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Abstract:	<p>Background Coral reefs house about 25% of marine biodiversity and are critical for the livelihood of many communities by providing food, tourism revenue, and protection from wave surge. These magnificent ecosystems are under existential threat from anthropogenic climate change. Whereas extensive ecological and physiological studies have addressed coral response to environmental stress, high-quality reference genome data are lacking for many of these species. The latter issue hinders efforts to understand the genomic and genetic basis of stress resistance and to design informed coral conservation strategies.</p> <p>Results We report genome assemblies from four key Hawaiian coral species, <i>Montipora capitata</i>, <i>Pocillopora acuta</i>, <i>Pocillopora meandrina</i>, and <i>Porites compressa</i>. These species, or members of these genera, are distributed worldwide and therefore of broad scientific and ecological importance. For <i>M. capitata</i>, an initial assembly was generated from short-read Illumina and long-read PacBio data, which was then scaffolded into 14 putative chromosomes using Omni-C sequencing. For <i>Poc. acuta</i>, <i>Poc. meandrina</i>, and <i>Por. compressa</i>, high-quality assemblies were generated using short-read Illumina and long-read PacBio data. The <i>Poc. acuta</i> assembly is from a triploid individual, making it the first reference genome of a non-diploid coral animal.</p> <p>Conclusions These assemblies are significant improvements over available data and provide invaluable resources for supporting multi-omics studies into coral biology, not just in Hawai'i, but also in other regions, where related species exist. The <i>Poc. acuta</i> assembly gives us, for the first time, a platform for studying polyploidy in corals, and its role in genome evolution and stress adaptation in these organisms.</p>	
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High-quality genome assemblies from key Hawaiian coral species

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1 **Abstract**

2

3 **Background**

4 Coral reefs house about 25% of marine biodiversity and are critical for the livelihood of many
5 communities by providing food, tourism revenue, and protection from wave surge. These
6 magnificent ecosystems are under existential threat from anthropogenic climate change. Whereas
7 extensive ecological and physiological studies have addressed coral response to environmental
8 stress, high-quality reference genome data are lacking for many of these species. The latter issue
9 hinders efforts to understand the genomic and genetic basis of stress resistance and to design
10 informed coral conservation strategies.

11 **Results**

12 We report genome assemblies from four key Hawaiian coral species, *Montipora capitata*,
13 *Pocillopora acuta*, *Pocillopora meandrina*, and *Porites compressa*. These species, or members
14 of these genera, are distributed worldwide and therefore of broad scientific and ecological
15 importance. For *M. capitata*, an initial assembly was generated from short-read Illumina and
16 long-read PacBio data, which was then scaffolded into 14 putative chromosomes using Omni-C
17 sequencing. For *Poc. acuta*, *Poc. meandrina*, and *Por. compressa*, high-quality assemblies were
18 generated using short-read Illumina and long-read PacBio data. The *Poc. acuta* assembly is from
19 a triploid individual, making it the first reference genome of a non-diploid coral animal.

20 **Conclusions**

21 These assemblies are significant improvements over available data and provide invaluable
22 resources for supporting multi-omics studies into coral biology, not just in Hawai‘i, but also in
23 other regions, where related species exist. The *Poc. acuta* assembly gives us, for the first time, a
24 platform for studying polyploidy in corals, and its role in genome evolution and stress adaptation
25 in these organisms.

26

27 **Keywords**

28 Coral; Scleractinia; *Montipora capitata*; *Pocillopora acuta*; *Pocillopora meandrina*; *Porites*
29 *compressa*; chromosome-level genome assembly; ploidy; triploid

30

31

32 **Background**

33 *Montipora capitata*, *Pocillopora acuta*, *Pocillopora meandrina*, and *Porites compressa* are
34 species of scleractinian corals that are widespread in the Hawaiian Islands, with *M. capitata* and
35 *Por. compressa* being dominant reef builders. These species are members of cosmopolitan
36 genera, with closely related taxa inhabiting reefs across the Great Barrier Reef and the Coral
37 Triangle [1-3], as well as other regions, such as *Pocillopora* in Panama [4]. In recent years, due
38 to their critical importance to Hawaiian reef ecosystems, the ease of accessibility of these species
39 to researchers working in the United States, and the growing threat that anthropogenic climate
40 change poses to global reef ecosystem, these four species have become the subject of many stress
41 (including thermal [5-7] and acidification [8, 9]), microbiome [10, 11], and population genomic
42 [12-15] studies (among many others). Given the significant interest in these species as models
43 for coral biology, there is a pressing need to generate high-quality reference data to provide a
44 solid foundation for future research.

45
46 A genome assembly for *M. capitata* was published in 2019 by our group [16] using Pacific
47 Biosciences (PacBio) RSII data. This assembly was significantly larger (886 Mbp) than any of
48 the other coral genomes available at that time (ca. 300-500 Mbp), and is larger than any
49 *Montipora* species genome [17, 18] that has since been published. The published assembly
50 contains a high number (>18% [19]) of duplicated BUSCO genes, suggesting the presence of
51 haplotigs (i.e., sequences derived from different homologous chromosomes) that were not
52 removed during the assembly process. There are currently published genomes for three
53 *Pocillopora* [4, 20, 21] species, none of which are from Hawai‘i. One of these is a *Poc. acuta*
54 isolate collected from Lombok, Indonesia [22] that was generated using Illumina short-read data.
55 This genome assembly is highly fragmented, consisting of 168,465 scaffolds, and whereas it
56 does have a scaffold N50 of 147 Kbp, the contig N50 is only 9,649 bp. The completeness of the
57 genes predicted in this genome is not high, with only 56% of the core eukaryotic genes [20]
58 identified in the reported “*ab initio*” predicted gene set. A second set of predicted genes inferred
59 using RNA-seq evidence (termed the “experimental” set) contains 93% of core eukaryotic genes,
60 however, this set does not have predicted open reading frames (i.e., it includes both coding and
61 non-coding genes), making it difficult to make a direct comparison with other published

62 genomes. There are currently three *Porites* species with published genomes [23-25], while they
63 are all of high completeness and reasonable contiguity, none are from Hawai‘i.

64

65 As the cost of genome sequencing, in particular, long-read methods continues to decrease,
66 opportunities arise to generate genome data from understudied species or species that have
67 genomes of lower quality that would benefit from the improvement gained from the newer
68 technologies. Furthermore, technologies such as Dovetail Omni-C, which provides long range
69 linkage information, enables the generation of genome assemblies that are at (or near)
70 chromosomal-level resolution. In this study, we generated an improved reference genome
71 assembly for our previously published Hawaiian *M. capitata* using long-read PacBio, short-read
72 Illumina, and newly generated Omni-C data, that is of chromosome-level resolution. The 14
73 largest scaffolds resulting from this assembly likely represent the 14 chromosomes predicted in
74 *Montipora* species [26]. We also generated, using PacBio HiFi data (i.e., circular consensus
75 corrected PacBio reads), high-quality genome assemblies for two *Pocillopora* and one *Porites*
76 species. The sequenced *Poc. acuta* isolate is a triploid, making it the first non-diploid coral
77 genome to be published.

78

79 **Data description**

80 **Sample collection and processing**

81 The four coral species targeted in this study were collected from Kāne‘ohe Bay, Hawai‘i. For *M.*
82 *capitata*, the initial PacBio and Illumina-based assembly was generated using sperm DNA (see
83 [16]). Input DNA for the Dovetail Genomics approach (<https://dovetailgenomics.com>), using the
84 Omni-C assay and workflow, was a bleached nubbin (a ~5 x 5cm fragment) from a colony that
85 was greatly reduced in algal symbionts (GPS coords: 21.474465, -157.834468; SRA BioSample:
86 SAMN21845729). This fragment was collected under Hawai‘i Department of Aquatic Resources
87 Special Activity Permit 2019-60, snap frozen in liquid nitrogen, and stored at -80°C before it was
88 shipped on dry ice to Dovetail Genomics (<https://dovetailgenomics.com>) for processing using
89 their Omni-C assay and workflow.

90

91 For *Poc. meandrina*, one nubbin (a ~5 x 5cm fragment) was collected from an adult colony from
92 Reef 13 (GPS coords: 21.450803, -157.794692) on 2020-09-05 (SRA BioSample:

93 SAMN21845732, SAMN21845733, and SAMN21845734) under DAR-2021-33, Amendment
94 No. 1 to HIMB. High molecular weight DNA was extracted using the QIAGEN Genomic-tip
95 100/G (Cat #: 10223), the QIAGEN Genomic DNA Buffer Set (Cat #: 19060), QIAGEN RNase
96 A (100mg/mL concentration: Cat #: 19101), QIAGEN Proteinase K (Cat #: 19131), and DNA lo-
97 bind tubes (Eppendorf Cat #: 022431021). In brief, a clipping of the coral fragment was placed in
98 a cleaned and sterilized mortar and pestle and ground to powder on liquid nitrogen. High
99 molecular weight DNA was then extracted according to the manufacturer's instructions for
100 preparation of tissue samples in the QIAGEN Genomic DNA Handbook (version 06/2015).
101 For *Poc. acuta*, one nubbin was collected from an adult colony from a reef next to the Hawai'i
102 Institute of Marine Biology (GPS coords: 21.436056, -157.786861) on 2018-09-05 (SRA
103 BioSample: SAMN22898959) under Special Activity Permit 2019-60. High molecular weight
104 DNA was extracted using the QIAGEN Genomic-tip 100/G approach outlined for *Poc.*
105 *meandrina* above. High molecular weight DNA from *Poc. meandrina* and *Poc. acuta* was sent to
106 DNA Link Sequencing Lab (<https://www.dnalinkseqlab.com>) for sequencing on their PacBio
107 Sequel 2 and Illumina NovaSeq 6000 platforms.

108

109 For *Por. compressa*, DNA was extracted from sperm released at 11 pm on 09 June 2017 from a
110 single colony in Kāne'ōhe Bay, O'ahu. Total genomic DNA was extracted using the CTAB
111 protocol and the DNeasy Blood and Tissue Kit (Qiagen, Germany) with subsequent clean-up
112 steps. Genomic data were generated using the PacBio RSII platform. To increase the sequence
113 quality of the assembly, a polishing step was done using the Arrow consensus caller. To this end,
114 we generated a total of 20 Gbp of high-throughput sequencing data (Illumina HiSeq2000; 100 bp
115 paired-end library) as follows. The whole-genome sequencing library of *Por. compressa* was
116 prepared using the Truseq Nano DNA Prep Kit (550bp) protocol following the manufacturer's
117 instructions. Randomly sheared genomic DNA was ligated with index adapters and purified. The
118 ligated products were size-selected for 300-400 bp and amplified using the adapter-specific
119 primers. Library quality was checked using a 2100 BioAnalyzer (Agilent Technologies, Santa
120 Clara, CA, USA).

121

122 **RNA Extractions**

123 RNA was extracted by clipping a small piece of coral using clippers sterilized in 10% bleach,
124 deionized water, isopropanol, and RNase free water, and then placed in 2 mL Fisherbrand™
125 Pre-Filled Bead Mill microcentrifuge tube containing 0.5mm glass beads (Fisher Scientific
126 Catalog. No 15-340-152) with 1000 µL of Zymo DNA/RNA shield. A two-step extraction
127 protocol was used to extract RNA and DNA, with the first step as a “soft” homogenization to
128 reduce shearing of RNA or DNA. Tubes were vortexed at high speed for 1 and 2 minutes for
129 *Poc. acuta* and *M. capitata* fragments, respectively. The supernatant was removed and
130 designated as the “soft extraction”. Second, an additional 500 µL of Zymo DNA/RNA shield was
131 added to the bead tubes and placed in a Qiagen TissueLyser for 1 minute at 20 Hz. The
132 supernatant was removed and designated as the “hard extraction”. Subsequently, 300 µL of
133 sample from both soft and hard homogenate was extracted with the Zymo Quick-DNA/RNA
134 Miniprep Plus Kit (Zymo Cat D7003) Protocol with the following modifications. RNA quantity
135 (ng_µL) was measured with a ThermoFisher Qubit Fluorometer, DNA quality was assessed
136 using gel electrophoresis, and RNA quality was measured with an Agilent TapeStation System.

137

138 **Haploid genome assembly of Hawaiian coral species**

139 The long-read genome sequencing data (PacBio) of the Hawaiian coral species were initially
140 assembled using CANU (v2.2; default options) [27]. The PacBio reads for *M. capitata* (78.3
141 Gbp; SRR17163565) and *Por. compressa* (63.3 Gbp; SRR12695159 – SRR12695166) were
142 generated using the PacBio RSII platform (‘-pacbio’ option for CANU assembler). The PacBio
143 reads for *Poc. meandrina* (311.8 Gbp; SRR16077713), and *Poc. acuta* (239.1 Gbp;
144 SRR16077715) were generated using PacBio HiFi platform (‘-pacbio-hifi’ option for CANU
145 assembler). An error correction step (nucleotide correction of assembly) using the initial
146 assemblies of *M. capitata* (1.2 Gbp; Supplementary Table S1), *Por. compressa* (1.0 Gbp), *Poc.*
147 *meandrina* (0.7 Gbp), and *Poc. acuta* (1.1 Gbp) was done using bowtie2 (v2.4.2; default options)
148 [31] and the Pilon program (v1.23; default options) [28] with the Illumina short-read sequencing
149 data (HiSeq2500: 27.4 Gbp of *M. capitata* [SRR8497577]; HiSeq 2000: 20.9 Gbp of *Por.*
150 *compressa* [SRR12695158]; NovaSeq 6000: 27.2 Gbp of *Poc. meandrina* [SRR16077712], and
151 23.0 Gbp of *Poc. acuta* [SRR16077714]). Before using the Illumina data, quality trimming and
152 adapter clipping of the raw reads were done using Trimmomatic (v0.39; default options) [29]. To
153 remove potential contaminant sequences, assembly results were analyzed using BLASTn (*e-*

154 value cutoff = $1.e^{-10}$ cutoff) analysis with the nr database (downloaded: Feb. 2019). To estimate
155 genome size and ploidy of the Hawaiian coral species, *k*-mer analysis was done using Jellyfish
156 (21-mer) [30] with the Illumina short-read data.

157 To reconstruct haploid genomes using the initial assemblies of the Hawaiian coral
158 species, we used the following protocol. First, we predicted repetitive DNA sequences in the
159 initial assemblies and constructed soft-masked assemblies. Repetitive DNA elements were
160 identified using the RepeatModeler pipeline (v2.0.1;
161 <http://www.repeatmasker.org/RepeatModeler/>) [31-33] which includes RECON (v1.08) and
162 RepeatScout (v1.0.6) as *de novo* repeat finding programs. We used the default options for l-mer
163 size and removed low-complexity and tandem repeats. To classify repeat content, the libraries
164 were constructed from giri repbase (<http://www.girinst.org>). The consensus sequences of repeat
165 families were used to analyze corresponding repeat regions with RepeatMasker (v4.1.1; default
166 options with soft-masked; <http://www.repeatmasker.org/>). The second step in the protocol was to
167 infer assemblies as haploid genomes using the HaploMerger2 (HM2) program (the latest release,
168 20180603) [34] and the soft-masked assemblies. The third step was validation of duplicated
169 eukaryotic core genes in the haploid genome assemblies using the Benchmarking Universal
170 Single-Copy Orthologs (BUSCO) program (v4.1.4; genome-based analysis with
171 eukaryota_odb10 dataset) [35]. The final step was to repeat the HM2 analysis until the number of
172 duplicated eukaryotic core genes decreased to under 1%, or the value could not be decreased any
173 further in the haploid assemblies (Supplementary Table S1). The purged assembly of *M. capitata*
174 was sent to Dovetail Genomics along with an additional coral fragment (see above) that was used
175 for high molecular weight DNA extraction for analysis using their Omni-C assay and HiRise
176 v2.2.0 assembly workflow. A total of 56.5 million read-pairs of Dovetail Genomics Omni-C
177 sequencing data (SRR16077716) were generated and used for scaffolding. This step produced a
178 final genome assembly that was at putative chromosome level resolution for *M. capitata*.

179

180 **Gene prediction and functional annotation**

181 Quality trimming and adapter removal of the RNA sequencing (RNA-seq) data in the Hawaiian
182 coral species (*M. capitata* [77.5 Gbp; SRR14729868 – SRR14729873, SRR14729878,
183 SRR14729881, SRR14729889, SRR14729890, SRR14729893, and SRR14729894], *Por.*
184 *compressa* [76.5 Gbp; SRR14729874 – SRR14729877, SRR14729879 – SRR14729880,

185 SRR14729882 – SRR14729888, SRR14729891, and SRR14729892], *Poc. acuta* [656.7 Gbp;
186 SRR14610884 – SRR14610890, SRR14610892 – SRR14610901, SRR14610903 –
187 SRR14610912, SRR14610914 – SRR14610923, SRR14610925 – SRR14610932,
188 SRR14610975, SRR14610977 – SRR14610986, SRR14610988 – SRR14610997, SRR14610999
189 – SRR14611008, SRR14611010 – SRR14611019, SRR14611021 – SRR14611030,
190 SRR14611033 – SRR14611042, SRR14611044 – SRR14611053, and SRR14611055 –
191 SRR14611057], and *Poc. meandrina* [10.6 Gbp; SRR16077711]) were done using Trimmomatic
192 (v0.39; default options) [29]. These data were assembled using Trinity v2.11 with the default
193 option of *de novo* transcriptome assembly [36, 37]. The trimmed RNA-seq raw reads, and the
194 assembled transcriptomes were aligned to the haploid genome assemblies using the STAR
195 aligner (v2.6.0c; default options for the raw reads), and the STARlong aligner (v2.6.0c; --
196 runMode alignReads --alignIntronMin 10 --seedPerReadNmax 100000 --seedPerWindowNmax
197 1000 --alignTranscriptsPerReadNmax 100000 --alignTranscriptsPerWindowNmax 10000),
198 respectively [38]. Based on each alignment (i.e., bam file), gene predictions were done using the
199 BRAKER2 pipeline (v2.1.5; <http://bioinf.uni-greifswald.de/bioinf/braker>) [39], which includes
200 GeneMark-ET [40] and AUGUSTUS [41] with default (automatically optimized) options. From
201 the two types (i.e., RNA-seq, and assembled transcriptome) of gene models from the Hawaiian
202 coral species, the best (longest) gene models were manually selected based on results of
203 BLASTp search (e -value cutoff = $1.e^{-5}$ cutoff). Functional annotation of gene models was done
204 using the NCBI Conserved Domain Search (CD-Search) [42], the eggNOG-mapper [43], and the
205 KEGG Automatic Annotation Server (KAAS) [44].

206

207 **Genomes of corals used for comparative analysis**

208 The genome assemblies and predicted genes from the four *Montipora* (*M. cactus* [17], *M.*
209 *capitata* from the Hawaiian Waiopae tide pools [18], *M. efflorescens* [17], and the previous
210 version of the Hawaiian *M. capitata* isolate [16] that we assembled in this study), three
211 *Pocillopora* (*Poc. damicornis* [4], *Poc. acuta* [from Indonesia] [22], and *Poc. verrucosa* [21]),
212 and four *Porites* (*Por. astreoides* [25], *Por. australiensis* [24], *Por. lutea* [23], and *Por. rus* [45])
213 species that have been sequenced were retrieved from their respective repositories
214 (Supplementary Table S2) and used for comparative analysis with the assemblies generated in
215 this study. The *M. cactus* and *M. efflorescens* genome assemblies (from

216 <https://marinegenomics.oist.jp> [17]) were filtered, retaining only scaffolds identified by Yuki, Go
217 [19] as not being haplotigs. The updated gene models from Yuki, Go [19] were used in place of
218 those available with the original assemblies. For species where just the gene models were
219 provided (in gff format), gffread v0.11.6 (-S -x cdsfile -y pepfile) [46] was used to infer the
220 protein and CDS sequences. Open Reading Frames (ORFs) were predicted in the RNA-Seq
221 based “experimental” genes predicted in the Indonesian *Poc. acuta* isolate [22], using
222 TransDecoder v5.5.0 (<https://github.com/TransDecoder/TransDecoder>); HMMER v3.1b2 was
223 used to search the candidate ORFs against the Pfam database (release 33.1; i-Evalue < 0.001)
224 and BLASTP (v2.10.1; -max_target_seqs 1 -evalue 1e-5) was used to search candidate ORFs
225 against the SwissProt database (release 2020_05), with the resulting homology information used
226 by TransDecoder to guide ORF prediction. Only the longest transcript per gene had ORFs
227 predicted and single-exon genes without strand information were assumed to be from the
228 forward/positive strand (TransDecoder will change the strand of single exon genes if required,
229 based on the results of ORF prediction).

230

231 **Genome size estimation**

232 The genome size and ploidy of the new (this study) and published *Montipora*, *Pocillopora*, and
233 *Porites* species (except the Indonesian *Poc. acuta* which does not have read data available to
234 download, *Por. rus* which only had reads from the holobiont [i.e., reads from the coral, algal
235 symbiont, and associated bacteria] available, and *Por. astreoides* which only had PacBio long
236 reads available) were estimated using the GenomeScope2 and Smudgeplot tools [47]. For each
237 species, the available short-reads genome sequencing data were retrieved from NCBI SRA
238 (Supplementary Table S2), trimmed using cutadapt v3.5 [48] (-q 20 --minimum-length 25 -a
239 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA -A
240 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT), and decomposed into *k*-mers using
241 Jellyfish [30] (v2.3.0; k=21). The *k*-mer frequency histogram produced by Jellyfish (using the
242 ‘jellyfish histo’ command) was imported into GenomeScope2 with a theoretical diploid model
243 fitted with the data (Fig. 1C, D, and F and Supplementary Fig. S1); a theoretical triploid model
244 was fitted with the Hawaiian *Poc. acuta* data (Fig. 1E and Supplementary Fig. S1F) because it
245 was found to be a triploid after initial analysis using Smudgeplot and GenomeScope2.
246 Smudgeplot was run using the *k*-mers extracted by Jellyfish (following the workflow from

247 <https://github.com/KamilSJaron/smudgeplot/wiki/manual-of-smudgeplot-with-jellyfish>), with
248 thresholds for the lower k -mer coverage cutoff (just after the minimum between the initial error
249 peak and the first major peak) and upper k -mer coverage cutoff (8.5 times the coverage of the
250 first major coverage peak) chosen for each species using the GenomeScope2 profile shown in
251 Supplementary Figure S1. The “smudge plots” shown in Supplementary Figure S1 were
252 generated using the haploid coverage values estimated by GenomeScope2. The cutoffs used
253 when running Smudgeplot for each species are shown in Supplementary Table S2.

254

255 **Conformation of sample ploidy**

256 The program nQuire [49] (retrieved 7/7/2021 from <https://github.com/clwgg/nQuire>), which uses
257 the frequency distribution of bi-allelic variant sites inferred from aligned reads to model the
258 ploidy of a sample, was used to verify the ploidy of the four genomes sequenced in this study.
259 Briefly, bowtie2 v2.4.4 (‘--very-sensitive --no-unal’) was used to align the trimmed (by cutadapt;
260 described previously) Illumina short-reads against their respective genome assemblies; aligned
261 reads were coordinate sorted using samtools v1.11 [50]. The aligned and sorted BAM files were
262 converted into “BIN” files using nQuire (‘nQuire create -q 20 -c 20 -x’), filtering for reads with a
263 minimum mapping quality of 20 and sites with a minimum coverage of 20. Denoised BIN files
264 were created using the “nQuire denoise” command run on the initial BIN files. The delta Log-
265 Likelihood values for each ploidy model (diploid, triploid, and tetraploid) was calculated by the
266 “nQuire lrdmodel” command for each of the initial and denoised BIN files. The lower the delta
267 Log-Likelihood value of a given model the better fit it is for the frequency distribution of the bi-
268 allelic variant sites extracted from the aligned reads; the ploidy of the sample is there for
269 assumed to be the ploidy model with the lowest delta Log-Likelihood value. The nQuire results
270 are shown in Supplementary Table S4.

271

272 **Assessment of completeness using BUSCO**

273 The “completeness” of the genome assemblies and predicted genes (published in this study and
274 from previous studies; Supplementary Table S3) were assessed using BUSCO v5.0.0 (‘--mode
275 genome’ and ‘--mode protein’, respectively) with the eukaryota_odb10 (release 2020-09-10) and
276 metazoa_odb10 datasets (release 2021-02-24) [51].

277

278 **Analysis of extra-chromosomal scaffolds**

279 The proteins predicted on the extra-chromosomal scaffolds (i.e., the scaffolds that do not
280 comprise the 14 putative chromosomes) in the *M. capitata* assembly were compared against the
281 proteins from the chromosomal scaffolds using BLASTP v1.10.1 [52]; the resulting hits were
282 filtered using an *e*-value cutoff $< 1 \times 10^{-5}$. Additional filtering steps were applied to produce two
283 sets of hits: for the first (lenient) set, hits were retained if they had a query coverage of $> 75\%$
284 and an identity $> 75\%$, with the single best (*e*-value-based) top hit kept for each query sequence;
285 for the second (stringent) set, hits were retained if they had a query coverage of $> 95\%$ and an
286 identity $> 95\%$, with the single best (*e*-value-based) top hit kept for each query sequence. The
287 lenient filtered top hits were used to determine if the extra-chromosomal scaffolds tend to encode
288 genes that have similarity to a single, or multiple, chromosomes. For this analysis, only proteins
289 with top hits to the chromosomal scaffolds (i.e., proteins with hits that have an *e*-value $< 1 \times 10^{-5}$,
290 query coverage $> 75\%$, and an identity $> 75\%$) were considered, and only scaffolds with multiple
291 proteins with top hits were considered.

292

293 **Data Validation and Quality Control**

294 ***Montipora capitata* genome assemblies**

295 The *M. capitata* assembly generated in the study (assembly version V3.0; hereinafter the “new”
296 Hawaiian *M. capitata* genome assembly) has fewer assembled bases (781 Mbp vs. 886 Mbp) and
297 scaffolds (1,699 vs. 3,043), and a vastly improved N50 (47.7 Mbp vs. 0.54 Mbp; Supplementary
298 Table S3), compared to the assembly of the same Hawaiian *M. capitata* isolate (hereinafter the
299 “old” Hawaiian *M. capitata* genome assembly) that was previously published by our group [16].
300 The 14 largest scaffolds in the new assembly, ranging in size from ~22 to ~69 Mbp, likely
301 represent the 14 chromosomes predicted in other *Montipora* species (Figs. 1A and B) [26]. These
302 putative chromosomes total 680 Mbp of assembled sequence, which is only slightly larger than
303 the estimated genome size of 644 Mbp (Fig. 1C; estimated by GenomeScope2 [47] using *k*-mers
304 of size 21 bp). The estimated genome size of the other published *Montipora* species is ~700
305 Mbp, whereas the estimated genome size of the new Hawaiian *M. capitata* genome is 644 Mbp
306 (although the assembly is a little larger; see discussion below). This suggests that species in the
307 genus *Montipora* have genomes that are marginally smaller than 700 Mbp in size.

308 The *M. capitata* isolate that was sequenced appears to be a diploid, with a good fit
309 between its *k*-mer frequency histogram and the theoretical diploid model implemented in
310 GenomeScope2 (black line in Fig. 1C and Supplementary Fig. S1A), and a clear “smudge”
311 (bright yellow region in Supplementary Fig. S1A) of *k*-mer pairs with a coverage of 2n and a
312 normalized coverage of 1/2; all of which suggests that the sample is diploid. nQuire also
313 predicted that the *M. capitata* sample was a diploid (i.e., the diploid model had the lowest delta
314 Log-Likelihood value; Supplementary Table S4), supporting the results of GenomeScope2 and
315 Smudgeplot.

316 Compared with the old assembly, the new *M. capitata* assembly has a slightly higher
317 BUSCO completeness for both the Metazoa (from 95.2% to 95.7%, respectively) and Eukaryota
318 (from 97.7% to 99.2%, respectively) datasets (Supplementary Table S3) but a significantly
319 reduced number of duplicated BUSCO genes for both the Metazoa (from 21.2% to 1.6%,
320 respectively) and Eukaryota (from 22.0% to 1.2%, respectively) datasets. The high number of
321 duplicated BUSCO genes in the old assembly is likely a result of haplotigs that evaded removal
322 during the assembly process; this problem appears to have been resolved in the new assembly.
323 Compared with the other published *Montipora* genomes, the new *M. capitata* assembly is the
324 most contiguous and complete to date, with a significantly higher N50 (47.7 Mbp compared to
325 the next best of 1.2 Mbp in *M. efflorescens*) and BUSCO completeness (e.g., 99.2% Eukaryota
326 dataset completeness compared to the next best of 92.1% in *M. cactus*). As the same PacBio and
327 Illumina libraries were used to construct the new and old assemblies, the significant
328 improvement observed in the new assembly is attributed to the use of a different hybrid assembly
329 approach, combined with the Dovetail Omni-C library preparation and scaffolding with the
330 HiRise (v2.2.0) software.

331

332 ***Pocillopora* genome assemblies**

333 The *Poc. acuta* genome assembly generated in this study (hereinafter the “Hawaiian *Poc. acuta*”)
334 is larger (408 Mbp) than *Poc. acuta* from Indonesia (352 Mbp) [22] (Supplementary Table S3)
335 and then its estimated genome size of 353 Mbp (Fig. 1E). The size of the *Poc. meandrina*
336 genome assembly generated in this study (377 Mbp) is comparable to the published Indonesian
337 *Poc. acuta* (352 Mbp) [22] and *Poc. verrucosa* (381 Mbp) [21] species, but is larger than *Poc.*
338 *damicornis* (234 Mbp) [4] (Supplementary Table S3). Although the latter is likely under-

339 assembled given its smaller size relative to the estimated genome size for that species. Moreover,
340 the estimated genome sizes for these species appears to be around 330-350 Mbp, with the
341 assemblies being 350-380 Mbp in size (excluding the Hawaiian *Poc. acuta* [further discussion
342 below]). This suggests that species in the genus *Pocillopora* have genomes that are ~350 Mbp in
343 size.

344 The Hawaiian *Poc. acuta* isolate that was sequenced is a triploid; the presence of three
345 major peaks in the *k*-mer frequency histogram (at ~17x, ~35, and ~51x) which fit the triploid
346 model implemented by GenomeScope2 (black line Fig. 1E and Supplementary Fig. S1F), and the
347 clear “smudge” (bright yellow region in Supplementary Fig. S1F) of *k*-mer pairs with a coverage
348 of ~3n and a normalized coverage of 1/3, all suggests that the sample is triploid. nQuire also
349 predicts that the *Poc. acuta* is a triploid (Supplementary Table S4), supporting the results of
350 GenomeScope2 and Smudgeplot. For *Poc. meandrina*, GenomeScope2 (Fig. 1D), Smudgeplot
351 (Supplementary Fig. S1E), and nQuire (Supplementary Table S4) all predict that the isolate that
352 was sequenced is a diploid.

353 The BUSCO completeness of the Hawaiian *Poc. acuta* genome is improved for both the
354 Metazoa (96.1%), and Eukaryota (98.5%) datasets (Supplementary Table S3) compared to the
355 Indonesian *Poc. acuta* assembly (89.4% and 91.4%, respectively) and the other *Pocillopora*
356 assemblies (~91-95% and 91-98%, respectively). However, the Hawaiian assembly does have a
357 slightly higher proportion of duplicated BUSCO genes (2.5% and 2.0% in the Metazoa and
358 Eukaryota datasets) compared with some (the Indonesian *Poc. acuta* and *Poc. damicornis*
359 genomes which have <1% in both datasets) but not all (the *Poc. verrucosa* genome which has
360 2.9% and 5.5%, respectively) of the published genomes. This is likely a result of the Hawaiian
361 *Poc. acuta* being a triploid; haplotig removal programs (i.e., HaploMerger2 [34]) are generally
362 designed for use with diploid species, so it is unsurprising that they were unable to fully
363 resolving the assembly given the added complexity associated with resolving assemblies of
364 higher ploidy genomes. Regardless, the Hawaiian *Poc. acuta* assembly is more contiguous (i.e.,
365 higher N50 and fewer scaffolds) than the other *Pocillopora* genomes and is the first assembly
366 generated from a non-diploid coral. The *Poc. meandrina* genome has a BUSCO completeness
367 (96.1% for the Metazoa and 98.8% for the Eukaryota datasets) that is just as high as the
368 Hawaiian *Poc. acuta* genome, but with fewer duplicated BUSCO genes (1.2% and 0.4%,
369 respectively), suggesting that this assembly has minimal retained haplotigs.

370

371 ***Porites compressa* genome assembly**

372 The size of the *Por. compressa* genome assembly generated in this study (593 Mbp) is similar to
373 the published *Por. australiensis* (576 Mbp) [24] and *Por. lutea* (552 Mbp) [23] genomes, and a
374 little smaller than *Por. astreoides* (677 Mbp). The estimated genome sizes for these species
375 appears to be around 525-550 Mbp (excluding *Por. astreoides*, *Por. lutea* and *Por. rus*), with the
376 assemblies coming in at around 550-600 Mbp. The high number of duplicated BUSCO genes in
377 the *Por. astreoides* assembly (11.5% and 14.9% for the Metazoa and Eukaryota datasets,
378 respectively) suggests that its larger assembly size (compared with the other *Porites* species) is
379 likely explained by retained haplotigs. The genome assembly (470 Mbp) and estimated genome
380 size (405 Mbp) of *Por. rus* is smaller than the other *Porites* isolates however, these data were
381 generated from holobiont samples (*i.e.*, samples with both coral, algal symbiont, and associated
382 bacteria DNA present) using a metagenomic binning strategy. The difference in this approach
383 compared with how the other *Porites* genomes were processed likely explain the difference
384 between the sizes. *Por. lutea* has an estimated genome size of 694 Mbp, which is significantly
385 larger than the other *Porites* species and its assembled genome. Whereas this suggests that the
386 *Por. lutea* genome is under-assembled (comprising only ~80% of the estimated genome) its
387 relatively high completeness (95.3% and 98.5% for the Metazoa and Eukaryota datasets,
388 respectively) suggests that the genome size has been overestimated, possibly driven by
389 sequencing error or other factors associated with sample preparation or collection from the field.
390 These results indicate that species in the genus *Porites* have genomes that are just under 600
391 Mbp in size. For *Por. compressa*, GenomeScope2 (Fig. 1F), Smudgeplot (Supplementary Fig.
392 S1I), and nQuire (Supplementary Table S4) all predict that the isolate sequenced is a diploid.

393 The BUSCO completeness of the *Por. compressa* assembly is slightly higher (95.5% for
394 the Metazoa and 99.2% for the Eukaryota datasets; Supplementary Table S3) compared to the
395 *Por. astreoides* (93.2% and 98.0%, respectively), *Por. australiensis* (91.6% and 94.9%,
396 respectively), *Por. lutea* (95.3% and 98.5%, respectively), and *Por. rus* (69.6% and 67.1%,
397 respectively) assemblies, but has a much higher N50 (4 Mbp) compared to the published species
398 (0.41, 0.55, 0.66, and 0.14 Mbp, respectively) and fewer scaffolds (608 vs. 3,051, 4,983, 2,975,
399 and 14,982, respectively). The published genome assemblies also have many more gaps (~0-29%
400 of assembled bases are 'N' characters) compared to *Por. compressa* (0%), demonstrating that the

401 new assembly is of equally high completeness compared to the published species, but with a
402 much higher contiguity.

403

404 **Predicted protein-coding genes**

405 For *M. capitata*, 54,384 protein-coding genes were predicted in the new assembly compared with
406 the 63,227 predicted in the old version (Supplementary Table S3). The reduction in the number
407 of predicted genes in the new *M. capitata* assembly, compared with the published version, is
408 likely driven by its reduced assembly size, with many of the missing genes likely arising from
409 haplotigs retained in the old assembly, that were removed in the new version. The BUSCO
410 completeness of the predicted genes is improved in the new assembly (95.2% of the Metazoa and
411 96.5% for the Eukaryota BUSCO datasets) compared with the old assembly (94.0% and 93.3%,
412 respectively), and the number of duplicated BUSCO genes is reduced in the new assembly (2.3%
413 and 1.2%, respectively) compared to the published (18.2% and 18.8%, respectively). The
414 predicted gene set from the new Hawaiian *M. capitata* assembly also has > 4.2% and > 3.5%
415 more complete BUSCO genes (from the Metazoa and Eukaryota datasets, respectively)
416 recovered compared to the other published isolates, demonstrating that the gene models
417 predicted in the new assembly are also highly complete. Whereas increase in the number of
418 genes predicted in the new Hawaiian *M. capitata* genome, compared with the published species,
419 could be attributed to differences in the workflows used to predicted the genes in these species
420 [53] however, it is also likely driven by the higher completeness and contiguity of the new
421 genome assembly.

422 There are 33,730 predicted protein-coding genes in the Hawaiian *Poc. acuta* and 31,840
423 in the *Poc. meandrina* genome assemblies (Supplementary Table S3), which is ~4,000–8,000
424 more than predicted in the other *Pocillopora* species. The number of complete BUSCO genes
425 from the Metazoa and Eukaryota BUSCO datasets is > 6% higher in the new Hawaiian *Poc.*
426 *acuta* and *Poc. meandrina* species than in the other *Pocillopora* species; the Hawaiian *Poc. acuta*
427 also has 29.6% and 31.3% (respectively) more complete BUSCO genes recovered than the
428 Indonesian *Poc. acuta*. The number of duplicated BUSCO genes is > 0.7% and > 2.3%
429 (respectively) higher in the Hawaiian *Poc. acuta* gene set compared with the published
430 *Pocillopora* species however, this was expected given the increased size of the genome
431 assembly. The proportion of fragmented BUSCO genes is > 0.9% and > 2% lower (Metazoa and

432 Eukaryota BUSCO datasets, respectively) lower in the Hawaiian *Pocillopora* species compared
433 with the published species. The average transcript length and the number of CDS per transcript
434 of the Hawaiian *Pocillopora* genes (~1,350 bp and ~6.6, respectively) are congruent with the
435 predicted genes of the published *Pocillopora* species (~1,100–1,900 bp and ~5.5–7.5,
436 respectively). This suggests that the higher number of predicted genes in the Hawaiian
437 *Pocillopora* species is not caused by the presence haplotigs in the genome assembly, although
438 this likely contributes to the slight higher number of duplicated BUSCO genes in the Hawaiian
439 *Poc. acuta*, or by the presence of fragmented genes models, since the number of fragmented
440 BUSCO genes and the gene statistics suggest that the majority of genes are full length.
441 Therefore, the higher number of predicted genes in this species can be (at least partially)
442 attributed to the more complete and contiguous genome assemblies of the Hawaiian *Pocillopora*
443 species relative to published species.

444 There are 44,130 predicted protein-coding genes in the Hawaiian *Por. compressa* genome
445 assembly (Supplementary Table S3), which is > 8,000 more genes than predicted in the *Por.*
446 *australiensis* (35,910) and *Por. lutea* (31,126) genomes, 4,677 more than in the *Por. rus* (39,453)
447 genome, and 20,506 less than in the *Por. astreoides* (64,636) genome. The number of complete
448 BUSCO genes from the Metazoa and Eukaryota BUSCO datasets is > 4% higher in *Por.*
449 *compressa* than in the published *Porites* species. The number of duplicated BUSCO genes in
450 *Por. compressa* is similar to *Por. lutea* and *Por. rus* but lower than in *Por. astreoides* and *Por.*
451 *australiensis*, and the number of fragmented BUSCO genes in *Por. compressa* is much lower (>
452 1.9% and > 5.1%, respectively) than in the published species. As with the previous Hawaiian
453 genomes, we attribute the higher number of predicted genes in this species to a more complete
454 and contiguous assembly, relative to the published data.

455

456 **Similarity between *Montipora capitata* chromosomal and extra-chromosomal scaffolds**

457 There are 1,685 scaffolds (totaling ~101 Mbp) in the new *M. capitata* assembly that were not
458 placed into the 14 putative chromosomes by the scaffolding software. Given that the size of the
459 14 chromosomal sequences totals ~680 Mbp, which is close to the estimated genome size of 644
460 Mbp, it is possible that the extra-chromosomal sequences represent retained haplotigs. To
461 explore this issue, we compared the predicted genes in the extra-chromosomal (6,545 protein-
462 coding genes) and chromosomal (47,839) scaffolds to determine how similar the protein content

463 is between the two sets of scaffolds and to see if the extra-chromosomal proteins tend to be
464 contained within a single chromosome (suggesting that the extra-chromosomal sequences are
465 likely retained haplotigs). Out of the 6,546 proteins encoded in the extra-chromosomal scaffolds,
466 3,896 (59.53%) have hits to chromosomal proteins with > 75% query coverage and > 75%
467 identity, and 1,623 (24.80%) have hits to chromosomal proteins with > 95% query coverage and
468 > 95% identity. This suggests that whereas the two sets of scaffolds encode many similar
469 (although not identical) proteins, the protein inventory of the extra chromosomal scaffolds only
470 partially overlaps with the gene inventory of the chromosomal scaffolds (we would expect them
471 to have a high level of overlap if they were haplotigs). Furthermore, the extra-chromosomal
472 scaffolds encode 12% of the total predicted genes but, when analyzed separately using BUSCO,
473 have only 1.9% of the Metazoa and 1.6% of the Eukaryota BUSCO genes recovered. This
474 conflict between the number of predicted genes in the scaffolds and the number of BUSCO
475 genes suggests that these scaffolds cannot be easily explained as simply unresolved haplotigs.
476 Finally, of the 3,896 proteins with top hits in the leniently filtered dataset (hit with > 75% query
477 coverage and > 75% identity), 2,748 (70.53%) were on scaffolds with other proteins with top hits
478 to different chromosomes. This suggests that the extra-chromosomal scaffolds have significant
479 structural differences when compared to the chromosomes. These results suggest that the extra-
480 chromosomal scaffolds do not comprise retained haplotigs however, given their significant size,
481 which increases the assembly size well above the estimated size, additional analyses will need to
482 be done to determine the placement of these sequences in the chromosomes and the genes that
483 they encode.

484

485 **Potential implications**

486 The substantial improvement in the contiguity and completeness of the assemblies and predicted
487 genes from the Hawaiian *M. capitata*, *Poc. meandrina*, *Poc. acuta*, and *Por. compressa* species
488 will enable many follow-up studies. The chromosome-level assembly of the *M. capitata* isolate
489 will not only serve as a key reference genome for future population studies focusing on this
490 species in Hawaii, but it will also enable more detailed studies on genome content (such as
491 repeats), gene content, and gene synteny with other species from reefs across the world. The *Poc.*
492 *acuta* genome, although not at chromosome-level resolution, is the most complete available for
493 this genus and will be a valuable model for not only comparative analysis, but for analysis of

494 ploidy in corals. As the first assembly ever generated from a non-diploid coral, this data will
495 open up new questions surrounding the role of ploidy in coral evolution and adaptation and how
496 this phenomenon is involved in the lifecycle of this species and potentially other *Pocillopora*
497 species, both in Hawai‘i and other reefs across the world. These questions are critical, because an
498 understanding of how changes in ploidy evolve in these corals, particularly in response to stress,
499 will help us model the response of these ecosystems to anthropogenic climate change, and may
500 even provide a new avenue of research for the development of stress resistant “super” corals.

501

502 **Data availability**

503 The Omni-C data generated from the Hawaiian *M. capitata* is available from the NCBI SRA
504 database, under BioProject PRJNA509219. The PacBio HiFi data generated from the Hawaiian
505 *Poc. meandrina* and *Poc. acuta* are also available from the SRA database under BioProject
506 PRJNA761443. The PacBio HiFi and Illumina data generated from the Hawaiian *Por. compressa*
507 are available from the SRA database under BioProject PRJNA663761. The genome assemblies
508 and predicted genes for the Hawaiian *M. capitata* is available from
509 <http://cyanophora.rutgers.edu/montipora/> (Version 3), for *Poc. acuta* from
510 http://cyanophora.rutgers.edu/Pocillopora_acuta/ (Version 2), *Poc. meandrina* from
511 http://cyanophora.rutgers.edu/Pocillopora_meandrina/ (Version 1), *Por. compressa* from
512 http://cyanophora.rutgers.edu/Porites_compressa/ (Version 1). The data from the other
513 *Montipora*, *Pocillopora*, and *Porites* species used in this study are available from their respective
514 repositories listed in Supplementary Table S2.

515

516 **Additional Files**

517 **Supplementary Figure S1.** GenomeScope2 (left) and Smudgeplot (right) profiles for (A)
518 Hawaiian *M. capitata* (this study), (B) Waiopae tide pools *M. capitata*, (C) *M. cactus*, (D) *M.*
519 *efflorescens*, (E) *Poc. meandrina* (this study), (F) Hawaiian *Poc. acuta* (this study), (G)
520 Indonesian *Poc. acuta*, (H) *Poc. verrucosa*, (I) *Por. compressa* (this study), (J) *Por.*
521 *australiensis*, and (K) *Por. lutea*. The profiles were computed for each species using 21-mers
522 generated from the trimmed short-read data listed in Supplementary Table S2.

523

524 **Abbreviations**

525 bp: base pairs
526 BUSCO: Benchmarking Universal Single-Copy Orthologs
527 Gbp: gigabase pairs
528 HM2: HaploMerger2
529 Kbp: Kilobase pairs
530 Mbp: megabase pairs
531 NCBI: National Center for Biotechnology Information
532 PacBio: Pacific BioSciences
533 SRA: Sequencing Read Archive
534

535 **Conflict of Interests**

536 The authors declare that they have no other competing interests.
537

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552

553 **Author contributions**

554 DB conceived the project with HMP and JL. TGS, JML, and YJJ did the bioinformatic analyses,
555 HSY provided sequencing resources, and HMP led the coral sample collection and processing

556 with EM. TGS wrote the manuscript draft with JML, and all authors commented on and
557 approved the submitted version.

558

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722

723 **Tables**

724 **Table S1:** Summary of coral assemblies before and after haplotype merging.

725

726 **Table S2:** Metadata for the genome and gene models downloaded for the coral species used for
727 comparative analysis.

728

729 **Table S3:** Comparison between the published *Montipora*, *Pocillopora*, and *Porites* genomes and
730 those generated in this study. All statistics were calculated in this study using the available
731 genome and gene models.

732

733 **Table S4:** Results from nQuire lrdmodel ploidy estimation for the Hawaiian coral genomes
734 analyzed in this study.

735

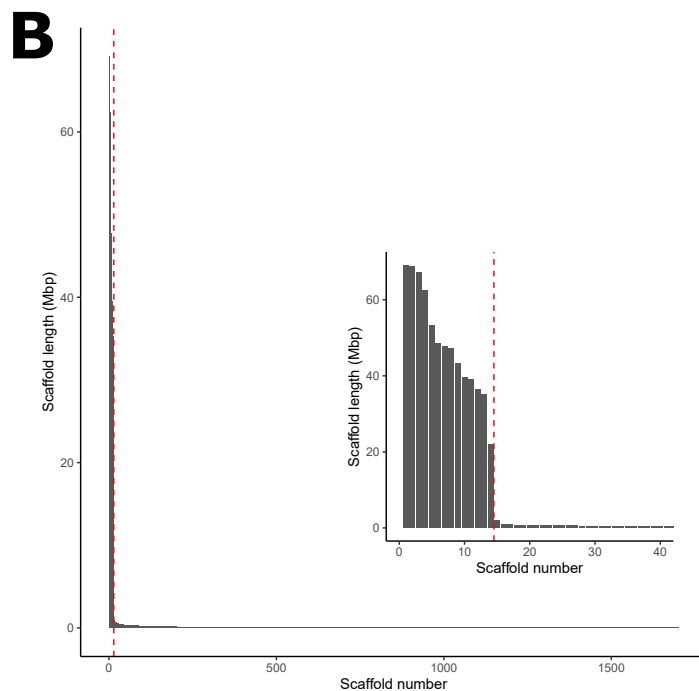
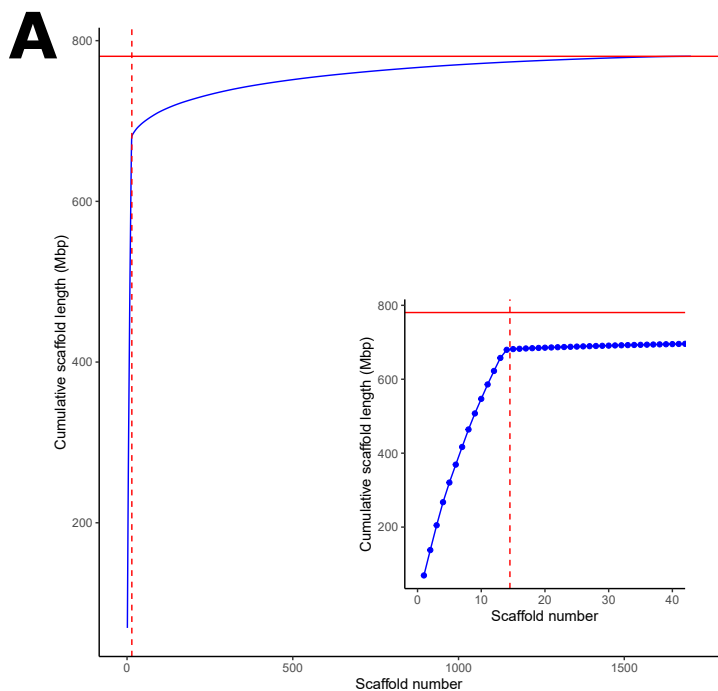
736

737 **Figure Legends**

738 **Figure 1:** (A) Cumulative and (B) individual length of scaffolds in the new Hawaiian *M.*
739 *capitata* genome assembly. Scaffolds were sorted by length in descending order; each point
740 along the x-axis of (A) and (B) represents a scaffold, with the longest scaffold being the first and
741 the shortest being the last on the x-axis of each plot. In (A) and (B) a zoomed-in section of the
742 larger plot is shown on the right highlighting the 40 largest scaffolds; a horizontal red line in (A)
743 shows the total assembled bases in the new genome and a vertical dashed line in (A) and (B) is
744 positioned after the 14th largest scaffold. GenomeScope2 linear *k*-mer distributions of the
745 Hawaiian (C) *M. capitata*, (D) *Poc. meandrina*, (E) *Poc. acuta*, and (F) *Por. compressa* species
746 with theoretical diploid (or triploid for *Poc. acuta*) models shown by the black lines. The
747 GenomeScope2 profiles were computed for each species using 21-mers generated from the
748 trimmed short-read data listed in Supplementary Table S2.

749

750 **Figure 2:** Results from BUSCO analysis run using the genomes and predicted genes from all
751 published (including this study) *Montipora*, *Pocillopora*, and *Porites* species, plus the old
752 version of the *M. capitata* genome that our group published in 2019 [16]. BUSCO results for
753 each species using the (A) Metazoa dataset (genome mode), (B) Eukaryota dataset (genome
754 mode), (C) Metazoa dataset (protein mode), and (D) Eukaryota dataset (protein mode).

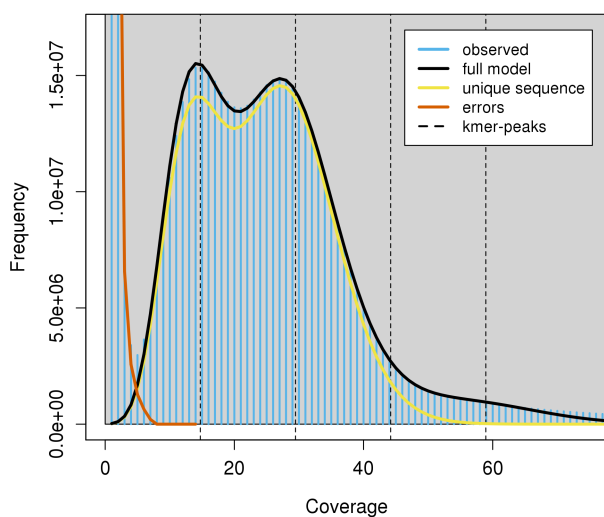


C

M. capitata

GenomeScope Profile

len:644,226,330bp uniq:50.9%
aa:98.6% ab:1.36%
kcov:14.7 err:0.228% dup:0.733 k:21 p:2

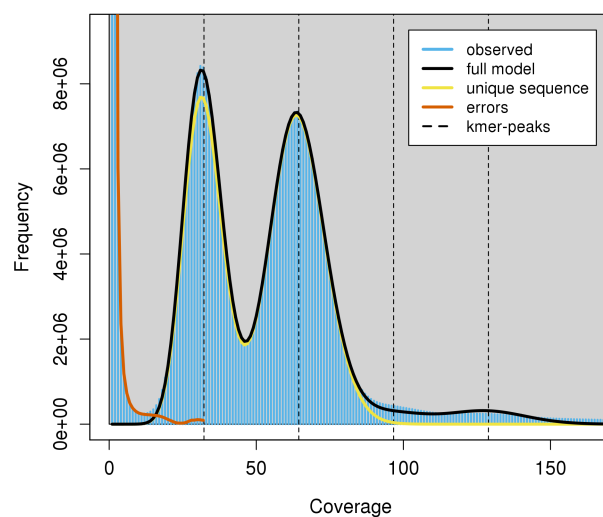


D

P. meandrina

GenomeScope Profile

len:349,144,232bp uniq:66.4%
aa:98.5% ab:1.5%
kcov:32.2 err:0.156% dup:0.337 k:21 p:2

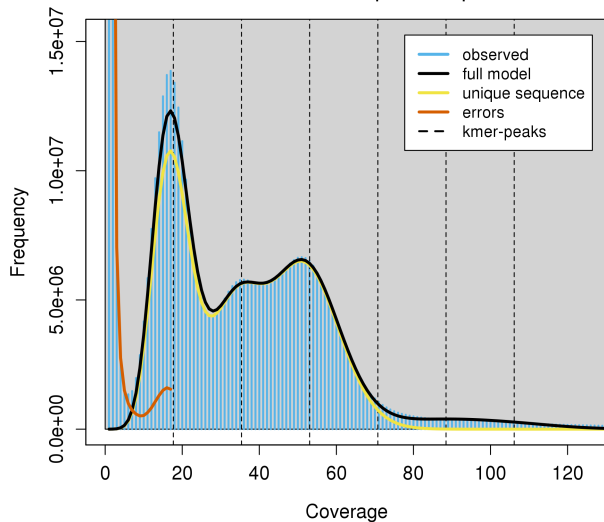


E

P. acuta

GenomeScope Profile

len:353,080,882bp uniq:65.3%
aaa:97.3% aab:2.4% abc:0.308%
kcov:17.7 err:0.228% dup:0.347 k:21 p:3



F

P. compressa

GenomeScope Profile

len:527,906,982bp uniq:58.7%
aa:98.2% ab:1.84%
kcov:13.9 err:0.303% dup:0.384 k:21 p:2

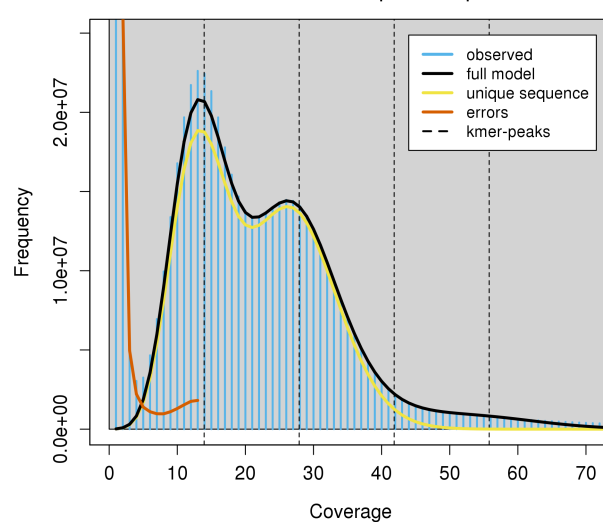
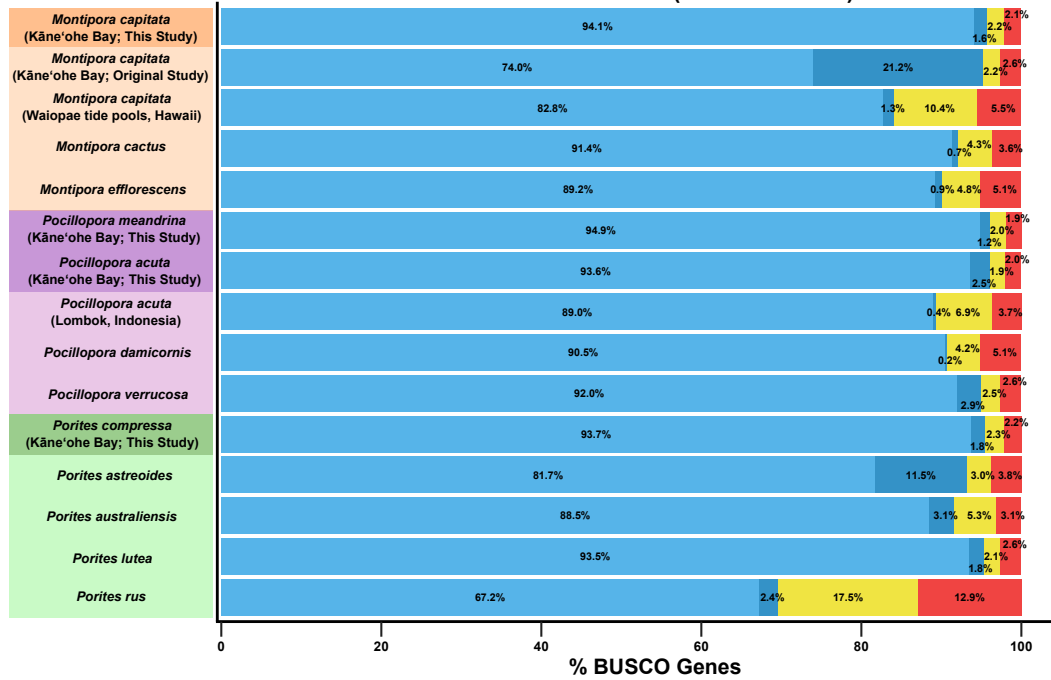
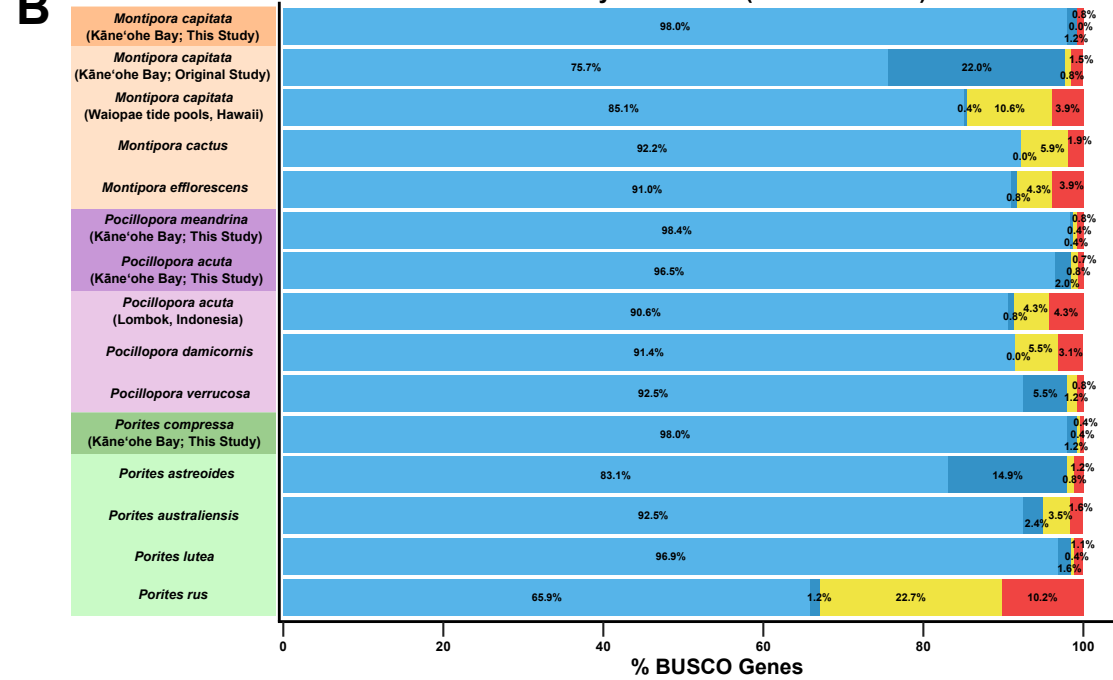


Figure 2

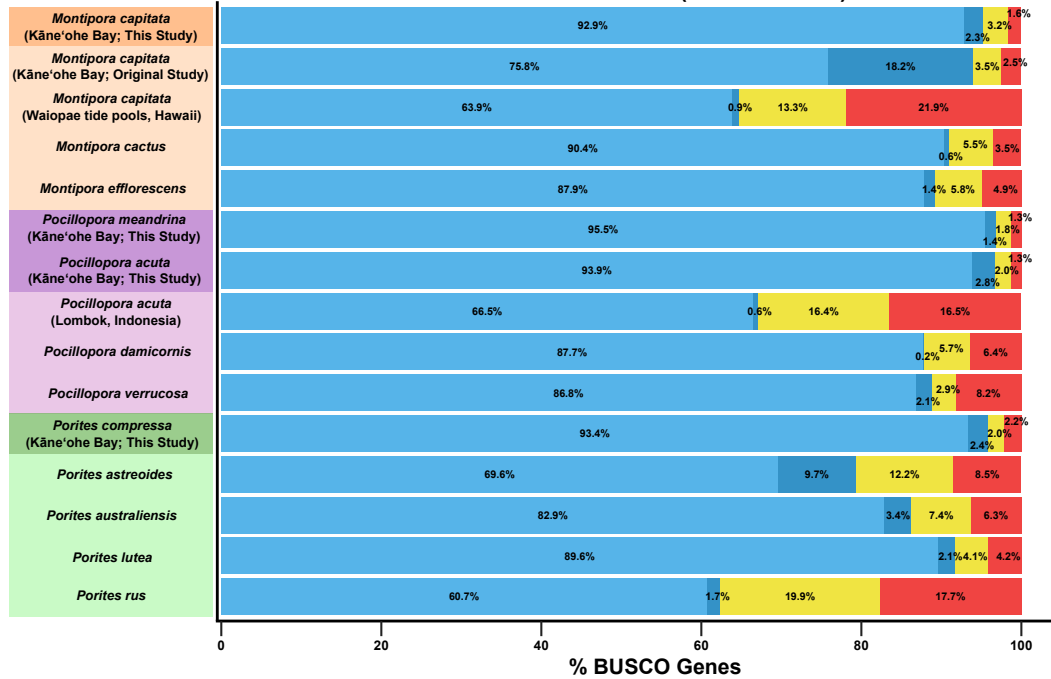
BUSCO Metazoa dataset (Genome mode)



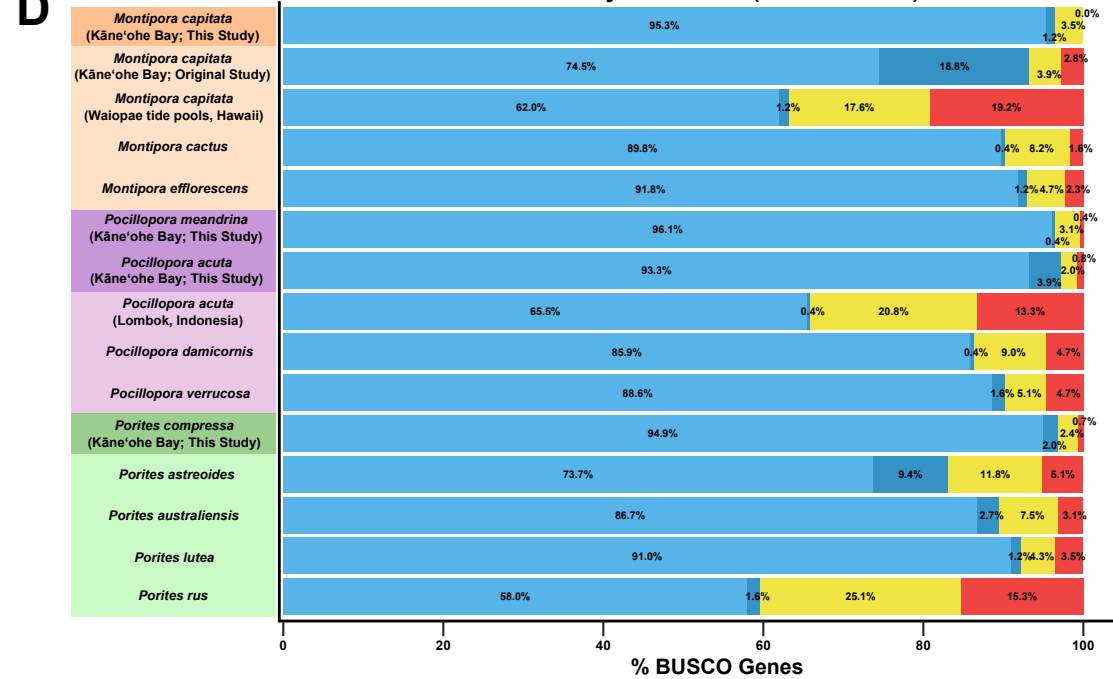
BUSCO Eukaryota dataset (Genome mode)




BUSCO Metazoa dataset (Protein mode)



BUSCO Eukaryota dataset (Protein mode)



Complete (C) and single-copy (S) Complete (C) and duplicated (D) Fragmented (F) Missing (M)



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