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Floating plastics and their associated biota in the Western South Atlantic

Ana L. d. F. Lacerda*^{1,2}, Joe D. Taylor³, Lucas d. S. Rodrigues^{1,2}, Felipe Kessler^{1,4}, Eduardo Secchi^{1,2,5}, Maíra C. Proietti^{1,2}

¹Projeto Lixo Marinho - Instituto de Oceanografia, Universidade Federal do Rio Grande-FURG, Rio Grande, Brazil; ²Programa de Pós-Graduação em Oceanografia Biológica, PPGOB, Universidade Federal do Rio Grande-FURG, Rio Grande, Brazil; ³School of Chemistry and Biosciences, University of Bradford, Bradford, West Yorkshire, United Kingdom; ⁴Escola de Química e Alimentos, Universidade Federal do Rio Grande-FURG, Rio Grande, Brazil;⁵Laboratório de Ecologia e Conservação da Megafauna Marinha-Ecomega, Universidade Federal do Rio Grande-FURG, Rio Grande, Brazil.

*corresponding author: analuzialacerda@gmail.com

1 **Abstract**

2 The lack of information about plastic pollution in many marine regions
3 hinders firm actions to manage human activities and mitigate their impacts. This
4 study conducted for the first time a quali-quantitative evaluation of floating
5 plastics and their associated biota from coastal and oceanic waters in South
6 Brazil. Plastics were collected using a manta net, and were categorized
7 according to their shape, size, malleability and polymer composition. Multi-
8 marker DNA metabarcoding (16S, and 18S V4 and V9 rRNA regions) was
9 performed to identify prokaryotes and eukaryotes associated to plastics. We
10 found 371 likely plastic particles of several sizes, shapes and polymers, and the
11 average concentration of plastics at the region was 4,461 items.km⁻² (SD ±
12 3,914). Microplastics (0.5 - 5 mm) were dominant in most sampling stations,
13 with fragments and lines representing the most common shapes. Diverse
14 groups of prokaryotes (20 bacteria phyla) and eukaryotes (41 groups) were
15 associated with plastics. Both the community composition and richness of
16 epiplastic organisms were highly variable between individual plastics but, in
17 general, were not influenced by plastic categories. Organisms with potential
18 pathogenicity (e.g. *Vibrio* species. and *Alexandrium tamarense*), as well as
19 potential plastic degraders (e.g. *Ralstonia*, *Pseudomonas*, and *Alcanivorax*
20 species), were found. The information generated here is pivotal to support
21 strategies to prevent the input and mitigate the impacts of plastics and their
22 associated organisms on marine environments.

23
24 **Keywords:** Plastic pollution; Plastisphere; Biofilm; DNA-metabarcoding;
25 Brazilian coast

26

27 **1. Introduction**

28 Although plastic pollution is receiving increasing scientific attention, there
29 are still many gaps in our understanding of factors that are crucial to solve this
30 issue, such as the abundance, characteristics and consequences of plastics in
31 the ocean (Wilcox et al., 2016; Beaumont et al., 2019; Rochman & Hoellein,
32 2020). Most plastics found at sea are from continental sources (land-based),
33 with a lower contribution from maritime (Li et al. 2016; Krantzberg 2019) and
34 atmospheric (Sridharan et al., 2021) sources; however, this can vary according
35 to location (Jang et al., 2014). Riverine systems are one of the main continental
36 sources of plastics to the ocean, and it has been estimated that between 1.15 -
37 2.41 million tons of plastics enter the ocean annually from rivers (Lebreton et
38 al., 2017). Among maritime sources, nautical activities such as fishing, shipping,
39 offshore mining and illegal dumping at sea are important contributors of plastics
40 (GESAMP, 2019), with an estimated 0.6 million tons entering the ocean
41 annually (Boucher & Billard, 2019). Most plastics at sea are small, being
42 classified as microplastics (0.001 - 5mm); those that are manufactured in this
43 size are classified as primary microplastics, whereas those that originate from
44 the breakdown of larger items are called secondary microplastics (Cole et al.,
45 2011).

46 Once in the ocean, plastics can cause habitat degradation and physically
47 impact the marine biota by entanglement, asphyxiation and ingestion (Gall &
48 Thompson, 2015; Kühn & van Franeker, 2020), which has been reported for
49 over 1200 species (Santos et al., 2021) – including several that are
50 commercially exploited (Neto et al., 2020). When ingested, plastics can cause

51 lesions or physical obstruction of the digestive tract, fecalomas, and false sense
52 of satiety (Kühn et al., 2015; Kumar et al., 2021); they can also transfer their
53 associated toxic compounds to animals (Derraik, 2002; Diepens & Koelmans,
54 2018; Provencher et al., 2018), with potential biomagnification of such
55 compounds to higher trophic levels (Teuten et al., 2009; Setälä et al., 2014;
56 Meyer-Rochowa et al., 2015). Entanglement in plastics can result in drowning,
57 lesions and infection, as well as restrict movements and foraging ability of
58 marine biota (Kühn et al., 2015; Kumar et al., 2021).

59 Plastics in the ocean also provide durable surfaces for the attachment of
60 many species, and can therefore support complex ecological communities
61 (Reisser et al., 2014; Kirstein et al., 2018; Amaral-Zettler et al., 2020). Mature
62 communities may have a wide range of associated bacteria (Pinto et al., 2019),
63 fungi (Lacerda et al., 2020), microalgae (Nava & Leoni, 2021), and several
64 metazoan groups (Reisser et al., 2014; Kirstein et al., 2018; Amaral-Zettler et
65 al., 2020). Floating plastics can transport organisms over large distances, with
66 unknown consequences that could include species introductions and
67 bioinvasions (Barnes 2002; Barnes & Milner 2005; Fazey and Ryan 2016; Rech
68 et al., 2016; Carlton et al., 2017). Biofilm-covered plastics can also appear and
69 smell like food items, stimulating their ingestion by other organisms (Amaral-
70 Zettler et al., 2015). Although the ecological impacts of plastic biofilms in the
71 ocean are still unclear, it is known that plastic-associated communities can
72 include primary producers, predators, symbionts and saprotrophic groups
73 (Amaral-Zettler et al., 2020; Lacerda et al., 2020). The prokaryotic and
74 eukaryotic groups that live in plastic biofilms can also include potential
75 pathogenic (Zettler et al., 2013; Kirstein et al., 2016; Amaral-Zettler et al., 2020)

76 or hydrocarbon-degrading organisms (Muthukumar et al., 2011; Paço et al.,
77 2017; Delacuvellerie et al., 2019; Oberbeckmann & Labrenz, 2020; Zhang et al.,
78 2021). Some pathogenic species are generally sparse in the open ocean, and
79 their association with plastics raises the concern as to whether the increasing
80 amount of plastics in the ocean provides greater opportunities for pathogens to
81 be transported and transmitted to hosts, leading to increased outbreaks of
82 disease (Bowley et al., 2020). It has been suggested that the characteristics of
83 plastics (e.g. polymer composition) influence the diversity of colonizing groups.
84 However, studies have shown contradictory evidences: while some show
85 significant difference in the species composition of distinct plastic polymers
86 (Debroas et al., 2017; Bhagwat et al., 2021), others conclude that plastic
87 composition does not determine its associated communities (Bryant et al., 2016;
88 Dussud et al., 2018; Oberbeckmann et al., 2020 and 2021). Indeed,
89 biogeography seems to be the most important factor driving the community
90 composition on plastics in aquatic systems (Oberbeckmann & Labrenz, 2020).

91 In the Western South Atlantic, studies on floating plastics are scarce; this
92 includes the Brazilian coastline, whose extensive length (~ 8,000 km) hinders
93 thorough monitoring of plastic pollution. In fact, studies on this subject in Brazil
94 were for many years focused on beaches (Wetzel et al., 2004; Ivar do Sul &
95 Costa, 2007; Portz et al., 2011; Carvalho & Baptista Neto, 2016; Ramos et al.,
96 2021) and estuaries (Possatto et al., 2015; Krelling & Turra, 2019), and there is
97 little information on plastics at the sea surface (Black et al., 2020; Videla &
98 Araujo, 2021; Lins-Silva et al., 2021). Additionally, few of these studies have
99 characterized the species inhabiting plastics at the region. To our knowledge,
100 only two studies in the open ocean of the Brazilian coast has assessed the

101 plastisphere through DNA analysis, one identifying the microbial communities of
102 plastics experimentally deployed in the deep sea (Agostini et al., 2021), and the
103 other focused on fungi from floating plastics (Lacerda et al., 2020); however, no
104 studies have used a DNA multibarcoding approach to identify both prokaryotes
105 and eukaryotes associated to plastics in surface waters of the Western South
106 Atlantic so far.

107 Only recently the Brazilian Ministry of the Environment created the
108 “National Plan to Combat Litter at Sea” (Ministério do Meio Ambiente, 2019),
109 which recommends the conduction of research on plastics at the Brazilian
110 coast. In addition, this action is also suggested in one of the Sustainable
111 Development Goals (SDG) established by the United Nations (SDG 14 - Life
112 Below Water). Most plastisphere studies worldwide have targeted prokaryotes
113 (Oberbeckmann & Labrenz, 2020; Wright et al., 2020a), with a very small
114 number focusing on eukaryotes, especially those on plastics from the open
115 ocean (Kirstein et al., 2018; Amaral-Zettler et al., 2020). Moreover, studies that
116 have evaluated the eukaryotic communities that live on plastic debris sampled
117 in the ocean clearly identify many taxa (Amaral-Zettler et al., 2020), which
118 reinforces the need to understand the holobiome of plastics, and their ecological
119 interactions in marine environments (Oberbeckmann et al., 2021). Therefore,
120 the aim of our study was to evaluate the abundance and characteristics of
121 plastics, including the diversity of their associated organisms, sampled at
122 surface oceanic waters in the Brazilian coastline. We hypothesized that 1) the
123 sea surface in South Brazil is highly polluted by plastics due to the input of both
124 continental (e.g. mismanaged waste, direct disposal by tourism) and maritime
125 sources (e.g. ship traffic, intense fishing activities); 2) there are numerous

126 prokaryotic and eukaryotic groups living associated with plastics from coastal
127 and oceanic regions along South Brazil, forming complex communities; and 3)
128 the characteristics of plastics influence the community composition of their
129 associated organisms at the region. Such information is pivotal for establishing
130 strategies to prevent inputs and mitigate the impacts of plastics on marine
131 ecosystems at local and global scales.

132

133 **2. Material and Methods**

134 *2.1 Sampling of plastics at the sea surface*

135 Plastics were collected at the ocean-air interface in October 2016 and
136 2017 at ten stations along the continental shelf off the coast of South Brazil,
137 with two inshore sampling locations (Supplementary material, Table 1), between
138 latitudes 26° S and 34° S (Figure 1A), as part of the TALUDE project. We
139 highlight that the two inshore locations were near the Patos Lagoon Estuary,
140 with a drainage basin of about 200,000 km² (Seeliger & Odebrecht, 2010), and
141 the Itajaí-Açu river, with an estuarine area of 15,000 km²
142 ([http://www.jornalmetas.com.br/valedasaguas/orio/a-maior-bacia-hidrografica-](http://www.jornalmetas.com.br/valedasaguas/orio/a-maior-bacia-hidrografica-de-sc)
143 [de-sc](http://www.jornalmetas.com.br/valedasaguas/orio/a-maior-bacia-hidrografica-de-sc)). Both estuaries have several urban and industrial centres along their
144 margins, with ports and intense fishing activities (Vasconcellos & Kalikoski,
145 2014). At each station, trawls using a Manta net (100 cm × 21 cm mouth, 330
146 µm mesh, Figure 1B) were performed in triplicate for 11-17 min each, at a
147 speed of 2.5 – 3.0 knots. After sampling, volume-reduced samples were
148 collected in a sterile sieve with the same mesh size (Figure 1C), and frozen in
149 aluminium bags at -20° C to preserve the DNA of plastic-associated biota.

150

151 Figure 1 here.

152

153 At the start and end of each trawl, we noted the geographical coordinates
154 and time. The trawled area was calculated based on trawl velocity (*trawl vel*,
155 considering 1 knot = 0.514 m.s⁻¹), time (*t*, in seconds) and the Manta net width
156 (1 meter), using the equation:

157

$$Area = trawl\ vel * t * 1$$

158 To estimate the concentration of plastics at the sea surface, for each
159 sampling point, the number of items found in the trawled areas was
160 extrapolated to items.km⁻², and the total average concentration was calculated.
161 We used an Analysis of Variance (ANOVA) to check for differences in plastic
162 concentrations in terms of categories and sampling regions (divided by states:
163 sampling points 1, 8, 9, 10 in Rio Grande do Sul - RS; sampling points 2, 3, 4,
164 5, 6, 7 in Santa Catarina - SC).

165 *2.2 Quanti-qualitative characterization of plastics*

166 In the laboratory, samples were thawed separately and placed in a sterile
167 container filled with artificial sterile salt water (salinity 35) for manual separation
168 of floating plastic pieces and biomass (Reisser et al., 2013). A trained observer
169 visually examined each sample for at least 2 hours. Synthetic polymers were
170 classified into paint particles and non-paint plastics (Song et al., 2014) (called
171 only paints and plastics, respectively, from here on). All items were picked up
172 with the naked eye using sterile forceps, and measured over their largest cross-
173 section (total length) using a digital caliper (0.01-150 mm). According to their
174 size, visible plastics ranging from ~ 0.5 mm up to 5.0 mm were classified as
175 microplastics, and those with 5 mm to 200 mm as mesoplastics, adapted from

176 Eriksen et al. 2014. We highlight that smaller particles could have been present,
177 but cannot be detected by this evaluation method. Plastics were also classified
178 according to their shape (fragment, foam, line, pellet and film) (GESAMP, 2019),
179 polymer composition and malleability (hard or flexible), where items were
180 considered flexible if they could be manually folded. The colours of paints and
181 plastics were estimated following the 12 basic colour terms of the Inter-Society
182 Colour Council, National Bureau of Standards/ISCC-NBS.

183 All plastic pieces were placed individually in a microcentrifuge tube with
184 absolute ethanol (reagent grade, MERK) to preserve the genetic material until
185 DNA extraction of plastic-associated organisms, and 32 were randomly chosen
186 for genetic analyses. The polymer composition of 117 samples (31.53% of all
187 particles) was determined through Fourier Transform Infrared Spectroscopy
188 (FTIR) with a SHIMADZU spectrometer, model Prestige 21, using a diffuse
189 reflectance module, 24 scans and 4 cm⁻¹ resolution. Of the 32 samples
190 destined for metabarcoding analysis, 21 were recovered from the PowerBead
191 extraction tube after DNA extraction for polymer analysis. FTIR procedures and
192 data analysis followed the standard practice ASTM E1252-98 (2013) (ASTM
193 international). We did not perform any FTIR or metabarcoding analysis for paint
194 particles.

195 *2.3 Profiling of plastic-associated organisms*

196 Plastic pieces were rinsed in sterile artificial seawater to remove loosely
197 associated organisms (organisms that co-occurred with plastics during
198 sampling) before DNA extraction. The total DNA of plastic biofilms was
199 extracted using a PowerSoil DNA extraction kit (Qiagen) (shown to be efficient
200 for this type of sample by Debeljak et al. (2017)), with some modifications from

201 the manufacturers' instructions: in the first step we added 10 μ l (1000U/ μ l) of
202 lysozyme (Debeljak et al., 2017), and in the last step the DNA was eluted in a
203 lower buffer volume (20-30 μ l) to increase DNA yield and concentration.

204 The quality and concentration of extracted DNA were checked by
205 spectrophotometry using a Biodrop DUO (Harvard Bioscience™). We used
206 primers 515f (5'-GTGYCAGCMGCCGCGGTAA-3') and 806r
207 (GGACTACNVGGGTWTCTAAT) to amplify the 16S V4 region (Walters et al.,
208 2016); TAREuk454 (5'-CAGCASCYGCAGTAATTCC-3') and TAREukRev3 (5'-
209 ACTTTTCGTTCTTGATYRA-3') to amplify the 18S V4 region (Stoeck et al.,
210 2010); and 1391f (5'-GTACACACCGCCCGTC-3') and EukB (5'-
211 TGATCCTTCTGCAGGTTACCTAC-3') to amplify the 18S V9 region (Amaral-
212 Zettler et al., 2009). PCR reactions and conditions for all molecular markers are
213 detailed in Supplementary Material (Table 2). The library preparation and
214 sequencing on the Illumina Mi-seq platform followed the methods described in
215 Lacerda et al. (2020).

216 *2.4 Analysis of metabarcoding data*

217 DNA sequences were analysed using a combination of USEARCH
218 v7.0.1090 (32Bit) (Edgar, 2010) and QIIME v 1.8.0 (Caporaso et al., 2010).
219 Forward and reverse reads were merged using USEARCH. Each primer set
220 was separated using Cutadapt (Martin, 2011), removing primers and adapters.
221 For each primer set, fastq files were quality filtered, removing reads with
222 expected error > 0.5 and short sequences < 200 bp. Reads were then truncated
223 to 250 bp, 370 bp and 150 bp for 16S, 18S V4 and 18S V9, respectively, and
224 converted to FASTA files. The FASTA files were dereplicated, abundance sorted
225 and had their singleton sequences removed. OTUs (Operational Taxonomic

226 Units) were clustered using the UPARSE clustering algorithm at 97% (Edgar,
227 2013). Chimeras were filtered using UCHIME (Edgar et al., 2011), OTUs were
228 mapped back to the original reads, and an OTU table was produced. Both 16S
229 and 18S sequences were classified against the SILVA 132 database (Quast et
230 al., 2013) using UCLUST (Edgar, 2010). OTUs with less than four sequence
231 reads per cluster were excluded from the downstream analyses. In addition,
232 prokaryotic OTUs present in less than three samples were also excluded from
233 the 16S dataset. Within the 16S dataset, contaminants such as Eukarya,
234 mitochondria, and unknown domain were removed; within both 18S datasets
235 some Eukarya sequences (Chloroplastida, some fungi species and large
236 metazoans such as Salpida, Chelicerata, Chilopoda, and Eutelostomi) were
237 also manually removed during analysis, since they likely represented
238 contamination. Abundant and frequent OTUs, as well as those identified as
239 being potentially pathogenic, hydrocarbon or plastic degraders were further
240 classified against the full NCBI database.

241 *2.5 Analyses of epiplastic communities*

242 The prokaryotic and eukaryotic communities obtained with each
243 molecular marker were analysed separately. To assess both alpha and beta
244 diversity, OTU tables were rarefied to 1,000 reads for 16S, 500 reads for 18S
245 V4 and 700 reads for 18S V9. Differences in alpha and beta diversity (OTUs
246 richness and community composition) among plastic categories (size, shape
247 and polymer composition) and locations (SC and RS) were evaluated.
248 Categories were considered as the following levels: size (fixed factor-
249 microplastic/mesoplastic), shape (fixed factor- fragment/line) and polymer
250 composition (fixed factor-

251 polyamide/polyethylene/polypropylene/polystyrene/polyurethane). A Kruskal-
252 Wallis test was performed to check for differences between OTU richness per
253 sample between plastic categories and locations for each marker. The beta
254 diversity was measured as the average distance from the individual plastic to
255 the category's median, using the binary Jaccard index (Anderson et al., 2006).

256 The Jaccard dissimilarity matrix was used to produce nonmetric
257 multidimensional scaling (NMDS) plots. Tests of multivariate homogeneity of
258 group dispersions (PERMDISP) were implemented to ensure that differences in
259 communities were not a within-group variation (Anderson, 2001). A
260 Permutational Multivariate Analysis of Variance (PERMANOVA), with fixed
261 factors and 9999 permutations was used to check if the community beta
262 diversity differs among plastic categories. A significance level of $p < 0.05$ was
263 considered, and all statistical analyses were done with the *vegan* package
264 (Oksanen et al., 2019) in R studio 1.1.456 (R Development Core Team). The
265 *ggplot2* package (Wickham, 2009) was used in R studio to build dot plots with
266 number of OTUs (richness), NMDS plots, as well as balloon plots showing the
267 frequency of occurrence of prokaryotes (phylum level) and eukaryotes
268 (separated by groups across the tree of life) for each molecular marker.

269 **3. Results**

270 *3.1 Plastic and paint concentration and types*

271 We found a total of 371 likely plastic particles of different sizes, shapes,
272 colours, malleability, and polymer compositions. The mean concentration of
273 plastics at the sea surface along South Brazil was 4,461 items.km⁻², varying
274 from 2,989 items.km⁻² at the oceanic station 5 to 19,267 items.km⁻² at the
275 coastal station 1. The two highest plastic concentrations were at stations

276 located in Rio Grande do Sul waters (stations 1 and 9, Figure 2 - I).
277 Microplastics (< 5mm) represented 68% of the plastic items and were dominant
278 in all the sampling stations, except at station 9; mesoplastics (5 - 200 mm)
279 represented 32% of the dataset. There was significant difference (ANOVA; $F =$
280 11.924, $p = 0.014$) between the concentration of micro and mesoplastics at the
281 Santa Catarina region (Figure 2 - I).

282

283 Figure 2 here

284

285 The most common plastic shape was fragment (65%), followed by line
286 (33%); other shapes (pellet, foam and film) represented less than 1% each of
287 the total. While fragments were dominant among microplastics (80%), lines –
288 the second most abundant plastic shape – dominated in the mesoplastic
289 category (70%). Pellet, foam and film were all microplastics. Sampling stations
290 had plastics of different shapes and sizes (Figure 2 - I and III), but microplastic
291 fragments prevailed in over half of the stations. There was significant difference
292 in the concentration of lines between the RS and SC regions ($p = 0.036$). None
293 of the stations had all plastic shapes, but station 8 had the highest variety
294 (fragment, line, foam and film) (Figure 2 - III).

295 In terms of malleability, most plastics were flexible (75%). The majority of
296 hard plastics were fragments, apart from one pellet. White/transparent, yellow,
297 blue, grey, orange, brown, black, green and red plastics were observed, with a
298 dominance of white/transparent and blue items (44% and 32%, respectively).
299 FTIR spectra of the 117 particles revealed that all were plastics, composed of
300 polyamide (PA, 48%), polyurethane (PU, 21%) polyethylene (PE, 9%),
301 polystyrene (PS, 9%), polypropylene (PP, 7%), polyethylene terephthalate (PET,

302 7%), cellulose acetate (CA, 3%), and ethylene-vinyl acetate (EVA, 1%) (Figure
303 2 - IV).

304 We found 613 paint chips in our samples, which corresponded to almost
305 twice the abundance of common plastics. The mean concentration of paint
306 fragments along South Brazil was 16,442 items.km⁻², varying from 3,243
307 items.km⁻² at station 10 to 31,843 items.km⁻² at station 2 (Figure 2 - II). Paint
308 chips displayed sizes ranging from 0.5 to 8 mm – with most particles falling in
309 the micro size class – and were green, orange, yellow, white, red and blue.

310 *3.2 Prokaryotic and eukaryotic diversity on plastics*

311 After sequence processing and quality filtering, the 16S dataset
312 contained 21 samples with 408,805 reads comprised into 444 prokaryotic
313 OTUs. The 21 plastic samples had Bacteria associated with biofilms and 17 had
314 Archaea OTUs. The Archaea group was composed by phyla Euryarchaeota and
315 Thaumarchaeota, but none of the Archaea OTUs had four or more reads per
316 sample, and thus were not included in the analysis.

317 The number of prokaryotic OTUs per sample varied from 30-288 OTUs
318 (mean 109 ± 12.8 SD). Representatives from 20 phyla of Bacteria were found
319 associated with plastics (Figure 3), with dominance of Proteobacteria over the
320 entire dataset (abundance of 64%, composed by Alpha, Delta and
321 Gammaproteobacteria), followed by Bacteroidetes (14%), Cyanobacteria and
322 Firmicutes (6% each). Six bacterial phyla presented abundances of between 1 -
323 3% (Verrucomicrobia, Planctomycetes, Epsilonbacteraeota, Actinobacteria,
324 Thermotogae and Chloroflexi), and the remaining phyla together represented
325 less than 2% of the total 16S dataset (Figure 3 - I). The prokaryotic community

326 of sampled plastics was highly similar in terms of OTU richness over the
327 latitudinal gradient (26° S – 34° S).

328

329 Figure 3 here

330

331 The four most abundant bacterial groups also showed a high frequency
332 of occurrence (Proteobacteria, Bacteroidetes, and Firmicutes FO 100%;
333 Cyanobacteria FO 95%) (Figure 3 - II). Within the Proteobacteria phylum,
334 Gammaproteobacteria was the most abundant group (47% abundance, FO
335 100%), followed by Alphaproteobacteria (16% abundance, FO 100 %) and
336 Deltaproteobacteria (less than 1% abundance, FO 71%). Within
337 Gammaproteobacteria, we found six *Oceanospirillales* OTUs, which had an
338 abundance of less than 1% in the 16S dataset. The most abundant prokaryotic
339 OTU, representing 23% of the dataset (OTU_1), was classified as uncultured
340 bacteria (uncultured *Ralstonia*) by SILVA, and it also matched many uncultured
341 bacteria from environmental samples (mostly sediments) on NCBI (Genbank
342 MN723154.1) (Table 1).

343 Some bacteria OTUs in our samples closely matched potential
344 pathogens such as *Vibrio parahaemolyticus* from shrimp (100%, Genbank
345 reference MT549171.1), and *Escherichia coli* from wastewater (100%, Genbank
346 reference CP055438.1), as well as many *Pseudomonas* species. We also found
347 bacterial groups previously described as plastics and/or hydrocarbon
348 degraders, such as polycyclic aromatic hydrocarbons (PAH), a common plastic-
349 associated contaminant (Adams et al., 2007; Diepens & Koelmans, 2018), as
350 *Ralstonia* sp. (Biki et al., 2021; Ryan et al., 2007), *Erythrobacter* species (Gao
351 et al., 2015), *Bacillus* species (Wright et al., 2020b), *Alcanivorax* and

352 *Arenibacter* species (Delacuvellerie et al., 2019; Sekiguchi et al., 2011),
353 *Pseudomonas* species (Balasubramanian et al., 2010), and *Colwellia* sp. and
354 *Oleibacter* sp. (Urbanek et al., 2018).

355 The most abundant OTUs had closest matches to sequences from
356 environmental sequence surveys in the full NCBI database, and some showed
357 low matches to any cultured representative (e.g. OTU_12, Table 1). Most OTUs
358 had matches to taxa observed in untreated water, as well as marine sediments
359 and coastal waters, including species associated with marine organisms such
360 as coral, fish, sponge, ascidian and shrimp (Table 1).

361

362 Table 1 here.

363

364 Two 18S markers were used to analyse the diversity of eukaryotic life on
365 the sampled plastics. After sequence processing and quality filtering, the 18S
366 V4 dataset contained 22 samples with 189,466 reads, whereas the 18S V9
367 dataset contained 28 samples with 876,534 reads, clustered into 337 and 655
368 OTUs, respectively. The number of OTUs per sample within the 18S V4 dataset
369 ranged from 5 - 65 (average 31 ± 3.2 SD), and within the 18S V9 from 18 - 231
370 (average 68 ± 8.1 SD). The 18S V4 and V9 markers detected different
371 taxonomic groups, with some exclusive groups in each dataset: Telonema and
372 Ichthyosporea were shown only with the 18S V4 marker, whereas Bryozoa,
373 Haptophyta, Excavata, Centrohelida, Picozoa, and Hemichordata (among
374 others) were present only within the 18S V9 dataset (Figure 4).

375 No eukaryotic group had FO 100% within the 18S V4 dataset:
376 Chlorophyta, Charophyta and Cnidaria had FO 95% each, followed by Fungi
377 with FO 91%, and Diatom, Dinoflagellata, and 'Other Stramenopiles' with FO

378 77% each. In addition, the groups Chrysophyta, Cercozoa, Ciliophora,
379 Nematoda, Chaetognatha, Syndiniales, and 'Other Alveolata' displayed FO >
380 50% each. The remaining groups identified by this marker had FOs lower than
381 50% each (Figure 4). The 18S V9 marker showed a different community
382 composition in terms of taxa diversity. Some groups presented higher
383 frequencies of occurrence: Fungi had FO 100%, followed by Chlorophyta with
384 FO 96%, and Diatom, 'Other Stramenopiles' and Cnidaria with FO 93% each,
385 while Cercozoa, Crustacea and Ciliophora had FO 86% each. Invertebrates
386 such as Tunicata, Nemotoda, Chaetognatha, and Mollusca occurred in over
387 50% of samples within the 18S V9 dataset (Figure 4).

388

389 Figure 4 here.

390

391 The most frequent eukaryotic OTUs detected by both molecular markers
392 also matched many uncultured eukaryotic OTUs from the marine environment
393 (seawater and sediments), as well as with cultured species from coastal zones
394 (e.g. coastal water, intertidal pools), and symbionts with marine sponge and
395 radiolarian (Table 2). Some harmful eukaryotes were found in the plastisphere
396 from the Western South Atlantic, such as dinoflagellates of genus *Alexandrium*,
397 whose species are known for being toxic to marine life and humans (Donald et
398 al., 2012). Fungi groups found in our samples were composed of some
399 parasites/pathogens, as well as hydrocarbon degraders – these groups are
400 detailed in Lacerda et al. (2020).

401

402 Table 2 here

403

404 Richness of OTUs per plastic fragment was variable between individual
405 plastic items, and some samples had low richness. There was no significant
406 difference in the OTU richness of plastics of distinct categories (Figure 5), as
407 well as according to location (Kruskal-Wallis, $p > 0.05$), except within the 16S
408 dataset where we found significant difference in bacterial OTUs richness
409 (Kruskal-Wallis, $p = 0.006$) between RS and SC. Likewise, the community
410 composition was highly variable between plastic items within each DNA marker
411 dataset (Figure 6). There was significant difference in community composition
412 according to size class within the 18S V9 dataset (PERMANOVA: $F = 1.276$, $p =$
413 0.04), and according to location in both the 18S V4 (PERMANOVA: $F = 1.988$, p
414 < 0.001) and V9 (PERMANOVA: $F = 1.495$, $p = 0.009$) datasets.

415

416 Figure 5 here.

417

418 Figure 6 here.

419

420 **4. Discussion**

421 *4.1 Concentration, types and potential sources of plastics and paint particles*

422 Studies conducted across the globe have revealed numerous types of
423 plastics floating in the ocean (Cózar et al., 2014; Eriksen et al., 2014; Cózar et
424 al., 2017; Avio et al., 2017), with a diversity of associated fauna (Reisser et al.,
425 2014; Goldstein et al., 2014; Carlton et al., 2017). However, the lack of studies
426 conducted in some regions hinders the mitigation of this problem, as it is hard to
427 remediate what is not known. Here we present the first description of the
428 concentration and characteristics – including associated prokaryotic and

429 eukaryotic communities detected through DNA metabarcoding – of floating
430 plastics in the open ocean of South Brazil.

431 We found plastics in a variety of sizes, shapes, colors, malleability and
432 polymer compositions, suggesting that they were used in different applications
433 (Rochman et al., 2019), and could be both continental and ocean-originated
434 (Pan et al., 2019). The dominance of microplastics, which is in accordance with
435 what has been reported in studies of floating plastics worldwide in all ocean
436 basins (Cozar et al., 2014; Eriksen et al., 2014), could be explained by the
437 higher numerical abundance of this size class due to the breakdown of larger
438 plastics in the marine environment (Browne et al., 2007; Andrady, 2011). Due to
439 the limitation of the method we used for plastic identification (naked eye
440 inspection of samples), we did not classify each item specifically into
441 primary/secondary microplastic categories. However, we can affirm that most
442 items were secondary microplastics based on their physical features. This
443 predominance of secondary over primary microplastics suggests that the
444 breakdown of plastics is likely occurring due to physical, chemical and biological
445 mechanisms such as solar U.V. radiation, abrasion through wave action, and
446 biofouling (Andrady, 2011; Cole et al., 2011; Rowland, 2014).

447 The mean concentration of floating plastics that we estimated for the
448 Southern Brazil (4,461 item.km⁻²) is within the estimated density range of plastic
449 pollution in the global ocean basins (from 1000 - 100000 items.km⁻²) (Eriksen et
450 al., 2014). This concentration is much lower than the mean concentration of
451 floating plastics in accumulation zones such as the North Pacific subtropical
452 gyre (> 700,000 items.km⁻²), as well as in the Mediterranean (> 800,000
453 items.km⁻²) (Eriksen et al., 2014), and Portuguese coasts (40,822.58 item.km⁻²)

454 (Rodrigues et al., 2020), but it is around two times higher than what was
455 reported for the Southern Ocean (Lacerda et al., 2019). However, we highlight
456 that it is difficult to compare with other studies, since a variety of sampling and
457 analysis are used when studying plastics in the ocean. In addition, the number
458 of replicates from each site could also be a limiting factor in such comparisons.

459 A recent study evaluated microplastics in northeastern Brazil, and
460 revealed polypropylene, polyethylene and nylon items contaminating estuarine,
461 coastal and shelf waters (Lins-Silva et al., 2021). These authors report that the
462 concentrations of microplastics were highest in the estuarine samples and
463 decreased oceanward, likely due to a higher input of plastics from continental
464 sources. We found high concentrations of plastics at stations close to the
465 mouths of the Patos Lagoon (station 1 - highest concentration) and Itajaí-Açu
466 (station 7 – third-highest concentration) estuaries, likely due to this large
467 contribution of continental sources of plastic waste. In fact, it has been
468 suggested that most plastic litter entering the oceans is the result of inadequate
469 waste management on land (UNEP, 2016), and rivers alone have been
470 estimated to carry millions of tonnes of plastic to the oceans every year
471 (Lebreton et al., 2017). The Patos Lagoon and Itajaí-Açu estuaries are located
472 near several urban and industrial development areas, with ports in their
473 estuarine portions. Considering that waste management in Brazil is highly
474 inefficient, with some regions lacking adequate waste collection, basic
475 sanitation, landfill sites and recycling facilities, with recycling rates being
476 generally low throughout the country (PNCLM 2019; Oliveira & Turra, 2015), we
477 suggest that these drainage basins are important carriers of plastics, and waste
478 in general, to the adjacent marine area.

479 Monofilament fishing lines were common in our samples and were the
480 dominant plastic type at sampling station 9, which had the second highest
481 concentration of plastics. At this station, more than 70% of plastics consisted of
482 meso-sized polyamide lines. As we mentioned previously, the manual sorting
483 evaluation method could have limited the identification of smaller particles from
484 all locations. Additionally, when smaller particles are biofouled or aggregate with
485 larger and denser particles, they can sink to deeper waters (Peeken et al.,
486 2018) that are not sampled with surface nets. However, considering that we
487 used the same method to analyse all sampling locations, it is also possible that
488 the dominance of meso over microplastics at this specific station – mostly made
489 of polyamide, the main material used in fishing nets – is indeed due to the
490 proximity of the source, since there are intense fishing activities close to station
491 9. This station is located at an area with an intense operation of gill, trawl and
492 seine net fisheries by the Southern Brazil fishing fleet (FURG/MPA, 2018). In
493 their evaluation of microplastics off Northeast Brazil, Lins-Silva et al. (2021) also
494 found higher concentrations of nylon fibres in samples from the continental shelf
495 than those from coastal/estuarine area. In this manner, it is important to act
496 locally with fishermen to create effective mitigation actions.

497 Characteristics such as size, color, and biofilm formation may also
498 indicate the time that plastics have spent in aquatic/marine environments
499 (GESAMP, 2019; Martí et al., 2020; Tu et al., 2020). As noted above, secondary
500 microplastics predominated in our samples, indicating the breakdown of larger
501 items; there was a dominance of white/light-colored plastics, which could
502 indicate discoloration over time due to weathering (Andrady, 2016). Additionally,
503 FTIR spectra showed that some plastics presented alterations in their primary

504 characteristics, also indicating that these particles had likely been subject to
505 weathering: the presence or absence of peaks and bands typically assigned to
506 oxygenated groups (-OH, -C=O, C-O-C) indicates different levels of degradation
507 (Jin et al., 2006). Additionally, the presence of biofilms containing a diversity of
508 groups (from microorganisms to invertebrates) corroborates that plastics were
509 in the ocean for some time, allowing the establishment of a well-developed
510 epiplastic community.

511 We found a high concentration of paint fragments in our samples, but
512 since the focus of this paper was on floating plastics we did not include these
513 paint chips into the estimates of floating plastics. There is still no consensus in
514 the scientific community as to whether paint particles should be included in such
515 estimates in the ocean, as they are denser than the seawater and are expected
516 to sink, but can be retained at the surface due to seawater surface tension
517 (Song et al., 2014). In addition, some of the paint fragments could have been a
518 result of sampling. Green, orange and white paint chips were possibly
519 originated from our research vessel (deck and hull), as the ship is painted in
520 these colours; additionally, yellow paint particles could be from the yellow
521 floaters of the manta net. However, red and blue paint fragments were not
522 present on any external structure of our ship or net, and we therefore infer that
523 these particles were already at the ocean surface, originating from other
524 continental or maritime sources.

525 Although still poorly understood, marine pollution by plastics in Southern
526 Brazil has been reported to impact marine mammals (Secchi & Zarzur, 1999)
527 birds and sea turtles (Bugoni et al., 2001; Tourinho et al., 2010; Rizzi et al.,
528 2019), as well as commercially exploited seafood such as fish, shrimp and

529 crabs (Vaske Jr. et al., 2009; Dantas et al., 2019; Neto et al., 2020; authors'
530 observation). The ingestion of plastics has been described as an evolutionary
531 trap (Santos et al., 2021), and we highlight that such ingestion can lead to
532 ecological and economic impacts at the region, especially if it affects fishery
533 resources.

534

535 *4.2 Epiplastic communities in the Western South Atlantic: diversity and* 536 *ecological impacts*

537 To date, relatively few studies have used molecular approaches to
538 analyse both the diversity of plastic-associated prokaryotes or eukaryotes in
539 environmental samples from the open ocean (Zettler et al., 2013; Bryant et al.,
540 2016; Debroas et al., 2017; Dussud et al., 2018; Amaral-Zettler et al., 2020),
541 and none have been performed in surface waters of the Western South Atlantic.
542 Proteobacteria, Cyanobacteria and Bacteroidetes were among the most
543 dominant bacterial taxa associated with plastics in this study. This was also
544 observed for open waters of the North Atlantic (Zettler et al., 2013) and North
545 Pacific oceans (Bryant et al., 2016), the Mediterranean coast (Davidov et al.,
546 2020), and the Chinese coast (Jiang., 2018). These groups were also common
547 in a global analysis of Bacteria in the plastisphere (Wright et al., 2020a). The
548 dominant eukaryotic groups we found were Diatoms, Ciliates, Dinoflagellates,
549 Radiolarian and Bryozoans, which are also among the most representative
550 groups living associated with floating plastics in the North Atlantic (Zettler et al.,
551 2013; Debroas et al., 2017) and North Pacific (Bryant et al., 2016) oceans. Our
552 previous study on Fungi showed that this group is also highly frequent in
553 plastics from the South Atlantic and Southern oceans (Lacerda et al., 2020).

554 There is still a lack of knowledge about “specific” organisms living on
555 different plastics in aquatic environments, and although some studies – mostly
556 those evaluating colonization – have reported that plastic types may drive the
557 community composition of their associated biota (Zettler et al., 2013; Kirstein et
558 al., 2018; Kirstein et al., 2019; Hansen et al., 2021), we did not observe this in
559 samples from the natural environment. As previously described for epiplastic
560 fungi in the Western South Atlantic (Lacerda et al., 2020), there was no
561 difference between OTU richness or community composition among plastic
562 polymers within prokaryotic and general eukaryotic datasets at the region. This
563 finding supports the suggestions of Oberbeckmann & Labrenz (2020) and
564 Oberbeckmann et al. (2021) that microorganisms opportunistically colonize
565 different plastic substrates, and are not specific to plastic polymers. However,
566 significant differences in community composition according to size class was
567 observed within the 18S V9 dataset, which we believe could be due to the
568 higher availability of physical space on mesoplastics for the settlement of larger-
569 sized taxa.

570 The community composition found with the 18S markers was also
571 significantly different according to location (RS versus SC). The two regions
572 have similar environmental conditions, but some slight differences exist in
573 temperature and rainfall (www.worlddata.info/america), as well as in productivity
574 (Boletim Estatístico da Pesca e Aquicultura, 2011), which could be driving
575 community differences as previously observed for microbial communities living
576 on plastics (Amaral-Zettler et al., 2015, 2020; Basili et al., 2020). For instance,
577 both prokaryotic and eukaryotic communities from the plastisphere in the North
578 Sea varied among geographic regions (between 51°31.497N and 53°31.918N)

579 (Oberbeckmann et al., 2016), and likewise the bacterial community composition
580 significantly varied between the Pacific and Atlantic ocean basin (Amaral-Zettler
581 et al., 2015). However, considering that RS and SC regions present quite similar
582 environments, we believe the difference we found could also be an artifact of
583 uneven sample sizes between locations (4 for RS and 18 for SC for 18S V4,
584 and 8 for RS and 20 for SC for 18S V9, respectively). We highlight that the
585 differences in biofilm composition among geographic regions, as well as
586 between experimental and environmental samples, should be considered
587 limiting factors when comparing data.

588 It has been well documented that potentially pathogenic *Vibrio* species
589 are associated with plastics (Zettler et al., 2013; Kirstein et al., 2016; Kesy et
590 al., 2021), and we found a diversity and abundance of *Vibrio* OTUs. We also
591 found OTUs in our samples that matched sequences of other potentially harmful
592 taxa (e.g. *Alexandrium tamarense*), but it is important to highlight that the
593 occurrence of *Vibrio* species and other harmful organisms on plastic biofilms
594 does not confirm their pathogenicity (Amaral-Zettler et al., 2020; Oberbeckmann
595 & Labrenz, 2020). The threat they pose to human and animal life is still not well
596 known, but once ingested, plastic biofilms could potentially lead to the
597 transmission of harmful organisms that can cause diseases to animals from low
598 to high trophic levels, since these materials (mainly microplastics) are easily
599 ingested over entire marine food webs (Setälä et al., 2014; Wilcox et al., 2016).

600 The ingestion of plastics by different marine species, including seafood,
601 has been recorded worldwide. In fact, eight species of commercially exploited
602 fish from South Brazil (Neto et al., 2020) have been shown to ingest plastics,
603 and the authors suggest that biofilm may favour ingestion by increasing

604 detectability/attractiveness of plastics. The interaction between plastic-
605 associated pollutants and plastic biofilms has not been described, but it is
606 known that pollutants could be biomagnified along marine trophic webs
607 (Carbery et al., 2018). Since many groups of primary producers live on plastic
608 surfaces, toxic compounds could bioaccumulate from the bottom of the food
609 web, posing serious risk to marine biota of different trophic levels, including
610 humans that consume seafood. It has been shown that plastics can host
611 potentially pathogenic organisms (Zettler et al., 2013; Keswani et al. 2016;
612 Kirstein et al. 2016; Bowley et al. 2020), but studies that evaluate the functional
613 genes associated with pathogenicity have shown contrasting results as to if they
614 pose (Bhagwat et al. 2021) or not (Oberbeckmann et al. 2021) a relevant risk to
615 human health. Additional studies are needed to clarify possible risks of the
616 plastisphere from different types of plastics, as well as from different geographic
617 regions and the open ocean.

618 Marine plastics can also host some fungal and bacterial groups known to
619 biodegrade these materials (Shah et al., 2008; Sangeetha et al., 2015; Paço et
620 al., 2017; Urbanek et al., 2018; Lacerda et al., 2020; Oberbeckmann & Labrenz,
621 2020). It was suggested that the Sphingomonadaceae (Proteobacteria) family is
622 one of the most important microplastic-associated group, due to their ability to
623 degrade hydrocarbons and form carotenoids, which protect bacterial cells from
624 oxidative stress caused by U.V. light at the sea surface (Oberbeckmann &
625 Labrenz, 2020). We found many OTUs of the Sphingomonadaceae family in the
626 biofilm of plastics from the Western South Atlantic, including *Erythrobacter* sp.
627 that is known for its ability to utilize polycyclic aromatic hydrocarbons (PAH)
628 (Gao et al., 2015), and we therefore reinforce that these groups should be

629 further investigated to evaluate their ability to degrade plastics and their
630 pollutants in the open ocean (Oberbeckmann & Labrenz, 2020).

631 The transport of species that live attached on artificial substrates such as
632 plastics is an important issue that should be deeply investigated, as it can lead
633 to changes in the structure and functioning of natural communities (Sridharan et
634 al., 2021), once invasive species become well established (Póvoa et al., 2021).
635 Mantellato et al. (2020) indicated that transport by rafting over long distances on
636 marine litter (including plastics) may be a mechanism of range expansion and
637 secondary introduction of two invasive species of corals, *Tubastraea coccinea*
638 and *T. tagusensis*, in Southern Brazil. Based on the high diversity of groups
639 within the plastisphere from the Western South Atlantic, which present different
640 characteristics throughout their different life stages (e.g. free living adults, but
641 with sessile spores, larvae or eggs), it is possible that at least some of these
642 groups could be successful in colonizing new environments with favourable
643 conditions.

644 Although we did not perform metabarcoding on paint chips, it was
645 recently shown that their biofilm communities seem to be distinct from common
646 microplastic biofilms (polypropylene, polyamide and polyvinyl chloride) in
647 brackish systems, but it is not yet known what drives these differences (Tagg et
648 al., 2019). Since ship coatings can be a prominent, even underestimated,
649 source of microplastic pollution in some marine environments (European
650 Commission DG, 2021), further work is needed to evaluate the ecology and
651 composition of biofilms on paint chips in the ocean.

652

653 *4.3 Final remarks*

654 In our work, the highest concentrations of floating plastics were observed
655 at stations close to the coast, near large drainage basins. This indicates that
656 continental sources are important contributors of plastics, and to better
657 understand the transport of plastics between land and sea, we suggest
658 monitoring and quantification of plastic pollution in the Patos Lagoon and Itajaí-
659 Açu estuaries should be conducted. This will aid in the search for effective
660 solutions for this problem. Additionally, high concentrations were found within a
661 high-use fishery area, where polyamide (nylon) meso-sized lines were
662 predominant, showing that fisheries are also an important source of plastics at
663 the region. In this manner, we suggest that governments and NGOs encourage
664 the implementation of sensibilization activities with fishers, tax incentives (e.g.
665 tax discounts) and/or environmental certifications for returning gear, as well as
666 incentives to properly dispose fishing gears on land.

667 The diversity of epiplastic communities, as well as their impacts, should
668 be further characterized via *in situ* studies conducted at different regions and
669 ocean compartments. Our study further highlights the utility of molecular
670 techniques in revealing the biodiversity associated with plastics, and shows that
671 a multi-marker approach is extremely important to detect different groups of
672 eukaryotic organisms, and consequently better depict the plastisphere.
673 Considering that the V9 region of the 18S marker allowed the identification of a
674 higher diversity of eukaryotic taxa, and showed greater amplification success,
675 we suggest that this molecular marker should be used in future studies on the
676 diversity of plastic-associated eukaryotic organisms. However, the greater read
677 length of the 18S V4 marker could provide a greater scope for more detailed
678 phylogenetic analysis. Omics approaches to identify the function of genes

679 present in the plastisphere should also be applied to investigate factors such as
680 pathogenicity and biodegradation potential of plastic-associated organisms from
681 environmental samples.

682 In summary, our study provides novel and fundamental regional
683 information on plastic concentrations and their associated communities, which
684 further integrates the current knowledge of global plastic pollution and the global
685 plastisphere. This will aid our efforts to act towards prevention and mitigation
686 strategies for plastic pollution, and to understand the broader ecology and
687 impacts of the plastisphere.

688

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713

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Figure Legends

Figure 1. A) Sampling stations of floating plastics along the South Brazilian coast; B) Manta net trawling at the ocean-air interface; C) Different types of plastics (1 - fishing line, 2 - chocolate wrapper, 3 - cigarette butt, and 4 - fragment) present in one sample.

Figure 2. (I) Plastic concentration (items/km²) at the sea surface of ten sampling stations along the Southern Brazilian coast (Rio Grande do Sul – RS and Santa Catarina – SC states), categorized according to their size as microplastic (< 5mm, light blue) and mesoplastic (5 - 200mm, dark blue); (II) Paint chip concentration (items/km²) at the sea surface of ten sampling stations along the Southern Brazilian coast (RS and SC states); (III) Relative abundance of plastic shapes (Frag = fragment, Line, Pellet, Foam and Film); (IV) Polymer composition of 117 sampled plastics: PA = Polyamide, PU = Polyurethane, PE = Polyethylene, PS = Polystyrene, PP = Polypropylene, PET = Polyethylene terephthalate, CA = Cellulose acetate, EVA = Ethylene-vinyl acetate (left panel), and examples of polymer FTIR spectra: a) degraded PA; b) degraded PS (right panel).

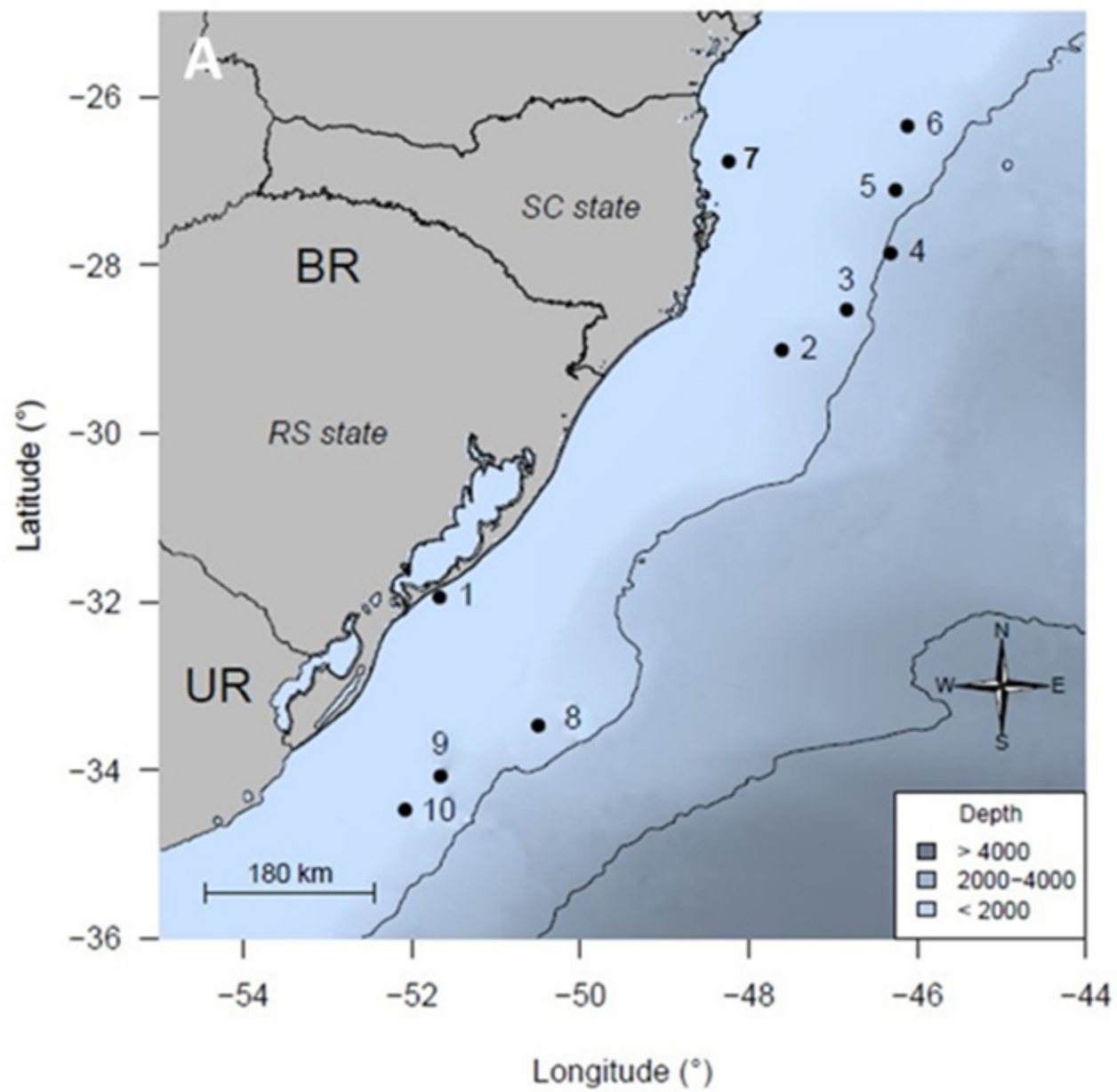
Figure 3. I) Relative abundance and II) Frequency of occurrence (FO %) of prokaryotes associated with floating marine plastics from Southern Brazil (Rio Grande do Sul – RS and Santa Catarina – SC), identified through high-throughput amplicon sequencing of a partial fragment of the 16S rRNA gene.

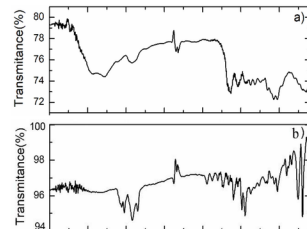
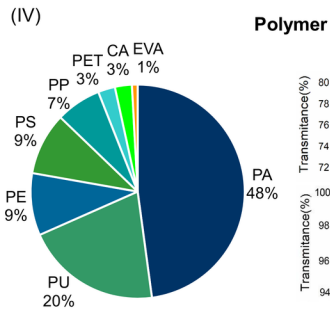
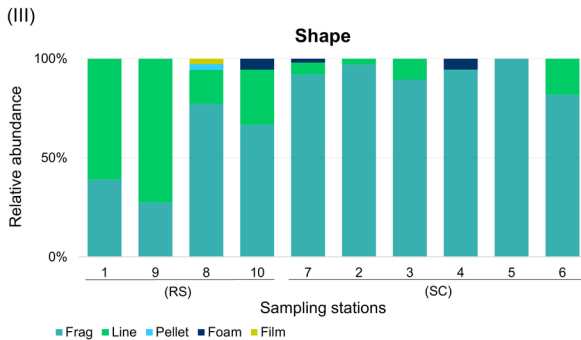
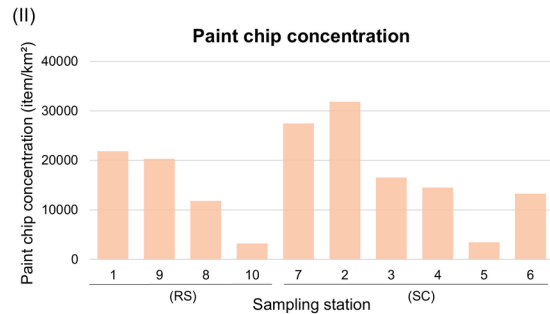
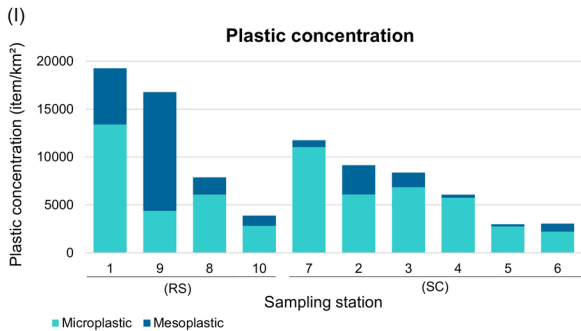
Figure 4. Frequency of occurrence (%) of eukaryotic groups associated with floating plastics from Southern Brazil, identified through high-throughput amplicon sequencing of partial fragments of 18S rRNA gene regions V4 and V9.

Figure 5. Mean number of OTUs per plastic sampled in Southern Brazil, according to plastic categories (size, shape and polymer composition) by location (RS – Rio Grande do Sul, SC – Santa Catarina), obtained from rarefied 16S (1,000 sequence/sample), 18S V4 (500 sequences/sample) and 18S V9 (700 sequences/sample) amplicon sequence libraries. Size: MESO (Mesoplastic) and MICRO (Microplastic); Shape: FRAGMENT and LINE; Polymer composition: PA

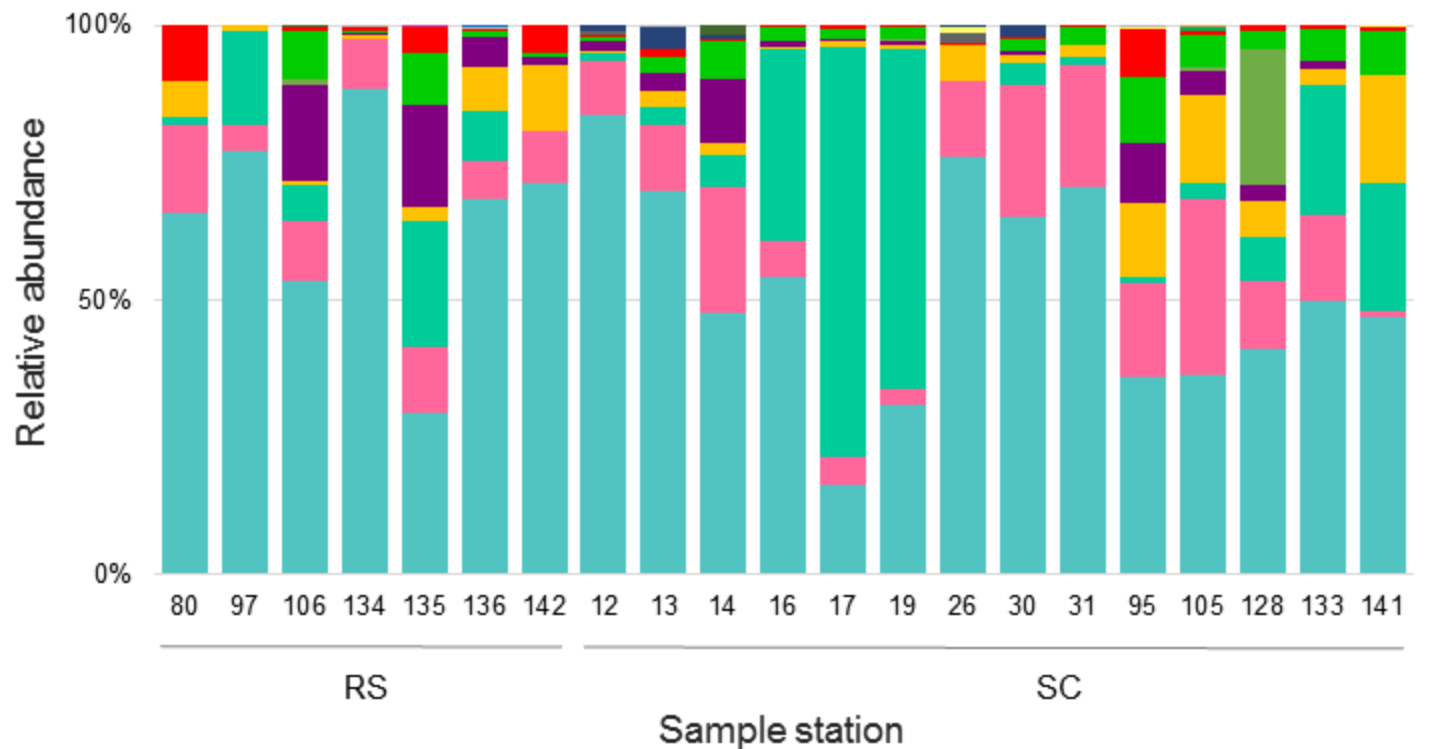
(Polyamide), PE (Polyethylene), PP (Polypropylene), PS (Polystyrene), and PU (Polyurethane).

Figure 6. Non-metric multidimensional scaling (NMDS) plots based on Jaccard distance matrix of prokaryotic and eukaryotic groups living associated with floating marine plastics in Southern Brazil, according to plastic size (micro and mesoplastic), shape (Foam, Fragments, Lines, Pellet), and polymer composition (CA: cellulose acetate, PA: polyamide, PE: polyethylene, PP: polypropylene, PS: polystyrene, PU: polyurethane).

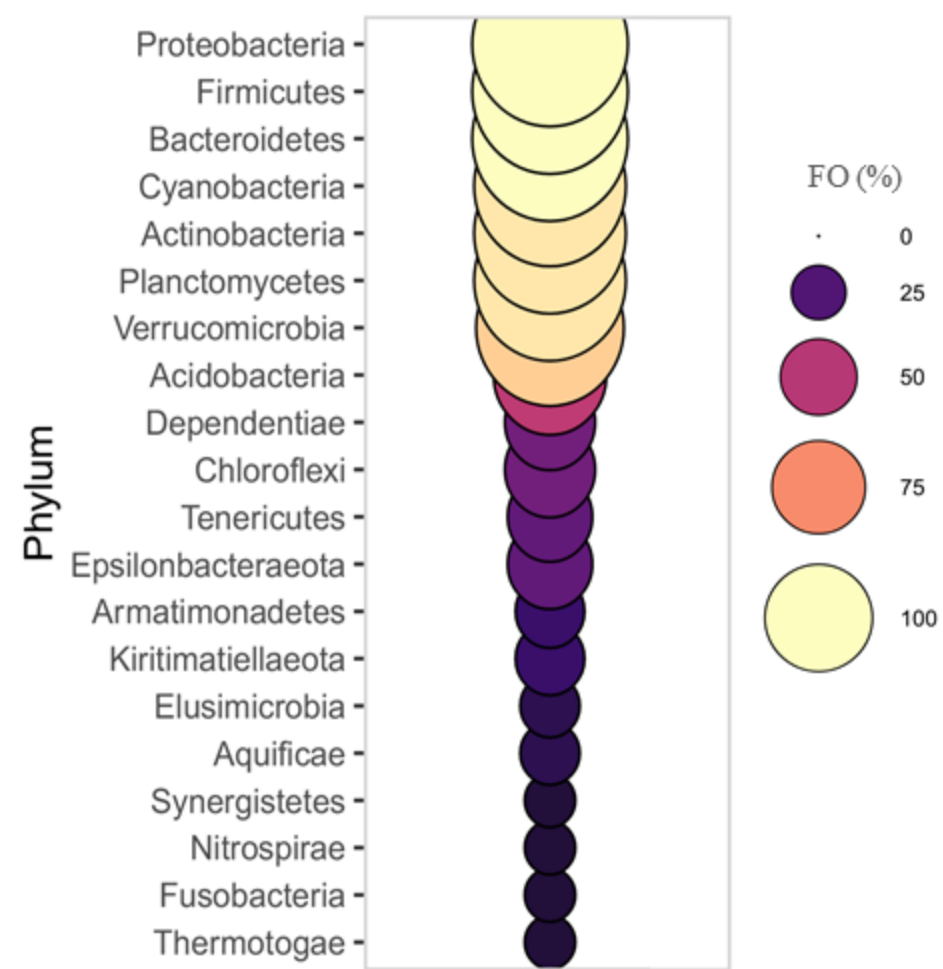


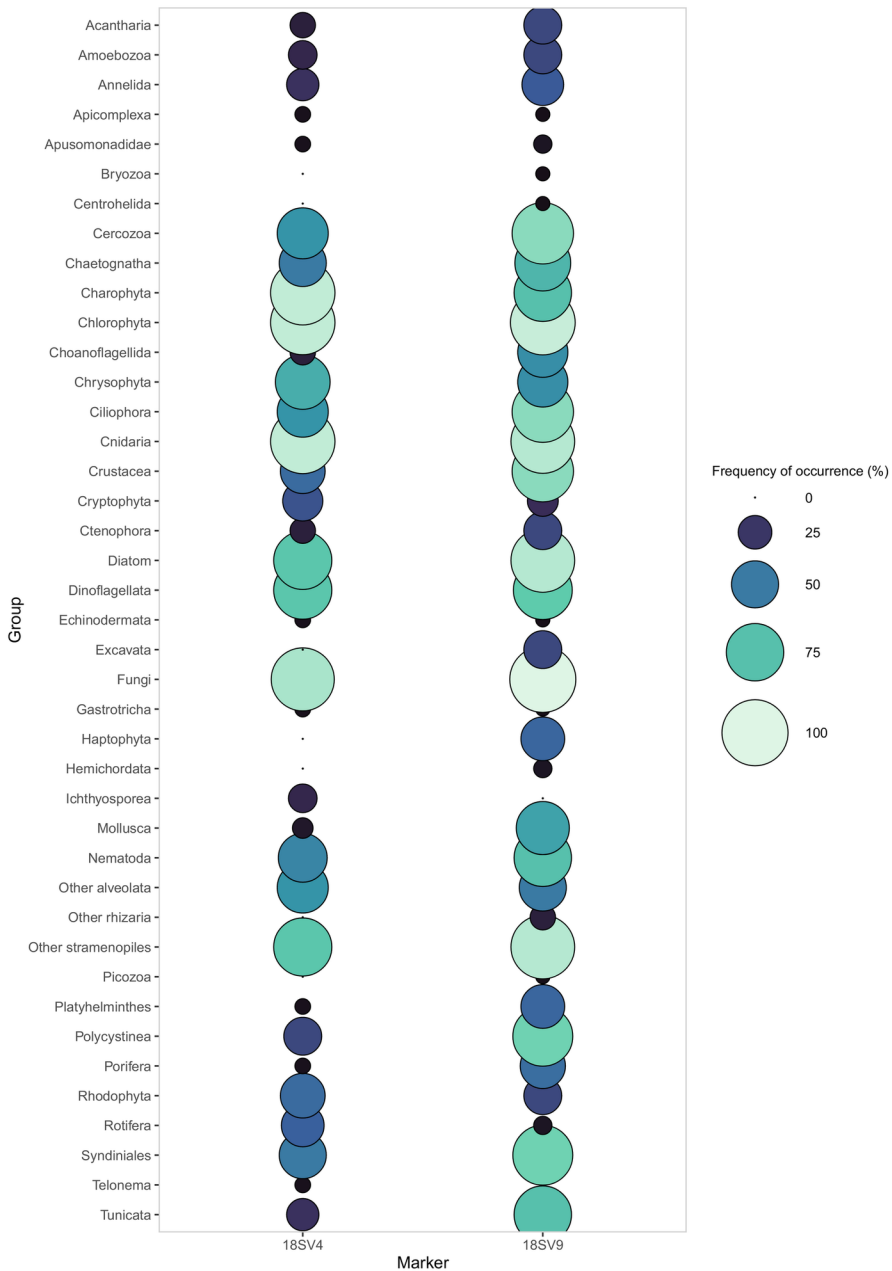


(I)

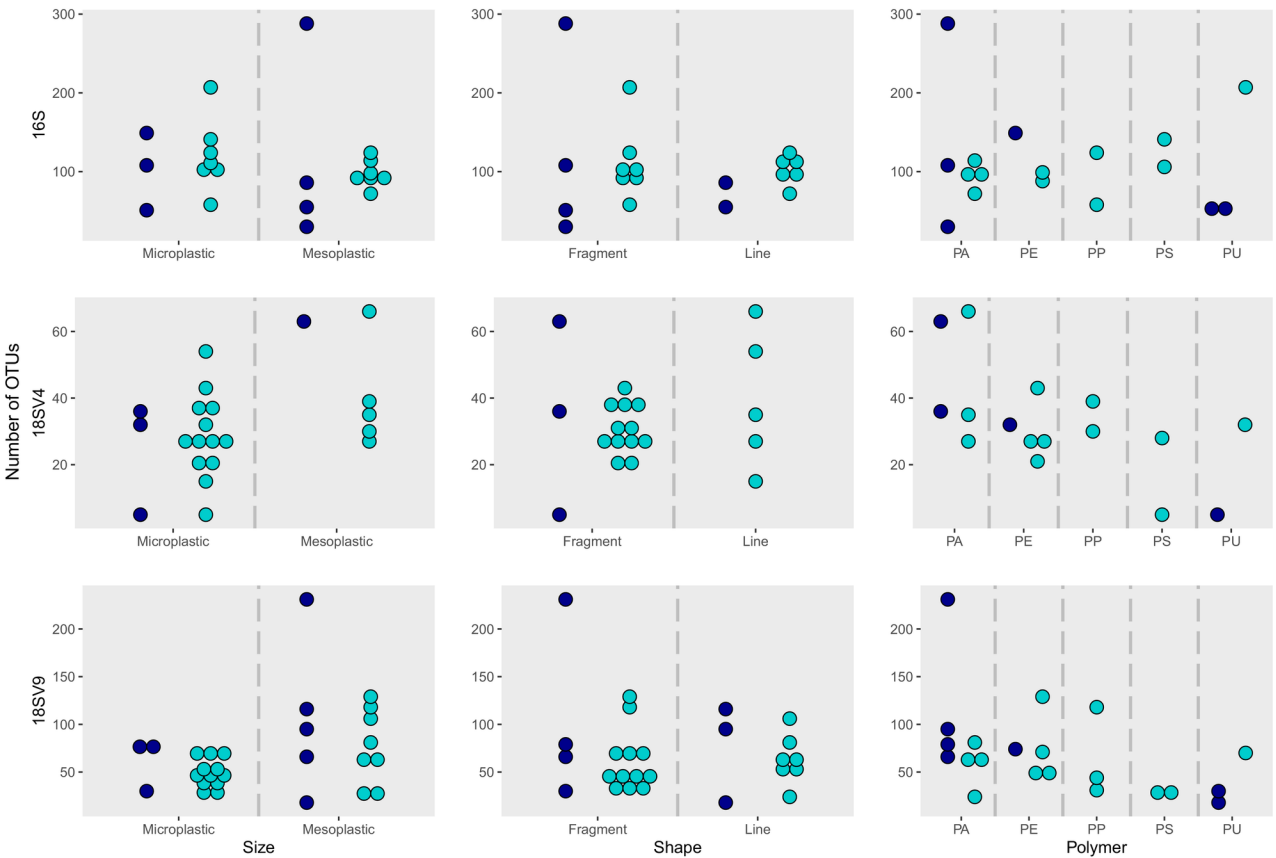


(II)





Province ● RS ● SC



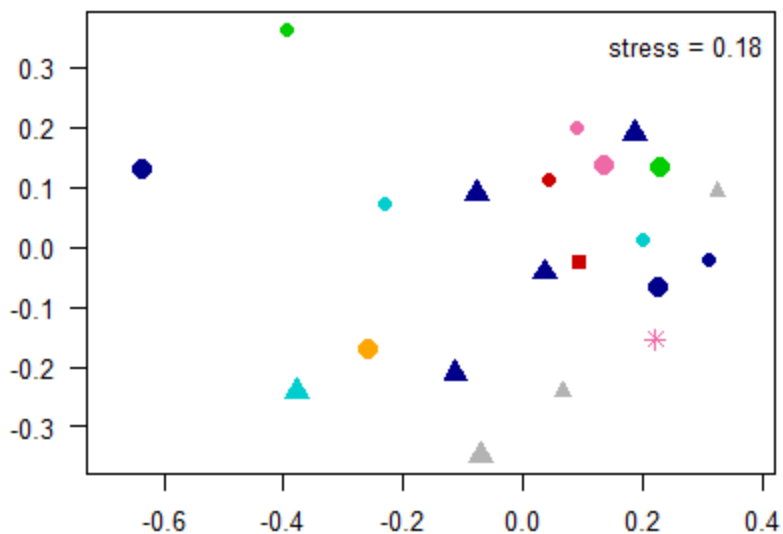
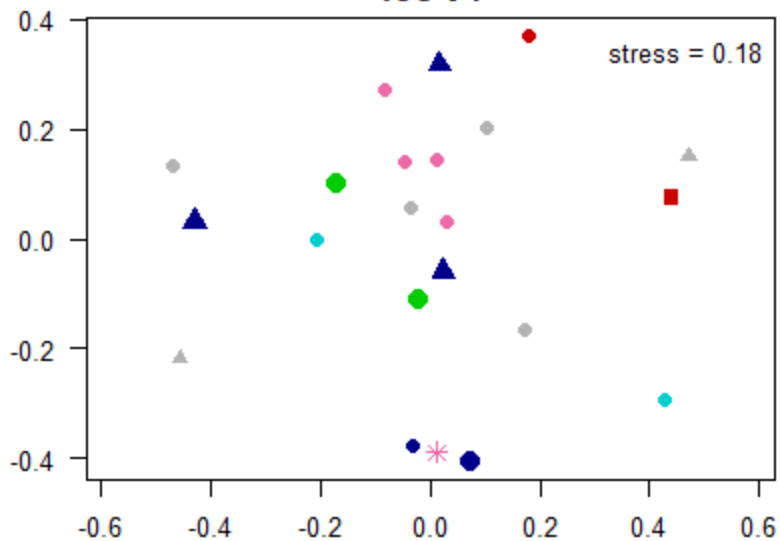
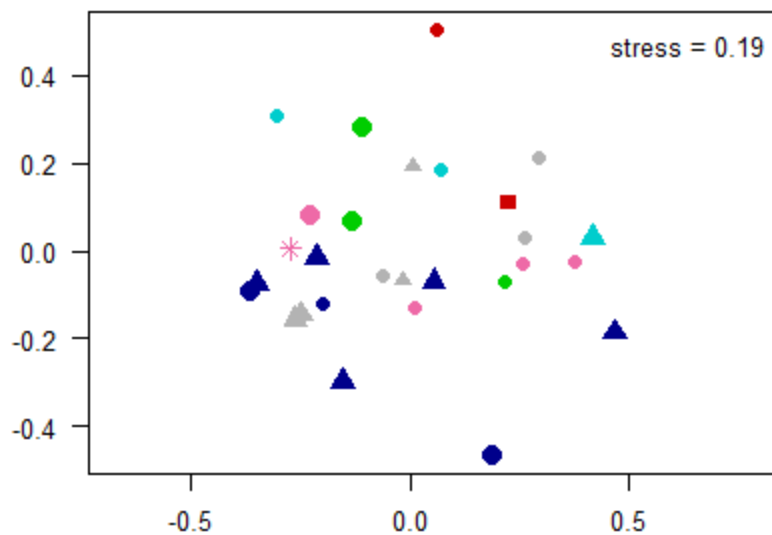
16S**18S V4****18S V9**

Table 1. Ten most abundant prokaryotic OTUs identified through 16S amplicon libraries from the marine plastisphere of the Western South Atlantic.

| | | | Environmental sample | | | Cultured | | | |
|------------|------|-----|----------------------------------|-----|-------------------|--|-----|-------------------|---------------------------------|
| OTU number | R.A. | FO% | Source | ID% | Genbank accession | Species | ID% | Genbank accession | Source/ Highlights |
| OTU_1 | 23% | 81 | Freshwater | 100 | MN072796.1 | <i>Ralstonia</i> sp. | 100 | MN723154.1 | Soil |
| OTU_5 | 6% | 67 | Soil | 100 | MT318452.1 | <i>Acinetobacter lwoffii</i> | 100 | MT323129.1 | Rainbow trout (fish) |
| OTU_6 | 5% | 90 | Marine sponge | 100 | MT464708.1 | <i>Synechococcus</i> sp. | 100 | KU867931.1 | Coastal water |
| OTU_2 | 5% | 76 | Water (marsh) | 100 | AY652491.1 | <i>Bacteroides</i> sp. | 99 | JQ317253.1 | Cat fish |
| OTU_12 | 3% | 24 | <i>Porites compressa</i> (Coral) | 90 | FJ930300.1 | <i>Alkalibacter saccharofermentans</i> | 85 | NR042834.1 | Soda Lake |
| OTU_32 | 2% | 81 | Polluted seawater | 100 | MW559885.1 | <i>Vibrio parahaemolyticus</i> | 100 | MW829316.1 | Shrimp and marine fish |
| OTU_9 | 2% | 43 | Floodplain lake water | 100 | MF439455.1 | <i>Alkalibacterium</i> sp. | 100 | MH044645.1 | <i>B. schlosseri</i> (ascidian) |
| OTU_22 | 2% | 62 | Marine water | 99 | KX935277.1 | <i>Salinimonas</i> sp. | 100 | CP064795.1 | Marine sediment |
| OTU_34 | 2% | 48 | Surface sewerage (China) | 100 | KC002482.1 | <i>Afiplia</i> sp. | 100 | EF371496.1 | Yellow Sea |
| OTU_21 | 2% | 52 | Coastal sewerage | 100 | LC496437.1 | <i>Loktanella</i> sp. | 100 | LR722710.1 | Coastal water |

Notes: OTU number; Relative Abundance (R.A.); Frequency of occurrence (FO%); Environmental Samples (Source of uncultured bacterium) and Cultured samples (Species); Similarity (ID%); Genbank accession number; and Source/Highlights.

Table 2. Ten most frequent eukaryotic OTUs identified through 18S amplicon libraries (18S V4 and V9 regions) from the marine plastisphere of the Western South Atlantic.

| 18S V4 | | | | | | | | | |
|------------|----------------|-----|------------------------|-----|-------------------|-----------------------------------|-----|-------------------|------------------------|
| OTU number | Group | FO% | Environmental Sample | | | Cultured | | | |
| | | | Source | ID% | Genbank accession | Species | ID% | Genbank accession | Source/ Highlights |
| OTU_19 | Cnidaria | 64 | Seawater | 100 | AY665134.1 | <i>Muggieae atlantica</i> | 100 | AY937337.1 | Seawater |
| OTU_41 | Chlorophyta | 55 | Freshwater | 100 | HQ191320.1 | <i>Ankyra judayi</i> | 99 | U73469.1 | Culture (SAG 17.84) |
| OTU_5 | Cnidaria | 55 | Seawater | 99 | KJ762819.1 | <i>Aeginopsis laurentii</i> | 100 | KY007604.1 | - |
| OTU_335 | Chlorophyta | 45 | Intertidal sediment | 99 | EF100243.1 | <i>Collinsiella tuberculata</i> | 95 | AY198125.1 | Intertidal pools |
| OTU_181 | Fungi | 45 | Seafloor | 100 | KR072832.1 | <i>Aspergillus restrictus</i> | 100 | EU723495.1 | Deep sea |
| OTU_25 | Dinoflagellata | 45 | Radiolarian (symbiont) | 100 | U52353.1 | <i>Brandtodinium nutricula</i> | 100 | MG905637.1 | Radiolarian (symbiont) |
| OTU_40 | Dinoflagellata | 45 | Tidal estuary | 100 | DQ386760.1 | <i>Karlodinium veneficum</i> | 94 | KY979983.1 | Coastal zone |
| OTU_77 | Chrysophyta | 45 | Marine environment | 98 | EF527168.1 | <i>Paraphysomonas</i> sp. | 100 | JQ967321.1 | Drainage ditch |
| OTU_28 | Chlorophyta | 41 | Seawater (Ross Sea) | 100 | KJ758236.1 | <i>Thalassiosira</i> sp. | 100 | MW722949.1 | - |
| OTU_2 | Rhodophyta | 41 | Seawater | 98 | AJ626846.1 | <i>Protomonostroma undulatum</i> | 99 | DQ821517.1 | Shaw Island |
| 18S V9 | | | | | | | | | |
| OTU_19 | Fungi | 93 | Marine sediment | 100 | GU474197.1 | <i>Aspergillus wentii</i> | 100 | AB002063.1 | Dried fish |
| OTU_3 | Cercozoa | 75 | Seawater | 100 | KF130578.1 | <i>Sphaeronectes haddocki</i> | 100 | KX421854.1 | Monterey Bay |
| OTU_21 | Cnidaria | 71 | Seawater | 100 | KF129695.1 | <i>Nanomia bijuga</i> | 100 | AY937324.1 | - |
| OTU_7 | Ciliophora | 64 | Gulf Stream 15m depth | 86 | KJ759360.1 | <i>Tintinnopsis</i> sp. | 82 | JX178854.1 | Coast (China) |
| OTU_24 | Cnidaria | 61 | Seawater | 100 | HM799922.1 | <i>Liriope tetraphylla</i> | 100 | KT722405.1 | Coast (Brazil) |
| OTU_97 | Fungi | 61 | Savanna soil | 99 | EU490070.1 | <i>Cladosporium halotolerans</i> | 100 | MN859971.1 | Marine sponge |
| OTU_18 | Chaetognatha | 57 | - | - | - | <i>Sagitta enflata</i> | 99 | LC581989.1 | Seawater (Japan) |
| OTU_489 | Fungi | 57 | Antarctica snow | 99 | KR131435.1 | <i>Aspergillus penicillioides</i> | 99 | AF548066 | Air |
| OTU_44 | Fungi | 57 | Seawater | 100 | JF826393.1 | <i>Wallemia mellicola</i> | 100 | AY741380.1 | Hypersaline water |
| OTU_33 | Dinoflagellata | 57 | Radiolarian (symbiont) | 100 | U52353.1 | <i>Brandtodinium nutricula</i> | 99 | U52357.1 | Sargasso Sea |

Notes: OTU number; Taxonomy identified by SILVA database (Group); Environmental Samples (Source) and Cultured samples (Species); Frequency of

occurrence (FO%); Similarity (ID%), Genbank accession number; and Source/Highlights.

