Transcriptional activity differentiates families of Marine Group II *Euryarchaeota* in the coastal ocean

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ABSTRACT

Marine Group II *Euryarchaeota* (*Candidatus* Poseidoniales) are abundant members of marine microbial communities. They are thought to be (photo)heterotrophs that metabolize components of dissolved organic matter (DOM) such as lipids and peptides, but little is known about their transcriptional activity. We mapped reads from metatranscriptomes collected off Sapelo Island, GA to metagenome-assembled genomes to determine the diversity of transcriptionally-active *Ca.* Poseidoniales. Summer metatranscriptomes had the highest abundance of *Ca.* Poseidoniales transcripts, mostly from the O1 and O3 genera within *Ca.* Thalassarchaeaceae (MGIIb). In contrast, transcripts from fall and winter samples were predominantly from *Ca.* Poseidoniaceae (MGIIa). Genes encoding proteorhodopsin, membrane-bound pyrophosphatase, peptidase/proteases, and part of the ß-oxidation pathway were highly transcribed across abundant genera. Highly transcribed genes specific to *Ca.* Thalassarchaeaceae included xanthine/uracil permease and receptors for amino acid transporters. Enrichment of *Ca.* Thalassarchaeaceae transcript reads related to protein/peptide, nucleic acid, and amino acid transport and metabolism, as well as transcript depletion during dark incubations, provided further evidence of heterotrophic metabolism. Quantitative PCR analysis of South Atlantic Bight samples indicated consistently abundant *Ca.* Poseidoniales in nearshore and inshore waters. Together, our data suggest *Ca.* Thalassarchaeaceae are important photoheterotrophs potentially linking DOM and nitrogen cycling in coastal waters.

39 **INTRODUCTION**

40 Since the initial discovery of Marine Group II (MGII) *Euryarchaeota* [1,2], definitive
41 determination of their physiology and ecological roles has remained challenging due to the lack
42 of a cultivated isolate. Nonetheless, as data describing MGII distributions throughout the oceans
43 have increased, several patterns have emerged: MGII are often highly abundant in the euphotic
44 zone and in coastal waters, reach high abundance following phytoplankton blooms, and are
45 largely comprised of two subclades, MGIIa and MGIIb [3,4]. Early metagenomic studies
46 provided the first evidence that MGII may be aerobic (photo)heterotrophs [5-7], a hypothesis
47 supported by incubation experiments [8-10] and by the gene content of diverse metagenome-
48 assembled genomes (MAGs) [11-14]. Two recent studies deepened our understanding of the
49 phylogenomics and metabolic potential of MGII by analyzing hundreds of MAGs, highlighting
50 clade-specific differences in genomic potential for transport and degradation of organic
51 molecules, light harvesting proteorhodopsins, and motility [15,16]. Here, we refer to MGII as the
52 putative order "*Candidatus* Poseidoniales," MGIIa and MGIIb as the putative families "*Ca.*
53 Poseidoniaceae" and "*Ca.* Thalassarchaeaceae," respectively, and putative genera as specified by
54 Rinke et al. [15]. We occasionally use "MGIIa" and "MGIIb" for consistency with previous
55 literature.

56 Metatranscriptomics is one strategy for gleaning information about microbial activity in
57 the environment. *Ca.* Poseidoniales transcripts can be abundant in marine metatranscriptomes,
58 suggesting transiently high transcriptional activity [17,18]. When metatranscriptome reads from
59 the Gulf of Aqaba were mapped to metagenomic contigs from the Mediterranean Sea, genes
60 involved in amino acid transport, carbon metabolism, and cofactor synthesis were highly
61 transcribed in the aggregate euryarchaeal community [19,20]. In another study, mapping deep-

62 sea metatranscriptome reads to novel *Ca.* Poseidoniales MAGs indicated transcription of genes
63 related to protein, fatty acid, and carbohydrate transport and metabolism, likely fueling aerobic
64 heterotrophy [21]. Finally, a metaproteomics study found that euryarchaeal transport proteins for
65 L-amino acids, branched-chain amino acids, and peptides were present throughout the Atlantic
66 Ocean [22]. Despite these advances, little is known about similarities or differences in gene
67 transcription between *Ca.* Poseidoniales and Thalassarchaeaceae.

68 We report MAG-resolved metatranscriptomic analyses of *Ca.* Poseidoniales in coastal
69 waters near Sapelo Island (GA, USA). Prior work suggested *Ca.* Poseidoniales are sporadically
70 active at Sapelo Island [23] and may comprise the majority of archaea in mid-shelf surface
71 waters of the South Atlantic Bight (SAB) [24]. Since other studies thoroughly described the
72 genomic content of *Ca.* Poseidoniales MAGs, our focus instead was determining which clades
73 were transcriptionally active and identifying highly or differentially transcribed genes. We used
74 two Sapelo Island MAGs [25] combined with recent marine MAG collections [15,16] to
75 competitively recruit reads from a metatranscriptomic time series [26] and an incubation
76 experiment [23] to determine which clades were active over time. We then used representative
77 MAGs from highly active genera to determine which *Ca.* Poseidoniales genes were transcribed.
78 Finally, we used quantitative PCR (qPCR) to measure the abundance of *Ca.* Poseidoniales 16S
79 rRNA genes in DNA samples throughout the SAB to assess the prevalence *Ca.* Poseidoniales in
80 this region.

81

82 **MATERIALS AND METHODS**

83 PHYLOGENOMICS

84 Phylogenomic analyses compared SIMO Bins 19-2 and 31-1 (ref. 25) to previously-
85 reported *Ca.* Poseidoniales MAGs [15,16]. Average nucleotide identity (ANI) was calculated
86 using fastANI [27] to compare the set of non-redundant MAGs from Tully [16] to 15 Port
87 Hacking MAGs [15] and the two SIMO MAGs; MAGs with ANI <98.5% were added to the
88 non-redundant set. Phylogenomic analysis was conducted using a set of sixteen ribosomal
89 proteins [28] within anvi'o v4 (ref. 29). All genomes were converted to contig databases and
90 ribosomal proteins were identified using HMMER [30]. These proteins were concatenated,
91 aligned using MUSCLE [31], and used to build a phylogenomic tree using FastTree [32] within
92 anvi'o.
93
94 COMPETITIVE READ MAPPING
95 We used competitive read mapping [33] to determine which *Ca.* Poseidoniales genera
96 were transcriptionally active in free-living Sapelo Island metatranscriptomes [23,26]. Analyses
97 of "field" communities included Gifford et al. metatranscriptomes (iMicrobe Accession
98 CAM_P_0000917) [26] and the T_0 metatranscriptomes from Vorobev et al. [23], while dark
99 incubation analyses included only Vorobev et al. samples (T_0 and T_{24}; NCBI BioProject
100 PRJNA419903). Temperature, salinity, dissolved oxygen, pH, and turbidity data corresponding
101 to metatranscriptome sampling times were downloaded from the NOAA National Estuarine
102 Research Reserve System website (http://cdmo.baruch.sc.edu; last accessed 16 July 2020).
103 Contigs from all MAGs from the phylogenomic analysis were used as a database for read
104 mapping using Bowtie2 v.2.2.9 (ref. 34) with the "very-sensitive" flag. Samtools v.1.3.1 (ref. 35)
105 was used to index resulting BAM files, which were then profiled and summarized in anvi'o.
106 Contig genus identity was imported to the anvi'o contig database as an external collection. The

107 number of transcripts L^{-1} was calculated by scaling the number of mapped reads by the volume
108 of water filtered and the recovery of internal standards (reported in [23,26]) as previously
109 described [36]. Seasonal transcript abundances were compared using a one-way ANOVA test in
110 R [37] with data log-transformed as necessary to improve normality. When ANOVA results were
111 significant, groupings were defined post-hoc with Tukey's Honest Significant Difference (HSD)
112 test using the agricolae R package [38].

113 Non-metric multidimensional scaling (NMDS) analysis of metatranscriptome hits was
114 conducted using the vegan R package [39]. NMDS input was a distance matrix constructed by
115 Hellinger-transforming the table of transcript hits and calculating Euclidean distance between
116 samples [40]. Genus vectors were calculated using the *envfit* command. Significance of
117 groupings were tested by permutational multivariate analysis of variance (the *adonis* command)
118 with 999 permutations.

119

120 MAG-SPECIFIC ANNOTATION AND TRANSCRIPT ANALYSIS

121 Gene-specific analyses focused on three MAGs: two from the SIMO collection (SIMO
122 Bin 19-2, Genbank: VMDE00000000; SIMO Bin 31-1, VMBU00000000; [25]) and one (RS440,
123 PBUZ00000000; [41]) binned from TARA Oceans metagenomes [42]. These MAGs represented
124 genera O1, O3, and M, respectively, which were highly abundant in metatranscriptomes (see Fig.
125 1). RS440 was selected due to a high number of transcripts recruited when genus M was
126 abundant (data not shown).

127 MAGs were annotated using the archaeal database in Prokka v.1.13 (ref. 43), using
128 DIAMOND [44] to search against all orthologous groups in eggNOG-mapper v.1 (refs. 45, 46),
129 and using the online BlastKOALA portal (https://www.kegg.jp/blastkoala/, last accessed 6

March 2019) [47]. Putative genes for carbohydrate-active enzymes, peptidases, and membrane transport proteins were identified using HMMER searches of dbCAN2 (HMMdb v.7) [48,49], *MEROPS* v.12.0 (ref. 50), and the Transporter Classification Database [51], respectively.

Transcript reads were mapped to MAGs (combined into a single database such that each read mapped to only one MAG) to identify *Ca.* Poseidoniales genes that were highly or differentially transcribed. Coverage was calculated by profiling BAM files in anvi'o and normalized to coverage per million reads (CPM) by dividing by the total number of reads per sample. For each MAG, "highly transcribed" genes were the 5% of putative genes with the highest median CPM across metatranscriptomes (SIMO Bin 19-2: 63 genes, SIMO Bin 31-1: 70 genes, RS440: 77 genes).

DESeq2 v.3.11 (ref 52) was used to identify genes from each MAG that were differentially transcribed when each genus was highly transcriptionally active. For each MAG, "treatment" samples in DESeq2 were those where the respective genus recruited ≥50% of *Ca.* Poseidoniales reads from the metatranscriptome. Thus, positive fold-change values are genes transcribed at higher levels when the genus is highly transcriptionally active (compared to other metatranscriptomes). DESeq2 was also used to identify differentially transcribed genes for each MAG between T_0 and T_{24} samples in high tide (HT) dark incubations [23]. Since T_{24} samples were the "treatment" condition in DESeq2, positive fold-change values here are genes transcribed at higher levels in T_{24} compared to T_0 samples. In all DESeq2 analyses, genes with Benjamini-Hochberg adjusted $p<0.1$ were counted as having significantly different transcription.

16S rRNA QUANTITATIVE PCR

152 DNA samples from SAB field campaigns in 2014 and 2017 [24,53] were used as
153 templates for qPCR reactions targeting the *Ca.* Poseidoniales 16S rRNA gene. Samples included
154 the variety of shelf habitats (inshore, nearshore, mid-shelf, shelf-break, and oceanic as previously
155 defined [24]; Fig. S1). Primers were GII-554-f [54] and Eury806-r [55] with cycling conditions
156 as previously reported [56] (Table S1). Reactions (25 µL, triplicate) used iTaq Universal Green
157 SYBR Mix (Bio-Rad, Hercules, CA) in a C1000 Touch Thermal Cycler/CFX96 Real-Time
158 System (Bio-Rad, Hercules, CA). Each plate included a no-template control and a standard curve
159 (serial dilutions of a linearized plasmid containing a previously-sequenced, cloned amplicon).
160 Abundance of *Ca.* Poseidoniales 16S rRNA genes was compared to published bacterial 16S
161 rRNA gene abundance from the same samples [24,53]. Regional variability of gene abundance
162 was assessed using a one-way ANOVA and a post-hoc HSD test as described above. Model II
163 regressions of log-transformed qPCR data were estimated using the lmodel2 R package [57] as
164 previously described [53]. All plots were constructed with anvi'o or the ggplot2 R package [58].
165

166 **RESULTS**

167 EURYARCHAEOTAL MAGs

168 SIMO Bins 19-2 and 31-1 were estimated as 82.5-92.3% and 77.5-96.2% complete,
169 respectively, with redundancy <0.6% [25]. Phylogenomics placed both in the putative family *Ca.*
170 Thalassarchaeaceae (MGIIb) and genera O1 (SIMO Bin 19-2) and O3 (SIMO Bin 31-1; Fig. S2).
171 Phylogenomic groupings were generally consistent with previous findings [15,16].

172 Both SIMO MAGs contained a proteorhodopsin gene. Presence of a methionine residue
173 at position 315 suggested absorption of green light [59,60], and both proteorhodopsin genes
174 grouped in "Archaea Clade B" [11,16,61] (Fig. S3). Both MAGs included partial or complete

175 pathways indicating aerobic heterotrophic growth, such as glycolysis, the TCA cycle, and
176 electron transport chain components (Table S2). Pathways for metabolism of compounds such as
177 fatty acids, peptides, and proteins were also present, as were transport systems and metabolic
178 pathways for amino acids and nucleotides.
179
180 DOMINANT GENERA IN FIELD METATRANSCRIPTOMES
181 There were significant seasonal differences in transcript recruitment by the combined set
182 of *Ca.* Poseidoniales MAGs ($F_{3,28}$=4.9, p=0.007): most summer samples had >10^{10} *Ca.*
183 Poseidoniales transcripts L^{-1}, significantly more than in other seasons (Fig. 1A,C; Table S3). The
184 diversity of transcriptionally-active *Ca.* Poseidoniales also changed seasonally. Genera O1 and
185 O3 accounted for most reads mapped from summer samples (typically 89.5-99.5% of *Ca.*
186 Poseidoniales reads), with most mapping to O1 (Fig. 1A,B). HT (and not low tide; LT)
187 metatranscriptomes from July 2014 also had a moderate fraction of reads (37.5-39.6%) mapped
188 to *Ca.* Poseidoniaceae. In contrast to summer samples, November 2008 and May 2009 transcripts
189 were predominantly O3, while those from February 2009 and October 2014 were mostly *Ca.*
190 Poseidoniaceae genera M, L1, or L2 (Fig. 1A, Table S3). NMDS analysis showed clear seasonal
191 groupings (PERMANOVA: r^2=0.693, p=0.001; Fig. S4).

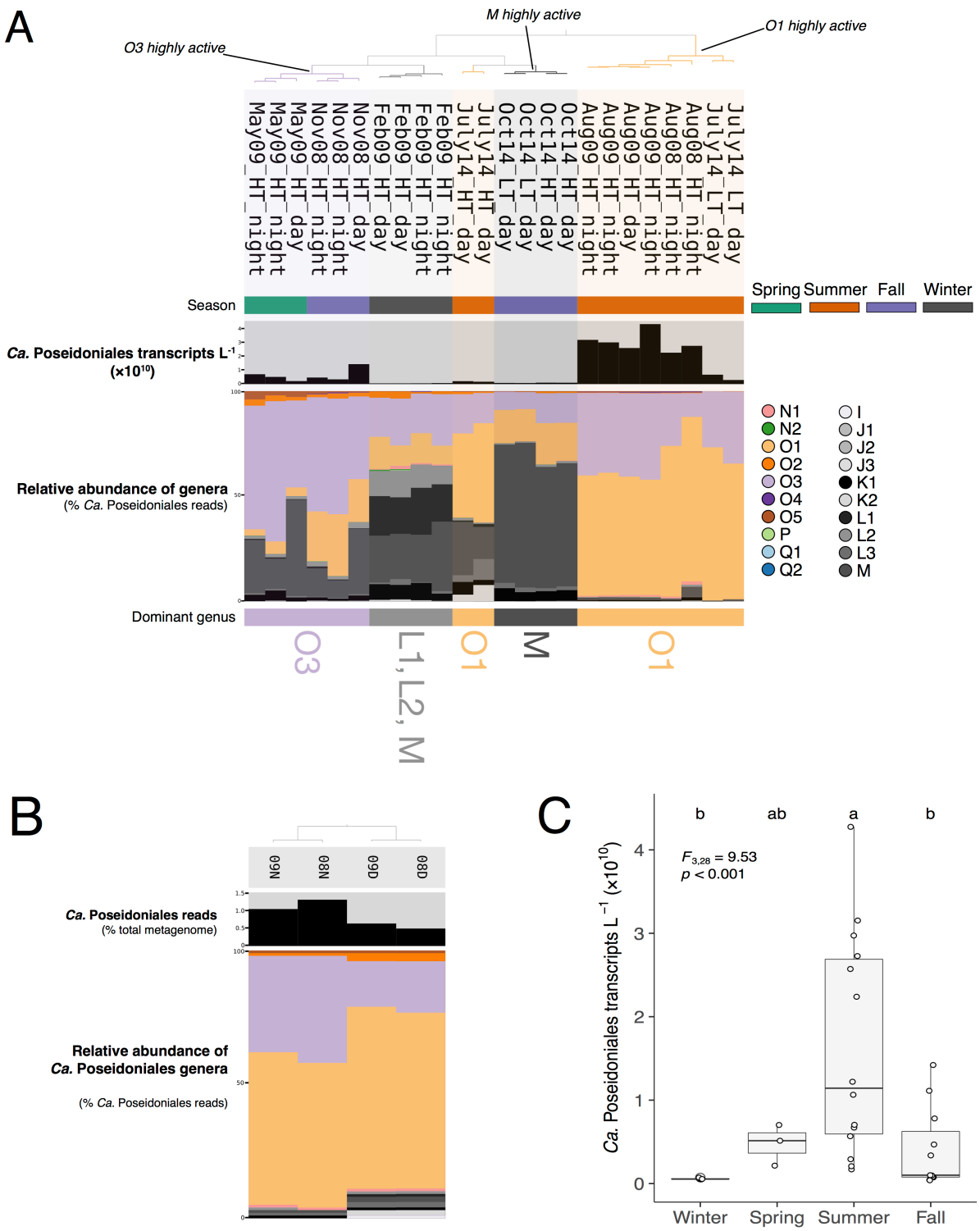

Fig. 1A) Relative abundance of *Ca.* Poseidoniales genera in Sapelo Island metatranscriptomes (*n*=24). The dendrogram (top) shows grouping by similarity. Season is indicated by color beneath sample names. The bar chart shows the abundance of *Ca.* Poseidoniales transcripts L^{-1} and the stacked bar charts show the relative abundance of genera (% total *Ca.* Poseidoniales transcripts), colored by genus. Dominant genera are indicated below the stacked bar chart. "Highly active" samples for each genus are marked and were used for analysis of differential transcription. **B)** Relative abundance of genera in Sapelo Island metagenomes (*n*=4), as described above. Since internal standards were not included in metagenomes, total *Ca.* Poseidoniales reads are shown as a percentage of the total metagenome. **C)** Boxplots of *Ca.* Poseidoniales reads L^{-1}, grouped by season (winter, *n*=4; spring, *n*=3; summer, *n*=10; fall, *n*=7). Values from individual metatranscriptomes are overlain. Results of an ANOVA are indicated; letters at the top indicate post-hoc groups according to Tukey's HSD test.

194 HIGHLY TRANSCRIBED *CA.* POSEIDONIALES GENES

195 Many highly transcribed *Ca.* Thalassarchaeaceae (MGIIb) genes were involved in
196 translation, transcription, replication/repair, or post-translation protein modification (Fig. 2).
197 Genes encoding proteins involved in energy production or conservation (ATPases, a
198 pyrophosphate-energized proton pump, and proteorhodopsin) were also highly transcribed.
199 Notably, the *aapJ* and *livK* genes, encoding substrate-binding proteins of L-amino acid and
200 branch-chain amino acid transporters, respectively, were among the most highly transcribed
201 genes in both *Ca.* Thalassarchaeaceae MAGs (Fig. 2, Table S2).

202 Many of the highly transcribed genes mapping to the *Ca.* Poseidoniaceae (MGIIa) MAG
203 were not highly transcribed by *Ca.* Thalassarchaeaceae, including genes encoding a carbamoyl
204 phosphate synthetase subunit (*carA*), a family 2 glycosyl transferase, chromosomal protein
205 MC1b, and a ftsX-like permease. The *carA* gene had the highest median coverage of *Ca.*
206 Poseidoniaceae genes across coastal metatranscriptomes (Fig. 2, Table S2). While both *Ca.*
207 Thalassarchaeaceae MAGs also contained the carbamoyl phosphate synthetase genes, neither
208 transcribed *carA* at high levels (Table S2).

209 Twelve genes were highly transcribed by *Ca.* Thalassarchaeaceae and not by *Ca.*
210 Poseidoniaceae, including genes encoding ATP synthase, transcription initiation factor IIB,
211 halocyanin, phytoene desaturase, protein translocase, xanthine/uracil permease, and receptors for
212 amino acid transporters (Fig. 2, Table S2). Other than those encoding ribosomal proteins, only
213 six genes were highly transcribed in all three MAGs: a chaperone protein, a ribonucleoside-
214 diphosphate reductase, translation elongation factor 1A, 3-hydroxyacyl-CoA dehydrogenase, a
215 membrane-bound pyrophosphatase (*hppA*), and proteorhodopsin (Fig. 2).

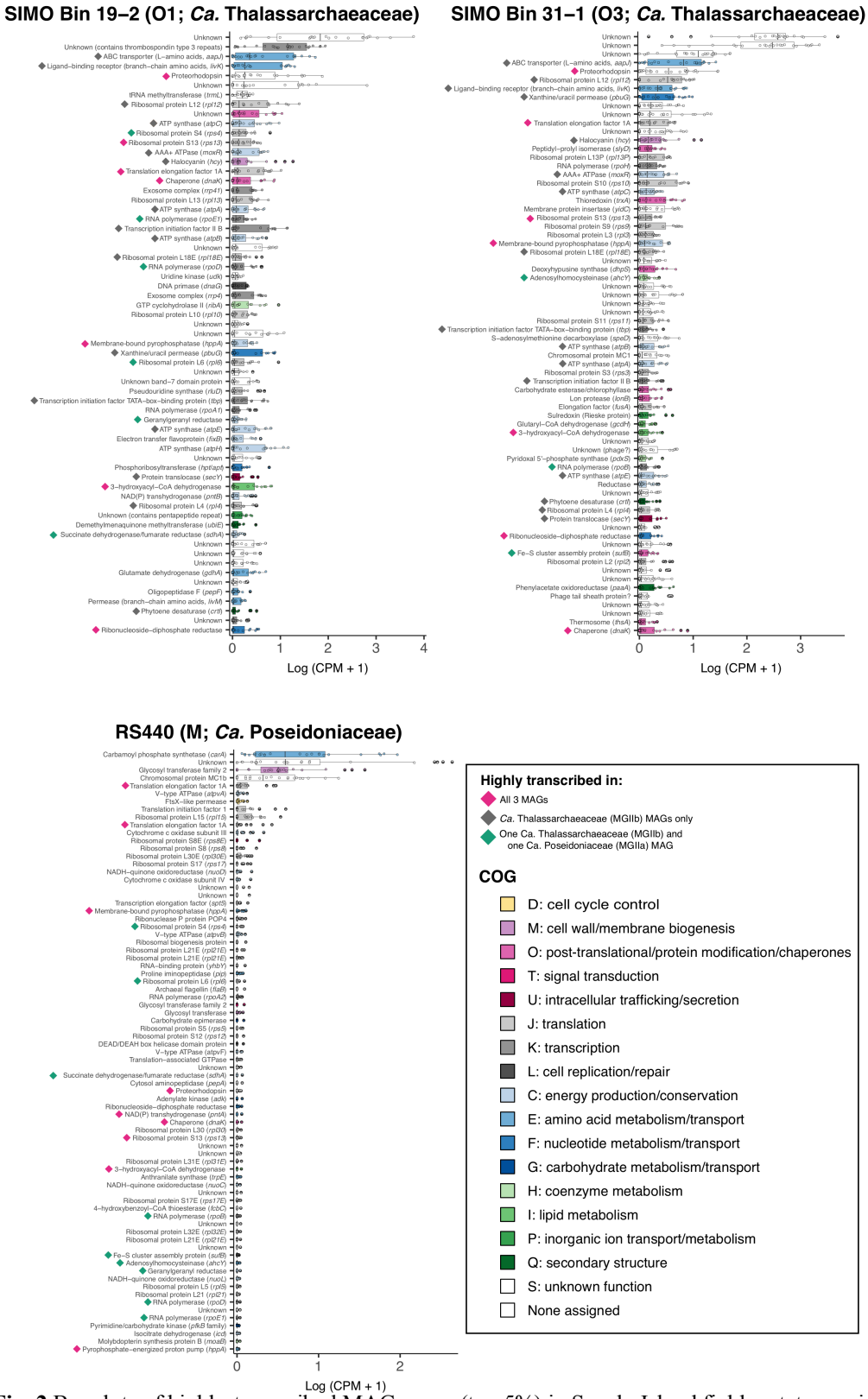

Fig. 2 Boxplots of highly-transcribed MAG genes (top 5%) in Sapelo Island field metatranscriptomes. Overlain points show CPM for individual metatranscriptomes (n=24). Shading indicates COG functional category assigned by eggNOG-mapper (genes assigned to group S were similar to proteins of unknown function in the COG database, while genes with no COG assignment did not match proteins in the COG database). Diamonds indicate genes highly transcribed in all MAGs (pink), in *Ca.* Thalassarchaeaceae (MGIIb) MAGs only (gray), or in the *Ca.* Poseidoniaceae (MGIIa) MAG and one *Ca.* Thalassarchaeaceae MAG (green).

DIFFERENTIAL GENE TRANSCRIPTION

We were interested in identifying genes with variable transcription levels when genera O1, O3, and M were highly transcriptionally active in the ocean. Twenty-three genes were differentially transcribed in O1-active samples (Fig. 3, Table S4), sixteen of which had higher abundance in O1-active metatranscriptomes compared to other metatranscriptomes. These highly transcribed genes encoded proteorhodopsin, two copper-containing redox proteins (halocyanin and plastocyanin), and proteins involved in lipid metabolism (3-hydroxyacyl-CoA dehydrogenase and oligosaccharyltransferase), nucleotide transport/metabolism (ribonucleotide-diphosphate reductase and xanthine/uracil permease), and amino acid transport (ligand-binding receptor for a L-amino acid transporter, *aapJ*). Differentially transcribed genes mapping to the O3 MAG were mostly depleted in O3-active metatranscriptomes and largely encoded proteins of unknown function; only the gene encoding ribosomal protein L12 was enriched in O3-active samples (Fig. S5, Table S4). Only four genes mapping to the M MAG were differentially transcribed in M-active metatranscriptomes. Annotated genes encoded chromosomal protein MC1b, an ATPase subunit, and a glycosyl transferase, which all had significantly fewer transcripts in M-rich samples (Fig. S6, Table S4). One gene of unknown function was enriched compared to other samples.

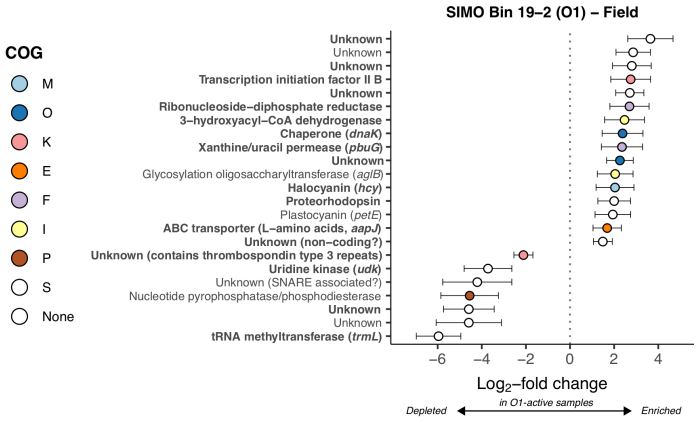

Fig. 3 Log_2-fold change of SIMO Bin 19-2 (genus O1) genes differentially transcribed in field metatranscriptomes where transcriptional activity of *Ca.* Poseidoniales was dominated by genus O1 (see Fig. 1, Table S3), calculated with DESeq2. Error bars show estimated standard error. Only genes with adjusted *p*-values <0.1 are shown. Color indicates COG functional category (see Fig. 2). Bold indicates genes in the top 5% median transcript coverage across field metatranscriptomes (Fig. 2).

DARK INCUBATION METATRANSCRIPTOMES

Incubation had little effect on transcription by the dominant genera in LT samples (Fig. 4, Table S3). In contrast, there were distinct shifts in transcriptionally active populations during incubations of all HT samples. July HT metatranscriptomes initially contained 60.4-62.5% *Ca.* Thalassarchaeaceae (MGIIb) while hits from the corresponding T_{24} samples were 98.1-99.3% *Ca.* Thalassarchaeaceae, due to increased transcript hits to genus O1 (Fig. 4, Table S3). Likewise, October 2014 HT samples initially contained 65.0-66.6% hits to *Ca.* Poseidoniaceae (MGIIa) but changed to 78.3-98.8% hits to *Ca.* Thalassarchaeaceae at T_{24} due to an increase in hits to O1 (Fig. 4).

DESeq2 identified 40 differentially transcribed genes mapping to the O1 MAG between HT T_0 and T_{24} metatranscriptomes. Four O1 genes had higher transcription at T_{24}, including xanthine/uracil permease (*pbuG*) and an amino acid transporter substrate-binding domain (*aapJ*; Fig. 5, Table S5). The 36 O1 genes transcribed at lower levels encoded proteins involved in repair of UV-damaged DNA, amino acid or nucleotide metabolism, coenzyme synthesis, peptidases or proteases, transcription, DNA replication, and lipid biosynthesis, as well as phytoene desaturase (*crtD*) and multiple subunits of pyruvate dehydrogenase (*pdhC*, *pdhA*). None of the genes mapping to the

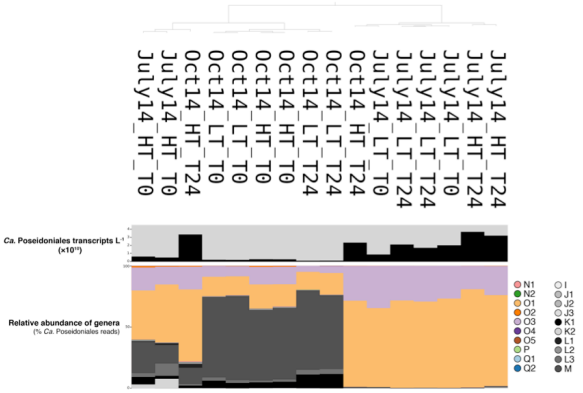

Fig. 4 Comparisons of competitive read mapping to *Ca.* Poseidoniales genera at the beginning and end of 24-hour incubations of Sapelo Island water conducted by [23]. The dendrogram (top) shows grouping by similarity. The bar chart below the dendrogram is the abundance of *Ca.* Poseidoniales transcripts L^{-1}. Stacked bar charts show the relative abundance of genera (% total *Ca.* Poseidoniales transcript reads), colored by genus.

264 O3 or M MAGs were transcribed at significantly different levels between HT incubation

265 timepoints (p>0.1 for all genes; Table S5).

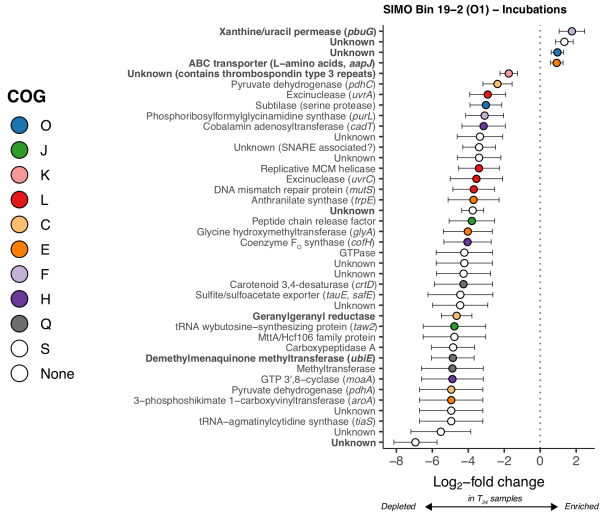

Fig. 5 Log_2-fold change of SIMO Bin 19-2 (genus O1) genes differentially abundant in T_{24} versus T_0 metatranscriptomes from Sapelo Island high tide waters. Error bars show estimated standard error. Only genes with adjusted p-values<0.1 are shown. Color indicates COG functional category (see Fig. 2). Bold indicates genes in the top 5% median transcript coverage across field metatranscriptomes (Fig. 2).

266

267 16S rRNA GENE ABUNDANCE

268 *Ca.* Poseidoniales 16S rRNA genes were detected in all SAB DNA samples (n=208),

269 with a range from 1.6×10^4 to 7.6×10^8 genes L^{-1} (Table S6). Standard curves for the *Ca.*

270 Poseidoniales assay always had r^2>0.99 (mean ± standard deviation: 0.99±0.001) and the mean

271 efficiency was 93.4% (±2.0%; Table S1). When data from all cruises were combined, *Ca.*

272 Poseidoniales genes were most abundant throughout inshore and nearshore waters and least

273 abundant in shelf-break and oceanic samples ($F_{4,204}$=18.5, p<0.001; Fig. 6A). There was a strong

274 linear relationship between log-transformed bacterial and *Ca.* Poseidoniales 16S rRNA gene

275 abundances, with bacterial abundances 2-3 orders of magnitude higher (Fig. 6B).

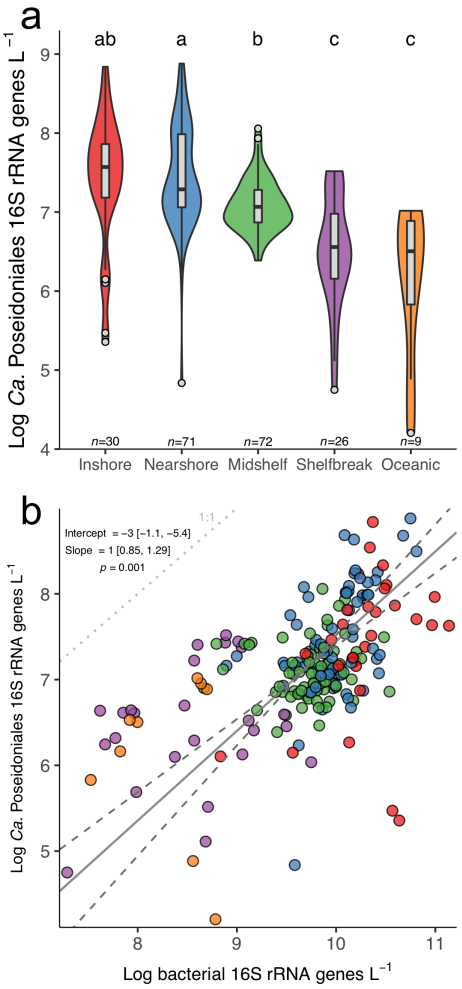

Fig. 6 A) Violin plots of log-transformed *Ca.* Poseidoniales 16S rRNA gene abundances across regions in the SAB, with overlain boxplots. Width of the violin plot corresponds to data probability density. Color denotes sampling region. Letters above boxes denote post-hoc grouping according to Tukey's HSD test. B) Scatterplot of bacterial and *Ca.* Poseidoniales 16S rRNA gene abundances in the SAB. The solid line shows the best fit of a model II (major axis) linear regression, with dashed lines showing a 95% confidence interval of the slope. Regression parameters are shown on the plot.

DISCUSSION

ABUNDANCE OF *CA.* POSEIDONIALES GENES IN THE SOUTH ATLANTIC BIGHT

The abundance of *Ca.* Poseidoniales 16S rRNA genes in the coastal SAB is among the highest measured in the ocean, with nearly 10^9 genes L^{-1} in some samples. Typical *Ca.* Poseidoniales abundance is 10^6-10^7 genes or cells L^{-1} in oligotrophic waters [62-66] and 10^7-10^8 genes or cells L^{-1} in coastal waters [9,54,56,67-69]. Greater gene abundance in inshore, nearshore, and mid-shelf waters indicates *Ca.* Poseidoniales are more abundant over the shallow shelf than further offshore (Fig. 6A), matching clone library data from the SAB [24] and data from the Central California Current and the Black Sea [9,68]. The DOM in productive, turbid

286 SAB coastal waters supports highly active heterotrophic microbial populations [70,71]. Our data
287 suggest large populations of *Ca.* Poseidoniales are part of this heterotrophic community, and the
288 correlation between *Ca.* Poseidoniales and bacterial abundance (Fig. 6B) suggests common
289 factors influence the abundance of these populations.
290

291 TRANSCRIPTIONALLY ACTIVE *CA.* POSEIDONIALES

292 While numerous studies have demonstrated high abundance of *Ca.* Poseidoniales in the
293 coastal ocean (e.g., [56,69]), little is known about which clades are transcriptionally active in
294 these regions. At Sapelo Island, the striking dominance of the *Ca.* Thalassarchaeaceae (MGIIb)
295 genera O1 and O3 in summer samples, which also contained the highest amount of aggregate *Ca.*
296 Poseidoniales transcripts (Fig. 1), indicates that *Ca.* Thalassarchaeaceae are highly-active during
297 the summertime. Outliers to this pattern were July 2014 HT samples, which contained abundant
298 *Ca.* Poseidoniaceae (MGIIa) transcripts (though *Ca.* Thalassarchaeaceae still comprised the
299 majority of their *Ca.* Poseidoniales reads). A previous study found relatively high salinity and
300 DOM enriched in marine-origin molecules over the mid-shelf SAB during July 2014 [72]. Our
301 data suggest *Ca.* Poseidoniaceae were relatively active over the shelf during this time and were
302 transported inshore during flood tides, leading to shifts in transcriptional diversity between LT
303 waters (dominated by *Ca.* Thalassarchaeaceae) and HT waters (which included a higher number
304 of *Ca*. Poseidoniaceae).

305 *Ca.* Poseidoniaceae (MGIIa) are the predominant euryarchaeal family in many coastal
306 ecosystems, particularly in summer (e.g., [9,14,56,73,74]), but it is unclear what general patterns
307 govern *Ca.* Poseidoniales distributions in coastal waters worldwide. Studies of *Ca.* Poseidoniales
308 ecology often focus on distributions with depth, typically finding abundant *Ca.*

309 Thalassarchaeaceae (MGIIb) in deeper waters and *Ca.* Poseidoniaceae (MGIIa) more prevalent
310 in euphotic waters (e.g., [12,54,75-77]). A recent mapping of global ocean metagenome reads
311 showed that coastal populations of *Ca.* Poseidoniales were primarily *Ca.* Poseidoniaceae, though
312 *Ca.* Thalassarchaeaceae MAGs recruited a substantial number of reads from some coastal
313 metagenomes [16]. Our data match this latter pattern, with *Ca.* Poseidoniales populations in
314 surface waters off Sapelo Island dominated by highly active *Ca.* Thalassarchaeaceae (Fig. 1).
315 The higher abundance of MAGs from genera O1 and O3 (also referred to as MGIIb.12 and
316 MGIIb.14) in some mesopelagic and coastal samples with relatively high temperature (~23–
317 30°C; [16]) may explain the unusual pattern found in SAB waters: these genera peaked in
318 summer at Sapelo Island, when water temperatures were 29–30°C (Table S2), suggesting they
319 may be adapted to growth at relatively low light and high temperature.
320

321 *CA.* POSEIDONIALES GENE TRANSCRIPTION PATTERNS

322 Sapelo Island metatranscriptome reads that mapped to *Ca.* Poseidoniales were analyzed
323 in three ways:

324 (1) We determined sets of "highly transcribed" genes mapping to MAGs of
325 transcriptionally-active *Ca* Poseidoniales genera (5% of MAG genes with the highest
326 median transcript coverage);
327 (2) We identified genes mapping to *Ca* Poseidoniales MAGs that were differentially
328 transcribed when its genus was highly active (≥50% of *Ca*. Poseidoniales transcripts
329 in a sample; Fig. 1, Table S2);
330 (3) We identified genes mapping to *Ca* Poseidoniales MAGs that were differentially
331 transcribed at the beginning versus end of dark incubations. Since dark incubation

separates indigenous microbes from light and sources of short-lived substrates, transcription of corresponding transporters and metabolic genes ceases during incubations as substrates are consumed. We therefore assume transcript depletion in T_{24} compared to T_0 metatranscriptomes indicates genes that were transcriptionally active in the field [23]. This interpretation was bolstered by significant transcript depletions for genes involved in repairing UV damage to DNA (*uvrA*, *uvrC*, and *cofH*; Fig. 5), an expected result given alleviation of UV stress in dark incubations.

In the following sections, we synthesize these analyses to discuss *Ca.* Poseidoniales transcriptional activity related to metabolism of labile DOM, transport/metabolism of amino acids and nucleotides, and basic energetic processes. Though lack of a cultivated representative limits the analysis to computationally-inferred functions, these data provide hypotheses regarding the activity of *Ca.* Poseideniales families in the coastal ocean.

PROTON GRADIENTS AND ELECTRON TRANSPORT

Our analysis revealed that *Ca* Poseidoniales genes involved in establishing transmembrane proton gradients were highly transcribed in our samples. Genes encoding proteorhodopsin were among the most highly transcribed by both *Ca.* Thalassarchaeaceae MAGs and were highly transcribed in O1-active samples (Figs. 2,3). Proteorhodopsins consist of a retinal chromophore linked to a transmembrane protein and use light energy to pump protons across the cell membrane [78]. The resulting energy can be coupled to ATP production or other chemiosmotic processes and often supports photoheterotrophy, though its function varies widely [61]. Proteorhodopsin genes are highly transcribed in the photic zone of both open ocean and

355 coastal waters (e.g., [79-81]) and our data indicate coastal *Ca.* Poseidoniales conform to this
356 pattern, consistent with recent evidence from other regions [20,82,83]. High transcription of
357 proteorhodopsin supports the photoheterotrophic lifestyle hypothesized for *Ca.* Poseidoniales
358 (e.g., [9,11,12,15,16]).

359 Since O1 proteorhodopsin transcript abundance did not differ between the beginning and
360 end of dark incubations (Fig. 5), light may not regulate *Ca.* Thalassarchaeaceae proteorhodopsin
361 transcription. However, depletion of O1 *crtD* (carotenoid 3,4-desaturase) transcripts during dark
362 incubation (Fig. 5) suggests light may regulate retinal synthesis. Whether proteorhodopsin
363 transcription responds to light varies among marine bacteria [60,84,85], and the function of
364 constitutive transcription is not straightforward: while some bacteria use proteorhodopsin to
365 produce ATP when carbon-limited [86], high amounts of proteorhodopsin in other bacteria can
366 physically stabilize membranes even when inactive [87]. The high proteorhodopsin transcription
367 in our data emphasizes, but provides little mechanistic clarification of, the physiological role for
368 proteorhodopsin in *Ca.* Poseidoniales (Table 1).

Table 1 Transcriptional traits shared or distinct among euryarchaeal families[1]

Putative function	Relevant gene(s)	Distribution	Evidence
Proteorhodopsin	Proteorhodopsin gene	Both families	Highly transcribed (Fig. 2); enriched when O1 active (Fig. 3)
Pyrophosphatase	*hppA*	Both families	Highly transcribed (Fig. 2)
Protease/peptidase	*pepF* , *lonB* , *pepA* , *pip* , carboxypeptidase A, subtilase	Both families	Highly transcribed (Fig. 2); depletion during dark incubation (Fig. 5)
ß-oxidation	3-hydroxyacyl-CoA dehydrogenase	Both families	Highly transcribed (Fig. 2); enriched when O1 active (Fig. 3)
Proteorhodopsin retinal synthesis	*crtI* , *crtD*	*Ca.* Thalassarchaeaceae only	Highly transcribed in SIMO MAGs (Fig. 2); depletion during dark incubation (Fig. 5)
Electron transport	Halocyanin gene	*Ca.* Thalassarchaeaceae only	Highly transcribed in SIMO MAGs (Fig. 2); enriched when O1 active (Fig. 3)
Amino acid transport/metabolism	*aapJ* , *livK* , *aroA* , *trpE*	*Ca.* Thalassarchaeaceae only	Highly transcribed (Fig. 2); depletion during dark incubation (Fig. 5)
Xanthine/uracil permease	*pbuG*	*Ca.* Thalassarchaeaceae only	Highly transcribed (Fig. 2); enriched when O1 active (Fig. 3); depletion during incubations (Fig. 5)
Amino acid synthesis	*carA*	*Ca.* Poseidoniaceae only	Highly transcribed (Fig. 2)
Carbohydrate synthesis	Family 2 glycosyl transferase	*Ca.* Poseidoniaceae only	Highly transcribed (Fig. 2)

[1]Putative *Ca.* Poseidoniales families are *Ca.* Poseidoniaceae (MGIIa; MAG RS440) and *Ca.* Thalassarchaeaceae (MGIIb; SIMO Bins 19-2, 31-1).

369

370 Numerous genes encoding putative electron transport proteins were highly transcribed by
371 at least one *Ca.* Poseidoniales family (Fig. 2). Like proteorhodopsin, a halocyanin gene was

372 among the most highly transcribed *Ca.* Thalassarchaeaceae genes and was enriched when genus
373 O1 was transcriptionally active (Fig. 3). Halocyanins are involved in the electron transport chain
374 and have been posited to increase the energy yield of aerobic respiration in *Ca.*
375 Thalassarchaeaceae to stimulate rapid growth [12]. The similar transcription patterns of
376 proteorhodopsin and halocyanin suggests proteorhodopsin activity in coastal *Ca.*
377 Thalassarchaeaceae may function to increase growth rates during respiration, aiding rapid
378 population growth when conditions permit.

379 The *hppa* gene from all *Ca* Poseidoniales MAGs was highly transcribed (Fig. 2; Table 1).
380 The gene putatively encodes a membrane-bound pyrophosphatase, which generates a proton
381 gradient via hydrolysis of pyrophosphate, a byproduct of numerous cellular processes [88]. In
382 metatranscriptomes from a phytoplankton bloom, enrichment of *hppA* transcripts suggested high
383 pyrophosphate-based energy conservation in oligotrophic waters [89]. Similarly, *hppA* was
384 abundant in metatranscriptomes from Lake Llebreta, particularly at night [90]. Although *hppA* is
385 widespread in MAGs from *Ca.* Poseidoniales [15] it has not been recognized as a potentially
386 important part of their metabolism. Our data suggest *Ca.* Poseidoniales may be capable of using
387 pyrophosphatase (along with proteorhodopsin) to generate a protonmotive force (Table 1).
388

389 POTENTIAL IMPORTANCE OF MARINE DOM IN *CA.* POSEIDONIALES METABOLISM

390 Although the T_0 metatranscriptomes from summer versus fall were dominated by
391 transcripts from different *Ca.* Poseidoniales families, a 24-hour dark incubation consistently
392 favored transcription by *Ca.* Thalassarchaeaceae when samples were collected at HT (Fig. 4).
393 This tidal stage-linked increase in *Ca.* Thalassarchaeaceae transcription could relate to
394 differences in DOM availability between HT and LT, consistent with numerous studies

395 implicating DOM in shaping *Ca.* Poseidoniales populations [9,10,91]. The HT Sapelo Island
396 DOM pool is primarily of marine origin while LT DOM is more riverine- and marsh-derived
397 [72], which may select for growth of different *Ca.* Poseidoniales families in the water masses
398 present at different tidal stages. Furthermore, the depletion of transcripts encoding two pyruvate
399 dehydrogenase subunits (*pdhA* and *pdhC*) during incubations (Fig. 5) suggests *Ca.* Poseidoniales
400 were metabolizing phytoplankton photosynthate *in situ*. Alternatively, these tidal stage-driven
401 transcriptional patterns may relate to differential light adaptation in populations originating in
402 offshore versus nearshore waters. Inshore populations, potentially adapted to life in turbid
403 waters, may increase transcription upon dark enclosure, whereas offshore populations
404 (transported shoreward during flood tide) may be adapted to clearer waters and reduce
405 transcription in dark conditions.

406 Multiple lines of evidence indicate coastal *Ca.* Poseidoniales were metabolizing proteins
407 and fatty acids. High transcription of genes encoding proteases or peptidases mapping to all *Ca.*
408 Poseidoniales MAGs (Fig. 2) suggests metabolism of proteins or peptides by both families
409 (Table 1). Furthermore, decreased transcription of protease genes mapping to the O1 MAG
410 during dark incubations (Fig. 5) suggests protein metabolism by *Ca.* Thalassarchaeaceae was
411 active *in situ* prior to incubation. While some of these genes could be involved in intracellular
412 recycling (particularly lon protease and cytosol aminopeptidase), active protein metabolism is
413 consistent with previous experiments demonstrating protein assimilation [10] and high
414 transcription of *Ca.* Poseidoniales peptidase genes in other marine regions [20,21].

415 In addition to genes encoding protein catabolism, high transcription of the 3-
416 hydroxyacyl-CoA dehydrogenase gene from all three MAGs (Fig. 2), and its enrichment in O1-
417 active field samples (Fig. 3), suggests the importance of fatty acid metabolism for both *Ca.*

418 Poseidoniales families (Table 1). This conclusion fits with the widespread occurrence of ß-
419 oxidation genes in *Ca.* Poseidoniales MAGs [15,16], as well as transcriptional data from the
420 deep ocean [21].
421
422 DISTINCT PATTERNS OF AMINO ACID AND NUCLEOTIDE UPTAKE AND
423 METABOLISM
424 Transcription of *livK* and *aapJ* appears to differentiate *Ca.* Poseidoniales families in the
425 coastal ocean (Table 1). These genes putatively encode ligand-binding receptors for ABC
426 transporters: *aapJ* for a general L-amino acid transporter and *livK* for a branched-chain amino
427 acid transporter [92]. Both are commonly present in *Ca.* Thalassarchaeaceae (MGIIb) but not *Ca.*
428 Poseidoniaceae (MGIIa) [16] and were among the most highly transcribed *Ca.*
429 Thalassarchaeaceae genes (Fig. 2). Previous studies noted high transcription of euryarchaeal *livK*
430 and *aapJ* genes in the water column of the Red Sea [19,20], at the Mid-Cayman Rise [21], and
431 throughout the Atlantic Ocean [22]. Our data suggest this activity was probably associated with
432 *Ca.* Thalassarchaeaceae.
433 The *aapJ* and *livK* genes were collocated with genes putatively encoding the full
434 transporters in the *Ca.* Thalassarchaeaceae MAGs (Table S1). Unfortunately, it is difficult to
435 guess their substrates from sequence data alone: AAP transporters are typically capable of
436 transporting a range of L-amino acids [92] while LIV transporters can be highly specific for
437 leucine, specific for branched-chain amino acids, or transport diverse amino acids [92-94]. In soil
438 bacteria grown under inorganic nitrogen limitation, elevated transcription of *aapJ* is linked to
439 organic nitrogen use [95], but it is unclear whether this mechanism translates to *Ca.*
440 Thalassarchaeaceae since amino acids could be used for numerous anabolic or catabolic

441 processes. In addition to these binding proteins, the depletion of transcripts from O1 genes
442 putatively involved in the shikimate pathway of aromatic amino acid synthesis (3-
443 phosphoshikimate 1-carboxyvinyltransferase and anthranilate synthase) during incubations (Fig.
444 5) suggests these archaea were synthesizing aromatic amino acids *in situ*.

445 The combination of high *pbuG* transcription by *Ca.* Thalassarchaeaceae (MGIIb) with the
446 high numbers of O1 *pbuG* transcripts in O1-active samples and dark incubation endpoints (Figs.
447 2,3,5) suggests an important role for xanthine/uracil permease (the putative product of *pbuG*) in
448 *Ca.* Thalassarchaeaceae metabolism. In some phytoplankton, *pbuG* is transcribed during
449 nitrogen-stressed growth [96-98], potentially allowing access to DON. However, *pbuG* and
450 xanthine dehydrogenase (*xdh*) are also transcribed when xanthine is catabolized by marine
451 bacteria [99]. Both *Ca.* Thalassarchaeaceae MAGs contain putative xanthine dehydrogenase
452 genes (*xdhC* and *yagS*; Table S1), suggesting the ability to catabolize xanthine (Table 1).

453 Transcription levels of *carA*, putatively encoding part of carbamoyl phosphate synthetase,
454 appears to be a distinct trait of *Ca.* Poseidoniaceae (MGIIa): while all three MAGs contained this
455 gene (Table S1), only *Ca.* Poseidoniaceae *carA* transcription was high. Since carbamoyl
456 phosphate synthetase is a key enzyme for arginine and pyrimidine synthesis from bicarbonate,
457 high *carA* transcription suggests these pathways may be important components of *Ca.*
458 Poseidoniaceae growth or survival.

459

460 **CONCLUSIONS**

461 Our metatranscriptomic data and associated experiments provide a window into the
462 activity of *Ca.* Poseidoniales families (formerly "MGIIa" and "MGIIb"). They indicate an
463 important role for *Ca.* Thalassarchaeaceae (MGIIb) as coastal photoheterotrophs, particularly in

warm waters. High transcription of proteorhodopsin and membrane-bound pyrophosphatase genes suggested common methods for establishing proton gradients. Furthermore, high transcription of genes involved in protein/peptide metabolism and ß-oxidation of fatty acids confirmed peptide and lipid metabolism as a common trait. However, high transcription of *Ca.* Thalassarchaeaceae genes encoding amino acid binding proteins and nucleotide transporters suggests uptake of these substrates may distinguish the two families. These data confirm the importance of DOM metabolism by *Ca.* Poseidoniales and suggest a potential role for organic nitrogen in *Ca.* Thalassarchaeaceae metabolism.

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