

Comparison of preservation and extraction methods on five taxonomically disparate coral microbiomes

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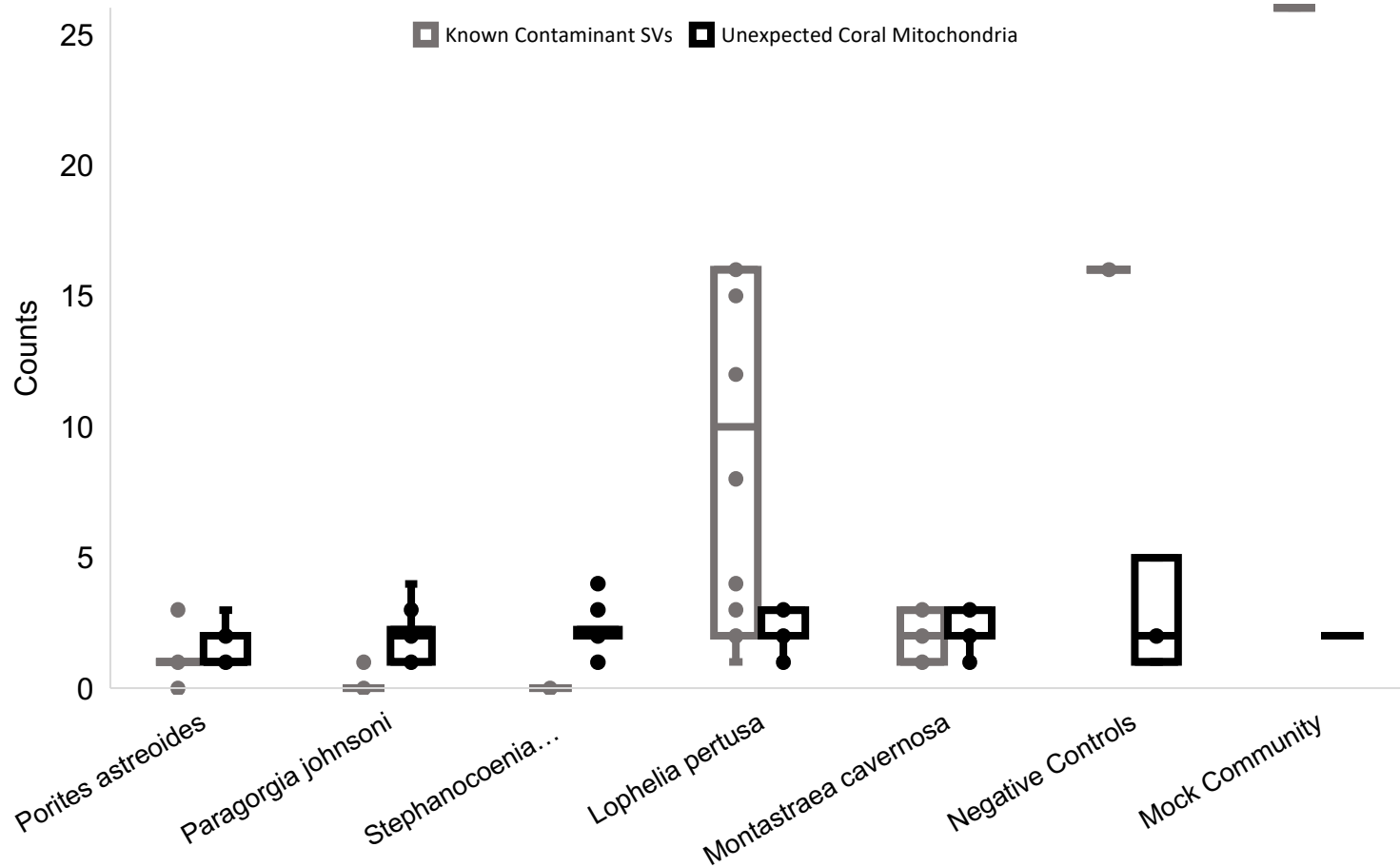
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Supplementary Material

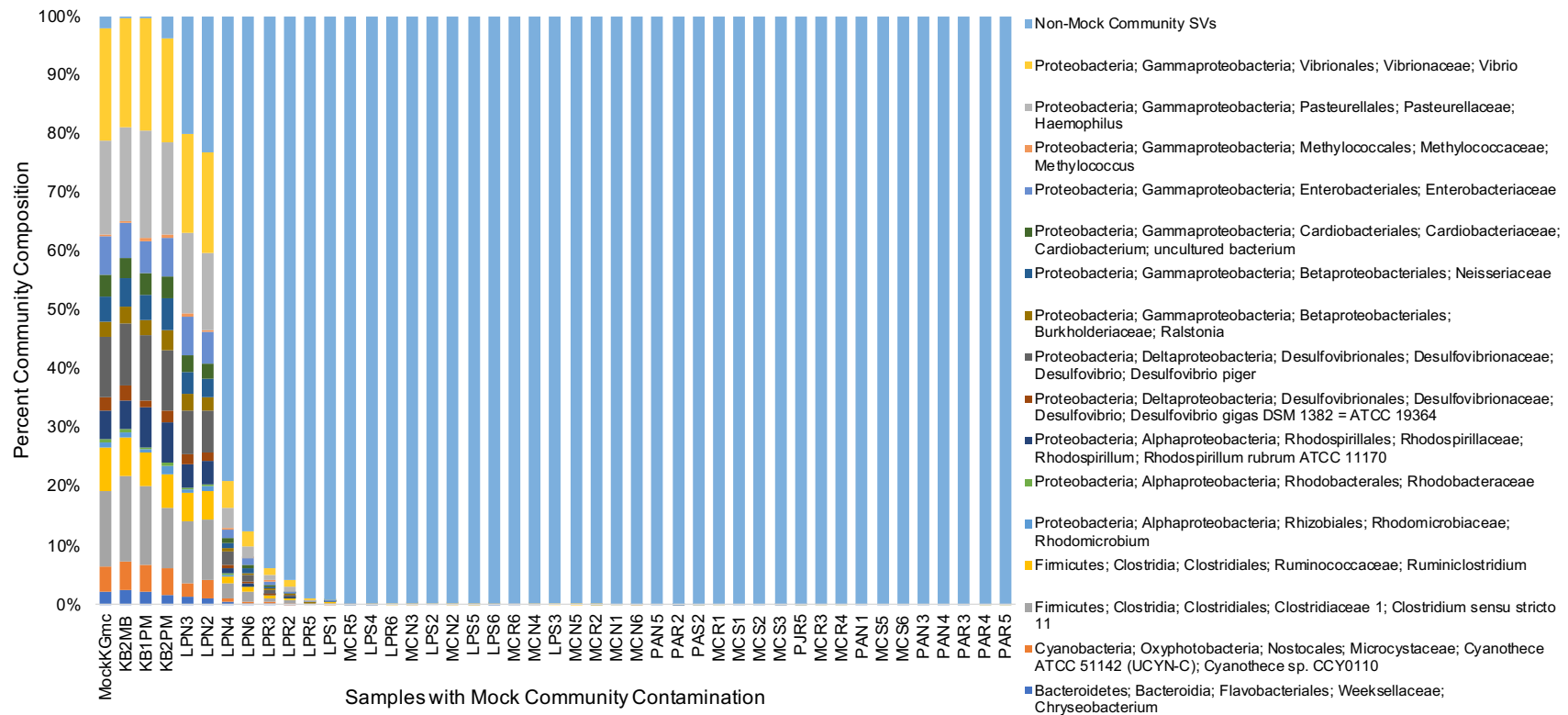
Supplemental figures and tables listed in order of appearance.

Supplemental Table 1. Mock community provided by sequencing vendor Glomics. Bacterial 16S rRNA gene copy numbers were calculated using Ribosomal Database Project's tool rrnDP (version 5.6). *indicates unknown copy numbers where the genus average was taken instead.

Strain	Genome Size (Mb)	16S Copies	QIIME2 taxonomic assignment	Counts from Rarefied Table
Cardiobacterium_hominis_ATCC_15826	2.6	3	Proteobacteria; Gammaproteobacteria; Cardiobacteriales; Cardiobacteriaceae; Cardiobacterium; uncultured bacterium	193
Chryseobacterium_gleum_ATCC_35910	2.6	5.5*	Bacteroidetes; Bacteroidia; Flavobacteriales; Weeksellaceae; Chryseobacterium	111
Clostridium_acetobutylicum_ATCC_824	3.9	11	Firmicutes; Clostridia; Clostridiales; Clostridiaceae 1; Clostridium sensu stricto 11	660
Clostridium_thermocellum_ATCC_27405	3.8	4	Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminiclostridium	388
Cyanothece_sp._ATCC_51142	4.9	2	Cyanobacteria; Oxyphotobacteria; Nostocales; Microcystaceae; Cyanothece ATCC 51142 (UCYN-C); Cyanothece sp. CCY0110	228
Desulfovibrio_gigas_DSM_1382_=_ATCC_19364	3.7	3.1*	Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio gigas DSM 1382 = ATCC 19364	110
Desulfovibrio_piger_ATCC_29098_strain_DSM_749	2.8	7	Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio piger	532
Haemophilus_parainfluenzae_ATCC_33392	2.1	6	Proteobacteria; Gammaproteobacteria; Pasteurellales; Pasteurellaceae; Haemophilus	824
Kingella_denitrificans_ATCC_33394	2.2	4*	Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Neisseriaceae	224
Methylococcus_capsulatus_str._Texas_=_ATCC_19069	3.3	2	Proteobacteria; Gammaproteobacteria; Methylococcales; Methylococcaceae; Methylococcus	23
Rahnella_aquatis_CIP_78.65_=_ATCC_33071	4.9	7	Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae	336
Ralstonia_pickettii_strain_ATCC_27511	4.8	2	Proteobacteria; Gammaproteobacteria; Betaproteobacteriales; Burkholderiaceae; Ralstonia	138
Rhodobacter_sphaeroides_ATCC_17025	3.2	3.5	Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae	30
Rhodocyclidium_vannielii_ATCC_17100	4	2	Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhodocyclidiaceae; Rhodocyclidium	44
Rhodospirillum_rubrum_ATCC_11170	4.4	4	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; Rhodospirillum; Rhodospirillum rubrum ATCC 11170	258
Vibrio_natriegens_NBRC_15636_=_ATCC_14048	5.1	13	Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio	994

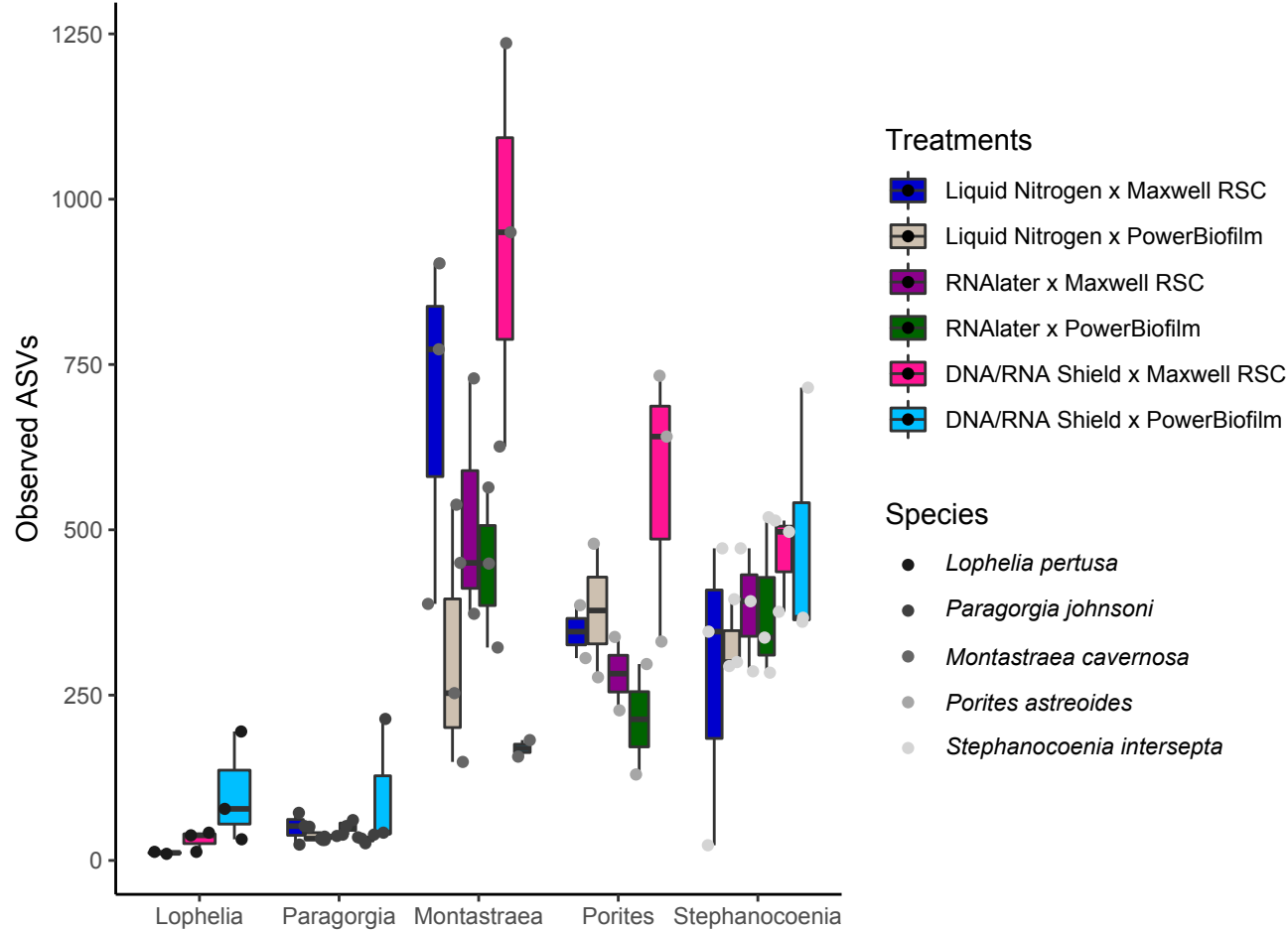


Supplemental Figure 1. Known contaminant sequence variants (SVs) and coral mitochondria identified as contaminants in each sample. Known contaminant SVs are defined as amplicon sequence variants (ASVs) detected in the mock community in addition to the expected sequences, or mock community members found within negative controls (extraction blanks) or coral samples. Coral mitochondrial sequences were identified by BLAST matches. Only mitochondria from the host coral were expected per sample, the rest are considered Unexpected Coral Mitochondria and are counted as number of non-host coral species detected (0-5). Coral mitochondrial data were unrarefied (and subsequently removed), and known contaminant SVs were obtained from the rarefied, curated table described in Materials and Methods.

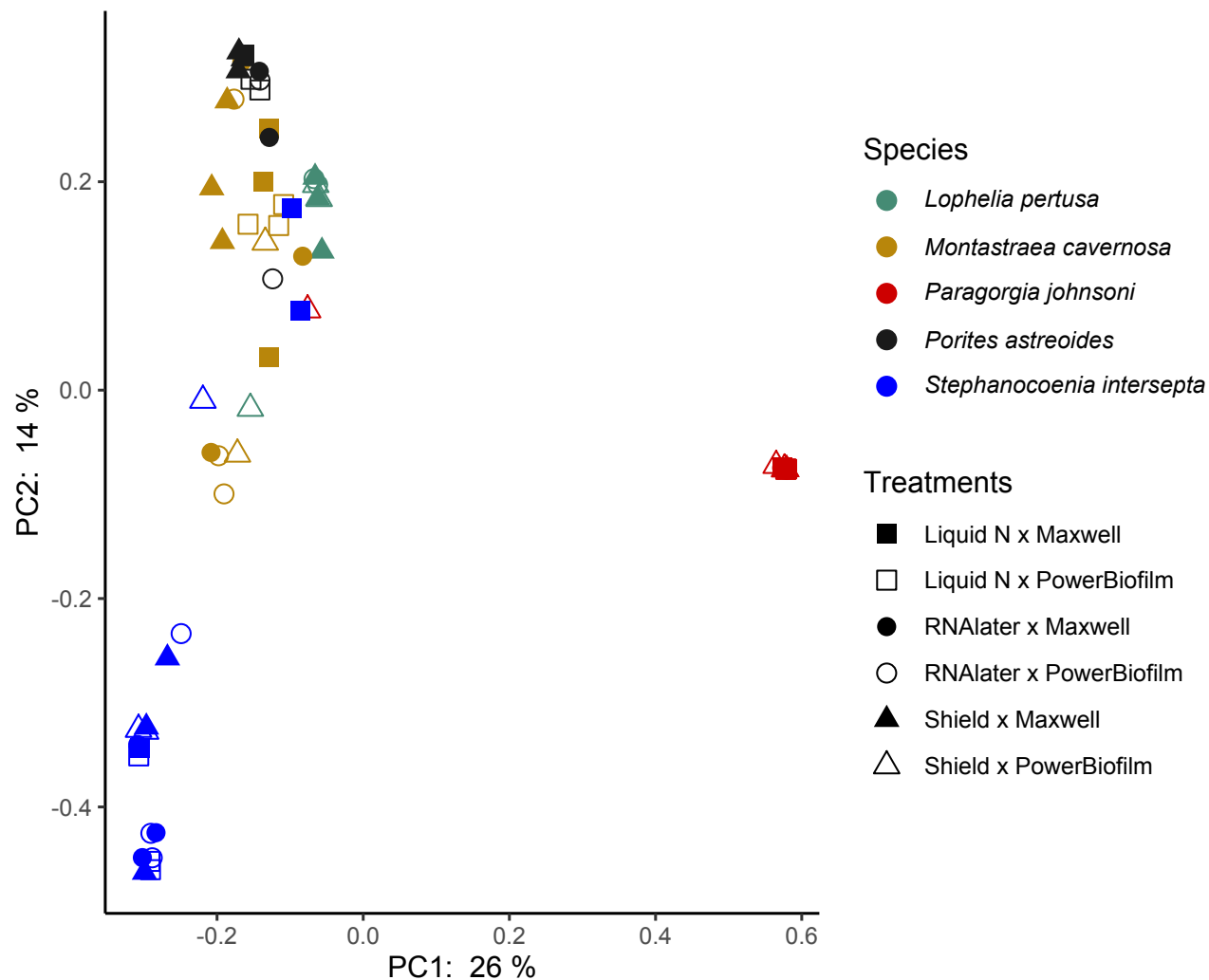


Supplemental Figure 2. Samples containing at least one mock community amplicon sequence variant (ASV) in the table rarefied to 5,203 sequences. Samples beginning with K are the three negative controls (kit extraction blanks) and were the most contaminated. Samples containing greater than 1% of mock community contamination were removed before further analysis (nine total, including the negative controls). Thirty-seven samples did not contain any ASVs from the mock community (not shown).

Supplemental Table 2. The final amplicon sequence variant (ASV) table used for diversity analyses. This table contains the 72 coral samples (out of the original 90) that passed quality controls. Each sample was rarefied to 5,203 sequences. This table is provided as a separate Excel file.



Supplemental Figure 3. Observed amplicon sequence variants (ASVs) for each coral species by treatment (preservative x extraction method). Treatments (from left to right for each coral): dark blue = liquid nitrogen x Maxwell RSC; gray = liquid nitrogen x PowerBiofilm; purple = RNAlater x Maxwell RSC; green = RNAlater x PowerBiofilm; pink = DNA/RNA Shield x Maxwell RSC; light blue = DNA/RNA Shield X PowerBiofilm.



Supplemental Figure 4. Principal coordinate plots based upon a Bray-Curtis dissimilarity matrix of all coral species and treatments (preservative x extraction method). Microbial communities clustered strongly according to coral species rather than treatment. Each coral species was analyzed for differences in treatments on an individual basis and presented in Figure 1. The single *Paragorgia johnsoni* sample (Shield x PowerBiofilm) that did not cluster with its species was removed from subsequent analysis, as it was an extreme outlier.

Supplemental Table 3. This table lists the amplicon sequence variants (ASVs) present in the mock community control and the three extraction kit controls that passed rarefaction to 5,203 sequences. The kit extraction blank KB1MB only had 3 sequencing reads and was removed. The 16 members of the mock community (listed in *Supplemental Table 1*) are shaded in dark gray. ASVs that were present in two or more coral samples and had read numbers in the samples that were 1 to 2 orders of magnitude higher than those of the controls were considered to be cross-talk from samples into the controls and have been indicated in light gray. The remaining 14 unshaded ASVs are likely actual reagent contaminants. This table is provided as a separate Excel file.

Supplemental Table 4. Significantly different amplicon sequence variants (ASVs) as identified by DESeq2 for each combination of coral species (*Lophelia pertusa*, *Paragorgia johnsoni*, *Montastraea cavernosa*, *Porites astreoides*, and *Stephanocoenia intersepta*), preservative (RNAlater, DNA/RNA Shield, and liquid nitrogen), and extraction method (Maxwell RSC and PowerBiofilm). See Table 3 for summary of results. This table is provided as a separate Excel file.