

## Supplementary Materials

### Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae.

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*Sampling and DNA isolation*

**Supplementary Table S1:** Coral skeleton samples and locality.

Sample	Country	Locality	Coral host
HV04438.R3	Papua New Guinea	Kavieng	Unidentified stony coral
HV04837.R3	Papua New Guinea	Kavieng	<i>Favites</i> sp.
HV04841.R3	Papua New Guinea	Kavieng	<i>Porites</i> sp.
HV04853.R3	Papua New Guinea	Kavieng	<i>Millepora</i> sp.
HV04854.R3	Papua New Guinea	Kavieng	<i>Goniastrea pectinata</i>
HV04857.R3	Papua New Guinea	Kavieng	<i>Porites</i> sp.
HV05003.R3	Papua New Guinea	Kavieng	<i>Goniastrea</i> sp.
HV05004b.R3	Papua New Guinea	Kavieng	<i>Platygyra</i> sp.
HV05004c.R3	Papua New Guinea	Kavieng	<i>Platygyra</i> sp.
HV05005.R3	Papua New Guinea	Kavieng	<i>Porites</i> sp.
HV05006a.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05006b.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05007.R3	Papua New Guinea	Kavieng	<i>Diploastrea heliopora</i>
HV05008.R3	Papua New Guinea	Kavieng	<i>Leptoseris</i> sp.
HV05009.R3	Papua New Guinea	Kavieng	<i>Favites russelli</i>
HV05010.R3	Papua New Guinea	Kavieng	<i>Madracis</i> sp.
HV05011.R3	Papua New Guinea	Kavieng	<i>Oxypora lacera</i>
HV05012.R3	Papua New Guinea	Kavieng	<i>Leptoseris striatus</i>
HV05013.R3	Papua New Guinea	Kavieng	<i>Pectinia</i> sp.
HV05014.R3	Papua New Guinea	Kavieng	<i>Leptoseris striata</i>
HV05015.R3	Papua New Guinea	Kavieng	<i>Porites profundus</i>
HV05016.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05017.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05019.R3	Papua New Guinea	Kavieng	<i>Favites halicora</i>
HV05020.R3	Papua New Guinea	Kavieng	<i>Pachyseris</i> sp.
HV05021.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05022.R3	Papua New Guinea	Kavieng	<i>Platygyra lamellina</i>
HV05023.R3	Papua New Guinea	Kavieng	<i>Porites australiensis</i>
HV05024.R3	Papua New Guinea	Kavieng	<i>Lobophyllia</i> sp.
HV05025.R3	Papua New Guinea	Kavieng	<i>Symphyllia valenciennesi</i>
HV05026.R3	Papua New Guinea	Kavieng	<i>Goniastrea edwardsii</i>
HV05028.R3	Papua New Guinea	Kavieng	<i>Stylophora pistillata</i>
HV05029.R3	Papua New Guinea	Kavieng	<i>Porites australiensis</i>
HV05031.R3	Papua New Guinea	Kavieng	<i>Pachyseris</i> sp.
HV05032.R3	Papua New Guinea	Kavieng	<i>Pertinia</i> sp.
HV05034.R3	Papua New Guinea	Kavieng	<i>Porites</i> sp.
HV05035.R3	Papua New Guinea	Kavieng	<i>Echinopora hirsutissima</i>
HV05037.R3	Papua New Guinea	Kavieng	<i>Merulina ampliata</i>
HV05038.R3	Papua New Guinea	Kavieng	<i>Pachyseris</i> sp.
HV05039.R3	Papua New Guinea	Kavieng	<i>Merulina ampliata</i>
HV05040c.R3	Papua New Guinea	Kavieng	<i>Porites</i> sp.
HV05041a.R3	Papua New Guinea	Kavieng	<i>Galaxea astreata</i>
HV05041b.R3	Papua New Guinea	Kavieng	<i>Galaxea astreata</i>
HV05042.R3	Papua New Guinea	Kavieng	<i>Diploastrea heliopora</i>
HV05043.R3	Papua New Guinea	Kavieng	<i>Echinopora hirsutissima</i>

HV05044.R3	Papua New Guinea	Kavieng	<i>Lobophyllia sp.</i>
HV05045.R3	Papua New Guinea	Kavieng	<i>Oxypora lacera</i>
HV05046.R3	Papua New Guinea	Kavieng	<i>Leptoseris troglodyta</i>
HV05047.R3	Papua New Guinea	Kavieng	<i>Leptoseris mycetoseroides</i>
PHV207.R1	Papua New Guinea	PNG	Unidentified stony coral
PHV237.R1	Papua New Guinea	PNG	Unidentified stony coral
PHV570.R1	Papua New Guinea	PNG	Unidentified stony coral
PHV882.R1	Papua New Guinea	PNG	Unidentified stony coral
VRM0028.R1	Australia	Western Australia	<i>Montipora sp.</i>
VRM0032.R1	Australia	Western Australia	<i>Montipora sp.</i>
VRM0036.R1	Australia	Western Australia	Unidentified stony coral
VRM0039.R2	Australia	Western Australia	<i>Cyphastrea sp.</i>
VRM0040.R2	Australia	Western Australia	<i>Goniastrea sp.</i>
VRM0042.R2	Australia	Western Australia	<i>Goniastrea sp.</i>
VRM0043.R2	Australia	Western Australia	<i>Leptoria sp.</i>
VRM0044.R2	Australia	Western Australia	Unidentified stony coral
VRM0045.R2	Australia	Western Australia	<i>Montastrea sp.</i>
VRM0046.R2	Australia	Western Australia	Unidentified stony coral
VRM0048.R2	Australia	Western Australia	<i>Goniastrea sp.</i>
VRM0051.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0052.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0053.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0054.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0055.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0056.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0057.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0058.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0059.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0060.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0061.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0062.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0063.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0064.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0065.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0066.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0067.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0068.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0069.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0070.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0071.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0072.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0073.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0074.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0076.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0077.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0078.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0079.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0080.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0081.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0082.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0083.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0084.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0085.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0086.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0087.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0090.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0091.R1	Australia	Western Australia	<i>Montipora sp.</i>
VRM0096.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0097.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0098.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0099.R1	Australia	Western Australia	<i>Porites sp.</i>

VRM0100.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0101.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0102.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0103.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0104.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0105.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0106.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0107.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0108.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0109.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0110.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0111.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0112.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0113.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0114.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0115.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0116.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0117.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0118.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0119.R2	Australia	Western Australia	<i>Porites sp.</i>
VRM0120.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0121.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0122.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0123.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0124.R1	Australia	Western Australia	<i>Porites sp.</i>
VRM0190.R1	Australia	Queensland	<i>Pocillopora sp.</i>

After collection, the coral tissue was removed with pliers and razor blades and samples were stored in RNAlater or 100% ethanol. The DNA was extracted using either: (1) a modified phenol-chloroform protocol — see Cremen *et al.* (2016); our only modification was to use phenol/chloroform/isoamyl alcohol [25:24:1] (instead of chloroform/isoamyl alcohol only) in the first extraction step. Or (2) the Wizard Genomic DNA Purification Kit (Promega). More amplification success and DNA yield was obtained using the kit with a small modification: incubating a piece of coral skeleton (ca. 80mm<sup>3</sup>) in the lysis buffer for 3 hours without grinding the sample, then proceeding with the manufacturer's instructions for plant tissue.

#### *Library preparation:*

The amplicons and respective primers used here were:

**16S rDNA:** We used either the 515f/806r (Caporaso *et al.*, 2012; Gilbert *et al.*, 2014) or the S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Klindworth *et al.*, 2013) primer pairs to PCR-amplify this marker. The amplicons generated by these 2 primer pairs overlap in the V3-V4 region, so we used only this overlapping region — the sequence length after trimming primer sequences was, on average, 225 base pairs.

**18S rDNA:** We used the NF1/18 Sr2b primer combination (Porazinska *et al.*, 2009).

**23S rDNA:** We used the algal specific primer pair p23SrV\_r1/p23SrV\_f1, which PCR-amplifies the Universal Plastid Amplicon (Presting 2006).

**tufA:** We used primers *tufAR* (Fama *et al.*, 2002) and a forward primer designed here for *Ostreobium* (Oq-tuf: ACN GGN CGN GGN ACN GT), which has several ambiguous bases in order to amplify a larger range of green algae species.

**Supplementary Table S2: 1<sup>st</sup> PCR primers design†**

	Tail 1	Ns (0-3bp)	Primer (e.g. 16S 515/806)
<b>Forward</b>	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	NNN	[GTGCCAGCMGCCGCGGTAA]
<b>Reverse</b>	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	NNN	[GGACTACHVGGGTWTCTAAT]

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**Supplementary Table S3: 2<sup>nd</sup> PCR oligonucleotides†.**

Forward Illumina adapter	F Indices	Tail_2
A*ATGATACGGCGACCACCGAGATCTACAC	TAGATCGC	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	CTCTCTAT	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	TATCCTCT	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	AGAGTAGA	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	GTAAGGAG	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	ACTGCATA	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	AAGGAGTA	TCGTCGGCAGCGTC
A*ATGATACGGCGACCACCGAGATCTACAC	CTAAGCCT	TCGTCGGCAGCGTC
Reverse Illumina adapter	R Indices	Tail_2
C*AAGCAGAAGACGGCATAACGAGAT	TAAGGCGA	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	CGTACTAG	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	AGGCAGAA	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	TCCTGAGC	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	GGACTCCT	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	TAGGCATG	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	CTCTCTAC	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	CAGAGAGG	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	GCTACGCT	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	CGAGGCTG	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	AAGAGGCA	GTCTCGTGGGCTCGG
C*AAGCAGAAGACGGCATAACGAGAT	GTAGAGGA	GTCTCGTGGGCTCGG

\*Indicates a phosphorothioate modification. † Oligonucleotide sequences © 2007-2012 Illumina, Inc. All rights reserved - Derivative works created by Illumina customers are authorized for use with Illumina instruments and products only. All other uses are strictly prohibited.

We amplified the four markers in separate reactions containing 0.2 mM dNTP mix, 0.5 µM forward and reverse primers, 2 mM MgCl<sub>2</sub>, 0.4 µg/µl Bovine Serum Albumin, 1× PCR buffer and 0.25U of Platinum *Taq* DNA Polymerase (Invitrogen). The first PCR round consisted of: initial denaturation step at 94°C for 5 min, followed by 25 cycles of denaturation (94°C for 30 s), annealing (45 s) and extension (72°C for 30 s) and a final extension step at 72°C for 5 min for the ribosomal DNA markers. Annealing temperature was set at 50°C for primer pair 515f/806r, 55°C for p23SrV\_r1/p23SrV\_f1 and S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 and 60°C for NF1/18Sr2b. Because *tufA* is a coding gene, it has higher mutation rates (especially at 3<sup>rd</sup> codon positions) when compared to ribosomal DNA, therefore a touchdown step and a lower

annealing temperature is required (55—48°C for 14 cycles followed by 24 cycles at 48°C). Unspecific amplification does occur, but those are excluded in the analysis pipeline (e.g. steps 5, 6 and 9 of the pipeline).

For the second PCR we used the following conditions: initial denaturation step at 94°C for 5 min, followed by 8 cycles of denaturation (94°C at 30 s), annealing (55°C at 30 s) and extension (72°C at 30 s) and a final extension step at 72°C for 5 min. We purified the samples using home-made magnetic beads as described in Rohland and Reich (2012) and quantified the libraries using the Qubit fluorometer (Invitrogen). We produced libraries for three runs: the first run containing 48 samples (of which 5 were PCR controls or mock extractions), the second and third runs contained 96 samples (including 7 and 8 controls, respectively). The libraries were sequenced with the Illumina MiSeq platform (V3 kit - 2×300 bp PE reads) at the Centre for Translational Pathology, University of Melbourne. The runs generated sequences for all samples that had a successful PCR amplification (*ca.* 1 uM or more). Not all samples successfully sequenced in these three runs are included in this study: here we included the 132 samples listed above, the remaining samples are part of a different study and will be published separately.

#### *Costs calculation:*

20 indexed oligos (Supplementary Table 3), with phosphorothioate modification, produced at 200 nmole scale by Bioneer Pacific = 425AUD (Including GST). Each 200 nmole oligonucleotide is sufficient for 800 reactions. Prices for plate orders.

Nextera kit with 96 Indices, 384 samples (FC-121-1012) = 1132 AUD.

Prices are from 2014.

1 Australian Dollar (AUD)  $\approx$  0.72 US Dollar (October 2015).

#### *Data processing pipeline:*

1. Remove the reverse complement of adapters from short amplicons. When the length of the reads is longer than the amplicon, you will get the reverse complement of the adapter sequenced in the 3' end of the read, which can influence the merging of the paired end sequences.
2. Separate genes into different files. With our library preparation design, the MiSeq run yields one file per sample, each containing all amplicons. The different amplicons are teased apart based on primers sequences in this step.
3. Trim 3' ends of reads (5 bases in forward reads and 20 bases in reverse reads) to improve consensus quality.

4. Merge forward and reverse reads using FLASH (Magoč and Salzberg, 2011).
5. Quality control: filter merged reads based on a quality threshold (average of 35 per merged read) using PRINSEQ (Schmieder and Edwards, 2011).
6. Trim primers from merged reads. Sequences that do not meet a minimum length threshold and/or do not have the exact primer sequence at the 3' and 5' ends are excluded from analysis in order to ensure quality (i.e., the sequence belong to the target gene) and global trimming (i.e., they start and end at the same position).
7. Format reads' identification and generate one file per gene containing all samples.
8. Run UPARSE pipeline (Edgar, 2013): dereplication, sort by size, cluster OTUs and produce OTU map. We chose UPARSE because other available software (e.g. Qiime and Mothur) seem to significantly overestimate the number of OTUs (Edgar, 2013). Based on the divergence of *tufA* among Bryopsidales we used a similarity threshold of 98% for OTU clustering in this marker, which is a conservative threshold for species level (i.e., most Bryopsidales species are more similar than that, so at 98% the OTUs will be somewhere between species and genus level). We choose the 97% threshold for the other markers for two main reasons: 1) there is not enough information in literature about the rDNA markers similarity among Bryopsidales species, on the contrary, it is known that they do not have phylogenetic signal to distinguish them; 2) our aim was to compare how the normally used markers (with their commonly used thresholds) perform in distinguishing algae species.
9. Alignment: we used PyNAST (Caporaso *et al.*, 2010a) to align the 16S and 18S rDNA sequences. This aligner requires a reference database with aligned sequences and lots of gaps in the alignment. Due to the lack of such reference databases for 23S rDNA and *tufA*, we chose MAFFT (Katoh *et al.*, 2002) to align 23S rDNA and *tufA*. The OTUs that failed to align were excluded from downstream analysis.
10. Assign taxonomy using the Naïve Bayesian Classifier (RDP) implemented in Qiime (Wang *et al.*, 2007; Caporaso *et al.*, 2010b). We used Greengenes and SILVA databases for the 16S and 18S rDNA sequences respectively. In order to produce an RDP-friendly database for the 23S rDNA and the *tufA*, we downloaded reference sequences from Genbank, used a phylogenetic similarity threshold (based on a UPGMA tree) to equalize the dataset (i.e. exclude repetitive species, which will bias the RDP classifier) and produced the reference dataset (one file with the sequences and another with taxonomic ranks). We used RDP taxonomic assignments to: i) infer the abundance of reads assigned to the main microbial groups (Figure 2); and ii) pre-filter OTUs to build a green algae phylogenetic tree: OTUs that were not classified as "Eukaryotic" (or "Chloroplast" in the 16S) were excluded from the phylogenetic analysis.
11. Filter OTUs found in negative controls (mock extractions and negative control PCRs). Although virtually no DNA was detected (with Qubit) in these controls, we added those samples to our library in order to detect any possible contaminant. But apart from cross contamination, sequencing errors can yield false-positives. Therefore some OTUs found in the controls could

be, for example, the most abundant *Ostreobium* sequences which should not be excluded. So we filtered the OTUs present in the controls, but only if they would represent less than 1% of the total number of reads. For the 18S dataset, OTUs matching Cnidaria and Dinophyceae were considered contaminants from the coral tissue and were also removed from the analysis.

12. Filter OTU table by minimum count (2) of reads per OTU per sample (filter\_observations\_by\_sample.py - <https://gist.github.com/adamrp/7591573>). Another quality control step to remove OTUs present with low abundance in the samples.
13. Filter rare OTUs (less than 5 reads) and produce final filtered OTU fasta file. This is the input for the phylogenetic analysis.
14. Produce final OTU table and statistics. These statistics are another sort of quality check, and the OTU table is necessary for beta-diversity/comparative analysis (which we do not do in this study). From here one can check the sequencing depth and proceed to Qiime's core\_diversity\_analysis.py, for example.

#### *Phylogenetic analysis:*

The short reads generated by high-throughput sequencing technologies remain an issue for phylogenetic analysis. To overcome this problem, we used longer larger parts of the sequenced genes (available on Genbank, generated by Sanger sequencing) and additional genes to reconstruct the backbone of the green algal phylogeny. That way, even though the relationship within OTU-only clades may not be well resolved due to short fragments, the position of these clades among the green algae phylogeny can be inferred with strong support. We concatenated genes of different species of the same genus when same-species-sequences were not available, therefore filling as much as possible the gaps in the alignment. We used only species for which there was a reference sequence (for species or genus) for the marker analyzed – for example: there is no *Ostreobium* 18S rDNA sequence available (Supplementary Table 4), therefore this taxon was not included in the phylogenetic analysis of the 18S OTUs. The phylogenetic trees (Figure 3 and Supplementary Figures 1-4) were built with the following markers:

- *tufA*-OTUs phylogeny: *tufA* (including OTUs) + *rbcL* + 18S rDNA = 4833 bp.
- 16S rDNA-OTUs phylogeny: *tufA* + *rbcL* + 18S rDNA + 16S rDNA (including OTUs) = 7251 bp.
- 18S rDNA-OTUs phylogeny: *tufA* + *rbcL* + 18S rDNA (including OTUs) = 5297 bp.
- 23S rDNA-OTUs phylogeny: *tufA* + *rbcL* + 18S rDNA + 23S rDNA (including OTUs) = 9489 bp.

**Supplementary Table S4:** Reference sequences (voucher and Genbank accession number, when available) used to reconstruct the green algae phylogeny.

Species	<i>tufA</i>	<i>rbcL</i>	18S rDNA	16S rDNA	23S rDNA
<i>Acetabularia acetabulum</i>	D11_HG518471	GB_HG518454.1	GB_AY165774		
<i>Acetabularia dentata</i>	HEC12349_XX	GB_AY177739.1	GB_Z33468		
<i>Acrochaete leptochaete</i>	MBLPoly1_AY454408	GB_AY303591			
<i>Acrosiphonia</i> sp.	GWS00746_HQ610			SAG127.80_FN563074.1	
<i>Acutodesmus obliquus</i>				UTEX.393_DQ396875	UTEX393_DQ396875
<i>Auxenochlorella protothecoides</i>				XX_KC843975	GB_KC843975
<i>Avrainvillea lacerata</i>	HV00599_FJ432651	GB_FJ432635.1		GB_FJ535833	
<i>Avrainvillea nigricans</i>	HV02664_XX	HV02664_XX		GB_FJ535834	
<i>Botophora</i> noID1	LL0095_XX				
<i>Bolbocoleon piliferum</i>	KMP01309a_KJ41191	GB_FJ715716.1	MA31b1_AY303599		
<i>Boodleopsis</i> sp.	H.0758_XX	H.0758_XX			
<i>Botryococcus braunii</i>				SAG.807.1_KM462884	SAG.807.1_KM462884
<i>Bryopsis duplex</i>	HV01238_XX				
<i>Bryopsis hypnoides</i>	GB_GQ892829	GB_GQ892829	GB_FJ715685	XX_GQ892829	GB_NC013359
<i>Bryopsis plumosa</i>	HV00880_XX	GB_AB038480	HV00880_FJ432630	West4718_LN810504	West4718_LN810504
<i>Bryopsis vestiata</i>	F004_XX				
<i>Callipsyigma wilsonis</i>	HV03983_XX	HV03983_XX	H.0890_XX		
<i>Caulerpa filiformis</i>	GB_CAD10730	GB_FR848349.1	GB_JF932262		
<i>Caulerpa obscura</i>	L.09.10.052_FR848335	GB_KF649941.1			
<i>Caulerpa racemosa</i>	GB_JN817682	GB_AB038485	GB_AF479702		
<i>Caulerpa sertularioides</i>					PGSTPM002_KF724396
<i>Caulerpa simpliciuscula</i>	L.09.10.048_FR848333	GB_FR848346.1			
<i>Caulerpa verticillata</i>	TS0072_KM186530	GB_FR668300.1	GB_JF932252		
<i>Caulerpella ambigua</i>	TS24_FJ432655	GB_FJ432638.1		GB_FJ535836	
<i>Cephaleuros parasiticus</i>	GB_AJM90125.1	GB_KM464711.1	GB_DQ399583		
<i>Chlamydomonas reinhardtii</i>	GB_DAA00908	GB_BK000554.2	GB_JN903984	GB_BK000554	GB_BK000554
<i>Chlorella mirabilis</i>				SAG.38.88_KM462865	GB_KM462865
<i>Chlorella</i> sp.				ArM0029B_KF554427	ArM0029B_KF554427
<i>Chlorella variabilis</i>					NC64A_KJ718922
<i>Chlorella vulgaris</i>	C-27_BAA57886	GB_CHLC27	GB_X13688		
<i>Chlorocladus australasicus</i>	PH660_XX	GB_AY177750.1	GB_Z33466		
<i>Chlorococcum oleofaciens</i>		GB_KM052785.1	GB_U41176		
<i>Chlorodesmis baculifera</i>	H.0880_XX	H.0880_XX			
<i>Chlorodesmis fastigiata</i>				GB_FJ535837	
<i>Chlorosarcina brevispinosa</i>				UTEX1176_KM462875	UTEX1176_KM462875
<i>Chlorosarcinopsis eremi</i>	UTEX1186_HQ246369	BCPJT1VF80_HQ246349	BCPJT1VF8_HQ246315		
<i>Choricystis</i> sp.	CAUP.H.1984_XX	GB_KM438409.1	GB_AY762605	SAG.17.98_KM462878	SAG.17.98_KM462878
<i>Cladophora pygmaea</i>			GB_FM205051		
<i>Cladophora rhodolithicola</i>			GB_FM205053		
<i>Cladophora socialis</i>			GB_AB971263		
<i>Cloniophora spicata</i>	ARS00769_KM676565	ARS00515_KM677025			
<i>Coccolobos verrucariae</i>		GB_AM260447.1	SAG1697_KM020110		
<i>Coccomyxa</i> sp.	C-169_HQ693844	GB_HQ693844.1		C.169_HQ693844	C-169_HQ693844
<i>Codium arabicum.2</i>	HV01432_XX	GB_EF107968.1			
<i>Codium arenicola</i>	HEC15968_KP685825	GB_JQ706329.1			
<i>Codium bursa.2</i>	G.113_XX	G.113_XX			
<i>Codium duthieae</i>	LT0248_JX463043	GB_EF107984.1	KZN2K4.1_FJ535848		
<i>Codium edule</i>					GB_EF426671.1
<i>Codium fragile</i>				GB_U08345.1	GWS002780_HQ603326.1
<i>Codium</i> sp.					GB_EF426672.1
<i>Collinsiella tuberculata</i>			WA3_AY198125		
<i>Cymopolia barbata</i>	XX_XX	XX_XX	HV00469_XX		
<i>Dasycladus vermicularis</i>	HV02661_XX	HV02661_XX			
<i>Derbesia</i> sp.	HADL602_XX				GWS008836_HQ603328.1
<i>Desmochloris halophila</i>				UTEX2073_HE610155	
<i>Desmococcus endolithicus</i>			GB_AJ431571		
<i>Diclostera acutus</i>				SAG.41.98_KM462885	SAG.41.98_KM462885
<i>Dictyochloropsis reticulata</i>				SAG.2150_KM462860	SAG.2150_KM462860
<i>Dunaliella salina</i>	CCAP19-18_GQ250046	GB_GQ250046.1	GB_EF473745	CCAP.19.18_GQ250046	
<i>Elliptochloris bilobata</i>				CAUP.H7103_KM462887	CAUP.H7103_KM462887
<i>Enteromorpha ovata</i>	XX_KC66149				
<i>Eremosphaera viridis</i>	SAG.228.1_XX	GB_KM438414.1	GB_AF387154		
<i>Ettlia pseudoalveolaris</i>				UTEX975_KM462869	UTEX975_KM462869
<i>Eugomontia sacculata</i>			CCMP1673_AY198123		
<i>Flabellia petiolata</i>	HV01202_XX	GB_FJ432640.1	GB_AF416389	GB_FJ535847	
<i>Floydella terrestris</i>	UTEX1709_ACZ58461	UTEX1709_NC014346	GB_D86498	UTEX1709_GU196268	UTEX1709_GU196268
<i>Fusochloris perforata</i>				SAG.28.85_KM462882	SAG.28.85_KM462882
<i>Gayralia</i> sp.	6969_JF680967				
<i>Geminella minor</i>				SAG.22.88_KM462883	SAG.22.88_KM462883
<i>Geminella terricola</i>				SAG.20.91_KM462881	SAG.20.91_KM462881
<i>Gloeotilopsis sterilis</i>	UTEX1704_KM462877	SAG888_KM020063	UTEX1704_KM462877	UTEX1704_KM462877	
<i>Gomontia polyrhiza</i>			MA24b1_AY278216		
<i>Gonium pectorale</i>				K3.F3.4_AP012494	K3-F3-4_AP012494
<i>Halicoryne wrightii</i>	HV00565_FJ535858	GB_AY177745.1	GB_AY165786		
<i>Halicystis</i> sp.	HV03191_XX	HV01207_XX			
<i>Halimeda borneensis</i>	HV00183b_AM049955	GB_FJ624514.1	PH534_AF525556		



<i>Halimeda copiosa</i>	H.0330_EF667065	GB_FJ624508.1	H.0330_AF525612		
<i>Halimeda cryptica</i>	HV00483_EF667057	GB_FJ624496.1	H.0237_AF407244		
<i>Halimeda cylindracea</i>	GB_AJF21965.1	GB_AJF21946.1	GB_AF525549.1		
<i>Halimeda discoidea.ip</i>	LPT0057_XX	GB_AB038488.1	SOC299_AF407254		
<i>Halimeda fragilis.l</i>	H.0125_EF667058	GB_FJ624498.1	H.0125_AF407245		
<i>Halimeda micronesica</i>	WLS184.0_EF667059	GB_FJ624499.1	H.0025_AF525578		
<i>Halochlorococcum moorei</i>	WA1.14B_AY454417		Wa14_AY198122		
<i>Helicosp. oridium sp.</i>				GB_DQ398104	GB_DQ398104
<i>Hemichloris antarctica</i>	UTEXEE124_KM464719.1	UTEXEE52_HQ317296.1	GB_FJ648517		
<i>Ignatius tetrasporus</i>				UTEX2012_FN563076	
<i>Jaoa prasina</i>	A_KM609	GB_JN102134.1	GB_JN102133		
<i>Jenufa minuta</i>			CAUPH8102_HM563744		
<i>Koliella corcontica</i>	SAG.24.84_XX	SAG.24.84_XX	GB_AJ306536	SAG.24.84_KM462874	SAG.24.84_KM462874
<i>Koliella longiseta</i>				UTEX339_KM462868	UTEX339_KM462868
<i>Kormannia leptoderma</i>	GWS004830_HQ6105				
<i>Leptosira terrestris</i>	UTEX333_ABO69293	GB_EF506945.1	GB_Z28973	UTEX333_EF506945	UTEX333_EF506945
<i>Lobosp.haera incisa</i>	CAUP.H.4301_XX	CAUP.H.4301_XX	GB_AY762602	SAG.2007_KM462871	SAG.2007_KM462871
<i>Marsupiomonas sp.</i>				NIES1824_KM462870	NIES1824_KM462870
<i>Marvania geminata</i>				SAG.12.88_KM462888	SAG.12.88_KM462888
<i>Micromonas sp.</i>				RCC299_FJ858267	RCC299_FJ858267
<i>Microthammon kuetzingianum</i>	CAUP.J.1201_XX	GB_KM438427.1	GB_AB488588	UTEX318_KM462876	UTEX318_KM462876
<i>Monomastix sp.</i>	OKE-1_ACK36861	GB_217314511_gb_FJ493457.1	GB_FJ493496	OKE.1_FJ493497	OKE-1_FJ493497
<i>Monostroma sp.</i>	GWS003626_HQ610262	GWS003626_HQ603497	LYGJM_HQ850570		
<i>Muriella zofingiensis</i>	SAG21114_HQ902932	SAG21114_HQ902940			
<i>Myrmecia israelensis</i>	UTEX1181_723456786	UTEX1181_723456786	UTEX1181_KM462861	UTEX1181_KM462861	
<i>Neocystis brevis</i>				CAUP.D802_KM462873	CAUP.D802_KM462873
<i>Nephroselmis astigmatica</i>				NIES.252_KJ746600	NIES.252_KJ746600
<i>Nephroselmis olivacea</i>	NIES484_AF137379	NIES484_AF137379	GB_FN562436	NIES.484_AF137379	GB_AF137379
<i>Ochlochaete hystrix</i>	MA1.8d1_AY454406				
<i>Oedogonium cardiacum</i>	SAG575-1b_ACC97263	GB_EU677193.1	GB_U83133	SAG.575.1b_EU677193	GB_EU677193
<i>Oltmannsiellopsis viridis</i>	NIES360_ABB81968	GB_DQ291132.1	GB_FN562431	NIES.360_DQ291132	GB_DQ291132
<i>Oocystis solitaria</i>	SAG83.80_ACC90812	GB_FJ968739.1	GB_AF228686	SAG.83.80_FJ968739	SAG.83.80_FJ968739
<i>Ostreobiaceae sp.</i>				GB_GU119643.1.1399	
<i>Ostreobiaceae sp.2</i>				GB_GU119844.1.1398	
<i>Ostreobiaceae sp.3</i>				GB_GU119848.1.1403	
<i>Ostreobiaceae sp.4</i>				GB_GU119621.1.1408	
<i>Ostreobiaceae sp.5</i>				GB_GU119562.1.1419	
<i>Ostreobiaceae sp.6</i>				GB_FJ203420.1.1418	
<i>Ostreobiaceae sp.7</i>				GB_FJ203501.1.1406	
<i>Ostreobium quekettii</i>	SAG699_XX	GB_AY004765.1		SAG699_XX	
<i>Ostreobium sp.</i>	H.0754_XX	GB_FJ535853.1			
<i>Ostreococcus tauri</i>	OTTH0595_CAL36350	GB_KC990831.1	GB_Y15841	OTTH0595_CR954199	OTTH0595_CR954199
<i>Pabia signiensis</i>				SAG.7.90_KM462866	SAG.7.90_KM462866
<i>Parachlorella kessleri</i>	SAG211-11g_ACQ90978	GB_FJ968741.1	GB_X56105	SAG.211.11g_FJ968741	SAG.211.11g_FJ968741
<i>Paradoxia multiseta</i>				SAG.18.84_KM462879	SAG.18.84_KM462879
<i>Parvocaulis parvulus</i>	HV01770_XX	GB_AY177741.1	GB_Z33471		
<i>Pedinomonas minor</i>	UTEXLB1350_ACQ90891	GB_FJ968740.1	GB_JN592588	UTEX.LB1350_FJ968740	UTEX-LB1350_FJ968740
<i>Pedinomonas tuberculata</i>					SAG.42.84_KM462867
<i>Pedinophyceae sp.</i>	YPF-701_AB561078				
<i>Pedobesia simplex</i>				GB_FJ535841.1.1486	
<i>Penicillus capitatus</i>	HV03128_XX	GB_FJ432641.1	H.0349_AF416404		
<i>Percursaria percursa</i>	UTEX143_AY454403	GB_AF387105.1	UTEX1423_AY303589		
<i>Phaeophila dendroides</i>	KMP01309b_KJ4119				
<i>Picocystis salinarum</i>	CCMP1897_AB561082	GB_AB491633.1	GB_FR865648	CCMP1897_KJ746599	CCMP1897_KJ746599
<i>Planctonema lauterborni</i>	SCCAP.K0187_XX	GB_KM438432.1	GB_AF387148	SAG.68.94_KM462880	SAG.68.94_KM462880
<i>Pleodorina starri</i>				NIES.1363_JX977846	NIES-1363_JX977846
<i>Prasinococcus sp.</i>	MBIC11011_AB561084	GB_AB491660.1	GB_AB058384	CCMP1194_KJ746597	CCMP1194_KJ746597
<i>Prasinoderma coloniale</i>				CCMP1220_KJ746598	CCMP1220_KJ746598
<i>Prasinophyceae sp.</i>	CCMP1205_AB561083	GB_AB491624.1	CCMP1205_U40921		CCMP1205_KJ746601
<i>Prasiola crispa</i>	P65_KF993457	GAL.W015724_JQ669724	GB_EF200532		
<i>Prasiolopsis sp.</i>	XX_XX	GB_KM464713.1	GB_AY762601	SAG.84.81_KM462862	SAG.84.81_KM462862
<i>Protomonostroma undulatum</i>	GWS00668_HQ61075	GB_HQ603507.1	18S_DQ821517		
<i>Prototheca wickerhami</i>				SAG.263.11_KJ001761	GB_KJ001761
<i>Pseudoclonium akinetum</i>	UTEX1912_AAV80665	UTEX1912_AAV80617	GB_DQ011230	UTEX.1912_AY835431	UTEX1912_AY835431
<i>Pseudochlorella pringsheimii</i>	CAUP.H.1990_XX	GB_KM438438.1	GB_X63520		
<i>Pseudochloris wilhelmii</i>				SAG.1.80_KM462886	SAG.1.80_KM462886
<i>Pseudochlorodesmis abbreviata</i>	TS64_FJ432660	GB_FJ432649.1			
<i>Pseudochlorodesmis sp.1</i>	HV01204_FJ432656	GB_FJ432643.1	HV01204_XX		
<i>Pseudococcomyxa simplex</i>	CAUP.H.102_XX	GB_KM438443.1	GB_FN298926		
<i>Pseudocodium devriesii</i>	JH1_AM909695	GB_AM909690.1			
<i>Pseudocodium floridanum</i>	DML58944_FJ607676	GB_AM909692.1	NSF.123_FJ432631		
<i>Pseudocodium natalense</i>	KZNB2250_FJ607679	GB_AM909693.1	KZNB2242_FJ432632		
<i>Pseudoderbesia sp.</i>	HV03339_LK0451				
<i>Pseudomuriella engadinensis</i>	UTEX58_HQ292755	UTEX58_HM770959	UTEX57_HM852441		
<i>Pseudoneochloris marina</i>	UTEX1445_AY4544				
<i>Pterosp. erma cristatum</i>	NIES626_AB561080	GB_U30281.PCU30281	GB_AJ010407		
<i>Pycnococcus provasolii</i>	CCMP1203_ACK36845	GB_FJ493498.1	GB_X91264	CCMP.1203_FJ493498	GB_FJ493498
<i>Pyramimonas parkeae</i>	CCMP726_ACJ71140	GB_FJ493499.1	GB_FN562443	CCMP.726_FJ493499	GB_FJ493499
<i>Rhipidosiphon javensis</i>				GB_FJ535843	
<i>Rhipilia coppejansii</i>	PIT1044_JQ082492	GB_JQ082482.1	G.453_XX		
<i>Rhipilia crassa</i>				GB_FJ535844	

<i>Rhipilia nigrescens</i>	HV00788_FJ432658	GB_FJ432646.1	HV00788_FJ432633		
<i>Rhipiliopsis gracilis</i>	HEC10439_XX	G_466_XX	HEC10439_XX		
<i>Rhipiliopsis howensis</i>	ASA001_XX	ASA001_XX			
<i>Rhipiliopsis madagascariensis</i>	TZ0333_XX	TZ0333_XX			
<i>Rhipiliopsis peltata</i>	H.0891_XX	H.0891.p2_XX	H.0891_XX		
<i>Rhipiliopsis profunda</i>	DML52138_XX	DML51973_FJ432647	DML51973_XX	GB_FJ535845	
<i>Rhipiliopsis reticulata</i>	DML67755_XX	DML68726_XX	GB_AF416386		
<i>Rhipiliopsis stri</i>	DML68740_XX	DML68740_XX			
<i>Rhipocephalus phoenix</i>				GB_FJ535846	
<i>Ruthnielsenia tenuis</i>	RNtenuis7_JQ30995				
<i>Scenedesmus obliquus</i>	UTEX393_ABD48278	UTEX393_ABD48257	GB_AJ249515		
<i>Schizomeris leibleinii</i>	UTEX-LB1288_HQ700713	UTEX-LB1288_HQ700713	GB_AF182820	UTEX.LB1228_HQ700713	UTEX.LB1228_HQ700713
<i>Spongomorpha aeruginosa</i>	GWS003854_HQ61078				
<i>Stichococcus sp.</i>	CAUP.J.1302_XX	GB_KM438447.1	GB_DQ275461	UTEX176_KM462864	UTEX176_KM462864
<i>Stigeoclonium helveticum</i>	UTEX441_ABF60202	GB_DQ630521.1	GB_U83131	UTEX.441_DQ630521	UTEX441_DQ630521
<i>Tetracystis aeria</i>		GB_EF113476.1	GB_U41175		
<i>Tetraselmis sp.</i>	NIES2432_AB561081		GB_U05039		
<i>Trebouxia aggregata</i>	SAG219-1d_EU123976	GB_EU123967.1	GB_EU123942		
<i>Trebouxiophyceae sp.</i>	MX-AZ01_JX402620	GB_JX402620.1		MX.AZ01_JX402620	
<i>Trentepohlia annulata</i>	GB_KM464717	GB_KM464712.1	SAG20.94_KM020077		
<i>Trochiscia hystrix</i>		GB_EF113480.1	SAG10380_KM020157		
<i>Tydemania expeditionis</i>	FL1151_LN810505	FL1151_LN810505	HV00873_FJ432634	FL1151_LN810505	FL1151_LN810505
<i>Udotea flabellum</i>	HV02674_XX	HV02674_XX	H.0415_AF407270		
<i>Ulothrix zonata</i>	UTEX745_AY45444	GB_AF499683.1	112011_JX491158		
<i>Ulva arasaki</i>	GB_AB561079	GB_AB097621.1	GB_AJ000040		
<i>Ulva fasciata</i>	GWS023543_JN029306	GB_AY422565	GB_DQ286547		
<i>Ulva shanxiensis</i>	SAS.06035_KJ617036				
<i>Ulva sp.</i>				UNA00071828_KP720616	UNA00071828_KP720616
<i>Ulvaria sp.</i>	GWS01793_KM55060				
<i>Ulvella endozoica</i>	UTEXB351_JQ30993				
<i>Ulvella sp.</i>	B_KM608	HB1306_KM226205	HB1306_KM226210		
<i>Ulvella waernii</i>	RN18981_JQ3099				
<i>Umbraulva japonica</i>	GWS018246_JN029346	C14_AB426255			
<i>Umbraulva sp.</i>	GWS03894_JN09359	GB_AB097612.1			
<i>Urospora wormskioldii</i>	GWS006374_HQ610441	GWS006374_HQ603676	Park1_AY476819		
<i>Volvox carteri</i>	UTEX2908_ACY06012	GB_EU755275.1	GB_AB542923		
<i>Watanabea reniformis</i>	CAUP.H.1932_XX	GB_KM464714.1	GB_U73991	SAG.211.9b_KM462863	SAG.211.9b_KM462863
<i>Xylochloris irregularis</i>	CAUP.H.7801_XX	GB_KM438451.1	GB_EU105209	CAUP.H7801_KM462872	CAUP.H7801_KM462872

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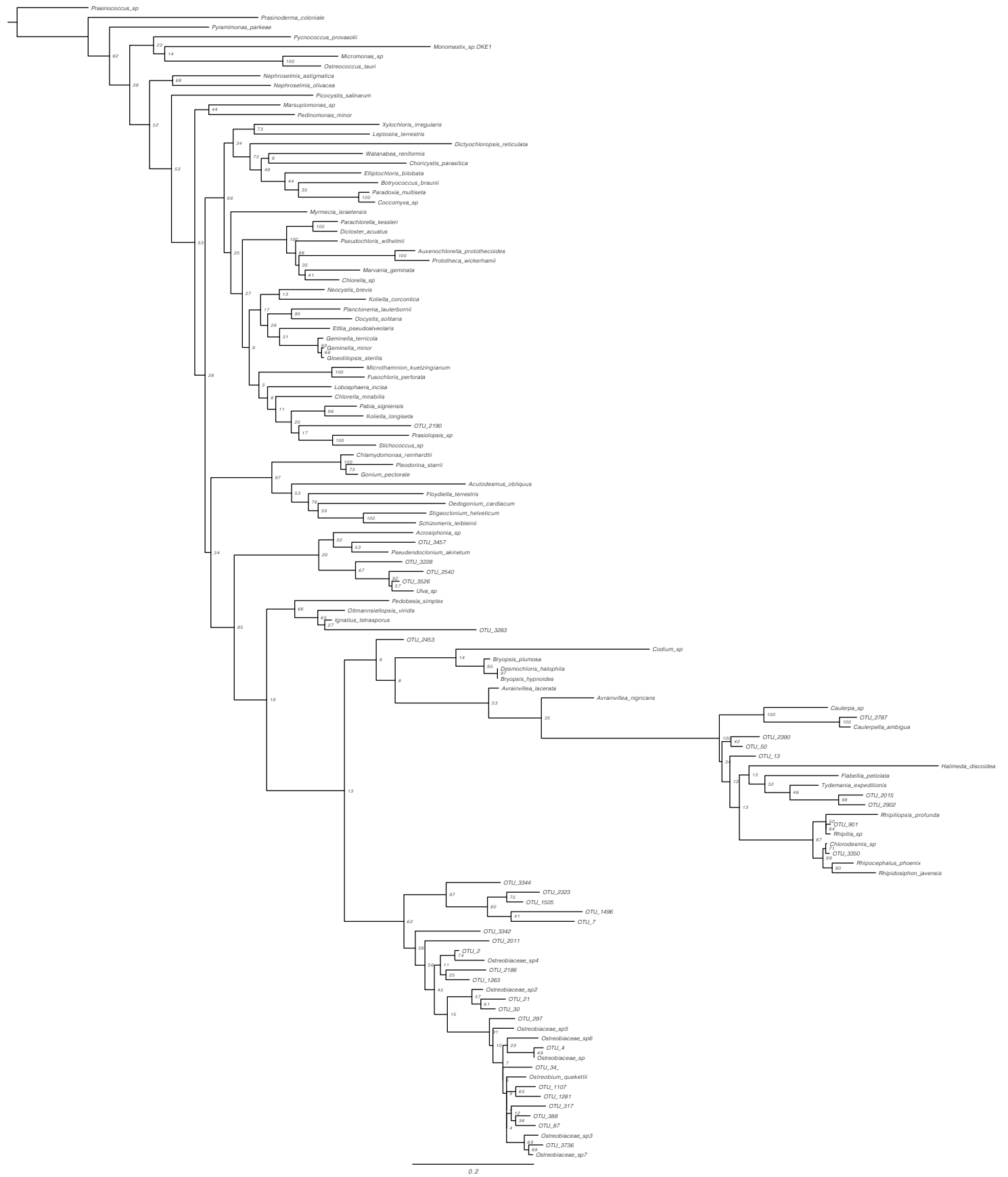
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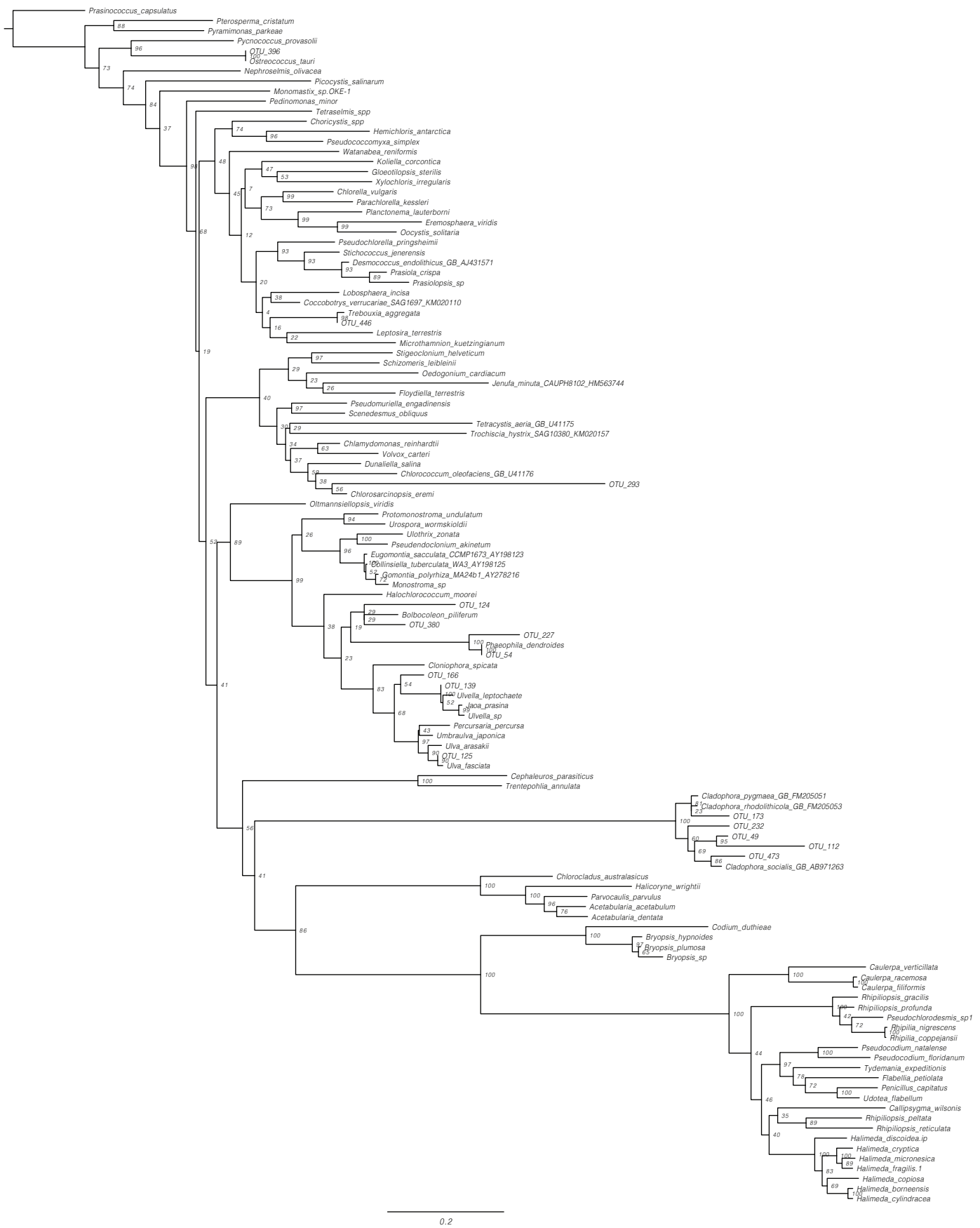
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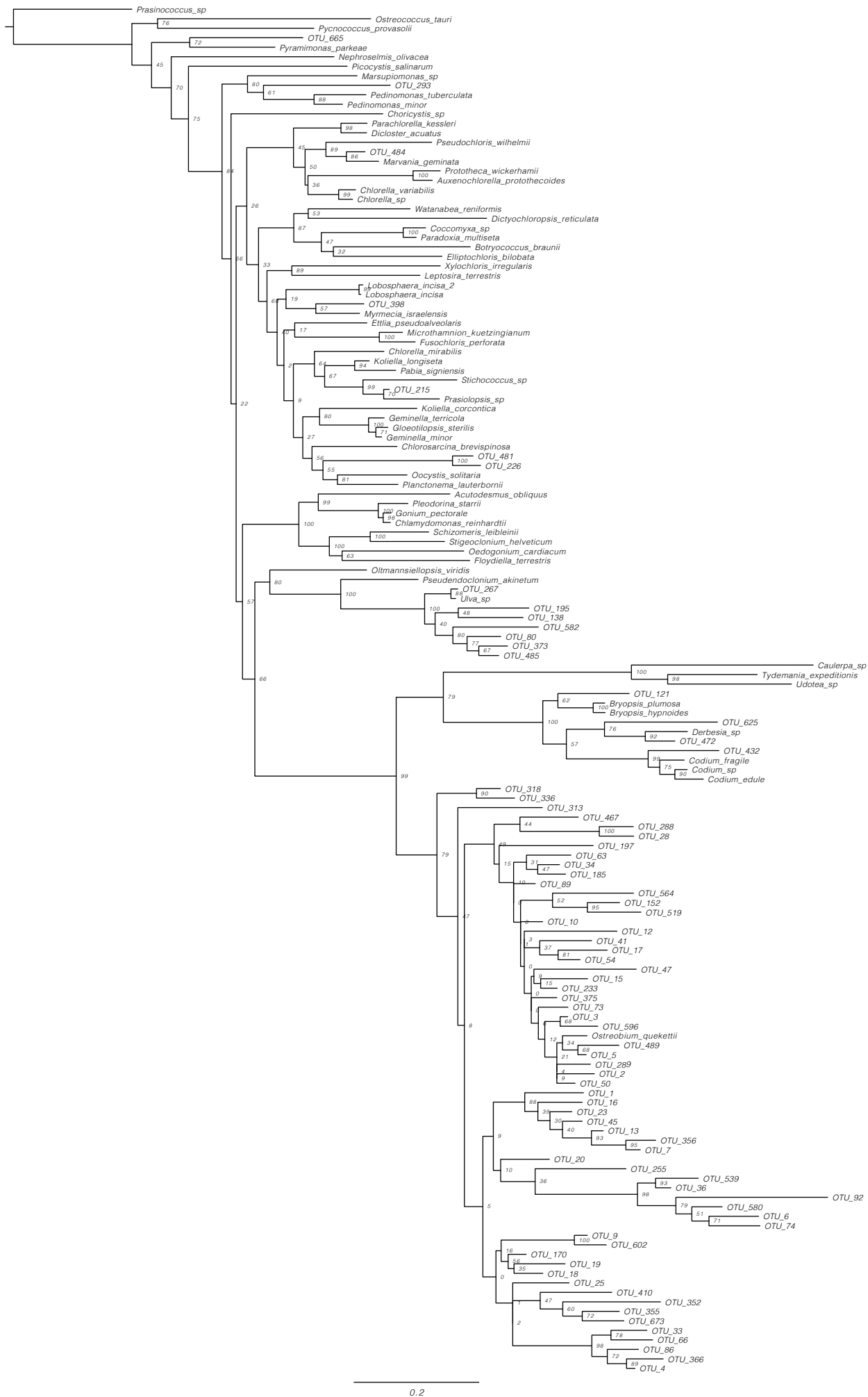
Supplementary Figure S1 – Maximum Likelihood phylogeny including *tufA* OTUs with bootstrap values.



Supplementary Figure S2 – Maximum Likelihood phylogeny including 16S rDNA OTUs with bootstrap values.



Supplementary Figure S3 – Maximum Likelihood phylogeny including 18S rDNA OTUs with bootstrap values



Supplementary Figure S4 – Maximum Likelihood phylogeny including 23S rDNA OTUs with bootstrap values