymond and Morgan-Kiss 2013; Raymond 2014). Future analysis of these gene sequences will help elucidate the origins of lipid metabolism in psychrophilic algae.

Adaptations or genetic drift?

Although many aspects of psychrophily are poorly understood, it is clear that it is a complex collection of traits and, thus, challenging to understand from an evolutionary standpoint. The study of distantly related psychrophiles (Siddiqui et al. 2013) has shown that psychrophily requires the combination of several traits, such as enzymes that function at low temperatures, increased membrane fluidity, and antifreeze compounds. However, our understanding of psychrophily is largely shaped from studies on bacteria and invertebrates, and the field as a whole is still in infancy, especially regarding photosyntheses at cold temperatures. Data on polar and alpine algae are slowly accumulating, providing a glimpse into the complex evolutionary patterns that govern psychrophily in photosynthetic species. Although detailed information for individual species is often limited, broadscale analyses are revealing common traits between diverse psychrophilic taxa. This, in turn, will help researchers understand which traits for cold tolerance are more easily acquired than others and whether they are acquired in a particular order.

As demonstrated above, closely related chlamydomonadalean psychrophiles exist in environments with extremely high light (e.g. *C. nivalis*) as well as those exposed to extreme shade and/or sustained darkness (e.g. UWO241). Although it is tempting to link the various physiological traits of these species with natural selection, one cannot ignore the effects of nonadaptive (i.e. neutral) processes, such as random genetic drift, recombination, and mutation, in shaping the physiological make-up of these organisms. Non-adaptive processes might be especially important for species with small population sizes, such as those existing in highly confined ecosystems. For instance, certain permanently ice-covered lakes in the Antarctic have been cut off from the atmosphere and other water bodies for thousands of years (Laybourne-Parry and Pearce 2007), including the lake from which UWO241 was isolated. As already mentioned, UWO241 is the only known green alga that lacks state transitions. Unlike UWO241, the psychrophilic trebouxiophyte *Chlorella* sp. BI isolated from an Antarctic transitory pond can dynamically regulate energy distribution between PSI and PSII (Morgan-Kiss et al. 2008). The lack of state transitions in UWO241 and its potential dependence on cyclic electron flow around PSI could simply be a consequence of neutral or slightly deleterious mutations being fixed by random genetic drift (Lynch 2007). Indeed, other novel evolutionary features of chlamydomonadalean algae, including the origins of multicellularity within this group, are believed to have arisen through nonadaptive evolution (Libby and Ratcliff 2014).

Life at the edge: future work

The origins and evolution of life in extreme environments has always intrigued scientists. Although the Earth abounds with extremophiles, including psychrophiles, few are currently used as model research organisms. Consequently, we know relatively little about the genetic and physiological features that allow species to endure what often appears to be the unendurable. Psychrophiles epitomize the existence of life at the edge. Today, with rapid climate change upon us, studying species that thrive under such severe conditions is more important than ever. In contrast to the study of model organisms, the study of non-model extremophiles has the potential to provide novel insights into mechanisms by which evolution can overcome the constraints imposed by extreme environmental conditions. Since the polar regions have been and will continue to be impacted by changing climate (IPCC 2007), organisms living life at the edge represent important biological markers for monitoring the impacts of climate change on biodiversity. Garnering information on the biodiversity and physiology of microbial primary producers and defining their role in the making and recycling of organic matter have been defined as priorities for polar science in the next decades (Kennicutt II and Chown 2014; Xavier et al. 2016).

Chlamydomonadalean algae as a group are ideal models for studying psychrophily, especially since they harbour several well-studied psychrophilic species, such as UWO241, *C. nivalis*, and *C.* sp. ICE-L. These algae are excellently placed for comparative investigations with photosynthetic, non-photosynthetic, and psychrophilic algae. The next logical step is to obtain complete genome and transcriptome sequences of these psychrophiles and compare them to those from other Chlamydomonadales. Comparative genomics of a "true" psychrophile with those from closely related mesophiles should reveal how photosynthetic algae function under extreme conditions. Key questions that could be answered include: What is the gene composition of a psychrophilic alga and how does it compare to its mesophilic counterparts? Are there unique genes or pathways present or absent from psychrophiles?

Other interesting avenues will be the comparison between closely related psychrophiles and psychrotrophs, such as comparisons between different strains of the snow alga *Chlamydomonas nivalis* (Lukeš et al. 2014). Exploring the loss of the ability to survive at mesophilic temperatures will give further insights into the evolution of psychrophily. Finally, we illustrated that psychrophiles originating from different habitats show a remarkable diversity in their adaptive characteristics despite their close phylogenetic relationships. Comparing such species will allow us to explore not only the evolution of psychrophily, but also some of the fundamental mechanisms of how stressful environments shape the genetic and physiological make-up of photosynthetic organisms. Addressing these and similar topics will surely improve our understanding of polar bioenergetics, metabolic fluxes, and the input that psychrophilic algae have on global biochemical processes.

However, exposing the complexities of psychrophilic algae will require much more than genome sequencing and bioinformatics. Detailed and sophisticated bench work will be needed to unravel the mysteries surrounding the lack of state transitions in UWO241, for example, or how so many photopsychrophiles can survive prolonged darkness. Population genetic analyses and mutation rate estimates of psychrophiles will be particularly interesting and could help researchers understand the evolutionary origins—be they adaptive or non-adaptive—of the various psychrophilic traits.

Acknowledgments MC is supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council (NSERC) of Canada. NPAH and DRS are each funded by a Discovery Grant from NSERC. NPAH is grateful for support through the Canadian Foundation for Innovation and the Canada Research Chairs Programme.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Aletsee L, Jahnke J (1992) Growth and productivity of the psychrophilic marine diatoms *Thalassiosira antarctica* Comber and *Nitzschia frigida* Grunow in batch cultures at temperatures below the freezing point of sea water. Polar Biol 11:643–647

An M, Mou S, Zhang X, Ye N, Zheng Z, Cao S, Xu D, Fan X, Wang Y, Miao J (2013a) Temperature regulates fatty acid desaturases at a transcriptional level and modulates the fatty acid profile in the Antarctic microalga *Chlamydomonas* sp. ICE-L. Bioresour Technol 134:151–157

An M, Mou S, Zhang X, Zheng Z, Ye N, Wang D, Zhang W, Miao J (2013b) Expression of fatty acid desaturase genes and fatty acid accumulation in *Chlamydomonas* sp. ICE-L under salt stress. Bioresour Technol 149:77–83

Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou SG, Allen AE, Apt KE, Bechner M et al (2004) The genome of the diatom *Thalassiosira pseudonana*: ecology, evolution, and metabolism. Science 306:79–86

Baldisserotto C, Ferroni L, Moro I, Fasulo MP, Pancaldi S (2005) Modulations of the thylakoid system in snow xanthophycean alga cultured in the dark for two months: comparison between microspectrofluorimetric responses and morphological aspects. Protoplasma 226:125–135

Becker S, Quartino ML, Campana GL, Bucolo P, Wiencke C, Bischof K (2011) The biology of an Antarctic rhodophyte, *Palmaria decipiens*: recent advances. Antarct Sci 23:419–430

Bidigare RR, Ondrusek ME, Kennicutt MC, Iturriaga R, Harvey HR, Hoham RW, Macko SA (1993) Evidence for a photoprotective function for secondary carotenoids of snow algae. J Psychol 29:427–434

Bielewicz S, Bell E, Kong W, Friedberg I, Priscu JC, Morgan-Kiss RM (2011) Protist diversity in a permanently ice-covered Antarctic lake during the polar night transition. ISME J 5:1559–1564

Blanc G, Duncan G, Agarkova I, Borodovsky M, Gurnon J, Kuo A, Lindquist E, Lucas S, Pangilinan J, Polle J et al (2010) The *Chlorella variabilis* NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. Plant Cell 22:2943–2955

Blanc G, Agarkova I, Grimwood J, Kuo A, Brueggeman A, Dunigan DD, Gurnon J, Ladunga I, Lindquist E, Lucas S et al (2012) The genome of the polar eukaryotic microalga *Coccomyxa subellipsoidea* reveals traits of cold adaptation. Genome Biol 13:1–12

Bligh J, Johnson KG (1973) Glossary of terms for thermal physiology. J Appl Physiol 35:941–961

Bravo L, Saavedra-Mella FA, Vera F, Guerra A, Cavieres LA, Ivanov AG, Hüner NP, Corcuera LJ (2007) Effect of cold acclimation on the photosynthetic performance of two ecotypes of *Colobanthus quitensis* (Kunth) Bartl. J Exp Bot 58:3581–3590

Brown SP, Olson BJSC, Jumpponen A (2015) Fungi and algae co-occur in snow: an issue of shared habitat or algal facilitation of heterotrophs. Arct Antarct Alp Res 47:429–749

Burki F (2014) The eukaryotic tree of life from a global phylogenomic perspective. Cold Spring Harb Perspect Biol 6:a016147. doi:10.1101/cshperspect.a016147

Chaux F, Peltier G, Johnson X (2015) A security network in PSI photoprotection: regulation of photosynthetic control, NPQ and O_2 photoreduction by cyclic electron flow. Front Plant Sci 6:875. doi:10.3389/fpls.2015.00875

Christmas NAM, Anesio AM, Sánchez-Baracaldo P (2015) Multiple adaptations to polar and alpine environments within cyanobacteria: a phylogenomic and Bayesian approach. Front Microbiol 6:1070. doi:10.3389/fmicb.2015.01070

Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. Nature 438:90–93

Curtis BA, Tanifuji G, Burki F, Gruber A, Irimia M, Maruyama A, Arias MC, Ball SG, Gile GH, Hirakawa Y et al (2012) Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. Nature 492:59–65

Dahal K, Knowles VL, Plaxton WC, Hüner NPA (2014) Enhancement of photosynthetic performance, water use efficiency and grain yield during long-term growth under elevated CO_2 in wheat and rye is growth temperature and cultivar dependant. Environ Exp Bot 106:207–220

Daugbjerg N, Marchant HJ, Thomsen HA (2000) Life history stages of *Pyramimonas tychotreta* (Prasinophyceae, Chlorophyta), a marine flagellate from the Ross Sea, Antarctica. Phycol Res 48:199–209

Delmont TO, Hammar KM, Ducklow HW, Yager PL, Post AF (2014) *Phaeocystis antarctica* blooms strongly influence bacterial community structures in the Amundsen Sea polynya. Front Microbiol 5:646. doi:10.3389/fmicb.2014.00646

Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309

Demmig-Adams B, Adams WW III (1996) Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. Planta 198:460–470

Derks A, Schaven K, Bruce D (2015) Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. Biochim Biophys Acta 1847:468–485

Dietz K-J (2015) Efficient high light acclimation involves rapid processes at multiple mechanistic levels. J Exp Bot 66:2401–2414

Dolhi JM, Maxwell DP, Morgan-Kiss RM (2013) Review: the *Antarctic Chlamydomonas raudensis*: an emerging model for cold adaptation of photosynthesis. Extremophiles 17:711–722

Dolhi JM, Teufel AG, Kong W, Morgan-Kiss RM (2015) Diversity and spatial distribution of autotrophic communities within and between ice-covered Antarctic lakes (McMurdo Dry Valleys). Limnol Oceanogr 60:977–991

Eddie B, Krembs C, Neuer S (2008) Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi sea ice. Mar Ecol Prog Ser 354:107–117

Feller G, Gerday C (2003) Psychrophilic enzymes: hot topic in cold adaptation. Nat Rev Microbiol 1:200–208

Figueroa-Martinez F, Nedelcu AM, Smith DR, Reyes-Prieto A (2015) When the lights go out: the evolutionary fate of free-living colorless green algae. New Phytol 206:972–982

Flaim G, Rott E, Frassanito R, Guella G, Obertegger U (2010) Eco-fingerprinting of the dinoflagellate *Borghiella dodgei*: experimental evidence of a specific environmental niche. Hydrobiologia 639:85–98

Gao Z, Li D, Meng C, Xu D, Zhang X, Ye N (2013) Survival and proliferation characteristics of the microalga *Chlamydomonas* sp. ICE-L after hypergravitational stress pretreatment. Icarus 226:971–979

Gómez I, Wulff A, Roleda MY, Huovinen P, Karsten U, Quartino ML, Dunton K, Wiencke C (2009) Light and temperature demands of marine benthic macroalgae and seaweeds in polar regions. Bot Mar 52:593–608

Gudynaite-Savitch L, Gretes M, Morgan-Kiss R, Savitch L, Simmonds J, Kohalmi S, Hüner NPA (2006) Cytochrome *f* from the Antarctic psychrophile, *Chlamydomonas raudensis* UWO241: structure, sequence, and complementation in the mesophile, *Chlamydomonas reinhardtii*. Mol Gen Genomics 275:387–398

Guiry MD, Guiry GM (2016) AlgaeBase. World-wide electronic publication. National University of Ireland, Galway. http://www.algaebase.org

Hanschen ER, Marriage TN, Ferris PJ, Hamaji T, Toyoda A, Fujiyama A, Neme R, Noguchi H et al (2016) The Gonium pectorale genome demonstrates co-option of cell cycle regulation during the evolution of multicellularity. Nat Commun 7:11370. doi:10.1038/ncomms11370

Hoffman PF, Schrag DP (2002) The snowball Earth hypothesis: testing the limits of global change. Terra Nova 14:129–155

Hoham RW, Duval B (2001) Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: Johns HG, Pomeroy JW, Walker DA, Hoham RS (eds) Snow ecology. Cambridge University Press, Cambridge, pp 186–203

Hüner NPA, Grodzinski B (2011) Photosynthesis and photoautotrophy. In: Moo-Young M (ed) Comprehensive biotechnology, 2nd edn. Elsevier, Amsterdam, pp 315–322

Hüner NPA, Öquist G, Sarhan F (1998) Energy balance and acclimation to light and cold. Trends Plant Sci 3:224–230

Hüner NPA, Bode R, Dahal K, Busch FA, Possmayer M, Szyszka B, Rosso D, Ensminger I, Krol M, Ivanov AG, Maxwell DP (2013) Shedding some light on cold acclimation, cold adaptation, and phenotypic plasticity. Botany 91:127–136

Hyde WT, Crowley TJ, Baum SK, Peltier RW (2000) Neoproterozoic 'snowball Earth' simulations with a coupled climate/ice-sheet model. Nature 405:425–429

IPCC (2007) Climate change 2007: The physical science basis. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jungblut AD, Vincent WF, Lovejoy C (2012) Eukaryotes in Arctic and Antarctic cyanobacterial mats. FEMS Microbiol Ecol 82:416–428

Kaczmarska I, Mather L, Luddington IA, Muise F, Ehrman JM (2014) Cryptic diversity in a cosmopolitan diatom known as *Asterionellopsis glacialis* (Flagilariaceae): implications for ecology, biogeography and taxonomy. Am J Bot 101:267–286

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science 284:654–657

Keeling PJ (2013) The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. Annu Rev Plant Biol 64:583–607

Keeling PJ, Burki F, Wilcox HM, Allam B, Allen EE, Amaral-Zettler LA, Armbrust EV, Archibald JM, Bharti AK, Bell CJ et al (2014) The marine microbial eukaryote transcriptome sequencing project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. PLoS Biol 12:e1001889. doi:10.1371/journal.pbio.1001889

Kennicutt MC II, Chown SL (2014) Polar research: six priorities for Antarctic science. Nature 512:23–25

Kirchhoff H (2013) Architectural switches in plant thylakoid membranes. Photosynth Res 116:481–487

Klochkova TA, Kwak MS, Han JW, Motomura T, Nagasato C, Kim GH (2013) Cold-tolerant strain of *Haematococcus pluvialis* (Haematococcaceae, Chlorophyta) from Blomstrandhalvoya (Svalbard). Algae 28:185–192

Koch F, Marcoval MA, Panzeca C, Bruland KW, Sanudo-Wilhelmy SA, Gobler CJ (2011) The effect of vitamin B_{12} on phytoplankton growth and community structure in the Gulf of Alaska. Limnol Oceanogr 56:1023–1034

Komárek J, Nedbalová L (2007) Green cryosestic algae. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 323–342

Kvíderová J (2011) Research on cryosestic communities in Svalbard: the snow alga of temporary snowfields in Petuniabukta, Central Svalbard. Czech Polar Rep 2:8–19

Laybourne-Parry J, Pearce DA (2007) The biodiversity and ecology of Antarctic lakes: models for evolution. Philos Trans R Soc Lond B 362:2273–2289

Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, Delwiche CF, De Clerck O (2012) Phylogeny and molecular evolution of the green algae. Crit Rev Plant Sci 31:1–46

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

Li W, Podar M, Morgan-Kiss RM (2016) Ultrastructural and single-cell-level characterization reveals metabolic diversity in microbial eukaryote community from an ice-covered Antarctic lake. Appl Environ Microbiol 82:3659–3670

Libby E, Ratcliff WC (2014) Ratcheting the evolution of multicellularity. Science 346:426–427

Liu C, Huang XH, Wang XL, Zhang XC, Li GY (2006a) Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. Phycologia 45:190–198

Liu S, Liu C, Huang X, Chai Y, Cong B (2006b) Optimization of parameters for isolation of protoplasts from the antarctic sea ice alga *Chlamydomonas* sp. ICE-L. J Appl Phycol 18:783–786

Liu Y, Ding Y, Jian J-C, Wu Z-H, Miao J-L (2011) Prokaryotic expression and its conditional optimization of glutathione reductase gene of antarctic *Chlamydomonas* sp. ICE-L. Oceanol Limnol Sin 42:817–821

Liu C, Wu G, Huang X, Liu S, Cong B (2012) Validation of housekeeping genes for gene expression studies in an ice alga *Chlamydomonas* during freezing acclimation. Extremophiles 16:419–425

Liu C, Wang X, Wang X, Sun X (2016) Acclimation of Antarctic *Chlamydomonas* to the sea-ice environment: a transcriptomic analysis. Extremophiles 20:437–450

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 Microbiol Ecol 89:303–315

Lynch M (2007) The frailty of adaptive hypotheses for the origins of organismal complexity. PNAS USA 104:8597–8604

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

Margesin R, Schinner F, Marx J-C, Gerday C (2007) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin

Matsuzaki M, Misumi O, Shin-i T, Maruyama S, Takahara M, Miyagishima S, Mori T, Nishida K, Yagisawa F et al (2004) Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature 428:653–657

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

McKay CP (2000) Thickness of tropical ice and photosynthesis on a snowball Earth. Geophys Res Lett 27:2153–2156

McKie-Krisberg ZM, Gast RJ, Sanders RW (2015) Physiological responses of three species of Antarctic mixotrophic phytoflagellates to changes in light and dissolved nutrients. Microb Ecol 70:21–29

Melis A (1998) Photostasis in plants. In: Williams TP, Thistle AB (eds) Photostasis and related phenomena. Plenum Press, New York, pp 207–220

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Maréchal-Drouard L et al (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318:245–250

Mock T, Hoch N (2005) Long-term temperature acclimation of photosynthesis in steady-state cultures of the polar diatom *Fragilariopsis cylindrus*. Photosynth Res 85:307–317

Morgan-Kiss RM, Ivanov AG, Hüner NPA (2002a) The Antarctic psychrophile, *Chlamydomonas subcaudata*, is deficient in state I–state II transitions. Planta 214:435–445

Morgan-Kiss R, Ivanov AG, Williams J, Mobashsher K, Hüner NPA (2002b) Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga. Biochim Biophys Acta 1561:251–265

Morgan-Kiss RM, Priscu JS, Pocock T, Gudynaite-Savitch L, Hüner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Morgan-Kiss RM, Ivanov AG, Modla S, Czymmek K, Hüner NPA, Priscu JC, Lisle JT, Hanson TE (2008) Identity and physiology of new psychrophilic eukaryotic green alga, *Chlorella* sp., strain BI, isolated from a transitory pond near Bratina Island, Antarctica. Extremophiles 12:701–711

Morgan-Kiss RM, Lizzote MP, Kong W, Priscu JC (2016) Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake. Limnol Oceanogr 61:3–13

Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167

Mou S, Xu D, Ye N, Zhang X, Liang C, Liang Q, Zhen Z, Zhuang Z, Miao J (2012) Rapid estimation of lipid content in an Antarctic ice alga (*Chlamydomonas* sp.) using the lipophilic fluorescent dye BODIPY505/515. J Appl Phycol 24:1169–1176

Mou S, Zhang X, Ye N, Miao J, Cao S, Xu D, Fan X, An M (2013) Analysis of Delta pH and the xanthophyll cycle in NPQ of the Antarctic sea ice alga *Chlamydomonas* sp. ICE-L. Extremophiles 17:477–484

Mou S, Zhang X, Miao J, Zheng Z, Xu D, Ye N (2015) Reference genes for gene expression normalization in *Chlamydomonas* sp. ICE-L by quantitative real-time RT-PCR. J Plant Biochem Biotechnol 24:276–282

Müller T, Bleiβ W, Martin CD, Rogaschewski S, Fuhr G (1998) Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. Polar Biol 20:14–32

Nakada T, Misawa K, Nozaki H (2008) Molecular systematics of Volvocales (Chlorophyceae, Chlorophyta) based on exhaustive 18s rRNA phylogenetic analyses. Mol Phylogenet Evol 49:281–291

Neale PJ, Priscu JC (1995) The photosynthetic apparatus of phytoplankton from a perennially ice-covered Antarctic lake: acclimation to an extreme shade environment. Plant Cell Physiol 36:253–263

Newsted WJ, Hüner NPA (1987) Major sclerotial polypeptides of psychrophilic fungi: temperature regulation of in vivo synthesis in vegetative hyphae. Can J Bot 66:1755–1761

Newsted WJ, Hüner NPA, Insell JP, Griffith M, van Huystee RB (1985) The effects of temperature on the growth and polypeptide composition of several snow mold species. Can J Bot 63:2311–2318

Nisbet EG, Fowler CMR (1999) Archaean metabolic evolution in microbial mats. Proc R Soc Lond B Biol 226:2375–2382

Panikov NS, Sizova MV (2006) Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to −35 °C. FEMS Microbiol Ecol 59:500–512

Pocock TH, Lachance M-A, Pröschold T, Priscu JC, Kim SS, Hüner NPA (2004) Identification of psychrophilic green alga from lake Bonney, Antarctica: *Chlamydomonas raudensis* Ettl. (UWO 241) *Chlorophyceae*. J Phycol 40:1138–1148

Pocock TH, Koziak A, Rosso D, Falk S, Hüner NPA (2007) *Chlamydomonas raudensis* (UWO241), Chlorophyceae, exhibits the capacity for rapid D1 repair in response to chronic photoinhibition at low temperature. J Phycol 43:924–936

Possmayer M, Gupta RK, Szyszka-Mroz B, Maxwell DP, Lachance M-A, Hüner NPA, Smith DR (2016) Resolving the phylogenetic relationship between *Chlamydomonas* sp. UWO241 and *Chlamydomonas raudensis* SAG 49.72 (Chlorophyceae) with nuclear and plastid DNA sequences. J Phycol 52:305–310

Prochnik SE, Umen J, Nedelcu AM, Hallmann A, Miller SM, Nishii I, Ferris P, Kuo A, Mitros T, Fritz-Laylin LK et al (2010) Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. Science 329:223–226

Quartino ML, Zaixso HE, Boraso de Zaixso AL (2005) Biological and environmental characterization of marine macroalgal assemblages in Potter Cove, South Shetland Islands, Antarctica. Bot Mar 48:187–197

Quesada A, Vincent WF (2012) Cyanobacteria in the cryosphere: snow, ice and extreme cold. In: Whitton BA (ed) Ecology of cyanobacteria II. Springer, Amsterdam, pp 387–399

Raven JA (1997) Phagotrophy in phototrophs. Limnol Oceanogr 42:198–205

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

Raymond JA, Kim HJ (2012) Possible role of horizontal gene transfer in the colonization of sea ice by algae. PLoS ONE 7:e35968. doi:10.1371/journal.pone.0035968

Raymond JA, Morgan-Kiss R (2013) Separate origins of ice-binding proteins in Antarctic *Chlamydomonas* species. PLoS ONE 8:e59186. doi:10.1371/journal.pone.0059186

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

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

Remias D, Pichrtova M, Pangratz M, Luetz 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. doi:10.1093/femsec/fiw030

Řezanka T, Nedbalová L, Kolouchova I, Sigler K (2013) LC-MS/APCI identification of glucoside esters and diesters of astaxanthin from the snow alga *Chlamydomonas nivalis* including their optical stereoisomers. Phytochemistry 88:34–42

Rochaix J-D (2014) Regulation and dynamics of the light-harvesting system. Annu Rev Plant Biol 65:287–309

Rothschild LJ (2008) The evolution of photosynthesis…again? Philos Trans R Soc Lond B 363:2787–2801

Schlichting CD (2008) Hidden reaction norms, cryptic genetic variation and evolvability. Ann N Y Acad Sci 1133:187–203

Schönknecht G, Weber APM, Lercher MJ (2014) Horizontal gene acquisitions by eukaryotes as drivers of adaptive evolution. BioEssays 36:9–20

Shikanai T (2007) Cyclic electron transport around photosystem I: genetic approaches. Annu Rev Plant Biol 58:199–217

Siddiqui KS, Williams TJ, Wilkins D, Yau S, Allen MA, Brown MV, Lauro FM, Cavicchioli R (2013) Psychrophiles. Annu Rev Earth Planet Sci 41:87–115

Szyszka-Mroz B, Pittock PP, Ivanov AG, Hüner NPA (2015) The Antarctic psychrophile *Chlamydomonas* sp. UWO241 preferentially phosphorylates a photosystem I–cytochrome b6/f supercomplex. Plant Physiol 169:717–736

Thingstad TF, Havskum H, Garde K, Riemann B (1996) On the strategy of "eating your competitor": a mathematical analysis of algal mixotrophy. Ecology 77:2108–2118

Tikkanen M, Aro AM (2014) Integrative regulatory network of plant thylakoid energy transduction. Trends Plant Sci 19:10–17

Umen JG, Olson BJSC (2012) Genomics of volvocine algae. Adv Bot Res 64:185–243

Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP (2015) Extremophilic micro-algae and their potential contribution to biotechnology. Bioresour Technol 184:363–382

Vincent WF, Mueller D, van Hove P, Howard-Williams P (2004) Glacial periods on early Earth and implications for the evolution of life. In: Seckbach J (ed) Cellular origin, life in extreme habitats and astrobiology. Springer, Dordrecht, pp 481–501

Wang DS, Xu D, Wang YT, Fan X, Ye NH, Wang WQ, Zhang XW, Mou SL, Guan Z (2015) Adaptation involved in nitrogen metabolism in sea ice alga *Chlamydomonas* sp. ICE-L to Antarctic extreme environments. J Appl Phycol 27:787–796

West NJ, Obernosterer I, Zemb O, Lebaron P (2008) Major differences of bacterial diversity and activity inside and outside of a natural iron-fertilized phytoplankton bloom in the Southern Ocean. Environ Microbiol 10:738–756

West-Eberhard MJ (2005) Developmental plasticity and the origin of species differences. PNAS USA 102:6543–6549

Wiencke C (1990) Seasonality of red and green macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601–607

Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. Bot Mar 37:247–259

Wu G, Liu C, Liu S, Cong B, Huang X (2010) High-quality RNA preparation for cDNA library construction of the Antarctic sea-ice alga *Chlamydomonas* sp. ICE-L. J Appl Phycol 22:779–783

Xavier JC, Brandt A, Ropert-Coudert Y, Badhe R, Gutt J, Havermans C, Jones C, Costa ES, Lochte K, Schloss IR, Kinnicutt MC II, Sutherland WJ (2016) Future challenges in Southern Ocean ecology research. Front Mar Sci 3:94. doi:10.3389/fmars.2016.00094

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686–693

Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, FitzJohn RG et al (2014) Three keys to the radiation of angiosperms to frozen environments. Nature 506:89–92

Zhang P, Liu S, Cong B, Wu G, Liu C, Lin X, Shen J, Huang X (2011) A novel omega-3 fatty acid desaturase involved in acclimation processes of polar condition from Antarctic ice algae *Chlamydomonas* sp. ICE-L. Mar Biotechnol 13:393–401

Zubkov MV, Tarran GA (2008) High bacterivory by the smallest phytoplankton in the North Atlantic Ocean. Nature 455:224–226