

**DISPERSAL & MULTIPLICITY CAPACITIES OF A TERRESTRIAL ALGAL GENUS  
*KLEBSORMIDIUM* (STREPTOPHYTA) IN POLAR REGIONS**

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**ABSTRACT**

In this paper, we examined the diversity and distribution of the very common, globally distributed green algal genus *Klebsormidium* across climatic zones, focusing on the polar regions. We tested whether (i) there is comparable diversity among the polar and temperate regions, and (ii) whether a spatial genetic differentiation occurs at the global scale. We collected a total of 58 Arctic, Antarctic and temperate strains, and genetically characterized them by sequencing the *rbcL* gene and two highly variable chloroplast markers. Our analyses revealed the presence of two different distribution patterns which are supposed to characterize both macroorganisms and protists. On the one hand, we demonstrated unlimited dispersal and intensive gene flow within one of the inferred lineages (superclade B).

**Keywords:** Genetic diversity; *Klebsormidium*; phylogeography

**INTRODUCTION**

The distribution of microalgae is a major topic of modern microbial research (Caron 2009; Gast 2015). Two opposite hypotheses have been proposed: the ubiquity model (Finlay, Esteban and Fenchel 1996; Finlay 2002), which emphasizes the cosmopolitan distribution of protists; and the moderate endemism model (Foissner 1999, 2006), which admits the existence of endemic species with limited distribution. Some authors proposed that the small organism size, large population sizes and high dispersal potential of eukaryotic microorganisms would lead to high gene flow across large geographical scales, resulting in an ubiquitous species distribution in suitable environments (Finlay 2002; Fenchel and Finlay 2004). Large population sizes would be expected to prevent local extinction and result in undisturbed population diversity (Fenchel and Finlay 2004), leading to high local genetic diversity (Mes 2008). In addition, intensive gene flow would constantly erase genetic diversity among populations, leading to a relatively low global diversity and undifferentiated populations (Fenchel and Finlay 2004).

Another consequence of the ubiquity hypothesis is that, as a result of global dispersal, latitudinal gradients in diversity should be weak or absent once ecological controls are factored out (Hillebrand and Azovsky 2001; Finlay and Fenchel 2004). However, there are only a few studies testing the presence of latitudinal gradients in eukaryotic microorganisms, moreover they are largely incongruent. Whereas Hillebrand and Azovsky (2001) showed that latitudinal gradients of species richness are largely absent for diatoms and presumably also for other unicellular and small multicellular organisms, the studies of Vyverman et al. (2007) and Siver and Lott (2012) showed these gradients on freshwater diatoms and silica-scaled chrysophytes, respectively. On the contrary, large organisms like plants and vertebrates show an obvious strong decrease of species richness towards the poles (see overview in Huston 1994). In addition, there should be significant differences in diversity between the poles due to the contiguous nature of the terrestrial Arctic landmass with a temperate landmass at lower latitudes. Conversely, terrestrial Antarctica is a large isolated continent with small outlying sub-Antarctic islands. There, high polar ecosystems are biologically unique, with a more central role for bryophytes, lichens and microbial photoautotrophs over that of vascular plants. The biggest diversity differences between the poles are known in vascular plants where 2218 species are recorded for the Arctic, but just two species for Antarctica (Pointing et al. 2015). Similar differences were also found in bryophytes and lichens, which constitute the major plant cover in the Polar Regions. Whereas ca 900 mosses and

liverworts have been described from the Arctic (Walker et al. 2005), Antarctica hosts only ca 125 species covering a small fraction of the total land area (Seppelt and Green 1998). For lichens, about 1750 species are known from the Arctic, with 8%–10% of these species being endemic (Dahlberg and Bultmann 2013). On the contrary, ca 380 lichen species have been recorded in Antarctica (Øvstedal and Smith 2001), with about 21% of these being endemic taxa (Hertel 1988; Sancho et al. 1999).

Based on their cosmopolitan distribution and high dispersal, the diversity of microbial photoautotrophs should be comparable through the various regions (Pearce et al. 2009). Indeed, the estimated total number of species occurring in the Arctic was comparable to the diversity estimated for Antarctica (Pointing et al. 2015). However, this assumption has never been tested directly for whole microbial communities. Usually, the composition of polar microbial flora has been investigated separately either in Antarctica (e.g. Seaburg et al. 1979; Broady 1986, 1996; Pankow, Haendel and Richter 1991; Broady and Smith 1994; Mataloni, Tell and Wynn-Williams 2000; Cavacini 2001; Fermani, Mataloni and Van de Vijver 2007) or the Arctic region (Kasˇ tovska´ et al. 2005, 2007; Stibal, Sˇ abacka´ and Kasˇ tovska´ 2006; Matu la et al. 2007). Similarly, the currently available molecular data are very fragmentary, consisting of a number of isolated taxonomic and ecophysiological studies on individual taxa, such as various diatom species (Sabbe et al. 2003; Vyverman et al. 2010; Souffreau et al. 2013), green algae (Lesser et al. 2002; Pocock et al. 2004; Pichrtova´ et al. 2013; Pichrtova´ , Ha´ jek and Elster 2014), xanthophytes (Broady, Ohtani and Ingerfeld 1997; Rybalka et al. 2009), ciliates (Petz et al. 2007), dinoflagellates (Montresor et al. 2003; Rengefors et al. 2008, 2015; Rengefors, Logares and Laybourn-Parry 2012) and lichen symbionts (Romeike et al. 2002; Ferna´ ndez-Menroza et al. 2011; Domaschke et al. 2012). The majority of studies suggested a bipolar distribution of the investigated microautotrophs (Darling et al. 2000; Montresor et al. 2003; Ferna´ ndez-Menroza et al. 2011; Domaschke et al. 2012). However, Petz et al. (2007) demonstrated that only 13% of ciliate species showed a bipolar distribution. Strunecky´ , Elster and Koma´ rek (2010) even observed no similarities between the poles when investigating the diversity of the cyanobacterial genus *Phormidium*. In the most recent evaluation of protist diversity in the Polar Regions, Wolf et al. (2015) found a rather small overlap between the Arctic and Antarctica, ranging from 5.5% to 14.5% depending on the group investigated.

There is still a fruitful debate concerning the endemicity of protist organisms in Polar Regions. For example, identical cyanobacterial taxa have been reported from the Arctic, Antarctica and alpine lakes (Jungblut et al. 2005), while the existence of endemic species has been proposed within the cyanobacterial genus *Phormidium* (Strunecky´ , Elster and Koma´ rek 2010). Some polar cyanobacteria occupying highly cryptic habitats, such as hypolithic substrates, may have been genetically isolated for an evolutionarily long time (Bahl et al. 2011). In diatoms, morphological studies suggested the existence of at least 40% endemic taxa in some Antarctic areas (Schmidt, Mausbacher and Muller 1990; Sabbe et al. 2003). Currently, combined molecular, ecological and morphological studies have indicated far greater microbial endemism than previously assumed (Vyverman et al. 2010). Souffreau et al. (2013) presumed that cosmopolitan Antarctic diatom species are in fact species complexes, possibly containing Antarctic endemics with lowtemperature preferences. However, in contrast to diatoms and cyanobacteria, the green algal component of microbial mats has remained virtually unstudied. The available data are largely restricted to morphological taxonomic inventories on the continent, such as Victoria Land (Cavacini 2001; Adams et al. 2006), the Antarctic Peninsula (Mataloni and Pose 2001) and maritime Antarctica (Fermani, Mataloni and Van de Vijver 2007; Zidarova 2007). Broady (1996) suggested that the vast majority of Antarctic terrestrial green algae are cosmopolitally distributed. However, this prediction has yet to be studied by modern molecular techniques.

In this study, we examined the diversity and distribution of the filamentous green algal genus *Klebsormidium* in the polar regions. The genus *Klebsormidium* is very common and diverse in temperate zones (Rindi and Guiry 2004; Rindi, Guiry and Lo´ pez-Bautista 2008; Rindi et al. 2011; Sˇ kaloud and Rindi 2013; Mikhailyuk et al. 2015; Rysˇ a´ nek, Hrcˇ kova´ and Sˇ kaloud 2015), but data about its occurrence in polar regions are still very scarce. The majority of its occurrence records comprises simple

notes about their presence in various algal assemblages (Mataloni, Tell and Wynn-Williams 2000; Cavacini 2001; Kas̃ tovska´ et al. 2005, 2007; Stibal, S̃ abacka´ and Kas̃ tovska´ 2006; Fermani, Mataloni and Van de Vijver 2007; Matu la et al. 2007).

The general aim of this study was to test whether there exists comparable diversity among the polar and temperate regions in green algal eukaryotic microorganisms. We used the genus *Klebsormidium* as a model evolutionary lineage of ubiquitous terrestrial protists. In addition, to test for the presence of spatial genetic differentiation at the global scale, we investigated the population structure of a selected globally distributed lineage by sequencing fast evolving cpDNA molecular markers.

## DISCUSSION

### DIVERSITY AND ABUNDANCE IN POLAR REGIONS

The genus *Klebsormidium* is one of the most abundant microautotrophs in various terrestrial and aerophytic habitats (Ettl and Gartner 1995; Lokhorst 1996; John 2002, 2003). In fact, species of this genus are regularly listed as among the most abundant organisms found during diversity assessments of various habitat types worldwide (e.g. Lukes̃ ova´ and Hoffmann 1996; Neustupa 2001; Hoffmann, Ector and Kostikov 2007; Langhans, Storm and Schwabe 2009; S̃ kaloud 2009; Schulz et al. 2015). Indeed, the recently published investigation of the *Klebsormidium* phylogeographic structure revealed its ubiquitous distribution on a global scale (Rys̃ a´ nek, Hrc̃ kova´ and S̃ kaloud 2015). All the above-mentioned studies thus imply the high global dispersal and comparable diversity estimates of the genus *Klebsormidium* through the various regions.

However, our investigation of newly isolated *Klebsormidium* strains revealed a conspicuously low genetic diversity in the polar regions as compared to the recently published DNA-based diversity assessments. Based on the molecular investigations of a number of isolated strains, Rindi et al. (2011) and S̃ kaloud and Rindi (2013) delimited a total of 22 well-supported clades belonging to the seven major superclades A–G. In their evaluation of *Klebsormidium* diversity in Northern temperate mixed forests, Rys̃ a´ nek, Hrc̃ kova´ and S̃ kaloud (2015) found a total of 44 unique *rbcL* genotypes, indicating a very high genotypic diversity in the dataset based on 15 sampling sites only. Most recently, Mikhailyuk et al. (2015) detected more than 25 ITS rDNA genotypes from 16 different localities in alpine soil crusts.

In contrast to the previously mentioned investigations, we recovered a total of only eight *rbcL* genotypes. Such low genetic diversity could be partly explained by a relatively small number of investigated strains. However, the abundance of *Klebsormidium* in polar regions is obviously very low, which makes very hard to obtain a considerably greater amount of isolated strains. In fact, despite our extensive sampling effort in both the Arctic and Antarctica, only 32 strains were successfully isolated. Indeed, the total number of samples we investigated (over 500) greatly exceeded the number of sampling sites investigated by both Rys̃ a´ nek, Hrc̃ kova´ and S̃ kaloud (2015) and Mikhailyuk et al. (2015). We even failed to isolate a single *Klebsormidium* clone in several samples, despite repeated inoculation of the samples to Petri dishes (Table S1, Supporting Information). Instead, a high number of *Xanthonema* colonies were obtained, indicating the good preservation of algal communities but a very low, undetectable abundance of *Klebsormidium* species in these samples. This low abundance is in concordance with many studies which focused on terrestrial algal assemblages in both Antarctica (Mataloni, Tell and Wynn-Williams 2000; Cavacini 2001; Fermani, Mataloni and Van de Vijver 2007) and the Arctic (Kas̃ tovska´ et al. 2005, 2007; Stibal, S̃ abacka´ and Kas̃ tovska´ 2006; Matu la et al. 2007). In these studies, *Klebsormidium* was usually reported as a rare taxon, exceeded in abundance by other microautotrophs, such as *Leptolyngbya*, *Phormidium*, *Xanthonema* and *Chlorella*.

Despite the above-mentioned low global abundance of *Klebsormidium* in polar regions, we presume that the

observed low genetic diversity can only partly be attributed to the effect of undersampling. Quite recently, Škaloud and Rindi (2013) investigated the ecological differentiation of Klebsormidium lineages based on the genetic characterization of a number of strains, including 27 newly isolated strains from the Czech Republic (central Europe). Although the area of the Czech Republic is incomparably smaller than that of polar regions, the genetic characterization of strains revealed the presence of 13 different genotypes belonging just into the single superclade E. Thus, using the comparable number of investigated strains (27 versus 32 strains), the diversity detected in a small temperate area significantly exceeds the total diversity found in both the Arctic and Antarctica (13 versus 8 genotypes). We therefore suppose that the observed low genetic diversity might be rather attributable to the overall low abundance of Klebsormidium in polar regions (Vogt, Beisner and Prairie 2010).

## UNDERSTANDING THE DISPERSAL CAPACITIES

Considering its cosmopolitan distribution and predominance in the polar regions, superclade B represents an ideal model for testing the dispersal capabilities of microorganisms on a global scale. To differentiate the particular populations, we sequenced highly variable spacers between the chloroplast genes, a method frequently used in population structure assessment of higher plants (Doorduyn et al. 2011; Hollingsworth, Graham and Little 2011). The most common haplotypes were shared across the arctic and temperate regions, indicating intensive gene flow and global dispersal. Such a high dispersal capacity explains the lack of differences in eco-physiological performance of seven superclade B strains isolated from the Arctic (LUC9, LUC11 and LUC14), Antarctica (LUC6, LUC7 and LUC8) and the temperate zone (LUC2), as reported by Elster et al. (2008). Seemingly, the intensive gene flow at a global scale may prevent adaptation of populations to the local environment (Kawecki and Ebert 2004; Whitaker 2006). However, our knowledge about local adaptation mechanisms of protists is severely limited and needs further investigations (Weisse 2008; Weisse et al. 2011; Rengefors et al. 2015).

While this population genetic investigation shows clear evidence of a high dispersal capability of superclade B, the absence of several genotypes in the polar regions points to the restricted distribution of the majority of Klebsormidium lineages. Such a pattern supports the moderate endemism model proposed by the contemporary protistologist (Foissner 1999, 2006; Gast 2015). Consequently, unlimited dispersal should be considerably limited in the majority of the lineages. Although filamentous, the great majority of Klebsormidium species easily disintegrate into fragments containing a few cells (Škaloud 2006).

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