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SAR11 clade microdiversity and activity during the early spring blooms off Kerguelen Island, Southern Ocean

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Running Title: SAR11 microdiversity in the Southern Ocean

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The present results expand current knowledge on the most abundant marine bacteria, the SAR11 clade, to the high nutrient low chlorophyll waters of the Southern Ocean. This report describes:

- SAR11 clade major contribution to the microbial community and carbon cycle, at the onset of the spring phytoplankton bloom induced by natural iron fertilization
- SAR11 clade microdiversity in a mosaic of phytoplankton blooms at different early bloom stages

The spatio-temporal partitioning of some of the SAR11 subclades revealed in this study suggests a niche specificity and periodic selection of the different SAR11 ecotypes in the Southern Ocean. Despite these changes in microdiversity the SAR11 population remained stable in abundance and activity across different bloom sites. These are significant observations to further understand the ecology of the SAR11 clade and its impact on biogeochemical cycles in the rapidly changing Southern Ocean.
Abstract

The ecology of the SAR11 clade, the most abundant bacterial group in the ocean, has been intensively studied in temperate and tropical regions, but its distribution remains largely unexplored in the Southern Ocean. Through amplicon sequencing of the 16S rRNA genes, we assessed the contribution of the SAR11 clade to bacterial community composition in the naturally iron fertilized region off Kerguelen Island. We investigated the upper 300 m at seven sites located in early spring phytoplankton blooms and at one High Nutrient Low Chlorophyll site. Despite pronounced vertical patterns of the bacterioplankton assemblages, the SAR11 clade had high relative abundances at all depths and sites, averaging 40% (±15%) of the total community relative abundance. Micro-autoradiography combined with CARD-FISH further revealed that the clade had an overall stable contribution (45-60% in surface waters) to bacterial biomass production (determined by $^3$H-leucine incorporation) during different early bloom stages. The spatio-temporal partitioning of some of the SAR11 subclades suggests a niche specificity and periodic selection of different subclades in response to the fluctuating extreme conditions of the Southern Ocean. These observations improve our understanding of the ecology of the SAR11 clade and its implications in biogeochemical cycles in the rapidly changing Southern Ocean.
Introduction

The SAR11 clade of the Alphaproteobacteria is one of the most abundant bacterioplankton in marine ecosystems (Morris et al., 2002; Carlson et al., 2009; Eiler et al., 2009) representing 25% or more of the total bacterial cells in seawater worldwide (Giebel et al., 2009; Brown et al., 2012; Sunagawa et al., 2015; Ortmann and Santos, 2016). Since its first discovery (Giovannoni et al., 1990), the clade’s spatial, vertical and seasonal patterns and links to ecosystem variables have been extensively studied and described (e.g. Carlson et al., 2009; Eiler et al., 2009; Brown et al., 2012; Morris et al., 2012; Vergin et al., 2013; Salter et al., 2014; Thrash et al., 2014; Ortmann and Santos, 2016). Conserved traits such as small cell size, small streamlined genomes, simplified regulatory systems, or efficient energy acquisition strategies seem to make members of this clade steady competitors that thrive in the minimal conditions provided by oligotrophic marine waters, and likely explain the clade’s remarkable success throughout the world’s oceans (reviewed in Giovannoni, 2017). Their ubiquitous abundances and specialized, atypical carbon substrate utilization profiles make SAR11 significant contributors to fluxes of carbon and other nutrients in the ocean (Giovannoni, 2017 and references therein). Beyond the existence of core characteristics shared by most members of the clade, detailed examination of SAR11 microdiversity has revealed several phylogenetic subclades that are consistently associated with specific environmental conditions (e.g. Field et al., 1997; Carlson et al., 2009; Vergin et al., 2013). These subclades seem to represent ecologically coherent populations or ecotypes. Subclade-specific traits and metabolic needs have been identified and likely play an important role in the ecological niche partitioning observed among the subclades (Grote et al., 2012; Thrash et al., 2014; Tsementzi et al., 2016; Haro-Moreno et al., 2020).
There are, however, fewer studies on the distribution and ecology of SAR11 subclades in the Southern Ocean (Brown et al., 2012; Liu et al., 2019; Haro-Moreno et al., 2020; Sow et al., 2022). The Southern Ocean is the largest High Nutrient Low Chlorophyll (HNLC) region in the world, a result of the low concentrations of the essential element iron (Fe) which limits primary production in surface waters (Blain et al., 2007; Pollard et al., 2009). Concentrations of dissolved organic carbon (DOC) in Southern Ocean surface waters are among the lowest of the global ocean (about 50 µM; Hansell et al. 2010), which in turns limits heterotrophic bacterial activity. Bacterial growth can also be co-limited by both Fe and DOC (Church et al., 2000; Obernosterer et al., 2015). Thus, in this environment, metabolic interactions shaping microbial communities are particularly complex, as diverse phytoplankton and heterotrophic bacterial taxa compete for Fe (Fourquez et al., 2016) while also relying on each other for key resources such as labile organic carbon and vitamins (Bertrand et al., 2007). Members of the SAR11 clade are highly adapted to oligotrophic waters in which nutrient concentrations are low, but they also have atypical metabolic requirements driven by genomic streamlining that hint at a strong dependency on metabolites synthesized by co-occurring microbes. They have also been shown to be dominant members of the microbial communities in other HNLC regions such as the Subarctic Pacific and Equatorial Pacific (e.g. Jing et al., 2013; West et al., 2016). For these reasons, the Southern Ocean provides an interesting environmental framework to investigate the activity and diversity of SAR11 and can contribute unique insight into the ecology of this marine bacterial clade.

Previously published work showed that in the Kerguelen area of the Southern Ocean, SAR11 populations can be abundant members of bacterial communities. The SAR11 clade was most successful in HNLC waters independent of season (West et al., 2008; Landa et al., 2016; Hernandez-Magana et al., 2021) where they also actively contribute to bacterial carbon uptake and cycling (Obernosterer et
...al., 2011; Fourquez et al., 2016; Sun et al., 2021). By contrast, in the naturally Fe fertilized waters off the Kerguelen plateau (Blain et al., 2007) the relative abundance of SAR11 revealed a pronounced seasonal pattern, starting with high relative abundances in the early bloom stage, followed by a drastic decrease after the spring phytoplankton bloom and an increase towards late summer (Liu et al., 2020). Whether or not different sublineages were found in HNLC waters and in the blooms or throughout the water column has implications for understanding the lifestyle and specific traits of SAR11 bacteria in these environments. In the present report, we follow up on the aforementioned studies and leverage the rich existing sample set to provide a detailed analysis of SAR11 activity, sublineage diversity and distribution patterns in this mosaic of phytoplankton blooms induced by natural Fe fertilization. Our results indicate that SAR11 populations remained abundant and active throughout the water column. Microdiversity analysis detected members from most known SAR11 sublineages and revealed distinct community composition at depth and across bloom stations.
The samples for the present study were collected in naturally Fe-fertilized and in HNLC waters off Kerguelen Island in early spring during the KEOPS2 cruise (Fig.S1). Concentrations of Chl a, bacterial abundance and bacterial heterotrophic production were overall higher in naturally Fe-fertilized mixed and intermediate waters as compared to the HNLC station R-2 (Table S1 and references within). However, among the Fe-fertilized sites, considerable variability in these biological parameters were observed (Table S1), reflecting spatial and temporal variability in the blooms development (Lasbleiz et al., 2016). By contrast, the major inorganic nutrients N and P, and DOC were similar across sites and characteristic for this region (Blain et al., 2015; Tremblay et al., 2015). In the present report, samples from eight stations and four sampled depths (20 to 300 m) were sorted into three water layers named mixed layer, intermediate layer and deep layer (Table S1), identified based on oceanographic parameters (Park et al., 2014).

We assessed the contribution of SAR11 to bacterial community using 16S rDNA amplicon sequencing at all stations and depth layers (Fig. 1). Additionally, we used CARD-FISH for 3 distinct Fe-fertilized bloom stations (A3.2; F-L and E-5) and the HNLC site R-2 (Table 1). Sequencing analysis showed that OTUs of the SAR11 clade represented on average 41±13% (and up to 62% in intermediate waters) of total sequences and were dominant at all stations down to 300 m regardless of the bloom regimes (Fig. 1). This observation expands our initial observations of generally high SAR11 relative abundances in surface waters (20 m) (Landa et al., 2016). The contribution of the SAR11 clade to bulk bacterial abundance based on CARD-FISH counts varied between 44.3±4% in HNLC waters and 33±10% at the three Fe-fertilized bloom stations A3.2, F-L and E-5, in the mixed and intermediate
layers (Table 1). Both microscopic and molecular methods provided comparable values that are in line with previous studies showing SAR11 abundances between 20 and 55% of the total bacterial communities in the same study area (West et al., 2008; Obernosterer et al., 2011; Hernandez-Magana et al., 2021) and in other regions of the Southern Ocean (Giebel et al., 2009; Tada et al., 2013). Both methods were generally in good agreement for most samples, however discrepancies between the two were observed in the deep-water samples (Table 1). The decrease in SAR11 relative abundances at 300 m compared to the mixed layer values was more pronounced for the CARD-FISH data than for the sequencing data. One limitation of the CARD-FISH approach is that cells with a low rRNA content are not always detected, while the PCR step required for 16S rRNA gene sequencing targets live, dormant or even dead cells. Another possible explanation for the observed differences between the two methods could be the existence of distinct deep SAR11 clades missed by the set of probes used in our study. This latter was likely not the case as our probes appeared to target all the subclades present.

Table 1

The success of SAR11 as the most abundant marine bacterial group suggests that they play an important role in organic matter fluxes. Here, micro-autoradiography combined with CARD-FISH showed that 11-84% of SAR11 cells were active in the upper 80 m (Table 1) and this fraction was generally lower below 150m. The average 48.2 ± 15.6% of total active cells as SAR11 in the upper 150 m, is similar to that observed previously in other, warmer oceanic regions, such as the North Atlantic and Mediterranean Sea where SAR11 contributed to 30-50% of the leucine-incorporating community (Malmstrom et al., 2004; Malmstrom et al., 2005; Laghdass et al., 2012) and higher than observed in other regions of the Southern Ocean (Straza et al., 2010; Tada et al., 2013). The contribution of SAR11 to bulk leucine incorporation was slightly lower than expected from their
contribution to abundance (Figure 2), a trend often observed in other regions (e.g., Elifantz et al., 2005; Alonso-Saez and Gasol, 2007; Alonso-Saez et al., 2008, Straza et al., 2010). A possible explanation is that SAR11 cells grow slower than other bacterial groups, and thus do not incorporate as much leucine as faster growing taxa. Interestingly and despite this seemingly moderate activity level of the SAR11 group, the taxon accounted for most of the leucine incorporating cell population, particularly in upper layers (Table 1). Overall, our data indicate that SAR11 are important contributors to carbon cycling across the studied region at this time of the year as a result of high abundances and sustained activity levels in various bloom conditions.

Figure 2

The present and previous studies (Giebel et al., 2009) highlight SAR11 to be major community members in non-productive Southern Ocean waters. Despite the differences in the environmental setting among stations (Table S1, Lasbleiz et al., 2016) the contribution of SAR11 to the total cells and to active cells appeared to be relatively constant. This is likely due to their successful adaptation to oligotrophic waters and to their capacity to take up efficiently organic substrates, among those some phytoplankton derived metabolites, such as very labile volatile molecules (Sun et al., 2011; Moore et al., 2020, 2022), while other taxa may favor high molecular weight DOM utilization (Malmstrom et al., 2005) as reported during the present cruise (Fourquez et al., 2016; Landa et al., 2018). So far, no known siderophores or heme uptake genes have been observed in SAR11 genomes (Hogle et al., 2016), suggesting that the success of SAR11 in these Fe-limited waters could further be due to other specific strategies related to uptake, storage and utilization of this limiting micronutrient (Beier et al., 2015; Debeljak et al., 2019; Sun et al., 2021). Pelagibacteraceae utilize predominantly inorganic Fe (Fe$^{3+}$, Hopkinson and Barbeau 2012; Debeljak et al., 2019); their metabolic activity is likely to be sustained
by the seasonally high concentrations of dissolved Fe prior to the phytoplankton bloom development (Quéroué et al., 2015). Using MICRO-CARD-FISH, it was indeed shown that SAR11 made up 25% of the community taking up Fe in surface waters at the sites investigated during the same cruise (Fourquez et al., 2016).

Figure 3

SAR11 subclades distribution over the studied area and dynamics during the onset of the spring bloom was further resolved to phylogenetic subclades partitioning. Overall, 47 OTUs were closely related to Pelagibacterales at 99% identity (covering 64310 reads, Fig. 3). The phylogenetic relationship between these OTUs and previously identified SAR11 subclasses (e.g. Field et al., 1997; Carlson et al., 2009; Vergin et al., 2013) showed that OTUs observed in this study separated between six different known subclades. Most of our reads (80±6%) belonged to subclade Ia, which is the most abundant and most studied SAR11 ecotype in the ocean (e.g. Giovannoni, 2017; Delmont et al., 2019). This cluster could be further separated in three subgroups (Fig. 3). The most abundant OTU in our dataset (KEOPS-6089, Fig. 3) represented 15% of the total relative abundance of all bacterial OTUs across all samples (on average 80% of all SAR11) and clustered in the Ia.1 group, with the first cultivated member of the clade: Pelagibacter HTCC1062 (Rappé et al., 2002). Ia. is the only subclade with Fe regulatory mechanisms for adaptation to Fe limitation (Smith et al., 2010; Gröte et al., 2012). Subclade Ia is also adapted to respond to phytoplankton derived one-carbon and volatile organic compounds (Sun et al., 2011; Halsey et al., 2017; Moore et al., 2020, 2022). Its capacity to efficiently utilize inorganic Fe and organic substances, or its low requirements of each to maintain cellular activity, may explain the success of this SAR11 Ia.1 clade in the region.

Figure 4
The SAR11 clades distribution as a function of depth showed that specific OTUs were more represented in different depth layers (Fig. 4). More specifically, subclades Ia, IIIa and IV were relatively more abundant in mixed and intermediate layers, while subclade Ib was more abundant in the deep layers (Fig. 4.A). Subclade Ib has also been reported in epi- and bathypelagic waters in the Red Sea (Jimenez-Infante et al., 2017). At the Atlantic time series site BATS, subclade Ib is usually found in mixed water in late spring and early summer, while Ic is found in deeper water (Vergin et al., 2013; Trash et al., 2014). Here, subclade Ic also increased in deeper waters, which may be related to specific adaptation to nutrient availability (Tsementzi et al., 2016; Ruiz-Perez et al., 2021).

Subgroup Ia.3 was more abundant in the deep layers, while Ia.1 and Ia.2 had higher relative abundances in mixed and intermediate layers (Fig. 4.B). Ia.1 has been observed in colder surface coastal waters (Rappe et al., 2002; Brown et al., 2012; Grote et al., 2012), and in other regions of the Southern Ocean (Haro-Moreno et al., 2020). Ia.3 has been reported in surface gyre and tropical waters (Brown et al., 2012; Grote et al., 2012; Delmont et al., 2019). Nevertheless, Ia.3 has also been observed in deep water of the Red Sea (Ngugi and Stingl, 2012).

The microdiversity of SAR11 subclades appeared more dynamic in surface waters (20m, Fig. 5), similar to the shift in overall surface bacterial community composition at the sites characterized by different early bloom stages (Landa et al., 2016). The different subclades showed evident patterns throughout the water column, with a shift in lineage identity and abundances at 300 m, compared to more similar mixed and intermediate layers (Fig. 4 and 5). This pronounced layer difference in microdiversity might be linked to the specific water masses circulation in the Southern Ocean. This
could also explain the general differences in microdiversity at station F-L, which is closest to the Polar Front and more influenced by Indian Ocean warmer waters.

SAR11 microdiversity also exhibited specific patterns linked to the bloom progression (Fig. 5). For instance, subclade IV was most abundant in more advanced bloom stage stations (A3.2, E4W and FL) where it probably benefited from specific relationships with phytoplankton cells (e.g. Becker et al., 2019; Tucker et al., 2021), such as public good secondary metabolites and volatile organic compounds produced by the phytoplankton (e.g. Giovannoni, 2017). Conversely, subclades Ic and IIa seemed most abundant in the non-bloom stations (R-2, HNLC station and E3, Fig. 5) with low organic carbon concentration. The bi-polar distribution of subclade IIa in surface waters has been previously reported (Kraemer et al., 2019); its adaptation to cold waters may explain its relatively stable distribution in less productive stations across the water column in the study area. Subclades Ia and Ib did not seem to respond to bloom stages (Fig. 5), which showcases their ability to maintain their metabolism regardless of conditions. Ia.2 appeared to be more abundant in the HNLC waters; this subgroup was the second most abundant in the region and did not cluster with published reference sequences (Fig. 3), suggesting that this group was locally adapted to less productive and Fe-limited waters of the Southern Ocean.

In conclusion, Pelagibacterales are highly adapted to the cold, organic carbon- and iron-limited waters of the Southern Ocean, which is consistent with the clade’s notorious ability to efficiently harvest limiting resources. The spatio-temporal partitioning of some of the SAR11 subclades revealed in this study followed observations made on niche specificity and periodic selection in other oceanic regions.
Nevertheless, the contribution of SAR11 to leucine incorporation was relatively stable across sites despite the variations in microdiversity, suggesting that subclades have a redundant impact on the carbon cycle or that one stable clade Ia1 was responsible for the overall activity. Investigating the metabolic potential of these SAR11 subclades are key to better understand the underlying mechanisms for their spatial distributions, and to further understand their evolution and ecological adaptation to the extreme conditions of the Southern Ocean, where they contribute substantially to the bacterial biomass and production and probably to other microbially mediated fluxes.
Acknowledgments

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The authors declare no conflict of interest.

References


Table 1: SAR11 clade contribution to bacterial abundance and bacterial leucine incorporation. Station R-2 represents the HNLC site while A3-2, F-L and E-5 represent Fe-fertilized sites at various bloom development stages (see Figure S1 and Table S1). Data are from this study unless specified, with * indicating surface data previously published in Fourquez et al. (2016). n.a.: not available. The percentage of active SAR11 was assessed by leucine uptake through Micro-CARD-FISH. The details of the method are described in (Fourquez et al., 2016). Briefly, 10 mL of seawater samples were incubated with radiolabeled leucine for 6-8h, fixed with paraformaldehyde and filtered onto 0.2 µm polycarbonate filters. The abundance of SAR11 was determined with catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) with the probes SAR11-152R, SAR11-441R, SAR11-542R and SAR11-732R (Morris et al., 2002). The micro-autoradiography development with photographic emulsion was exposed for 2-3d. After development the proportion of substrate active SAR11 were determined as the proportion of probe positive cell with silver grains.

<table>
<thead>
<tr>
<th>Station</th>
<th>Depth (m)</th>
<th>BA(1) (x10^5 cells ml^-1)</th>
<th>% of BA cells as SAR11(2)</th>
<th>SAR11 abundance (x 10^4 cells ml^-1)</th>
<th>% of active SAR11(3) cells</th>
<th>% of total active cells as SAR11</th>
<th>% relative abundance of SAR11 OTUs(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-2</td>
<td>20</td>
<td>2.30</td>
<td>49 ± 7*</td>
<td>11.2</td>
<td>51 ± 9*</td>
<td>60 ± 5</td>
<td>47</td>
</tr>
<tr>
<td>R-2</td>
<td>60</td>
<td>2.95</td>
<td>42 ± 4</td>
<td>12.4</td>
<td>38 ± 5</td>
<td>50 ± 3</td>
<td>n.a.</td>
</tr>
<tr>
<td>R-2</td>
<td>150</td>
<td>2.87</td>
<td>42 ± 2</td>
<td>12.1</td>
<td>42 ± 3</td>
<td>65 ± 6</td>
<td>14</td>
</tr>
<tr>
<td>R-2</td>
<td>300</td>
<td>1.30</td>
<td>19 ± 0</td>
<td>2.47</td>
<td>15 ± 3</td>
<td>23 ± 4</td>
<td>13</td>
</tr>
<tr>
<td>A3-2</td>
<td>20</td>
<td>2.70</td>
<td>35 ± 12*</td>
<td>9.45</td>
<td>12 ± 3*</td>
<td>52 ± 10</td>
<td>36</td>
</tr>
<tr>
<td>A3-2</td>
<td>80</td>
<td>3.53</td>
<td>43 ± 1</td>
<td>15.2</td>
<td>84 ± 3</td>
<td>58 ± 2</td>
<td>39</td>
</tr>
<tr>
<td>BA: Bacterial abundance determined by flow cytometry (data as published in Christaki et al., 2014)</td>
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<tr>
<td>Positive cells hybridized with SAR11 clade probes (*: marks 20 m depth data published in Fourquez et al., 2016)</td>
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<tr>
<td>Micro-autoradiography positive cells showing leucine incorporation (*: marks 20 m depth data published in Fourquez et al., 2016)</td>
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<td>Based on 16s rDNA sequencing (the community-level analysis of the 16S sequencing data from 20m-depth samples can be found in Landa et al., 2016)</td>
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Figure legends

Figure 1: Relative abundance (%) of the main bacterial taxonomic groups over the three depth layers. A total of 31 samples from eight different stations were analyzed for bacterial community composition by 454 pyrosequencing of the V1-V3 regions of the 16S rRNA gene. Each bar shows the average contribution of the specified groups across: 12, 11, 8 samples for the mixed, intermediate and deep layer respectively. Filtration, extraction, sequencing procedures and denoising of the sequences are described in Landa et al. (2016). Clean reads were subsequently processed using the Quantitative Insight Into Microbial Ecology pipeline (QIIME v1.7; (Caporaso et al., 2010b)). Reads were clustered into (OTUs) at 99% pairwise identity using Uclust and representative sequences from each bacterial OTU were aligned to Greengenes reference alignment using PyNAST (Caporaso et al., 2010a). All singletons and operational taxonomic units (OTUs) present in only one sample were removed. Taxonomy assignments were made using the Ribosomal Database Project (RDP) classifier (Wang et al., 2007) against the database Greengene 13_8 (McDonald et al., 2012) and SILVA 128 (Quast et al., 2013). The data were deposited in the Sequence Read Archive (SRA) database under accession number SRP041580.

Figure 2: Contribution of SAR11 clade to total ³H-leucine incorporating cells versus contribution of SAR11 clade to total bacterial abundance. The solid line indicates a 1:1 relationship.

Figure 3: Phylogenetic relationships of SAR11 OTUs: Maximum likelyhood tree of OTUs closely related to SAR11 clade. Only SAR11 OTUs representing more than 0.1% of the total SAR11 reads are included. Reference sequences from previously published SAR11 subclades
identifications are indicated in blue italic. Bootstrap values (n=1000) are indicated at nodes; scale bar represents changes per positions. SAR11 maximum likelyhood tree was computed with Mega7 (Tamura et al., 2013).

**Figure 4:** Layer distribution of SAR11 subclades (A) and subclades Ia (B): % relative abundance of SAR11 related OTUs representing more than 0.1 % of all SAR11 OTUs. Average of SAR11 relative abundance across all stations are shown, with error bars representing standard deviation between stations.

**Figure 5:** Vertical distribution of SAR11 subclades under different bloom conditions (integrated weight average of SAR11 relative abundance to total community, note different z scales, Grey line represent the limit of the mixed layer depth).
Figure 1

Dinasquett et al. SAR11 clade distribution in the Southern Ocean
Figure 2
Dinasquet et al.  

**SAR11 clade distribution in the Southern Ocean**

**Figure 3**

**Subclade Ia**
- KEOPS_5985
- KEOPS_6089
- KEOPS_5988
- KEOPS_6276
- KEOPS_6273
- KEOPS_8296
- HTCC1062 (AF310192)
- HTCC1062 (AF310191)
- KEOPS_8494
- KEOPS_8554

**Subclade Ib**
- KEOPS_6744
- KEOPS_5765
- HTMB5 (CP003809)
- KEOPS_51
- HTMB1521 (LT840189)
- HTCC7211 (NZ_DS95298)
- KEOPS_6768
- KEOPS_6120

**Subclade Ic**
- KEOPS_8543
- KEOPS_5936
- KEOPS_6163
- KEOPS_6617
- KEOPS_6608
- KEOPS_8588
- KEOPS_2482
- KEOPS_6734
- KEOPS_1793
- KEOPS_56
- KEOPS_5202
- KEOPS_8538
- KEOPS_8
- KEOPS_5361
- KEOPS_2902

**Subclade IIa**
- KEOPS_155
- SAR191 (U73649)
- KEOPS_1655
- KEOPS_35
- KEOPS_8586
- KEOPS_5346

**Subclade IIb**
- Ma-NH04 (AB195883)
- KEOPS_161
- SAR203 (U71255)
- SAR211 (U71256)
- KEOPS_2275
- KEOPS_7087
- KEOPS_7454
- KEOPS_9181
- KEOPS_8399
- KEOPS_1711
- KEOPS_8939
- KEOPS_8280
- KEOPS_8589
- KEOPS_8538
- KEOPS_8531

**Subclade IIIa**
- KEOPS_127
- MCC9663 (CP002511)
- LD12 (299997)
- KEOPS_17751
- KEOPS_25

**Subclade IIIb**
- SPOTSAPR01 (DQ009555)
- KEOPS_17751
- SPOTSAPR01 (DQ009555)
- KEOPS_17751

**Subclade IV**
- Polaribacter irgensii
- Brachibacterium casei

**Subclade V**

**La.1** (40481 reads; 8 OTUs)

**La.2** (12717 reads; 14 OTUs)

**La.3** (2925 reads; 6 OTUs)
Figure 4

% of relative abundance of SAR11 related OTUs

A.

B.
Figure 5
Supplementary Table and Figures

**Table S1:** Depth layer classification, environmental and bacterial parameters for the sampled stations. (n.a.: not available, HLNC: High nutrients low chlorophyll station). The wind mixed layer depth (MLD) as determined based on a difference in sigma of 0.02 to the surface value, as for instance described in de Boyer Montégut *et al.* (2004). The samples were then attributed to one of the three categories: surface (MLD), intermediate (transition zone between MLD and winter water) and deep (winter water) based on the physical properties as published in Park *et al.* (2014). The sampling was conducted during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study 2) cruise from October to November 2011 on board the R/V Marion Dufresne in the Kerguelen region (FigS1 and see Fig. 1 in Landa *et al.*, 2016). A total of seven stations (A3.2, E stations and F-L) were sampled in the naturally iron-fertilized regions east of the Kerguelen Islands and a reference station (R-2) was sampled in high nutrient low chlorophyll waters (HNLC) located west of the islands. E1, E3, E4-E and E5 were sampled temporally in a quasi-Lagrangian manner. For each station four depths were sampled according to CTD profiles.

<table>
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<th>Station group</th>
<th>Station</th>
<th>Depth (m)</th>
<th>layer</th>
<th>Chl. <em>a</em> (µg L⁻¹)*</th>
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<th>Bacterial production (ng C L⁻¹ h⁻¹)*</th>
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*data from Christaki et al. 2014*
Supplementary Figure S1: KEOPS2 study area. The sampling was conducted during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study 2) cruise from October to November 2011 on board the R/V Marion Dufresne in the Kerguelen region (FigS1 and see Fig. 1 in Landa et al., 2016). A total of seven stations (A3.2, E stations and F-L) were sampled in the naturally iron-fertilized regions east of the Kerguelen Islands and a reference station (R-2) was sampled in high nutrient low chlorophyll waters (HNLC) located west of the islands. For each station four depths were sampled according to CTD profiles. Chl $a$ (color scale), surface velocity fields (arrows), the polar front (PF, black line), and the position of the different stations: The Chl $a$ rich stations: A3, on the Kerguelen plateau; F-L and E-4W north and south of the polar front; and “E” stations sampled in a quasi-Lagrangian manner (E-1, E-2, E-3, E-4E, and E-5) within a complex meander south of the polar front. The reference HNLC station (R-2) is not shown as it is out of the area of the map (66.692743 E longitude,
50.38954 N latitude). Map is courtesy of Y. Park and colleagues. To note: the chlorophyll content represented on the map corresponds to the last week of the KEOPS2 cruise.

References

