

## RESEARCH/REVIEW ARTICLE

# Protists in the polar regions: comparing occurrence in the Arctic and Southern oceans using pyrosequencing

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18S rDNA; bipolar; next-generation sequencing; phytoplankton; polar regions; protist distribution

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E-mail: christian.wolf@awi.de**Abstract**

In the ongoing discussion of the distribution of protists, whether they are globally distributed or endemic to one or both of the polar regions is the subject of heated debate. In this study, we compared next-generation sequencing data from the Arctic and the Southern oceans to reveal the extent of similarities and dissimilarities between the protist communities in the polar regions. We found a total overlap of operational taxonomic units (OTUs) between the two regions of 11.2%. On closer inspection of different taxonomic groups, the overlap ranged between 5.5% (haptophytes) and 14.5% (alveolates). Within the different groups, the proportion of OTUs occurring in both regions greatly differed between the polar regions. On the one hand, the overlap between these two regions is remarkable, given the geographical distance between them. On the other hand, one could expect a greater overlap of OTUs between these regions on account of the similar environmental conditions. The overlap suggests a connection between the polar regions for at least certain species or that the evolutionary divergence has been slow, relative to the timescales of isolation. The different proportions of common OTUs among the groups or regions may be a result of different life cycle strategies or environmental adaptations.

To access the supplementary material for this article, please see supplementary files under Article Tools online.

The distribution of microbes is the subject of much debate. For prokaryotes, there is a consensus for a largely global dispersal of species (Bano et al. 2004; Pommier et al. 2007). For the eukaryotic fraction (protists), global distribution versus endemism is heatedly discussed (Finlay & Fenchel 2004; Lachance 2004; Medlin 2007). There are bipolar species that do not occur in intervening latitudes (Sul et al. 2013). The investigation of bipolarity among microbes recently gained more interest. Jungblut et al. (2012) found a limited overlap between Antarctic and Arctic microbial mat communities. Pawlowski et al. (2008) found that populations of monothalamous foraminifers from the Arctic and Antarctic differed genetically and represented distinct species.

The polar oceans are subject to similar physical extremes, such as 24 h of sun in the polar summer and

24 h of darkness during the polar winter (for a great portion of the Southern Ocean). Low temperatures at the beginning of the polar winter lead to sea-ice formation. During the polar spring, ice algal blooms followed by ice melt and phytoplankton production support organisms higher in the food chain. One basic difference between the two oceans is the freshwater input (Ghiglione et al. 2012). Surrounded by landmasses, the Arctic Ocean receives freshwater from several large rivers and glacial meltwater. In contrast, the Southern Ocean surrounds the continent of Antarctica and is supplied with freshwater only through glacial meltwater.

Here, we present a comparison of next-generation sequencing data from the Arctic and Southern oceans. We want to answer two questions. (i) Are there operational taxonomic units (OTUs) that are present in both

polar oceans and if so in what proportion? (ii) Are there any differences in the proportion of OTUs occurring in both polar regions between different taxonomic groups and the two regions? Since we did not include samples from temperate regions, we are aware that we are not able to draw definitive conclusion about true bipolarity. Therefore, the OTUs found in both regions could also have a global distribution. However, our data set can be compared with other pyrosequencing data sets to complete the information about the distribution of the OTUs.

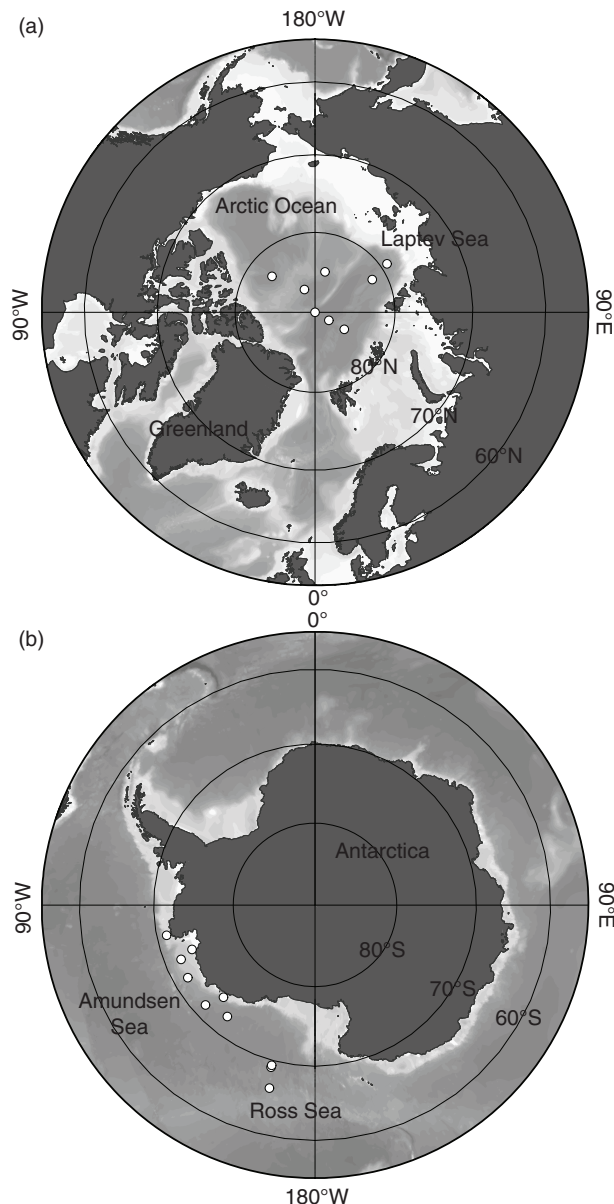
## Material and methods

### Study sites and sampling

For the analysis, we used eight surface water samples from the Arctic Ocean (Fig. 1a) and 10 surface water samples from the Pacific sector of the Southern Ocean (Fig. 1b). All samples are part of other studies containing detailed information about the sampling procedure and data handling (Wolf et al. 2013; Kiliyas et al. 2014; Wolf et al. 2014). All samples were collected in polar waters (north of 79°N or south of 66°S) and in late summer. Arctic Ocean samples were taken at the surface with 12 L Niskin bottles deployed on a rosette. The Southern Ocean samples were collected using the ship pumping system (membrane pump), located at the bow at 8 m depth below the surface. All samples were immediately fractionated by filtering them on Isopore Membrane Filters (Milipore, Billerica, MA, USA) with a pore size of 10 µm, 3 µm and 0.4 µm (Arctic Ocean) or 0.2 µm (Southern Ocean). Filters were stored at -80°C until analysis in the laboratory.

### DNA extraction, polymerase chain reaction amplification and 454-pyrosequencing

The DNA was extracted with the E.Z.N.A.™ SP Plant DNA Kit (Omega Bio-Tek, Norcross, GA, USA) as described by Wolf et al. (2014). For subsequent 454-pyrosequencing, the V4 region of the 18S rRNA gene was amplified with the primer set 528F (5'-GCG GTA ATT CCA GCT CCA A-3') and 1055R (5'-ACG GCC ATG CAC CAC CCA T-3') (modified from Elwood et al. 1985). Details about the procedure, the polymerase chain reaction (PCR) reaction mixture and the reaction conditions were described by Kiliyas et al. (2013). Pyrosequencing was performed on a Genome Sequencer FLX system (Roche, Basel, Switzerland) by GATC Biotech AG (Konstanz, Germany).



**Fig. 1** Locations of sampling sites (white dots) in the (a) Arctic Ocean and the (b) Southern Ocean.

### Data analysis

Raw sequence reads were processed as described by Wolf et al. (2014) to obtain high-quality reads. Briefly, reads shorter than 300 bp and longer than 670 bp, reads with more than one uncertain base (N), chimeric reads, and reads belonging to metazoans were removed. The remaining high-quality reads of all 18 samples were placed into a reference tree, containing about 1250 high-quality sequences of Eukarya from the SILVA reference database (SSU Ref 111) (Quast et al. 2013), using the PhyloAssigner pipeline (Vergin et al. 2013). Reads were

divided into major taxonomic groups and for each group, the reads were clustered (furthest neighbour algorithm) into OTUs at the 98% similarity level using the DNASTAR software Lasergene 10. In the past, the 97% similarity level has tended to be the most suitable to reproduce original eukaryotic diversity (Behnke et al. 2011) and also has the effect of bracing most of the sequencing errors (Kunin et al. 2010). However, the similarity level is still under debate. The known intragenomic SSU polymorphism level strongly varies between different taxonomic groups. In dinoflagellate species it can range up to 2.9% (Miranda et al. 2012). Consequently, different taxonomic groups may require different similarity levels, which hampers the choice for an appropriate similarity level for environmental samples. We took a more conservative approach and used the 98% similarity level, as previously used for eukaryotes by Comeau et al. (2011), to avoid an overestimation of similarities between the two polar regions. OTUs comprised of only one sequence (singletons) were removed. Consensus sequences were generated for each OTU and their taxonomical affiliation was determined using the PhyloAssigner pipeline and individual reference databases for each major taxonomic group, containing about 382 (haptophytes) to 9052 (alveolates) high-quality sequences of the particular group from the SILVA reference database (SSU Ref 111). The compiled reference database is available on request in ARB-format. The raw sequences were deposited at GenBank's Short Read Archive (SRA) under accession numbers SRA056811, SRA057133 and SRA064761.

## Results

We compared a total of 590616 high-quality reads. The number of reads for both regions was quite similar with 317478 for the Southern Ocean and 273138 for the Arctic Ocean. The classification of the reads into the major taxonomic groups resulted in 114292 reads for haptophytes, 42556 reads for chlorophytes, 144914 reads for stramenopiles and 256823 reads for alveolates. The remaining 32031 reads affiliated with other groups (e.g., cryptophytes, rhodophytes) or their affiliation was uncertain (the position in the database tree was too high to be assigned to one group). The number of reads for each group greatly differed between the two regions (Table 1). About 35–53% of all reads were identified as singletons and excluded for the final comparison.

We determined 23637 OTUs in all samples, of which 11.2% occurred in both polar regions (Fig. 2a). For the haptophytes, we found nearly twice the number of OTUs in the Southern Ocean than in the Arctic Ocean

**Table 1** Number of reads and defined operational taxonomic units (OTUs) assigned to the major taxonomic groups.

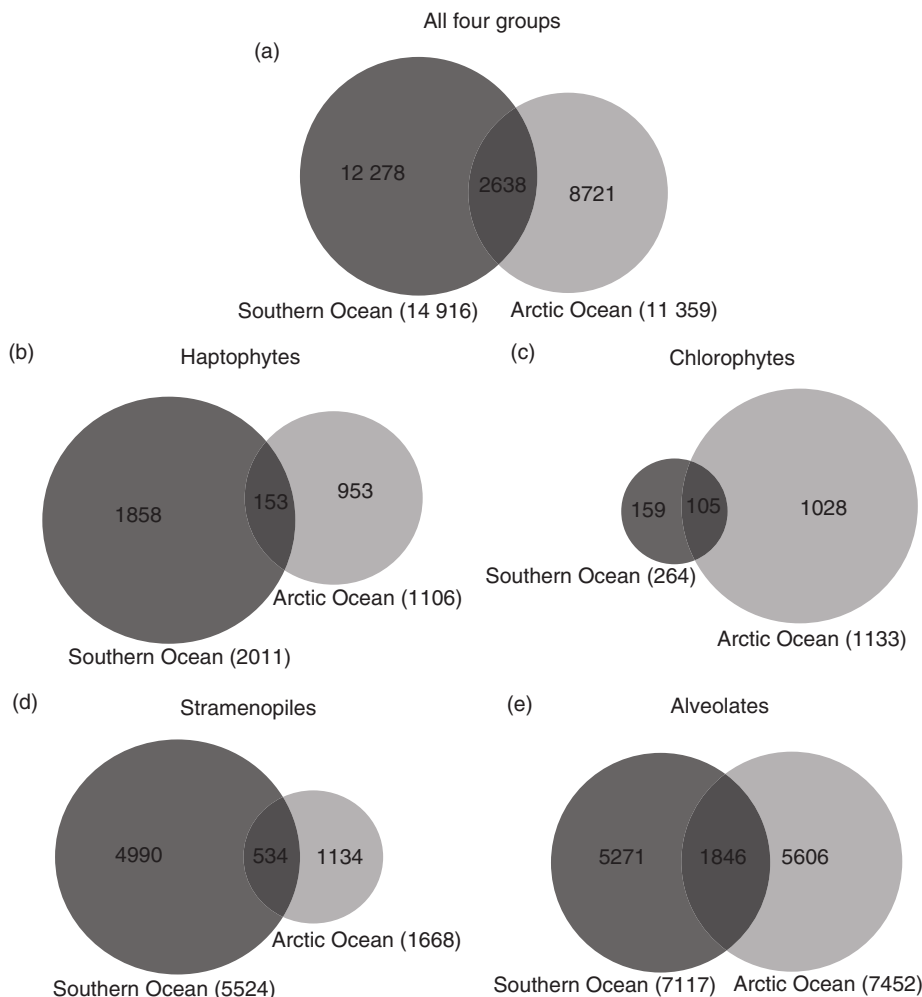
	Arctic Ocean total reads/ defined OTUs	Southern Ocean total reads/ defined OTUs
Haptophytes	14682/1106	58624/2011
Chlorophytes	22160/1133	2674/264
Stramenopiles	15077/1668	78668/5524
Alveolates	57048/7452	62768/7117

(Fig. 2b). The overlap in haptophyte OTUs between the two regions was 5.5%. The number of chlorophyte OTUs also differed strongly between both regions (Fig. 2c). The Arctic Ocean presented a 10-fold higher number of chlorophyte OTUs compared to the Southern Ocean. The proportion of chlorophyte OTUs occurring in both regions was 8.1%. In contrast, the stramenopiles showed five times more OTUs in the Southern Ocean (Fig. 2d). The overlap in stramenopile OTUs between the two regions was 8%. Among the alveolates, the number of determined OTUs in the two regions was very similar (Fig. 2e). The overlap of alveolate OTUs between the Southern Ocean and Arctic Ocean (14.5%) was greatest among all four groups.

Only two OTUs that occurred in both regions were also abundant (OTUs with >1% of total reads) in both regions (Fig. 3a–d). One of these OTUs affiliated with the alveolates (unc. Alveolate) and one with the genus *Micromonas* (*Mamiellales* 1). All other OTUs occurring in both regions originated from the rare biosphere (OTUs with <1% of total reads) in at least one region.

The proportion of common OTUs (occurring in both regions) among haptophytes was nearly twice as high in the Arctic Ocean (Fig. 4). In contrast, the proportion of common OTUs among chlorophytes was four times greater in the Southern Ocean. Within the chlorophytes, the *Mamiellales* showed a common OTU proportion of 65% in the Southern Ocean, whereas in the Arctic Ocean the proportion was only 9%. The proportion of common OTUs among the stramenopiles was three times higher in the Arctic Ocean. Here, the diatoms showed a 10-fold and the pelagophytes a seven-fold higher proportion of common OTUs. For the marine stramenopiles (MAST), we determined similar proportions of common OTUs in both regions. Overall, alveolates showed similar common OTU proportions in both regions. The biggest differences between the two regions were constituted by Syndiniales. Here, the proportions of common OTUs were always higher in the Southern Ocean (up to 10-fold).

The abundant OTUs were compared with the National Center for Biotechnology Information nucleotide database using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) and the best match originating from the warmest region was used to provide an insight



**Fig. 2** Venn diagrams showing the overlap of operational taxonomic units (98% similarity level) between the two polar regions for (a) all four groups; (b) haptophytes; (c) chlorophytes; (d) stramenopiles; and (e) alveolates.

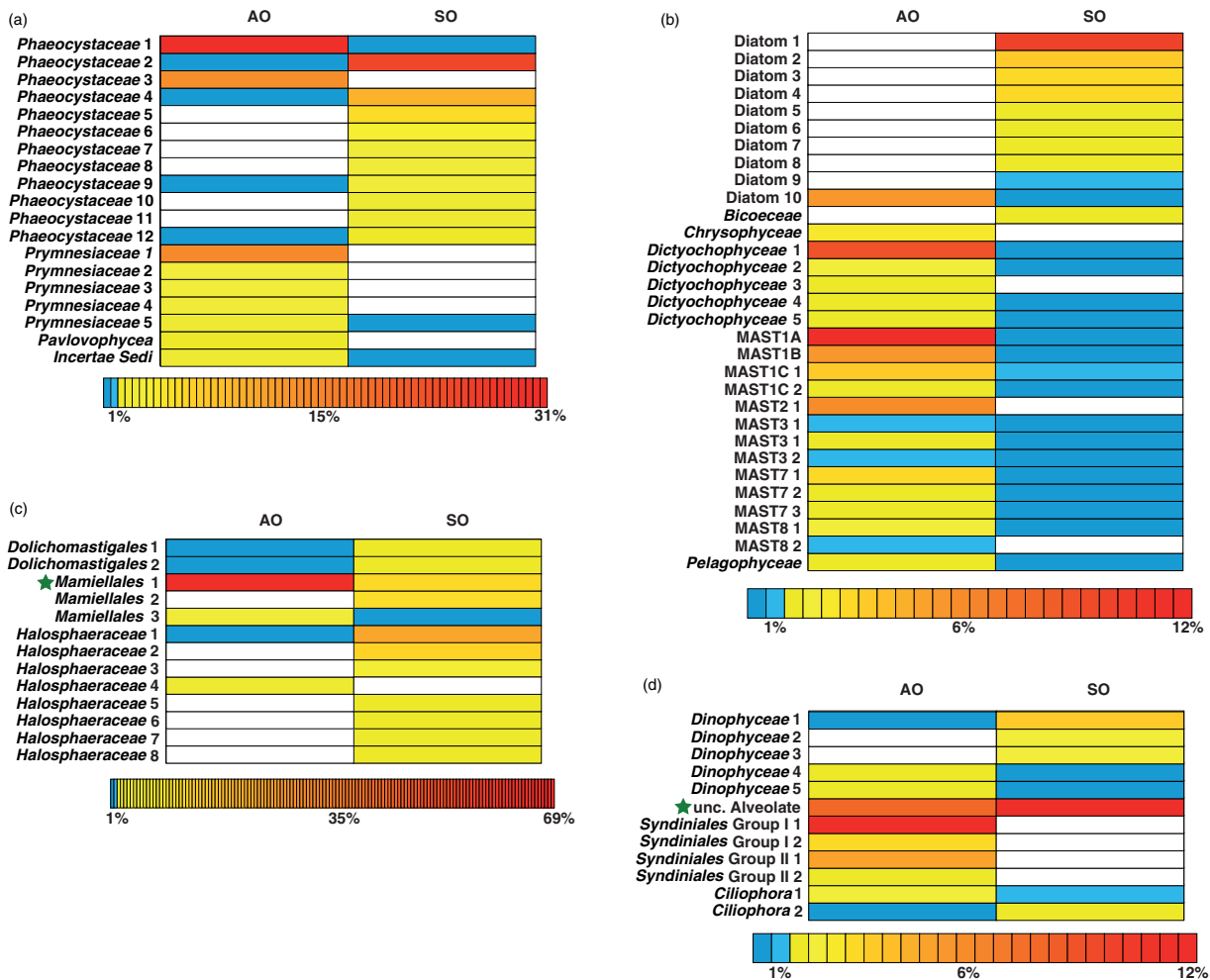
into their potential global distribution (Supplementary Table S1). The majority of haptophyte OTUs (63%) and nearly all OTUs assigned to *Phaeocystaceae* (92%) were restricted to the polar regions. Among the stramenopiles, the diatoms were the most limited to the polar regions (60%), whereas the rest were largely also found in more temperate regions. The chlorophyte OTUs were mostly associated with temperate representatives, except for the Mamiellales OTUs, which were restricted to polar regions. Most of the representatives affiliated with the OTUs belonging to alveolates originated from temperate and tropic regions and only 25% were restricted to polar regions.

## Discussion

In the present study, we compared pyrosequencing data sets from both polar oceans. Samples were taken in both regions from the surface during summer, which allows

for a suitable comparison as the sequencing resulted in similar read counts. We focused our comparison on the two polar regions and on the OTU level. We decided against calculating diversity indices in our study because the numeration of single organisms is a prerequisite. The results of 454-pyrosequencing data are semi-quantitative and due to the differences in gene copy numbers among different species (Zhu et al. 2005) we cannot state that every read represents one organism.

Our first question, whether a number of OTUs is present in both polar oceans, was positively answered. One-tenth of the revealed OTUs were found in both areas. Given that a large distance geographically separates these two marine regions, this overlap between them is remarkable. On the other hand, considering the similar environmental conditions, one could expect a greater overlap of OTUs between these regions. This relatively small overlap could be explained by the restricted sampling opportunity in



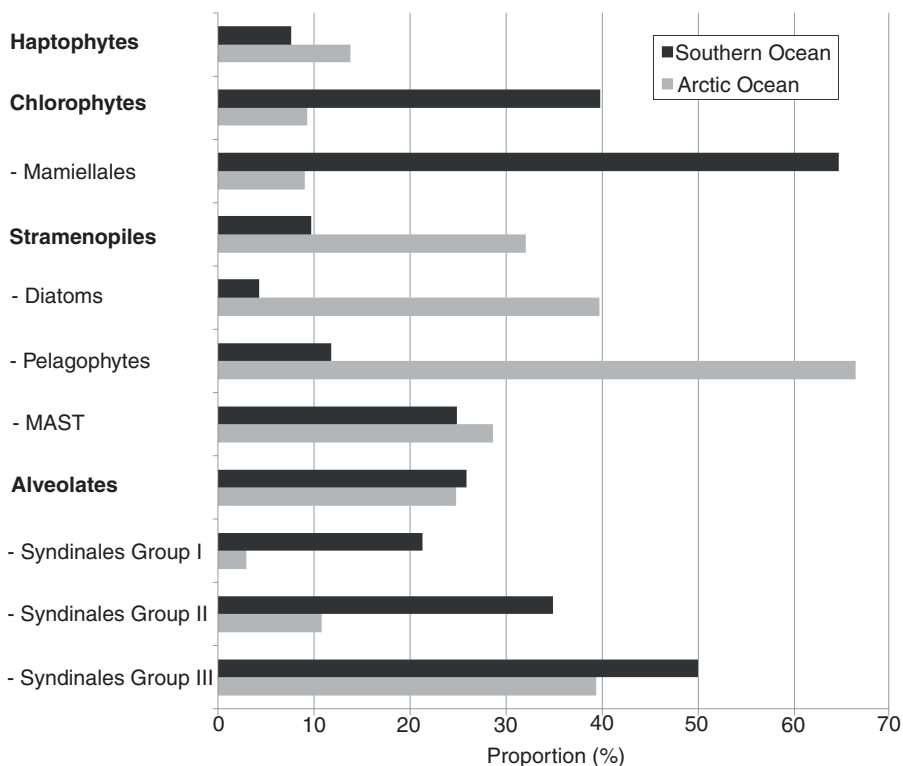
**Fig. 3** Colour-coded matrix plot, illustrating the relative read abundance of abundant operational taxonomic units (OTUs) (abundance  $\geq 1\%$ ) for (a) haptophytes; (b) stramenopiles; (c) chlorophytes; and (d) alveolates in the Arctic Ocean (AO) and the Southern Ocean (SO). White boxes indicate the absence of the respective OTU. The green stars indicate the two OTUs that occurred in the abundant biosphere in both regions.

the Southern Ocean, confined only to the Pacific sector while our Arctic Ocean samples covered different water masses. Including samples from the Atlantic sector of the Southern Ocean would probably increase the detection of common OTUs between both regions. Another consideration is that the samples may not have been exhaustively sequenced, resulting in a lower number of available sequences for overlapping possibilities.

The detected common OTUs suggest that there may be a connection between the polar regions for at least certain species. Dispersion from one pole to the other may happen via long-range transport processes, including wind and the ocean conveyor belt. Such long-distance transportation has been reported for bacteria (Hughes et al. 2004) and land plants (Muñoz et al. 2004). Shipping traffic may also account for the dispersal of protists across the oceans, principally for species that form spores or

resting cysts (Jungblut et al. 2012). Darling et al. (2000) showed that trans-tropical gene flow between Arctic and Antarctic populations of planktonic foraminifers must have occurred in the past. The exchange might have happened during cooling periods associated with glacial cycling. In warmer periods, the tropical ocean acts as a barrier, promoting diversification (Darling et al. 2004). This may also be true for certain species of haptophytes, chlorophytes, stramenopiles and alveolates, which we have investigated. The slow evolutionary divergence, relative to the timescales of isolation (Jungblut et al. 2012) could also partly explain the overlap of OTUs between the two polar regions. Therefore, in the future, lower similarities between protist communities in both polar regions might be observed.

Another outcome from our analysis was that most similarities between the two polar regions were found in



**Fig. 4** Proportion of common operational taxonomic units (occurring in both polar regions) within the different groups.

the rare biosphere, suggesting that ecosystem functioning in both polar regions is supported by different species. No statements about temporal patterns can be drawn from our investigation because we only had a snapshot during polar summers in both regions. The rare biosphere might serve as a seed pool and certain species might become abundant during other times of the year when environmental conditions are favourable. Rarity may also be an evolutionary trait (Logares et al. 2014) and the avoidance of competition, predation, and parasitism might be advantages of a low-abundance life (Pedrós-Alió 2006).

In a previous study investigating protist diversity in the central Arctic Ocean (Kiliyas et al. 2014), dinoflagellates (i.e., Syndiniales), haptophytes (i.e., *Phaeocystis* sp.) and chlorophytes (i.e., *Micromonas* sp.) dominated the assemblages (Supplementary Table S2). Samples with similar sea-ice concentrations showed a high accordance in their community composition, indicating that sea ice has a stronger influence than nutrient availability. In another previous study focusing on protist community structure in the Southern Ocean (Wolf et al. 2014), dinoflagellates, haptophytes and diatoms dominated the assemblages (Supplementary Table S3). Here, we found that different water masses harboured different protist communities: small-sized protists dominated the northern part of

the investigated area and big-sized protists the south. In the Amundsen Sea, we observed a high occurrence of dinoflagellates, haptophytes and diatoms (Wolf et al. 2013) (Supplementary Table S4). We found characteristic offshore and inshore protist communities, with dinoflagellates more abundant offshore and diatoms inshore. In the current study, the Southern Ocean was readily distinguishable from the Arctic Ocean in terms of the number of OTUs for each group. We found more OTUs affiliated with haptophytes and stramenopiles in the Southern Ocean, which is consistent with high abundances of *Phaeocystis* and diatoms found in earlier studies (Alderkamp et al. 2012; Mills et al. 2012; Wolf et al. 2013). High abundances of chlorophytes were reported in the Arctic Ocean (Lovejoy et al. 2006; Lovejoy et al. 2007), which agrees with the higher amounts of OTUs belonging to this group in the Arctic Ocean that we found. In both regions, we found similar numbers of OTUs affiliated with alveolates. This last group consists of dinoflagellates, Syndiniales and ciliates and is mostly heterotrophic, mixotrophic or parasitic (Sherr & Sherr 2007). This indicates that the distribution of alveolates may not be as influenced by nutrient availability as that of other protists, which jibes with their presence in the polar regions.

Our second question, whether there are any differences in the proportion of OTUs occurring in both regions between different taxonomic groups and the regions, was also positively answered. Alveolates showed the highest proportion of common OTUs, fitting well to the fact that they form resting cysts (e.g., Bravo et al. 2010), which are more easily transported over long distance across the oceans. Also, as stated previously, the distribution of alveolates is less affected by abiotic factors, in contrast to autotrophs. In general, the polar region that showed the lower number of OTUs (within one group) had the highest proportion of common OTUs. We suggest that the region with the higher number of OTUs could serve as source for dispersion across the ocean. Another explanation may be that the diversification of species goes faster in the region with the larger number of OTUs. The Syndiniales, a parasitic group within the dinoflagellates (Guillou et al. 2008), showed more OTUs restricted to the Arctic samples. We found representatives of the Syndiniales group I–V in the Arctic Ocean, but only group I–III in the Southern Ocean (data not shown). Our findings suggest that the conditions for Syndiniales (e.g., host availability) in the north have led to a greater diversification.

Our first insight into the potential global distribution of the investigated abundant OTUs showed that most of the OTUs restricted to polar regions were autotrophic (e.g., *Phaeocystaceae*, diatoms, *Mamiellales*). In contrast, most of the heterotrophs (e.g., MAST, alveolates) also showed similarities to representatives from warmer regions. This strengthens our suggestion that heterotrophs are less affected by abiotic factors and are not as limited in their global distribution as autotrophs. Most of the OTUs revealed in our study were restricted to one polar region. The limited occurrence is confirmed by other studies investigating microbial distribution. Logares et al. (2012) found that not all MASTs are equally represented at each geographic location they investigated. Edgcomb et al. (2013) found distinct communities of eukaryotes in microbialites from Australia and Bahamas.

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