LIMNOLOGY
and
OCEANOGRAPHY: METHODS

ASLO

Limnol. Oceanogr.: Methods 13, 2015, 74–80

doi: 10.1002/lom3.10008

Characterizing variability in marine protist communities via ARISA fingerprints—a method evaluation

E.S. Kilias,* C. Wolf, K. Metfies
Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Polar Biological Oceanography, Bremerhaven, Germany

Abstract

It is important to characterize and understand the diversity of marine protists because of their relevance for ecosystem functioning. In the era of molecular science, diversity studies have received renewed attention. High-throughput, cost-intensive next generation sequencing provides deep insight in protist diversity but limits the volume of studied samples. Protist observations with high spatiotemporal resolution, therefore, require a quick and cost-effective tool to channelize the large sample volume and help select representatives for diversity studies. In this study, we evaluated the validity of "Automated Ribosomal Intergenic Spacer Analysis" (ARISA) as a means of estimating variability in marine protist communities. The evaluation was based on statistical correlation of ARISA data and 454-pyrosequencing data from samples collected in the Southern Ocean and Arctic Ocean. Here, we provide evidence that differences in ARISA profiles reflect taxon-specific differences observed in 454-pyrosequencing data sets. Calculated similarity indices for the ARISA profiles and 454-pyrosequencing data of 27 marine protist samples revealed strong agreements between the results of both methods regarding the extent of variability among protist communities. We suggest that ARISA might become an important tool for surveillance of differences in marine protist communities with high spatiotemporal resolution. Furthermore, it might serve as a preselection tool to identify representative samples in large data sets.

Marine protists are single-celled organisms and important constituents of the marine environment, composing much of the genetic diversity in the eukaryotic domain. They are forming the base of the marine food-web and include important classes such as stramenopiles, chlorophytes, chrysophytes, haptophytes, dinophytes, and ciliates that in turn cover different nutritional strategies such as autotrophy, heterotrophy, and mixotrophy. Protists are well suited to serve as indicators of environmental change, because their population dynamics are closely coupled to environmental conditions. The use of marine protist observations to assess the impact of environmental change on marine ecosystems faces various challenges. For one, the distribution of marine protists is spatially heterogeneous, or "patchy". Thus, protist observations are needed at high spatiotemporal resolution when studying the dynamics of protist communities in a changing environment. Furthermore, species that significantly contribute to marine protist communities are present in every plankton size fraction (micro-, nano-, and picoplankton), and yet surveillance of small-size species is extremely challenging due to their small cell size and morphological monotony. Quick and cost-effective approaches, therefore, have to be applied to allow analyses of large sample numbers in all marine protist size classes.

Molecular fingerprints, e.g., automated ribosomal intergenic spacer analysis (ARISA), might serve these needs. ARISA is a quick and cost-effective method that is independent of the size or morphology of target organisms. The method bases on the comparison of fragment lengths of a specific DNA region, the internal transcribed spacer region (ITS; Baldwin et al. 1995). The ITS region is located between the small (18S) and large (28S) subunits of the rRNA and defined by its great length heterogeneity (Baldwin 1992). Amplification and size separation of the ITS region involves the use of fluorescently labeled primers and electrophoresis. The composition of differently-sized fragments in a sample acts as a characteristic fingerprint of a microbial community that allows qualitative comparison of their compositions. In the past, most published ARISA-based studies focused on prokaryotes and fungi (e.g., Danovaro et al. 2009; Smith et al. 2010). Recently, a number of field studies used ARISA to assess differences in the compositions of marine protist communities (Fechner et al. 2010; Kilias et al. 2013; Wolf et al. 2013). To our knowledge, there is as yet no publication that statistically assesses the validity of ARISA for surveillance of variability in marine protist communities. ARISA data sets do not directly provide information on species

*Correspondence: estelle.kilias@awi.de

Table 1. Expedition, duration, location, and sample volume of the analyzed water samples.

Expedition	Duration	Region	Samples
ARK25/2	30 Jun 2010–29 Jul 2010	Fram Strait	6
ANT26/3	29 Jan 2010–5 Apr 2010	Ross/Amundsen Sea	6/7
ARK26/3	5 Aug 2011–6 Oct 2011	Central Arctic Ocean	8

composition and abundance or the diversity in a sample. Numerous fragments of the same length can be obtained from multiple taxa, providing ambiguous taxonomic information (Caron et al. 2012). Other fragments may not be detected despite their presence in a rich community (Bent et al. 2007; Dunbar et al. 2001). Finally, there is a lack of comprehensive information on the ITS length variability of several marine protist taxa. As a consequence, the validity of estimates of marine protist community variability from ARISA has to be evaluated statistically.

Currently, the most appropriate method for such an evaluation is statistical comparison with community data based on sequencing of molecular marker regions, e.g., ribosomal genes. Next generation sequencing (NGS) of ribosomal genes allows high resolution, taxon-specific assessments of protist communities, including their smallest size fractions and the rare biosphere (Margulies et al. 2005; Wolf et al. 2013; Kilias et al. 2014*a*). Including rare protists in the evaluation of ARISA is important because the method provides qualitative data on the composition of marine protist communities, which can also include rare species. Therefore, the evaluation method should reflect the protist community as comprehensively as possible.

In this study, we evaluate the potential of ARISA to reflect the variability of marine protist communities by comparing the similarity distances of the ARISA community profiles with the similarity distances of the taxon composition determined by 454-pyrosequencing. The study is based on a total of 27 polar samples that were selected based on the protist community structure and the environmental condition, showing preferably differences in both. The samples are part from previous studies, addressing other scientific questions using ARISA and 454-pyrosequencing on polar protist communities (Kilias et al. 2013; Wolf et al. 2013; Kilias et al. 2014*b*). We compiled the data sets from these studies to generate a number of NGS data sets that was sufficient for a meaningful statistical evaluation of the two different methods.

Materials and Procedures

The sampling was performed during three expeditions of the RV Polarstern (Table 1). Fourteen samples were taken in the northern hemisphere (Fram Strait and central Arctic Ocean) and 13 samples originated from the Southern Ocean (Fig. 1). Information on the sampling procedure (e.g., size

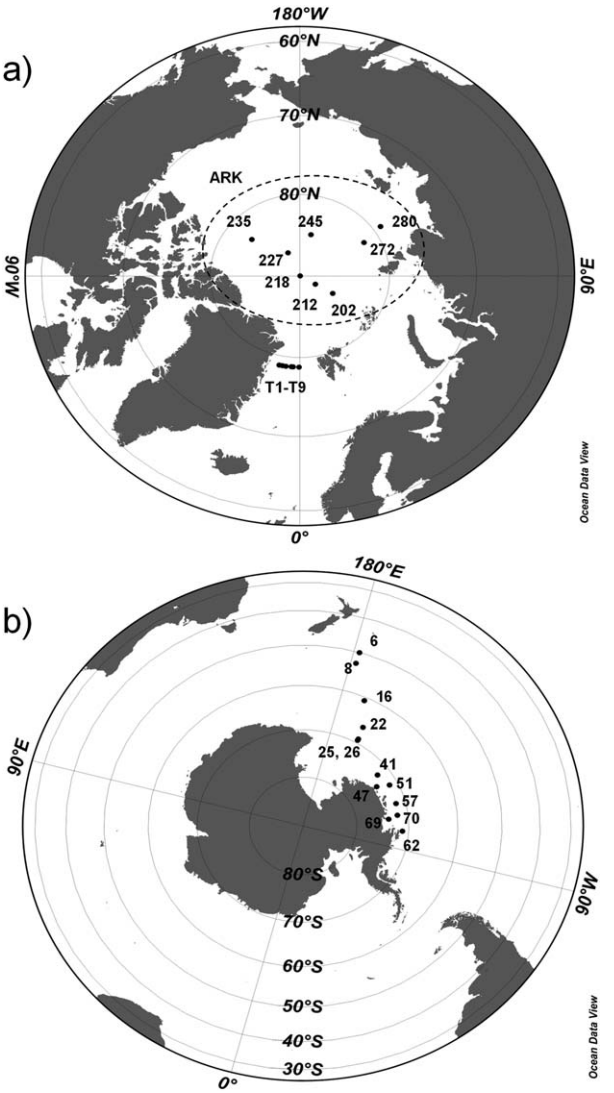

Fig. 1. Map of the sampling stations in (a) the northern and (b) the southern hemisphere.

fractionated filtering) is given in Kilias et al. (2013) (Arctic samples) and Wolf et al. (2013) (Southern Ocean samples).

DNA extraction from environmental samples was performed with the E.Z.N.A TM SP Plant DNA Kit Dry Specimen Protocol (Omega Bio-Tek) following the manufacturer's instructions.

Amplification of a ~ 670 bp long fragment of the 18S rDNA containing the V4 region for pyrosequencing was performed using the primer-set 528F (GCG GTA ATT CCA GCT CCA A) and 1055R (ACG GCC ATG CAC CAC CAC CCA T) (Elwood et al. 1985). Polymerase chain reaction (PCR) reaction mixture and protocol were used as described below for ARISA. PCR products of each size fraction of each sample were pooled by mixing equal volumes. Pyrosequencing was performed on a 454 GS FLX sequencer (Roche, Germany) by an external company (GATC Biotech GmbH, Germany).

Table 2. Distribution of the ITS1 fragments within the dataset.

Polar region	Average fragment number	Maximum fragment number	Minimum fragment number	Unique fragment number
Fram Strait	100	115 (T5)	93 (T7)	19
Ross Sea	39	40 (ANT26)	38 (ANT16 to 25)	7
Amundsen Sea	28	38 (ANT41)	13 (ANT51)	12
Arctic Ocean	70	85 (ARK280)	45 (ARK202)	22

Data processing, including a quality check and clustering of pyrosequences into operational taxonomic units (OTUs), followed the procedure of Wolf et al. (2013). Briefly, this involved the removal of chimeric reads, reads shorter than 300 bp and longer than 670 bp, reads with more than one uncertain base (N) and metazoan reads. Afterward, sequences of all samples were subsampled to the lowest sequence number (ARK218; 4246) and clustered at a 97% identity threshold. Consensus sequences of each OTU were placed with PhyloAssigner (Vergin et al. 2013) into a reference tree built from 1250 high quality eukaryotic sequences from the SILVA reference database (SSU Ref 111). Distances between the samples were calculated using the Jaccard and Bray–Curtis index implemented in the R package vegan (Oksanen et al. 2011). Multidimensional scaling (MDS) plots were computed and possible clusters were determined using the package's hclust function. An analysis of similarity (ANOSIM) was performed to test whether the resulting clusters differ significantly. The null hypothesis, therefore, is that there are no differences between the samples of the various groups. This is supported when R, scaling between +1 and −1, is close to the upper limit and the *p*-value is less than 0.05.

Amplification of the ITS 1 region for ARISA was performed in triplicates with the primers 1528F (5′-GTAGGT GAA CCT GCA GAA GGA TCA-3′) (modified after Medlin et al. (1988)), labeled with dye 6-FAM (6-carboxyfluorescein), and ITS2 (5′-GCT GCG TTCTTC ATC GAT GC-3′) (White et al. 1990). DNA isolates of each size fraction from each sample were pooled prior to amplification. PCR reagents were mixed as follows: 1 μl of DNA extract, 1× HotMaster Taq Buffer containing 2.5 mM Mg^{2+} (5 Prime), 0.8 mM dNTP-mix (Eppendorf, Germany), 0.2 mM of each Primer, and 0.4 U of HotMaster Taq DNA polymerase (5 Prime) in a final volume of 20 μl. PCR cycling conditions included an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 1 min and extension at 72°C for 3 min, and a final extension at 72°C for 10 min.

The fragment sizes of amplicons in the ITS1 region were determined by capillary electrophoresis with an ABI 310 Prism Genetic Analyzer (Applied Biosystems). Analysis of the electropherograms was carried out with GeneMapper v4.0 software (Applied Biosystems). We applied a threshold of 50 bp for peaks to exclude fragments originating from primers or primer dimers. Binning was carried out in R (R development Core Team 2008) to remove background noise and obtain sampled-by-bin operational taxonomic unit tables (Ramette 2009). The resulting data were converted to a presence/absence matrix (Supporting Information Table S1). Distances between the samples were calculated, using the Jaccard index implemented in vegan. As before, MDS plots were computed and possible clusters were determined using vegan's hclust function. An ANOSIM was performed to test, whether the resulting clusters differ significantly. A Mantel test (10,000 permutations) was used to test the correlation of the protist community structure distance matrices obtained by ARISA (Jaccard) and pyrosequencing (Jaccard and Bray Curtis). A key benefit of the Mantel test is that it proceeds from a distance matrix and, therefore, can be applied to different kinds of variables. This is important for this study where data based on fragment size (ARISA) are compared to the sequence itself (454-pyrosequencing). The Mantel test was implemented in the R package ade4 (Dray and Dufour 2007).

Assessment

Automated ribosomal intergenic spacer analysis

The analysis of the ITS1 region length heterogeneity resulted in 321 different fragments that ranged between 50 bp and 496 bp. The average fragment number per sample was 59. In general, numbers of ARISA fragments were lower for the Southern Ocean samples than for the Arctic Ocean samples. The average numbers of amplified fragments were 28 from the Amundsen Sea samples, 39 from the Ross Sea samples, 70 from the samples collected in the central Arctic Ocean, and 100 from the Fram Strait samples (Table 2). The maximum number of fragments (115) was amplified from the sample collected at station T5 in Fram Strait and the minimum (13) at ANT51 in the Amundsen Sea. Jaccard-based ordination analysis of the ARISA profiles grouped the samples into four clusters (Fig. 2a). One cluster included all samples from the Fram Strait except T9, which was grouped in the second cluster of all central Arctic Ocean samples. The third cluster was solely composed of samples from the Amundsen Sea, while the fourth cluster included both Ross and Amundsen Sea samples. The ANOSIM confirmed the grouping into four clusters, with $R = 0.9$ and $p = 0.01$ (Table 3).

Pyrosequencing

Clustering of pyroreads over an identity threshold of 97% resulted in 4076 different OTUs. The average number of OTUs per sample was 400. In contrast to ARISA, OTU numbers were greater in the Southern Ocean than in the Arctic samples. The average numbers of OTUs determined were 444 in the Ross Sea, 480 in the Amundsen Sea, 335 in Fram Strait, and 346 in the central Arctic Ocean samples. An

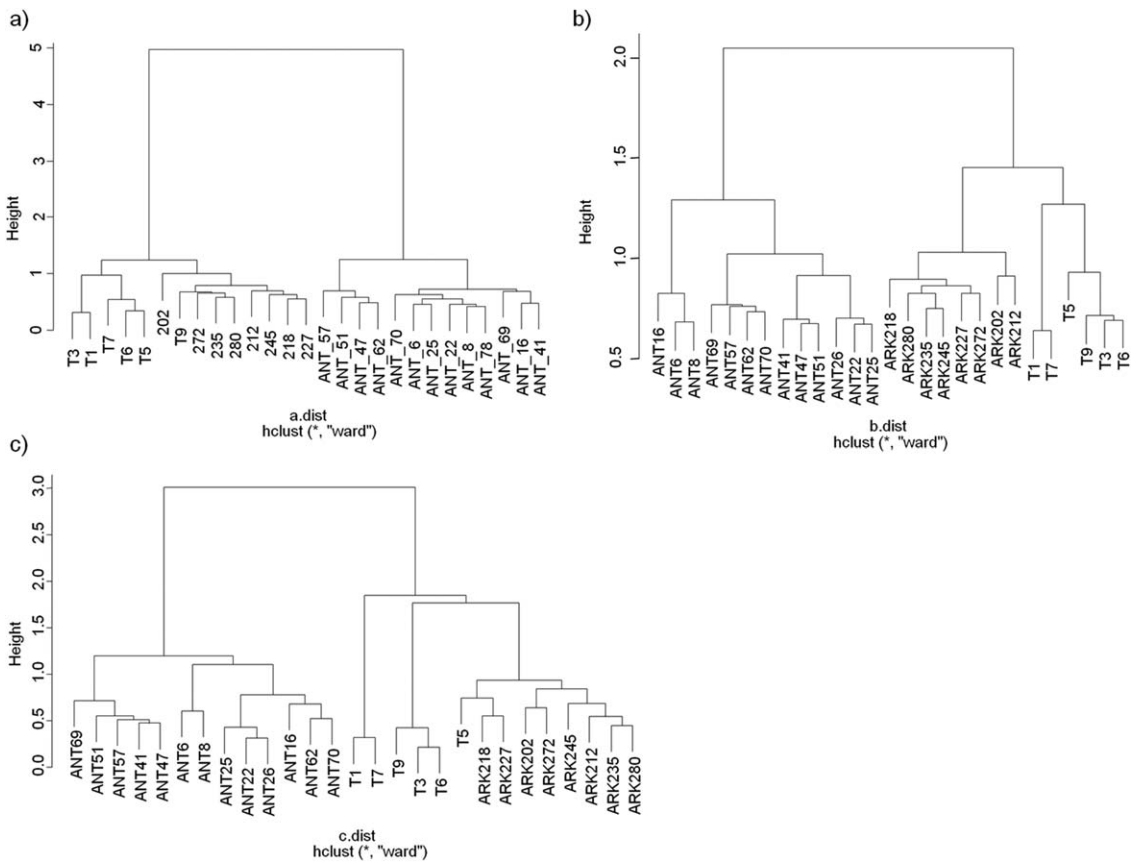

Fig. 2. Clustering dendrogram of all 27 water samples, calculated for (a) ARISA using Jaccard distances (R = 9.3 e -05); (b) 454-pyrosequencing using Jaccard distances (R = 0.07); and (c) 454-pyrosequencing using Bray Curtis distances (R = 0.07).

Table 3. Statistical comparison of ARISA and 454-pyrosequencing data.

	ARISA (Jaccard)	454-pyroseq. (Jaccard)	454-pyroseq. (Bray Curtis)
ANOSIM			
R-value	0.9	0.95	0.78
p-value	0.01	0.01	0.001

	ARISA/454-pyroseq. (Jaccard)	ARISA/454-pyroseq. (Bray Curtis)
Mantel-test		
Observed correlation (*R*)	0.64	0.82
p-value	0.0001	0.0001

ordination analysis of the pyroread distribution was performed using the Jaccard and Bray Curtis index. The Jaccard index provides information on qualitative dissimilarities, while the Bray Curtis index calculates dissimilarities on the basis of quantitative information.

Using the Jaccard index, distances between the samples clustered into four groups in accordance with the ARISA data (Fig. 2b). The first group includes all samples from Fram Strait, the second all samples from the central Arctic Ocean (with ARK202 and 212 as outliers), the third all samples from the Amundsen Sea along with three from the Ross Sea, and the fourth contains three samples from the Ross Sea. The ANOSIM strengthened the grouping, with R = 0.95 and p = 0.01.

Using the Bray Curtis index on the same data changed the distance clustering to show six clusters (Fig. 2c). The first cluster included two samples (T1 and T7) from Fram Strait, while the second was composed of the other Fram Strait samples (T3, T6, and T9). All central Arctic Ocean sample and T5 formed the third cluster. The fourth included four samples from the Ross Sea (ANT16 to 26) with two from the Amundsen Sea (ANT62 and 70), while the fifth included ANT6 and 8. The last cluster included samples from the Amundsen Sea (ANT41 to 57 and ANT69). The clustering was backed up with R = 0.78 and p = 0.001 (ANOSIM).

Mantel test

A Mantel test was computed to investigate the correlation of the different ordinations calculated by the different

methods (ARISA and pyrosequencing) and/or indices (Jaccard and Bray Curtis). Differences of all treatments were significantly correlated, with $R = 0.64$ and $p = 1$ e -04 for the ARISA and pyrosequencing grouping by Jaccard and the ARISA and pyrosequencing grouping by Bray Curtis, $R = 0.82$ and $p = 1$ e -04 (Table 3).

Discussion

Long-term information on the composition of marine protists is needed at high spatiotemporal resolution to monitor the impact of changing environmental conditions on marine ecosystems. Generating this information requires analyzing protist community compositions in large numbers of marine field samples. ARISA is a quick and cost-effective method that can be used to approach the variability in marine protist communities in large sample sets. The overall costs for ARISA comprise less than five Euro per sample and triplicate, while the sequencing costs of one sample with 454-pyrosequencing is a hundred times higher, ranging around 400 Euro (including adapter integration and a 360,000–520,000 read yield). However, the sequencing costs can vary between sequencing companies and depend on the offered read number. ARISA has the advantages of being suited for analyses of large numbers of samples because of its relatively low cost and, because it is a molecular method, it is insensitive to the morphological monotony and small cell sizes of the target organisms.

Recently, ARISA has been used in various projects of our research group to determine spatial variability among marine protist communities in the Southern and Arctic oceans (Kilias et al. 2013; Wolf et al. 2013, 2014). In all of these studies, the clustering of the ARISA profiles was shown to be closely correlated with ambient environmental conditions. Based on this observation, we hypothesized that the ARISA profiles reflect environmentally influenced differences in marine protist communities. ARISA profiles were used as a preselection tool to identify representative samples of different water masses. Subsequently, these representatives were subjected to NGS-sequencing of the 18S rDNA V4-region to determine the water masses' protist compositions. Related to the high costs of 454-pyrosequencing, numbers of representative samples never exceeded eight per study. As a consequence, the value of any statistical dissimilarity comparison (ARISA vs. 454-pyrosequencing) in the individual studies would be limited. To generate a statistically meaningful data set, we compiled all 454-pyrosequencing and ARISA data sets from the previous studies into one larger data set of 27 samples.

In this data set, ARISA fragment numbers were not statistically correlated to the number of OTUs observed by 454-pyrosequencing. On average, the number of ARISA fragments observed per sample was around two orders of magnitude less than the number of OTUs observed by 454-pyrosequencing. This observation suggests that one ARISA fragment might represent more than one taxon (Caron et al. 2012). Similar observations have been made for fungi (Gillevet et al. 2009). One of the main reasons for this finding is the information content of the different classifying attributes. Sequence length on its own includes less information for OTU characterization than the sequence of nucleotides itself. Consequently, the risk of missing species with similar sequence lengths is greater for ARISA.

It should nonetheless be acknowledged that the analysis in the two methods was based on different regions with different resolutions, such as the ITS1 (ARISA) and V4 region (18S rDNA). The hypervariable V4 region is known to resolve even intraspecific variation (e.g., ecotypes) (Kilias et al. in press) which can increase the OTU yield.

The grouping of the ARISA profiles (Jaccard index) was compared to the grouping of the 454-pyrosequencing data, once calculated using the Jaccard and once using the Bray–Curtis index. The main difference between the indices is that the Jaccard index is based on presence/absence patterns while the Bray–Curtis index additionally includes abundance information. Grouping of the ARISA profiles was highly correlated to the grouping of the 454-pyrosequencing data sets, independent of which index was applied. The correlation was stronger when the Jaccard index was used for both treatments. The correlation was weaker but still significant when the Bray–Curtis index replaced the Jaccard index for the 454-pyrosequencing data. This indicates that the limited information of fragment length is sufficient to reflect differences and similarities in community structure. We further suggest that rare taxa, which are only detected by 454-pyrosequencing, do not greatly contribute to community structure differences. Our finding is in agreement with Caron et al. (2012) who stated that fragment analyses provide a snapshot of a subset of the dominant taxa within a community. This snapshot of abundant taxa appears to be sufficient to define natural community structure differences.

Overall, the tight correlation of ARISA data with 454-pyrosequencing data suggests that ARISA might serve as a quick and cost-efficient tool to assess variability in marine protist communities. The method might form the basis of a cost-efficient analysis program with high spatiotemporal resolution and might contribute to overcoming the challenges of observing patchily-distributed marine protist communities. We demonstrated that ARISA profiles reflect the variability of marine protist communities determined by 454-pyrosequencing. As a consequence, ARISA data might also serve as a basis on which to select representative samples from larger data sets for subsequent detailed taxon-specific NGS-sequencing of ribosomal genes. Therefore, we suggest using ARISA if the study aims to survey community structure differences according to changing environmental parameters while 454-pyrosequencing is the tool of choice when the study focuses on changes in protist groups or species (e.g., key species).

Comments and Recommendations

The main focus of this study was to evaluate the validity of ARISA molecular fingerprinting as a method of reflecting variability in marine protist communities. Our data suggest that ARISA might be very well suited to reflect this variability. It is a cost-efficient method that has strong potential to be used for analyses of large numbers of samples. ARISA could be used as a preselection tool to identify representative samples in large data sets.

References

Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the compositae. Mol. Phylogenet. Evol. **1**: 3-16. doi:10.1016/1055-7903(92)90030-K

Baldwin, B. G., M. J. Sanderson, J. M. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue 1995. The ITS region of nuclear ribosomal DNA—a valuable source of evidence on angiosperm phylogeny. Ann. Mo. Bot. Gard. **82**: 247-277.

Bent, S. J., J. D. Pierson, and L. J. Forney. 2007. Measuring species richness based on microbial community fingerprints: The emperor has no clothes. Appl. Environ. Microbiol. **73**: 2399-2399. doi:10.1128/AEM.02383-06

Caron, D. A., P. D. Countway, A. C. Jones, D. Y. Kim, and A. Schnetzer. 2012. Marine protistan diversity, p.467-493. *In* C. A. Carlson and S. J. Giovannoni [eds.], Annual reviews of marine science, Annual Reviews.

Danovaro, R., C. Corinaldesi, G. M. Lung, M. Magagnini, E. Manini, and A. Pusceddu. 2009. Prokaryote diversity and viral production in deep-sea sediments and seamounts. Deep Sea Res. Part II **56**: 738-747. doi:10.1016/j.dsr2.2008.10.011

Dray, S., and A. B. Dufour. 2007. The ade4 package: Implementing the duality diagram for ecologists. J. Stat. Softw. **22**: 1-20.

Dunbar, J., L. O. Ticknor, and C. R. Kuske. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. Appl. Environ. Microbiol. **67**: 190-197. doi:10.1128/AEM.67.1.190-197.2001

Elwood, H. J., G. J. Olsen, and M. L. Sogin. 1985. The small-subunit ribosomal RNA gene sequence from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. Mol. Biol. Evol. **2**: 399-410.

Fechner, L.C., F. Vincent-Hubert, P. Gaubert, T. Bouchez, C. Gourlay-France, and M. H. Tusseau-Vuillemin. 2010. Combined eukaryotic and bacterial community fingerprinting of natural freshwater biofilms using automated ribosomal intergenic spacer analysis. FEMS Microbiol. Ecol. **74**: 542-553. doi:10.1111/j.1574-6941.2010.00968.x

Gillevet, P. M., M. Sikaroodi, and A. P. Torzilli. 2009. Analyzing salt-marsh fungal diversity: Comparing ARISA fingerprinting with clone sequencing and pyrosequencing. Fungal Ecol. **2**: 160-167. doi:10.1016/j.funeco.2009.04.001

Kilias, E., E.-M. Nöthig, C. Wolf, and K. Metfies. 2014a. Picoeukaryote plankton composition off West Spitsbergen at the entrance to the Arctic Ocean. J. Eukaryot. Microbiol. doi:10.1111/jeu.12134.

Kilias, E., G. Kattner, C. Wolf, S. Frickenhaus, and K. Metfies. 2014b. A molecular survey of protist diversity through the central Arctic Ocean. Polar Biol. **37**: 1271-1287. doi:10.1007/s00300-014-1519-5

Kilias, E., C. Wolf, E.-M. Nöthig, I. Peeken, and K. Metfies. 2013. Protist distribution in the western Fram Strait in summer 2010 based on 454-pyrosequencing of 18S rDNA. J. Phycol. **49**: 996-1010. doi:10.1111/jpy.12109

Margulies, M., and others. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature **437**: 376-380. doi:10.1038/nature03959

Medlin, L., H. J. Elwood, S. Stickel, and M. L. Sogin. 1988. The characterization of enzymatically amplified eukaryotic 16S like rRNA coding regions. Gene **71**: 491-499. doi:10.1016/0378-1119(88)90066-2

Oksanen, J., and others. 2011. Vegan: Community ecology package, R package version 1.17, 6th ed.

Ramette, A. 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Appl. Environ.Microbiol. **75**: 2495-2505. doi:10.1128/AEM.02409-08

Smith, J. L., J. E. Barrett, G. Tusnady, L. Rejto, and S. C. Cary. 2010. Resolving environmental drivers of microbial community structure in Antarctic soils. Antarct. Sci. **22**: 673-680. doi:10.1017/S0954102010000763

Vergin, K. L., and others. 2013. High-resolution SAR11 ecotype dynamics at the Bermuda Atlantic time-series study site by phylogenetic placement of pyrosequences. ISME J. **7**: 1322-1332. doi:10.1038/ismej.2013.32

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White [eds.], PCR protocols: A guide to methods and applications. Academic Press.

Wolf C., S. Frickenhaus, E. S. Kilias, I. Peeken, and K. Metfies. 2013. Regional variability in eukaryotic protist communities in the Amundsen Sea. Antarct. Sci. **25**: 741-751. doi:10.1017/S0954102013000229

Wolf, C., S. Frickenhaus, E. S. Kilias, I. Peeken, and K. Metfies. 2014. Protist community composition in the Pacific sector of the Southern Ocean during austral summer 2010. Polar Biol. **37**: 375-389. doi:10.1007/s00300-013-1438-x

Acknowledgments

This study was accomplished within the Young Investigator Group PLANKTOSENS (VH-NG-500), funded by the Initiative and Networking Fund of the Helmholtz Association. Furthermore, we thank the captain and crew of the RV Polarstern for their support during the cruises. We are especially indebted to S. Frickenhaus, F. Kilpert, and B. Beszteri for their bioinformatical support and very grateful to A. Nicolaus and K. Oetjen for excellent technical support in the laboratory.

Submitted 30 April 2014
Revised 15 October 2014
Accepted 17 December 2014

Associate editor: Paul F. Kemp