

1 Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: New approaches to  
2 long-standing problems

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46 **Abstract**

47 Anthozoans (e.g., corals, anemones) are an ecologically important and diverse group of  
48 marine metazoans that occur from shallow to deep waters worldwide. However, our  
49 understanding of the evolutionary relationships among the ~7500 species within this class is  
50 hindered by the lack of phylogenetically informative markers that can be reliably sequenced  
51 across a diversity of taxa. We designed and tested 16,308 RNA baits to capture 720  
52 Ultraconserved Element loci and 1,071 exon loci. Library preparation and target enrichment was  
53 performed on 33 taxa from all orders within the class Anthozoa. Following Illumina sequencing  
54 and Trinity assembly, we recovered 1,774 of 1,791 targeted loci. The mean number of loci  
55 recovered from each species was  $638 \pm 222$ , with more loci recovered from octocorals ( $783 \pm$   
56  $138$  loci) than hexacorals ( $475 \pm 187$  loci). Phylogenetically informative sites ranged from 26-  
57 49% for alignments at differing hierarchical taxonomic levels (e.g., Anthozoa, Octocorallia,  
58 Hexacorallia). The percent of variable sites within each of three genera (*Acropora*, *Alcyonium*,  
59 and *Sinularia*) for which multiple species were sequenced ranged from 4.7-30%. Maximum  
60 likelihood analyses recovered highly resolved trees with topologies matching those supported by  
61 other studies, including the monophyly of the order Scleractinia. Our results demonstrate the  
62 utility of this target-enrichment approach to resolve phylogenetic relationships from relatively  
63 old to recent divergences. Re-designing the baits with improved affinities to capture loci within  
64 each sub-class will provide a valuable toolset to address systematic questions and further our  
65 understanding of the timing of diversifications in the class Anthozoa.

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## 69 **Introduction**

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71 Anthozoan cnidarians play critical roles in many marine ecosystems. The class contains  
72 ~7,500 extant species (i.e., soft corals, sea fans, stony corals, black corals, and anemones) that  
73 live worldwide in a variety of marine habitats—from tropical shallow waters to the cold deep  
74 abyss (Daly *et al.* 2007). Many anthozoans serve as foundation species by creating habitat and  
75 supporting a diversity of invertebrates and fishes, including obligate symbiotic associates. In  
76 addition, scleractinian (stony) corals can create massive biogenic structures and engineer entire  
77 reef-based ecosystems. In fact, coral reefs are some of the most diverse and valuable marine  
78 ecosystems across the globe, supporting a high concentration of the oceans' biodiversity  
79 (Carpenter *et al.* 2008). Yet, coral reefs are disappearing at an alarming rate, the health of many  
80 coral habitats is declining, and the extinction risk of reef-building corals is increasing due to  
81 human impacts, including anthropogenic climate change (Gardner *et al.* 2003; Pandolfi *et al.*  
82 2003; Carpenter *et al.* 2008; Hughes *et al.* 2017). With global ocean change occurring at  
83 unprecedented rates (Levitus *et al.* 2000; Gruber *et al.* 2012; Schmidtko *et al.* 2017), it would be  
84 helpful to understand the timing of divergence events among particular clades and the history of  
85 morphological character (e.g., skeletal type) evolution within the Anthozoa to identify possible  
86 connections between those evolutionary events and paleoclimate conditions. However, to  
87 advance our understanding of the order and timing of these events, we need to generate a robust,  
88 well-resolved phylogeny for the group.

89 Classification of Anthozoa has traditionally been based on morphological characters such  
90 as skeletal morphology, colony organization and soft-tissue anatomy of the polyps (Daly *et al.*  
91 2007), including the arrangement of internal mesenteries (Fautin and Mariscal 1991). Long-  
92 standing views have recognized the anthozoan sub-classes Octocorallia and Hexacorallia as

93 reciprocally monophyletic (Daly *et al.* 2007), a view also supported by recent phylogenomic  
94 analyses of 10s to 100s of genes (Zapata *et al.* 2015; Pralong *et al.* 2017). Within each sub-class,  
95 however, molecular phylogenetic studies have revealed widespread homoplasy in morphological  
96 characters and widespread polyphyly at the ordinal, sub-ordinal, family, and genus levels (e.g.,  
97 Fukami *et al.* 2008; McFadden *et al.* 2010; Rodríguez *et al.* 2014; Daly *et al.* 2017).  
98 Consequently, deep flaws exist in our understanding of the phylogenetic relationships among and  
99 within anthozoan orders. Attempts to resolve the deep phylogenetic relationships among  
100 anthozoans using molecular data have largely been unsuccessful due to relatively slow  
101 evolutionary rates of mitochondrial genomes (Shearer *et al.* 2002; Hellberg 2006; Huang *et al.*  
102 2008; Forsman *et al.* 2009), lack of signal in rDNA (Berntson *et al.* 2001; Daly *et al.* 2003) and  
103 difficulty identifying and developing PCR primers for single-copy nuclear genes that can be  
104 amplified across the entire class (McFadden *et al.* 2011).

105         Within most anthozoan orders, there is also a lack of phylogenetic resolution at the  
106 species level. This may be due to incomplete lineage sorting in gene trees, insufficient data due  
107 to the small number of currently available markers, hybridization, and/or lack of morphological  
108 synapomorphies in taxonomy (McFadden *et al.* 2010, 2011, 2017; Prada *et al.* 2014; Rodríguez  
109 *et al.* 2014; Grajales and Rodríguez 2016; Daly *et al.* 2017). Currently available markers are  
110 insufficient at resolving species boundaries for the majority of anthozoans. For octocorals, an  
111 extended mitochondrial barcode (*COI+igr1+mtMutS*) has proven useful for revealing cryptic  
112 species and delimiting species boundaries within some clades; however, the divergence criterion  
113 proposed (McFadden *et al.* 2011) to elucidate these boundaries is low (>0.5% p-distance) and  
114 often no genetic divergence is observed among congeneric species (McFadden *et al.* 2011,  
115 Dueñas *et al.* 2014, Pante *et al.* 2015). The low genetic variability in the mitochondrial genome

116 has been attributed to a unique mis-match repair enzyme (*mtMutS*) that potentially repairs  
117 mutations (Bilewitch and Degnan 2011) thereby causing reduced mitochondrial sequence  
118 variation in octocorals when compared to other metazoans (Shearer *et al.* 2002). Mitochondrial  
119 sequence variation is also low in the hexacorals (Hellberg *et al.* 2006; Daly *et al.* 2010), creating  
120 difficulties in resolving species boundaries using traditional mitochondrial barcodes (i.e., *cox1*,  
121 COI Barcode of Life, Hebert *et al.* 2003; Shearer and Coffroth 2008). Although several studies  
122 have resolved species boundaries using a nuclear ITS marker (e.g., Medina *et al.* 1999; Pinzon  
123 and LaJeunesse 2011), using ITS poses problems as it is not a single-locus marker (Vollmer and  
124 Palumbi 2004) and there are often high levels of intra-specific variation (Van Oppen *et al.* 2000).  
125 Methods that allow for collecting and analyzing hundreds or thousands of loci across shallow  
126 and deep levels of divergence are sorely needed.

127         While there are numerous pathways to find new informative loci (see McCormack *et al.*  
128 2013a), target enrichment of ultraconserved elements (UCEs) (Faircloth *et al.* 2012) has proven  
129 robust in inferring species histories of both vertebrates [e.g., fishes (Faircloth *et al.* 2013), birds  
130 (McCormack *et al.* 2013b), reptiles (Crawford *et al.* 2012), and mammals (McCormack *et al.*  
131 2012)] and invertebrates [e.g., arachnids (Starrett *et al.* 2016), hymenopterans (Branstetter *et al.*  
132 2017), and coleopterans (Baca *et al.* 2017)] across shallow to deep timescales. UCEs occur in  
133 high numbers throughout genomes across the tree of life, including Cnidaria (Ryu *et al.* 2012),  
134 making them easy to identify and align among divergent species (Faircloth *et al.* 2012). As the  
135 name implies, UCEs are highly conserved regions of the genome, but the flanking regions  
136 surrounding UCEs are more variable and phylogenetically informative (Faircloth *et al.* 2012).  
137 Some advantages of using target enrichment of UCEs include that 100s to 1000s of loci can be  
138 sequenced at a relatively low cost from a wide range of taxa (Faircloth *et al.* 2012); they can be

139 generated from 100 year old, formalin-preserved museum specimens and specimens with  
140 degraded DNA (McCormack *et al.* 2016; Ruane and Austin 2017); and they have proven useful  
141 at resolving evolutionary questions across both shallow and deep time scales (Smith *et al.* 2013;  
142 McCormack *et al.* 2013b; Manthey *et al.* 2016). Similar approaches using target-enrichment of  
143 coding regions, or exon-capturing (Bi *et al.* 2012; Ilves and Lopez-Fernandez 2014; Hugall *et al.*  
144 2016), have also proven valuable in phylogenomics.

145 We used all available genomes and transcriptomes to design a set of target-capture baits  
146 for enriching both UCEs and exons for use in anthozoan phylogenetics. Herein, we discuss how  
147 loci were targeted and baits were designed. Using an *in silico* analysis, we demonstrate that these  
148 loci recover the established sub-class and ordinal relationships among anthozoans. Finally, we  
149 test the utility of these baits *in vitro* using 33 species from across both sub-classes of Anthozoa.

150

## 151 **Materials and Methods**

### 152 *Available Genomes and Transcriptomes*

153 Genomic and transcriptomic data were gathered from various sources for use in bait  
154 design and *in silico* testing (Table S1). All data were masked for repetitive regions,  
155 retroelements, small RNAs, and transposons using Repeat Masker open-4.0 (Smit *et al.* 2015).  
156 The N50 was calculated for each genome using stats.sh in the BBtools package (Bushnell 2015).  
157 We then constructed 2bit files for all genomes and transcriptomes (faToTwoBit, BLAT Suite,  
158 Kent 2002) and simulated 100 bp paired reads from each genome and transcriptome using the  
159 program art\_illumina (Huang *et al.* 2012). All programs and parameters used for the entire  
160 workflow can be found in Supplemental File 1.

161

## 162 *Identification of UCE Loci and Bait Design*

163 We used the open-source program PHYLUCE (Faircloth *et al.* 2016) and followed the  
164 workflow in the online tutorial (<http://phyluce.readthedocs.io/en/latest/tutorial-four.html>), with a  
165 few modifications to identify conserved regions and design baits to target these regions for  
166 downstream next-generation sequencing (Faircloth 2017). To find UCEs, we first aligned an  
167 average of 34 million simulated reads from each of the four exemplar taxa, *Acropora digitifera*,  
168 *Exaiptasia pallida*, *Renilla muelleri*, and *Pacifigorgia irene*, to a base genome, *Nematostella*  
169 *vectensis*, using stampy v. 1 (Lunter and Goodson 2011), with a substitution rate set at 0.05.  
170 *Nematostella vectensis* ('*nemve*') was chosen as the base genome for the primary bait design  
171 because it is one of the most well assembled and annotated anthozoan genomes. Approximately  
172 1.3% of the reads mapped to the *nemve* genome; the resulting alignment file was transformed  
173 from SAM format into BAM format (samtools, Li *et al.* 2009) and then transformed into a BED  
174 formatted file (BEDtools, Quinlan and Hall 2010). These BED files were sorted by  
175 scaffold/contig and then by position along that scaffold/contig. We then merged together the  
176 alignment positions in each file that were close (<100 bp) to one another using bedtools. In  
177 addition, sequences that included masked regions (>25%) or ambiguous (N or X) bases or were  
178 too short (<80 bp) were removed using `phyluce_probe_strip_masked_loci_from_set`. These steps  
179 resulted in BED files containing regions of conserved sequences shared between *nemve* and each  
180 of the exemplar taxa for further analysis. An SQLite table was created using  
181 `phyluce_probe_get_multi_merge_table`, and included 70,312 loci that were shared between pairs  
182 of taxa.

183 We queried the SQLite table and output a list of 1,794 conserved regions shared between  
184 *nemve* and the other four exemplar taxa using `phyluce_probe_query_multi_merge_table`. This

185 list plus `phyluce_probe_get_genome_sequences_from_bed` was used to extract the conserved  
186 regions from the *nemve* genome. These regions were buffered to 160 bp by including an equal  
187 amount of 5' and 3' flanking sequence from the *nemve* genome. Another filter was performed at  
188 this stage to remove sequences < 160 bp, sequences with > 25% masked bases, or sequences  
189 with ambiguous bases. A temporary set of sequence capture baits was designed from the loci  
190 found in this final FASTA file. Using `phyluce_probe_get_tiled_probes`, we designed the bait set  
191 by tiling 120 bp baits over each locus at 3x density (baits overlapped in the middle by 40 bp).  
192 This temporary set of baits was screened to remove baits with >25% masked bases or high  
193 (>70%) or low (<30%) GC content. Any potential duplicates were also removed using  
194 `phyluce_probe_easy_lastz` and `phyluce_probe_remove_duplicate_hits_from_probes_using_lastz`.  
195 Bait sequences were considered duplicates if they were  $\geq 50\%$  identical over  $\geq 50\%$  of their  
196 length.

197 The temporary bait set (2,131 baits, targeting 1,787 loci) was aligned back to *nemve* and  
198 the four exemplar taxa using `phyluce_probe_run_multiple_lastzs_sqlite`, with an identity value of  
199 70% (the minimum sequence identity for which a bait could be an accepted match to the  
200 genome) and a minimum coverage of 83%. From these alignments, baits that matched multiple  
201 loci were removed. We then extracted 180 bp of the sequences from the alignment files and input  
202 the data into FASTA files using `phyluce_probe_slice_sequence_from_genomes`. A list  
203 containing 710 loci shared between at least three of the taxa was created. Based on this list of  
204 710 loci, the anthozoan UCE bait set was re-designed to target these 710 loci using  
205 `phyluce_probe_get_tiled_probe_from_multiple_inputs`, *nemve*, and the four exemplar genomes.  
206 Using this script, 120-bp baits were tiled (3X, middle overlap) and screened for high (>70%) or  
207 low (<30%) GC content, masked bases (>25%), and duplicates. This bait set included a total of



208 5,459 non-duplicated baits targeting 710 anthozoan loci. All above methods were repeated to add  
209 more baits to produce additional octocoral-specific baits and capture octocoral-specific loci. We  
210 re-ran the analyses using *R. muelleri* as the base genome and *P. irene*, *Paragorgia*  
211 *stephencairnsi*, and *Antillogorgia bipinnata* as the exemplar taxon to add 1,317 baits targeting an  
212 additional 168 UCE loci to the dataset.

213

#### 214 *Identification of Exon Loci and Bait Design*

215 To design baits to target exon regions, the above methods were repeated using available  
216 transcriptome data. An average of 7 million reads from five exemplar transcriptome-enabled taxa  
217 (*A. digitifera*, Cerianthidae, *Edwardsiella lineata*, *Gorgonia ventalina*, and *Paramuricea* sp.)  
218 were simulated and an average of 5.8% of these reads per species were aligned to the *nemve*  
219 transcriptome. After we converted the alignments to BED files, merged overlapping reads, and  
220 filtered data for short loci and repetitive regions, 44,215 conserved sequences were added to an  
221 SQLite database. We queried this database and selected 3,700 loci that were shared between  
222 *nemve* and the additional five exemplar taxa. Following a second screening for masked regions,  
223 high/low GC content, and duplicates, a temporary exon bait set (5,661 baits) targeting 3,633  
224 exon loci was designed. The temporary baits were re-aligned to the transcriptomes of *nemve* and  
225 the additional five exemplar anthozoans to ensure we could locate the loci. A set of 906 loci that  
226 were shared by *nemve* and the additional five exemplar anthozoans were added to an SQLite  
227 database. We re-designed the exon bait set to target these 906 exon loci using  
228 `phyluce_probe_get_tiled_probe_from_multiple_inputs`, *nemve*, and the five exemplar  
229 transcriptomes. This bait set included a total of 8,080 non-duplicated baits targeting 906 loci  
230 across all anthozoans. To add more octocoral-specific baits and loci, we then repeated the

231 methods with *Paramuricea* sp. as the base transcriptome and *Anthomastus* sp., *Corallium*  
232 *rubrum*, *Eunicea flexuosa*, *G. ventalina*, Keratoisidinae sp., and *Nepthyigorgia* sp. as the  
233 exemplar taxa to add 4,914 baits targeting an additional 407 loci to the dataset.

234

#### 235 *Final Bait Screening*

236 All of the bait sets were screened against one another to remove duplicates ( $\geq 50\%$   
237 identical over  $>50\%$  of their length), allowing us to create a final non-duplicated Anthozoa bait  
238 set. We also screened these baits (70% identity, 70% coverage) against the *Symbiodinium*  
239 *minutum* genome by using `phyluce_probe_run_multiple_lastzs_sqlite` and  
240 `phyluce_probe_slice_sequence_from_genomes` and removed loci that matched the symbiont.  
241 Bait names in the final bait FASTA file begin with ‘uce-’ if designed using genomes to target  
242 UCEs and ‘trans-’ if designed using transcriptomes to target exons.

243

#### 244 *In Silico Test*

245 *In silico* tests were performed to check how well the designed baits aligned to existing  
246 genomes and transcriptomes. First, `phyluce_probe_run_multiple_lastzs_sqlite` was used to align  
247 the UCE baits to the nine 2-bit formatted genomes and an outgroup genome (*Hydra*  
248 *magnipapillata*) and the exon baits to the 24 2-bit formatted transcriptomes (Table S1). An  
249 identity value of 50% was chosen for alignments. For each bait test, the matching FASTA data  
250 were sliced out of each genome or transcriptome, plus 200 bp of 5’ and 3’ flanking regions,  
251 using `phyluce_probe_slice_sequence_from_genomes`. This resulted in an average of  $429 \pm 178$   
252 SD (44 to 599 per species) UCE loci and  $497 \pm 230$  SD (206 to 857) exon loci per anthozoan  
253 species (Table 1). To do a final screen for duplicates, loci were matched to baits using

254 phyluce\_assembly\_match\_contigs\_to\_probes, with a minimum coverage of 67% and minimum  
255 identity of 80%. Here, an average of  $355 \pm 166$  SD (25 to 529 per species) non-duplicate UCE  
256 loci and  $354 \pm 210$  SD (106 to 670) non-duplicate exon loci were recovered per anthozoan  
257 species (Table 1). Each locus was exported into a FASTA file and aligned with MAFFT (Katoh  
258 et al. 2002) using phyluce\_align\_seqcap\_align with default parameters.

259 The resulting alignments were trimmed using GBlocks (Castresana 2000, Talavera and  
260 Castresana 2007). We created two final datasets using  
261 phyluce\_align\_get\_only\_loci\_with\_min\_taxa, in which all locus alignments contained at least 4  
262 of the 10 taxa for the genome data and 9 of the 24 taxa for the transcriptome data. We then  
263 concatenated the resulting alignments into separate supermatrices; one containing UCE loci from  
264 10 genome-enabled taxa and the other containing exon loci from the 24 transcriptome-enabled  
265 taxa. Maximum likelihood (ML) inference was conducted on each supermatrix using RAxML v8  
266 (Stamatakis 2014). This analysis was carried out using rapid bootstrapping, which allows for a  
267 complete analysis (20 ML searches and 200 bootstrap replicates) in one step.

268

#### 269 *In Vitro Test*

270 Following the *in silico* test, the list of designed baits was sent to MYcroarray for  
271 synthesis. MYcroarray further screened and removed baits that either had repetitive elements or  
272 the potential to cross-hybridize (0.007% total baits removed). We then tested the bait set on 33  
273 anthozoan specimens (Table 2), with both sub-classes and all major orders and sub-orders (for  
274 Octocorallia) represented.

275 DNA was extracted using a Qiagen DNeasy Blood & Tissue kit, Qiagen Genra Kit, or a  
276 CTAB extraction protocol (McFadden et al. 2006). DNA quality was assessed using a Nanodrop

277 spectrophotometer, with 260/280 ratios ranging from 1.8-2.1 and 260/230 ratios ranging from  
278 1.4-3.2. The initial concentration of each sample was measured with a Qubit 2.0 fluorometer. For  
279 the majority of samples, we then sheared 600 ng DNA (10 ng per  $\mu\text{L}$ ) to a target size range of  
280 400-800 bp using sonication (Q800R QSonica Inc. Sonicator). For eight samples (Table 2), we  
281 sheared 35  $\mu\text{L}$  (115-372 ng, average 217 ng) of EDTA-free DNA using enzymes from the Kapa  
282 HyperPlus (Kapa Biosystems) library preparation kit. These samples were mixed on ice with 5  
283  $\mu\text{L}$  of Kapa Frag buffer and 10  $\mu\text{L}$  of the Kapa Frag enzyme and put on a pre-cooled ( $4^{\circ}\text{C}$ )  
284 thermocycler prior to incubation for 10-15 min at  $37^{\circ}\text{C}$  to achieve a target size range of 400-800  
285 bp. After shearing, DNA was run out on a 1% agarose gel (120V, 60 min). Small DNA  
286 fragments were removed from each sample (250 ng DNA) using a generic SPRI substitute  
287 (Rohland and Reich 2012; Glenn *et al.* 2016) bead cleanup (3X). DNA was re-suspended in 25  
288  $\mu\text{L}$  double-distilled water (ddH<sub>2</sub>O).

289         Details of library preparation and target enrichment can be found in Supplemental File 2.  
290 Briefly, library preparation (Kapa Biosystems) was carried out on the majority of DNA samples  
291 (Table 2) using a Kapa Hyper Prep protocol. For the subset of the samples for which DNA was  
292 sheared using enzymes (Table 2), we followed the protocol in the Kapa Hyper Plus enzyme-  
293 shearing library preparation kit (Kapa Biosystems). Universal Y-yoke oligonucleotide adapters  
294 and custom iTru dual-indexed primers were used in library preparations (Glenn *et al.* 2016). For  
295 target enrichment, the MYcroarray MyBaits were diluted in 1/2 (250 ng) of the standard (500 ng)  
296 MyBaits reaction, using 2.5  $\mu\text{L}$  of the baits and 2.5  $\mu\text{L}$  of ddH<sub>2</sub>O for all samples. Different bait  
297 strengths were tested on a set of six samples (Table 2): full bait strength (500 ng), 1/2 bait  
298 strength (250 ng), 1/4 bait strength (125 ng), and 1/8 strength (63 ng). One pool of enriched  
299 libraries was sent to Oklahoma Medical Research Facility for sequencing on 2/3 of a lane of

300 Illumina HiSeq 3000 (150bp PE reads). The remaining 1/3 of the sequencing lane was used for  
301 unrelated samples with non-overlapping indexes.

302

### 303 *Post-Sequencing Analyses*

304 De-multiplexed Illumina reads were processed using PHYLUCÉ following the workflow  
305 in the online tutorial (<http://phyluce.readthedocs.io/en/latest/tutorial-one.html/>), with a few  
306 modifications (Suppl. File 1). The reads were first trimmed using the Illumiprocessor wrapper  
307 program (Faircloth 2012) with default values and then assembled using Trinity v. 2.0 (Haas *et al.*  
308 2013). We also assembled the data using Abyss 2.0 (Simpson *et al.* 2009) with a kmer value of  
309 31. UCE and exon bait sequences were then separately matched to the assembled contigs (70%  
310 identity, 70% coverage) using `phyluce_assembly_match_contigs_to_probes` to locate the loci.  
311 Loci were then extracted using `phyluce_assembly_get_match_counts` and  
312 `phyluce_assembly_get_fastas_from_match_counts`, exported into separate FASTA files and  
313 aligned with default parameters using `phyluce_align_seqcap_align`, which uses MAFFT. Loci  
314 were internally trimmed using GBlocks.

315 Data matrices of locus alignments were created using  
316 `phyluce_align_get_only_loci_with_min_taxa`, in which each locus had either 25% or 50%  
317 species occupancy. Concatenated locus alignments consisted of exon loci only, UCE loci only,  
318 and all loci. The number of phylogenetically informative sites was calculated for each alignment  
319 across various taxonomic datasets. The script `phyluce_align_get_informative_sites` was used on  
320 the following taxonomic datasets: Anthozoa+genome+outgroup (33 taxa used in *in vitro* test,  
321 plus nine genome-enabled taxa and the outgroup *H. magnipapillata*), Anthozoa (33 taxa used in  
322 *in vitro* test), Hexacorallia only (17 taxa used in *in vitro* test), and Octocorallia only (16 taxa

323 used in *in vitro* test). The total number of variable sites, total number of phylogenetically  
324 informative sites and number of phylogenetically informative sites per locus were calculated. We  
325 also calculated the total number of variable sites and the number of variable sites per locus for  
326 alignments containing species in each of three genera: *Acropora* (*A. digitifera*, *A. millepora*, *A.*  
327 *muricata*), *Alcyonium* (*A. acaule*, *A. digitatum*, *A. haddoni*), and *Sinularia* (*S. slieringsi*, *S.*  
328 *lochmodes*, *S. maxima*). For the three *Acropora* species, we used loci from one target-capture  
329 enrichment sample and from the two *Acropora* genomes that were available.

330 ML was conducted on each alignment (exon loci only, UCE loci only, and all loci) for the  
331 Anthozoa+genome+outgroup taxon set using RAxML v8. This analysis was carried out using  
332 rapid bootstrapping, which allows for a complete analysis (20 ML searches and 200 bootstrap  
333 replicates) in one step.

334

## 335 **Results**

### 336 *Identification of Loci and Bait Design*

337 A total of 16,308 baits were designed to capture 1,791 anthozoan loci with 4 to 10 baits  
338 targeting each locus. The principal UCE bait set included 5,513 baits designed to target 720 loci.  
339 The principal exon bait set included 10,795 baits to target 1,071 loci. Four loci that matched  
340 genomic regions in *Symbiodinium minutum* were removed from the dataset. These loci, however,  
341 were also detected in azooxanthellate anthozoans, such as *Chrysogorgia tricaulis*.

342

### 343 *In Silico Test*

344 We generated two alignment matrices, one consisting of the exon loci taken from the  
345 transcriptome-enabled taxa and the other one consisting of the UCE loci taken from the genome-

346 enabled taxa. The alignment matrix generated with the UCE loci, which included the *H.*  
347 *magnipapillata* outgroup, had a total of 522 loci, with a trimmed mean locus length of 373 bp  
348 (95% CI: 8.4) and a total alignment length of 138,778 bp. The alignment matrix generated with  
349 the exon loci included 407 loci, with a trimmed mean locus length of 462 bp (95% CI: 5.8) and a  
350 total length of 219,339 bp. The ML phylogenies generated from these alignments recovered the  
351 previously established sub-class and ordinal relationships within Anthozoa (Fig. 1). The  
352 phylogeny generated with the UCE loci had 100% support at all the nodes (Fig. 1a) whereas the  
353 phylogeny generated with the exon loci had complete support at the majority (86%) of the nodes  
354 (Fig. 1b).

355

#### 356 *In Vitro Test*

357 The number of reads obtained from Illumina sequencing ranged from 460,724 to  
358 17,283,798 reads per sample (mean: 5,938,769  $\pm$  3,407,199 SD reads) across all bait strengths  
359 and Kapa kits tested. Quality and adapter trimming lead to the removal of 1.8 to 10.5% reads  
360 from each sample, resulting in a mean of 5,486,800  $\pm$  2,092,161 SD trimmed reads per sample  
361 (Tables 2, S2, S3). Trimmed reads were assembled into 4,699 to 327,623 contigs per sample  
362 (mean: 92,076  $\pm$  65,772 SD contigs) with a mean length of 384  $\pm$  27 bp (range: 224 to 32,406  
363 bp) using Trinity (Tables 2 and S3). Coverage averaged 2.5 to 9.9X per contig. No differences in  
364 numbers of contigs or reads were evident between the individuals prepared using the two  
365 different Kapa kits (Hyper Prep or Hyper Plus) at 1/2 bait strength or between the different bait  
366 strengths used (1/4, 1/8, 1/2, full) (Fig. S1, Tables 2 and S3). Using Abyss, trimmed reads were  
367 assembled into 43,428 to 763,227 contigs per sample with a mean length of only 179  $\pm$  24 bp.

368 Because contig sizes were much smaller from Abyss than those assembled via Trinity, remaining  
369 analyses were done on the Trinity-assembled data.

370 A total of 713 UCE loci and 1,061 exon loci (1,774 total loci out of 1,791 targeted UCE  
371 loci) were recovered from the assembled contigs. Mean length of UCE contigs was  $598 \pm 158$  bp  
372 (range: 224 to 3,995 bp) and mean length of exon contigs was  $593 \pm 156$  bp (range: 224 to 4,500  
373 bp). No differences in numbers of loci recovered were evident between the two different Kapa  
374 kits (Hyper Prep or Hyper Plus) at 1/2 bait strength or between the individuals subjected to the  
375 four different bait strengths used (Fig. S1, Tables 2 and S3). The number of loci recovered from  
376 each species using a Kapa Hyper prep kit with 1/2 bait strength was highly variable, ranging  
377 between 172 to 1034 total loci per sample (mean:  $638 \pm 222$  loci) (Tables 2 and S3), although  
378 few loci (172) were recovered from the sample with the fewest contigs (15,433). More loci were  
379 recovered from octocorals (mean:  $783 \pm 138$  loci, range: 569-1036 loci) compared to hexacorals  
380 (mean:  $475 \pm 187$  loci, range: 172-786 loci), even after removing the sample with the fewest loci  
381 ( $498 \pm 172$  loci).

382 Alignment lengths, locus number and length, and the number of phylogenetically  
383 informative sites varied depending upon percent (25 or 50%) of taxon occupancy per locus and  
384 type of taxonomic dataset (Anthozoa+genome+outgroup, Anthozoa, Hexacorallia, Octocorallia)  
385 included in the GBlocks trimmed alignments (Table 3). The average percentage of  
386 phylogenetically informative sites across all alignments was 39%. For the comparisons within  
387 each of three genera (*Acropora*, *Alcyonium*, *Sinularia*), 382 to 426 loci were retained in the  
388 100% alignment matrices (Table 4). Mean % variable sites per locus ranged from 4.7 to 30%,  
389 with the most variation found in the *Alcyonium* dataset and the least found within *Acropora*.



390 Percent variation per locus ranged from 0 to 55%, with only one non-polymorphic locus found in  
391 the *Acropora* dataset.

392 Tree topologies were mostly congruent between the 25% and 50%  
393 Anthozoa+genome+outgroup data matrices using all loci (Fig. 2., Fig. S2). By rooting to the  
394 outgroup *H. magnipapillata*, monophyly for the currently established anthozoan subclasses and  
395 the hexacoral orders was recovered in both taxon occupancy data matrices. As expected, the  
396 octocoral order Pennatulacea was found nested within Alcyonacea. Only a few branches shifted  
397 between the two data matrices. *Acropora digitifera* was sister to *A. muricata* in the 50% dataset,  
398 but sister to *A. millepora* in the 25% dataset. In Octocorallia, both *Cornularia pabloi* and  
399 *Erythropodium caribaeorum* shifted positions between datasets. In addition, bootstrap support  
400 was higher in the 25% Anthozoa+genome+outgroup tree (Fig. 2) compared to the 50% dataset  
401 tree. At most nodes in the 25% dataset tree, bootstrap support was 97-100%; only three nodes  
402 had lower bootstrap support (59-82%, Fig. 2).

403 Lower bootstrap support also occurred in trees created with only the exon loci or the  
404 UCE loci (Fig. S3), but tree topologies were mostly congruent with the few exceptions noted  
405 above (Fig. S3). We also found that the cerianthids were sister to all other anthozoans in both  
406 25% and 50% exon datasets, but sister to hexacorals in the UCE locus datasets. *Zoanthus* cf.  
407 *pulchellus* was sister to the actinarians in the 25% exon dataset, but sister to a clade containing  
408 Actiniaria, Antipatharia, Corallimorpharia, and Scleractinia in all other datasets (Fig S3).

409

## 410 **Discussion**

411 Our results demonstrate the utility of the target-capture enrichment approach for inferring  
412 phylogenomic relationships in the class Anthozoa. To date, a few studies based on transcriptomic

413 data have recovered well-supported phylogenomic relationships within Anthozoa, but these  
414 studies were based on only a handful ( $\leq 15$ ) of taxa (Zapata *et al.* 2015; Lin *et al.* 2016, Pratlong  
415 *et al.* 2017) and were limited in scope. In general, phylogenomic studies based on transcriptomic  
416 data have provided well-supported and well-resolved phylogenies based on 100s to 1000s of  
417 orthologs (Dunn *et al.* 2008; Kocot *et al.* 2011; Zapata *et al.* 2015). However, obtaining these  
418 types of sequencing data can be relatively expensive and requires high-quality RNA, two  
419 limitations that hinder the transcriptomic-approach for large datasets. In addition, it is often not  
420 feasible to obtain RNA from rare taxa or taxa that have not been properly preserved for  
421 transcriptomics, such as museum specimens. In our study, we show that the sequence-capture  
422 approach for both UCEs and exons can be used to capture genome-scale data in anthozoans. To  
423 date, this approach has not been applied to anthozoans or to marine invertebrates more generally  
424 (except Hugall *et al.* 2016). We successfully designed a novel bait set based on existing  
425 transcriptomes and genomes, and captured 1,774 loci from a diversity of anthozoans spanning  
426 >500 million years of divergence (Peterson *et al.* 2004). This target-enrichment approach has the  
427 capability to resolve a wide range of divergence levels, from deep- (orders, sub-orders) to  
428 shallow-level (species) evolutionary relationships. This novel genomic resource can help to  
429 advance studies of systematics, divergence-time estimation, character evolution, and species  
430 delimitation in the species-rich class Anthozoa.

431

#### 432 *In Vitro Test Results*

433 The newly designed bait set successfully enriched 713 UCE loci and 1,061 exon loci  
434 across a diversity of anthozoans. These loci had an average of 39% phylogenetically informative  
435 sites, comparable to the arachnid (30% PI sites, Starrett *et al.* 2016) UCE dataset, which targeted

436 ~1,000 loci. The large range of loci recovered per anthozoan species (172 to 1036 loci) was also  
437 similar to the arachnid results (170 to 722 loci). We note that the number of loci recovered from  
438 octocorals was much higher than what was recovered from hexacorals. This result is perhaps  
439 because we added more octocoral-specific baits to the final bait set. And as we added more  
440 octocoral-specific baits, we removed baits that were potential paralogs; the majority of these  
441 were designed based on the hexacorals. As was done for the hymenopteran UCE bait set  
442 (Branstetter *et al.* 2017), we need to re-design the baitset and include additional octocoral-  
443 specific baits and hexacoral-specific baits to increase the success of locus capture. We will also  
444 design separate octocoral- and hexacoral-specific bait sets so that additional loci specific to each  
445 sub-class can be targeted. Nevertheless, this first bait design and *in vitro* results from 33 taxa  
446 demonstrate the promising utility of the target-capture method for resolving anthozoan  
447 relationships across deep divergence levels.

448         The number of variable sites found at loci recovered from within three genera  
449 demonstrates that this is also a promising approach to delimit species boundaries. Within all  
450 three genera examined, variable sites ranged up to 55% per locus, with a mean variation across  
451 all loci of 4.7, 5.5, and 30% in *Sinularia*, *Acropora*, and *Alcyonium*, respectively. The high  
452 variation seen within *Alcyonium* is consistent with unpublished data (C. McFadden, unpubl. data)  
453 that suggest the three species are perhaps different genera. For *Sinularia*, average divergence  
454 estimates are also higher (~10X) than what has been demonstrated in other studies using  
455 mitochondrial barcoding markers (McFadden *et al.* 2009). In fact, a 0.5% divergence level at an  
456 extended mitochondrial barcode (mtMutS+igrI+COI) was proposed as a conservative criterion  
457 for species delimitation (McFadden *et al.* 2011, 2014). Similarly, low divergence estimates at  
458 mitochondrial barcoding markers have been found among hexacoral congeners (Shearer and

459 Coffroth 2008; Brugler *et al.* 2013; Gonzalez-Muñoz *et al.* 2015). Thus, these UCE and exon  
460 loci are promising for resolving species boundaries, although we still need to determine the level  
461 of intraspecific variation. Recently, restriction-site associated sequencing (RADSeq) has been  
462 successfully used to delimit species within anthozoan genera (e.g., Combosch and Vollmer 2015;  
463 Pante *et al.* 2015; Herrera *et al.* 2016; McFadden *et al.* 2017; Johnston *et al.* 2017). Although  
464 RADSeq is an effective approach for delimiting species and population structure, addressing  
465 deeper-level relationships is not feasible due to locus drop out (Althoff *et al.* 2007; McCormack  
466 *et al.* 2013a). Our UCE and exon locus datasets may serve as an alternative resource to address  
467 species-boundary questions while allowing for data to be combined and examined across deeper  
468 levels.

469         Because this was the first time the target-enrichment UCE approach had been tested on  
470 anthozoans, we compared different concentrations of baits and different library preparation kits  
471 to determine whether or not particular methods would recover more loci. We found no  
472 differences in the number of loci recovered using different concentrations of baits in the  
473 hybridization and enrichment protocols. This bait-strength test suggested that the number of  
474 hybridizations obtained from one standard reaction could, at least, be doubled. We also found no  
475 differences between the two different Kapa kits used. The enzymatic DNA shearing that can be  
476 performed with the Kapa Hyper Plus kit may be useful for researchers who do not have access to  
477 a sonicator.

478         Following trimming and aligning of conserved loci, the mean locus length was much  
479 shorter (~190 bp) compared to the mean length of un-trimmed loci (~600 bp). Therefore, some of  
480 the loci included in the ML analyses were relatively short (< 100 bp), particularly in the  
481 Anthozoa+genome+outgroup dataset. In alignments between highly divergent taxa (such as

482 between hexacorals and octacorals), numerous poorly aligned positions and divergent positions  
483 were filtered with GBlocks. In contrast, the locus size was considerably higher within genera  
484 (~525 bp) because of fewer poorly aligned and divergent positions. Perhaps re-performing the  
485 GBlocks internal trimming with less stringent parameters would increase the size of loci in  
486 alignments of divergent taxa. Alternatively, by not using GBlocks in the pipeline, we could  
487 increase the size of loci and perhaps the accuracy of tree inference (Tan *et al.* 2015). Stringent  
488 alignment filtering, as done with GBlocks, can not only increase the proportion of unresolved  
489 branches, but can also lead to well-supported branches that are in fact incorrect (Tan *et al.* 2015).  
490 Different methods of aligning and filtering data will be explored in future work.

491 The phylogenies produced from the *in vitro* data were highly supported despite low  
492 overall taxon occupancy (>25 or 50% matrices) and inclusion of short loci. Bootstrap support at  
493 most nodes was >97% in both trees, although there were a few nodes that had low support and a  
494 few branches that shifted between datasets, particularly in the Octocorallia. In addition to  
495 stringent filtering as discussed above, sources of incongruence and low bootstrap support could  
496 include compositional bias, saturation, violations of model assumptions (Jeffroy *et al.* 2006)  
497 and/or missing data. Missing data, however, are generally not problematic if there are a  
498 reasonable number of informative characters (see Streicher *et al.* 2015). Rather, incongruence  
499 and low support at a few nodes is perhaps due to incomplete taxon sampling (Wiens 2005; Wiens  
500 and Tiu 2012). Although a diversity of taxa from across the clades were selected for *in vitro*  
501 analyses, several lineages were not represented, particularly in the Octocorallia. Outgroup choice  
502 and taxon evenness can also impact topology and clade support in UCE phylogenomics  
503 (Branstetter *et al.* 2017). Future efforts will need to incorporate more thorough taxon sampling.

504 In general, the inferred phylogenetic relationships corresponded to those found in  
505 previous studies (Zapata *et al.* 2015; Rodríguez *et al.* 2014), although there were a few  
506 exceptions. One exception was the position of *Cornularia pabloi*. This stoloniferan octocoral  
507 was nested within the clade containing sea pens (Pennatulacea) and calcaxonians (*C. tricaulis*,  
508 Keratoisidinae sp.), but this species has been found to be sister to the rest of the octocorals based  
509 on mitochondrial data (McFadden and vanOfwegen 2012). The superfamily Actinostoloidea  
510 (*Sicyonis* sp., *Stomphia* sp.) was recovered as sister to superfamily Actinioidea  
511 (*Actinostella* sp., *Isosicyonis alba*) differing from a combined mitochondrial and nuclear rDNA  
512 dataset (Rodríguez *et al.* 2014), which instead recovered Actinostoloidea as sister to both  
513 Actinioidea and Metridioidea (*Lebrunia danae*, *E. pallida*, *Bunodeopsis* sp.). Furthermore, trees  
514 in our study were rooted to *H. magnipapillata*, based on the results of Zapata *et al.* (2015);  
515 however, the unrooted trees indicated that *H. magnipapillata* was sister to the Octocorallia, a  
516 relationship that has been noted in mitochondrial data (Park *et al.* 2012, Kayal *et al.* 2013), but  
517 not supported by phylogenomic analyses (Zapata *et al.* 2015). Zapata *et al.* (2015) also found  
518 that the position of the order Ceriantharia was phylogenetically unstable. Similarly, our results  
519 indicated that the placement of Ceriantharia changed between the different datasets. The  
520 topologies resulting from exon data placed the ceriantharians as sister to the anthozoans, a  
521 relationship also supported by mitochondrial data (Stampar *et al.* 2014). Trees from UCE loci  
522 had ceriantharians as sister to hexacorals, a relationship also supported by combined  
523 mitochondrial and nuclear rDNA data (Rodríguez *et al.* 2014). Future work must include  
524 different outgroup choices (i.e., sponges), while closely examining the distribution and strength  
525 of phylogenetic signal. This will help clarify the source of incongruence and resolve which loci  
526 strongly influence the resolution of a given ‘contentious’ branch (Shen *et al.* 2017).

527 Whether or not scleractinians are monophyletic has been a controversial topic as a result  
528 of different phylogenetic analyses. In 2006, Medina *et al.* reported that scleractinians were  
529 polyphyletic with corallimorpharians. The “naked coral hypothesis” was thus proposed,  
530 suggesting that corallimorpharians arose from a scleractinian ancestor that had undergone  
531 skeletal loss during paleoclimate conditions when the oceans experienced increased CO<sub>2</sub>  
532 concentrations (Medina *et al.* 2006). Since that study, other studies based on transcriptomic data  
533 (Lin *et al.* 2016), rDNA (Fukami *et al.* 2008), and mitochondrial data (Fukami *et al.* 2008; Park  
534 *et al.* 2012, Kayal *et al.* 2013; Kitahara *et al.* 2014) recovered a monophyletic Scleractinia with  
535 corallimorpharians as the sister clade. Our results also recovered a monophyletic Scleractinia;  
536 thus supporting the conclusions of others that corallimorpharians are not naked corals. However,  
537 increased sampling of robust, complex, and basal scleractinians is necessary to conclusively  
538 address this issue.

539

#### 540 *Future Research Directions*

541 The *in silico* and *in vitro* tests of the novel bait set demonstrate that the target-enrichment  
542 approach of UCEs and exons is a promising new genomic resource for inferring phylogenetic  
543 relationships among anthozoans. Using this bait set, target-capture enrichment of the UCE and  
544 exon loci from at least 192 additional anthozoans is currently underway to further our  
545 understanding of character evolution and systematics of the clade. Adding more taxa will likely  
546 increase the accuracy of the phylogenetic inference. We also plan to sequence additional  
547 outgroup taxa, including medusozoan cnidarians and sponges to help address whether or not  
548 octocorals are sister to hexacorals or medusozoans and resolve the position of ceriantharians.  
549 Finally, we plan to re-design the bait sets to create hexacoral- and octocoral-specific bait sets.

550 We will include additional baits to increase the capture efficiency of loci that were targeted in  
551 this study, while adding more loci that are specific to each sub-class. This target-enrichment  
552 approach provides a promising genomic resource to resolve phylogenetic relationships at deep to  
553 shallow levels of divergence, considerably advancing the current state of knowledge of  
554 anthozoan evolution.

555

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565

## 566 **Author Contributions**

567

568 AMQ, CSM, ER, and BCF conceived and designed this study. AMQ designed the baits,  
569 conducted library preparation, target enrichment, and data analyses, and wrote the initial draft of  
570 the manuscript. BCF developed protocols and guided AMQ in laboratory and bioinformatic  
571 analyses. LFD helped with preliminary analyses. MB, ER, and CSM extracted DNA. ICB, DMD,  
572 SF, SH, SL, DJM, CP, GRB, CRP, and JAS provided genomic or transcriptomic data for  
573 analysis. TB provided samples. All authors edited and approved the final version of this  
574 manuscript.

575

576

## 577 **Data Accessibility**

578 Tree and alignment files: Data Dryad Entry XXXX

579 Raw Data: SRA Genbank

580 Anthozoan bait set: Data Dryad Entry XXXX

581 Scripts: Supplemental file 1

582

## 583 **References**

584

585 Althoff, D. M., Gitzendanner, M. A., & Segraves, K. A. (2007). The utility of amplified  
586 fragment length polymorphisms in phylogenetics: a comparison of homology within and  
587 between genomes. *Systematic Biology*, 56(3), 477-484.

588



- 589 Baca, S. M., Alexander, A., Gustafson, G. T., & Short, A. E. (2017). Ultraconserved elements  
590 show utility in phylogenetic inference of Adephaga (Coleoptera) and suggest paraphyly of  
591 '*Hydradephaga*'. *Systematic Entomology*, doi:10.1111/syen.12244  
592
- 593 Berntson, E. A., Bayer, F. M., McArthur, A. G., & France, S. C. (2001). Phylogenetic  
594 relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA  
595 sequences. *Marine Biology*, 138(2), 235-246.  
596
- 597 Bi, K., Vanderpool, D., Singhal, S., Linderth, T., Moritz, C., & Good, J. M. (2012).  
598 Transcriptome-based exon capture enables highly cost-effective comparative genomic data  
599 collection at moderate evolutionary scales. *BMC Genomics*, 13(1), 403.  
600
- 601 Bilewicz, J. P., & Degnan, S. M. (2011). A unique horizontal gene transfer event has provided  
602 the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an  
603 unusual self-contained function. *BMC Evolutionary Biology*, 11(1), 228.  
604
- 605 Blumenstiel, B., Cibulskis, K., Fisher, S., DeFelice, M., Barry, A., Fennell, T., ... & Maquire, J.  
606 (2010). Targeted Exon Sequencing by In-Solution Hybrid Selection. *Current Protocols in Human*  
607 *Genetics*, 18-4.  
608
- 609 Branstetter, M. G., Longino, J. T., Ward, P. S., & Faircloth, B. C. (2017). Enriching the ant tree  
610 of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other  
611 Hymenoptera. *Methods in Ecology and Evolution*. 8: 768–776. doi:10.1111/2041-210X.12742  
612
- 613 Brugler, M. R., Opresko, D. M., & France, S. C. (2013). The evolutionary history of the order  
614 Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear  
615 DNA: implications for black coral taxonomy and systematics. *Zoological Journal of the Linnean*  
616 *Society*, 169(2), 312-361.  
617
- 618 Bushnell, B. (2015). BBMap short-read aligner, and other bioinformatics tools. Available from:  
619 [sourceforge.net/projects/bbmap](http://sourceforge.net/projects/bbmap).  
620
- 621 Carpenter, K. E., Abrar, M., Aeby, G., Aronson, R. B., Banks, S., Bruckner, A., ... & Edgar, G. J.  
622 (2008). One-third of reef-building corals face elevated extinction risk from climate change and  
623 local impacts. *Science*, 321(5888), 560-563.  
624
- 625 Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in  
626 phylogenetic analysis. *Molecular Biology and Evolution*, 17(4), 540-552.  
627
- 628 Combosch, D. J., & Vollmer, S. V. (2015). Trans-Pacific RAD-Seq population genomics  
629 confirms introgressive hybridization in Eastern Pacific Pocillopora corals. *Molecular*  
630 *Phylogenetics and Evolution*, 88, 154-162.  
631
- 632 Crawford, N. G., Faircloth, B. C., McCormack, J. E., Brumfield, R. T., Winker, K., & Glenn, T.  
633 C. (2012). More than 1000 ultraconserved elements provide evidence that turtles are the sister  
634 group of archosaurs. *Biology Letters*, 8(5), 783-786.  
635

- 636 Daly, M., Fautin, D. G., & Cappola, V. A. (2003). Systematics of the hexacorallia (Cnidaria:  
637 Anthozoa). *Zoological Journal of the Linnean Society*, 139(3), 419-437.  
638
- 639 Daly, M., Brugler, M. R., Cartwright, P., Collins, A. G., Dawson, M. N., Fautin, D. G., ... &  
640 Romano, S. L. (2007). The phylum Cnidaria: a review of phylogenetic patterns and diversity 300  
641 years after Linnaeus. *Zootaxa*, 1668, 127–182.  
642
- 643 Daly, M., Gusmão, L., Reft, A., & Rodríguez, E. (2010) Phylogenetic signal in mitochondrial  
644 and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative Comparative Biology*  
645 50(3), 371-388.  
646
- 647 Daly, M., Crowley, L. M., Larson, P., Rodríguez, E., Saucier, E. H., & Fautin, D. G. (2017).  
648 Anthopleura and the phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). *Organisms*  
649 *Diversity & Evolution*, 1-20.  
650
- 651 Dueñas, L. F., Alderslade, P., & Sánchez, J. A. (2014). Molecular systematics of the deep-sea  
652 bamboo corals (Octocorallia: Isididae: Keratoisidinae) from New Zealand with descriptions of  
653 two new species of Keratoisis. *Molecular Phylogenetics and Evolution*, 74, 15-28.  
654
- 655 Dunn, C. W., Hejnal, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., ... & Sørensen,  
656 M. V. (2008). Broad phylogenomic sampling improves resolution of the animal tree of  
657 life. *Nature*, 452(7188), 745-749.  
658
- 659 Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., & Glenn,  
660 T. C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple  
661 evolutionary timescales. *Systematic Biology*, 61(5), 717-726.  
662
- 663 Faircloth, B. C., Sorenson, L., Santini, F., & Alfaro, M. E. (2013). A phylogenomic perspective  
664 on the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved elements  
665 (UCEs). *PLoS One*, 8(6), e65923, doi:10.1111/2041-210X.12754.  
666
- 667 Faircloth, B.C. (2016). PHYLUCE is a software package for the analysis of conserved genomic  
668 loci. *Bioinformatics*, 32(5), 786-788.  
669
- 670 Faircloth, B.C. (2017). Identifying conserved genomic elements and designing universal bait sets  
671 to enrich them. *Methods in Ecology and Evolution*.  
672
- 673 Fautin, D.G., & Mariscal, R.N. (1991). *Cnidaria: Anthozoa* (Vol. 2, pp. 267-358). New York:  
674 Wiley-Liss.  
675
- 676 Forsman, Z.H., Barshis, D.J., Hunter, C.L. and Toonen, R.J., 2009. Shape-shifting corals:  
677 molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC evolutionary*  
678 *biology*, 9(1), p.45.  
679
- 680 Fukami, H., Chen, C. A., Budd, A. F., Collins, A., Wallace, C., Chuang, Y. Y., ... & Knowlton,  
681 N. (2008). Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most

682 families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). PloS  
683 one, 3(9), e3222.

684

685 Gardner, T. A., Côté, I. M., Gill, J. A., Grant, A., & Watkinson, A. R. (2003). Long-term region-  
686 wide declines in Caribbean corals. *Science*, 301(5635), 958-960.

687

688 Glenn, T. C., Nilsen, R., Kieran, T. J., Finger, J. W., Pierson, T. W., Bentley, K. E., ... & Reed,  
689 K. (2016). Adapterama I: universal stubs and primers for thousands of dual-indexed Illumina  
690 libraries (iTru & iNext). *BioRxiv*, 049114.

691

692 González-Muñoz, R., Simões, N., Mascaró, M., Tello-Musi, J. L., Brugler, M. R., & Rodríguez,  
693 E. (2015). Morphological and molecular variability of the sea anemone *Phymanthus crucifer*  
694 (Cnidaria, Anthozoa, Actiniaria, Actinoidea). *Journal of the Marine Biological Association of the*  
695 *United Kingdom*, 95(1), 69-79.

696

697 Grajales, A., & Rodríguez, E. (2016). Elucidating diversity within the Aiptasiidae, a widespread  
698 cnidarian-dinoflagellate model system (Cnidaria: Anthozoa: Actiniaria: Metridioidea). *Molecular*  
699 *Phylogenetics and Evolution*, 94(A), 252-263.

700

701 Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., & Plattner, G. K. (2012). Rapid  
702 progression of ocean acidification in the California Current System. *Science*, 337(6091), 220-  
703 223.

704

705 Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., ... & Regev,  
706 A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform  
707 for reference generation and analysis. *Nature Protocols*, 8(8), 1494-1512.

708

709 Hebert, P. D., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: cytochrome c  
710 oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society*  
711 *of London B: Biological Sciences*, 270(Suppl. 1), S96-S99.

712

713 Hellberg, M. E. (2006). No variation and low synonymous substitution rates in coral mtDNA  
714 despite high nuclear variation. *BMC Evolutionary biology*, 6(1), 24.

715

716 Herrera, S., & Shank, T. M. (2016). RAD sequencing enables unprecedented phylogenetic  
717 resolution and objective species delimitation in recalcitrant divergent taxa. *Molecular*  
718 *Phylogenetics and Evolution*, 100, 70-79.

719

720 Huang, D., Meier, R., Todd, P. A., & Chou, L. M. (2008). Slow mitochondrial COI sequence  
721 evolution at the base of the metazoan tree and its implications for DNA barcoding. *Journal of*  
722 *Molecular Evolution*, 66(2), 167-174.

723

724 Huang, W., Li, L., Myers, J. R., & Marth, G. T. (2012). ART: a next-generation sequencing read  
725 simulator. *Bioinformatics*, 28(4), 593-594.

726

- 727 Hugall, A. F., O'Hara, T. D., Hunjan, S., Nilsen, R., & Moussalli, A. (2016). An exon-capture  
728 system for the entire class Ophiuroidea. *Molecular biology and evolution*, 33(1), 281-294.  
729
- 730 Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D.,  
731 Baird, A. H., ... & Bridge, T. C. (2017). Global warming and recurrent mass bleaching of  
732 corals. *Nature*, 543(7645), 373-377.  
733
- 734 Ilves, K. L., & López-Fernández, H. (2014). A targeted next-generation sequencing toolkit for  
735 exon-based cichlid phylogenomics. *Molecular Ecology Resources*, 14(4), 802-811.  
736
- 737 Jeffroy, O., Brinkmann, H., Delsuc, F., & Philippe, H. (2006). Phylogenomics: the beginning of  
738 incongruence?. *TRENDS in Genetics*, 22(4), 225-231.  
739
- 740 Johnston, E.C., Z.H. Forsman, J. François Flot, S. Schmidt-Roach, J.H. Pinzón, I.S.S. Knapp,  
741 and R.J. Toonen. (2017). A genomic glance through the fog of plasticity and diversification in  
742 Pocillopora. *Scientific Reports* 7,  
743
- 744 Kayal, E., Roure, B., Philippe, H., Collins, A. G., & Lavrov, D. V. (2013). Cnidarian  
745 phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, 13(1), 5.  
746
- 747 Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: a novel method for rapid  
748 multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, 30(14),  
749 3059-3066.
- 750
- 751 Kent, W. J. (2002). BLAT—the BLAST-like alignment tool. *Genome research*, 12(4), 656-664.  
752
- 753 Kim, E., Lasker, H. R., Coffroth, M. A., & Kim, K. (2004). Morphological and genetic variation  
754 across reef habitats in a broadcast-spawning octocoral. *Hydrobiologia*, 530(1-3), 423-432.  
755
- 756 Kitahara, M. V., Lin, M. F., Forêt, S., Huttley, G., Miller, D. J., & Chen, C. A. (2014). The  
757 “naked coral” hypothesis revisited—evidence for and against scleractinian monophyly. *PLoS*  
758 *One*, 9(4), e94774.  
759
- 760 Kocot, K. M., Cannon, J. T., Todt, C., Citarella, M. R., Kohn, A. B., Meyer, A., ... & Halanych,  
761 K. M. (2011). Phylogenomics reveals deep molluscan relationships. *Nature*, 477(7365), 452-456.  
762
- 763 Levitus, S., Antonov, J. I., Boyer, T. P., & Stephens, C. (2000). Warming of the world  
764 ocean. *Science*, 287(5461), 2225-2229.  
765
- 766 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... & Durbin, R. (2009).  
767 The sequence alignment/map format and SAMtools. *Bioinformatics*, 25(16), 2078-2079.  
768
- 769 Lin, M. F., Chou, W. H., Kitahara, M. V., Chen, C. L. A., Miller, D. J., & Forêt, S. (2016).  
770 Corallimorpharians are not “naked corals”: insights into relationships between Scleractinia and  
771 Corallimorpharia from phylogenomic analyses. *PeerJ*, 4, e2463.  
772

- 773 Lunter, G., & Goodson, M. (2011). Stampy: a statistical algorithm for sensitive and fast mapping  
774 of Illumina sequence reads. *Genome Research*, 21(6), 936-939.  
775
- 776 Manthey, J. D., Campillo, L. C., Burns, K. J., & Moyle, R. G. (2016). Comparison of target-  
777 capture and restriction-site associated DNA sequencing for phylogenomics: a test in cardinalid  
778 tanagers (Aves, Genus: *Piranga*). *Systematic Biology*, 65(4), 640-650.  
779
- 780 McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T., & Glenn,  
781 T. C. (2012). Ultraconserved elements are novel phylogenomic markers that resolve placental  
782 mammal phylogeny when combined with species-tree analysis. *Genome research*, 22(4), 746-  
783 754.  
784
- 785 McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013a).  
786 Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular*  
787 *Phylogenetics and Evolution*, 66(2), 526-538.  
788
- 789 McCormack, J. E., Harvey, M. G., Faircloth, B. C., Crawford, N. G., Glenn, T. C., & Brumfield,  
790 R. T. (2013b). A phylogeny of birds based on over 1,500 loci collected by target enrichment and  
791 high-throughput sequencing. *PLoS One*, 8(1), e54848.  
792
- 793 McCormack, J. E., Tsai, W. L., & Faircloth, B. C. (2016). Sequence capture of ultraconserved  
794 elements from bird museum specimens. *Molecular Ecology Resources*, 16(5), 1189-1203.  
795
- 796 McFadden, C. S., Alderslade, P., Van Ofwegen, L. P., Johnsen, H., & Rusmevichientong, A.  
797 (2006). Phylogenetic relationships within the tropical soft coral genera *Sarcophyton* and  
798 *Lobophytum* (Anthozoa, Octocorallia). *Invertebrate Biology*, 125(4), 288-305.  
799
- 800 McFadden, C. S., Van Ofwegen, L. P., Beckman, E. J., Benayahu, Y., & Alderslade, P. (2009).  
801 Molecular systematics of the speciose Indo-Pacific soft coral genus, *Sinularia* (Anthozoa:  
802 Octocorallia). *Invertebrate Biology*, 128(4), 303-323.  
803
- 804 McFadden, C. S., Sánchez, J. A., & France, S. C. (2010). Molecular phylogenetic insights into  
805 the evolution of Octocorallia: a review. *Integrative and Comparative Biology*, 50, 389-410.  
806
- 807 McFadden, C. S., Benayahu, Y., Pante, E., Thoma, J. N., Nevarez, P. A., & France, S. C. (2011).  
808 Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology*  
809 *Resources*, 11(1), 19-31.  
810
- 811 McFadden, C. S., & van Ofwegen, L. P. (2012). Stoloniferous octocorals (Anthozoa,  
812 Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus  
813 of Clavulariidae, and a new species of *Cornularia* (Cornulariidae). *Invertebrate*  
814 *Systematics*, 26(4), 331-356.  
815
- 816 McFadden, C. S., Brown, A. S., Brayton, C., Hunt, C. B., & van Ofwegen, L. P. (2014).  
817 Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular

- 818 proxies agree with morphological estimates of species richness in Palau. *Coral Reefs*, 33(2), 275-  
819 286.
- 820
- 821 McFadden, C. S., Haverkort-Yeh, R., Reynolds, A. M., Halász, A., Quattrini, A. M., Forsman, Z.  
822 H., Benayahu, Y., & Toonen, R. J. (2017). Species boundaries in the absence of morphological,  
823 ecological or geographical differentiation in the Red Sea octocoral genus *Ovabunda*  
824 (Alcyonacea: Xeniidae). *Molecular Phylogenetics and Evolution*, 112, 174-184.
- 825
- 826 Medina, M., Weil, E., & Szmant, A. M. (1999). Examination of the *Montastraea annularis*  
827 species complex (Cnidaria: Scleractinia) using ITS and COI sequences. *Marine*  
828 *Biotechnology*, 1(1), 89-97.
- 829
- 830 Medina, M., Collins, A. G., Takaoka, T. L., Kuehl, J. V., & Boore, J. L. (2006). Naked corals:  
831 skeleton loss in Scleractinia. *Proceedings of the National Academy of Sciences*, 103(24), 9096-  
832 9100.
- 833
- 834 Pandolfi, J. M., Bradbury, R. H., Sala, E., Hughes, T. P., Bjorndal, K. A., Cooke, R. G., ... &  
835 Warner, R. R. (2003). Global trajectories of the long-term decline of coral reef  
836 ecosystems. *Science*, 301(5635), 955-958.
- 837
- 838 Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S. C., Boisselier, M. C., & Samadi, S.  
839 (2015). Use of RAD sequencing for delimiting species. *Heredity*, 114(5), 450-459.
- 840
- 841 Park, E., Hwang, D. S., Lee, J. S., Song, J. I., Seo, T. K., & Won, Y. J. (2012). Estimation of  
842 divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the  
843 fossil record. *Molecular Phylogenetics and Evolution*, 62(1), 329-345.
- 844
- 845 Paz-García, D. A., Hellberg, M. E., García-de-León, F. J., & Balart, E. F. (2015). Switch  
846 between morphospecies of *Pocillopora* corals. *The American Naturalist*, 186(3), 434-440.
- 847
- 848 Peterson, K.J., Lyons, J.B., Nowak, K.S., Takacs, C.M., Wargo, M.J. & McPeck, M.A. (2004).  
849 Estimating metazoan divergence times with a molecular clock. *Proceedings of the National*  
850 *Academy of Sciences*, 101(17), 6536-6541.
- 851
- 852 Pinzon, J. H., & LaJeunesse, T.D. (2011). Species delimitation of common reef corals in the  
853 genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis  
854 ecology. *Molecular Ecology*, 20(2), 311-325.
- 855
- 856 Prada, C., DeBiase, M. B., Neigel, J. E., Yednock, B., Stake, J. L., Forsman, Z. H., ... &  
857 Hellberg, M. E. (2014). Genetic species delineation among branching Caribbean *Porites*  
858 corals. *Coral Reefs*, 33(4), 1019-1030.
- 859
- 860 Pratlong, M., Rancurel, C., Pontarotti, P., & Aurelle, D. (2017). Monophyly of Anthozoa  
861 (Cnidaria): why do nuclear and mitochondrial phylogenies disagree? *Zoologica Scripta*, 46(3),  
862 363-371.
- 863

- 864 Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing  
865 genomic features. *Bioinformatics*, 26(6), 841-842.  
866
- 867 Rodríguez, E., Barbeitos, M. S., Brugler, M. R., Crowley, L. M., Grajales, A., Gusmão, L., ... &  
868 Daly, M. (2014). Hidden among sea anemones: the first comprehensive phylogenetic  
869 reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group  
870 of hexacorals. *PLoS One*, 9(5), e96998.  
871
- 872 Ruane, S., & Austin, C. C. (2017). Phylogenomics using formalin-fixed and 100+ year-old  
873 intractable natural history specimens. *Molecular Ecology Resources*, doi:10.1111/1755-  
874 0998.12655  
875
- 876 Ryu, T., Seridi, L., & Ravasi, T. (2012). The evolution of ultraconserved elements with different  
877 phylogenetic origins. *BMC Evolutionary Biology*, 12(1), 236.  
878
- 879 Schmidtko, S., Stramma, L., & Visbeck, M. (2017). Decline in global oceanic oxygen content  
880 during the past five decades. *Nature*, 542(7641), 335-339.  
881
- 882 Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial  
883 DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11(12), 2475-2487.  
884
- 885 Shearer, T. L., & Coffroth, M. A. (2008). DNA BARCODING: Barcoding corals: limited by  
886 interspecific divergence, not intraspecific variation. *Molecular Ecology Resources*, 8(2), 247-  
887 255.  
888
- 889 Shen, X. X., Hittinger, C. T., & Rokas, A. (2017). Contentious relationships in phylogenomic  
890 studies can be driven by a handful of genes. *Nature Ecology & Evolution*, 1, 0126.  
891
- 892 Simpson, J. T., Wong, K., Jackman, S. D., Schein, J. E., Jones, S. J., & Birol, I. (2009). ABySS:  
893 a parallel assembler for short read sequence data. *Genome Research*, 19(6), 1117-1123.  
894
- 895 Smit, AFA, Hubley, R & Green, P. (2015). RepeatMasker Open-4.0.  
896 <<http://www.repeatmasker.org>>.  
897
- 898 Smith, B. T., Harvey, M. G., Faircloth, B. C., Glenn, T. C., & Brumfield, R. T. (2014). Target  
899 capture and massively parallel sequencing of ultraconserved elements for comparative studies at  
900 shallow evolutionary time scales. *Systematic Biology*, 63(1), 83-95.  
901
- 902 Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of  
903 large phylogenies. *Bioinformatics*, 30(9), 1312-1313.  
904
- 905 Stampar, S. N., Maronna, M. M., Kitahara, M. V., Reimer, J. D., & Morandini, A. C. (2014).  
906 Fast-evolving mitochondrial DNA in Ceriantharia: a reflection of hexacorallia paraphyly? *PLoS*  
907 *One*, 9(1), e86612.  
908

909 Starrett, J., Derkarabetian, S., Hedin, M., Bryson, R. W., McCormack, J. E., & Faircloth, B. C.  
910 (2016). High phylogenetic utility of an ultraconserved element probe set designed for  
911 Arachnida. *Molecular Ecology Resources*, 17(4), 812-823, doi:10.1111/1755-0998.12621  
912  
913 Streicher, J. W., Schulte, J. A., & Wiens, J. J. (2015). How should genes and taxa be sampled for  
914 phylogenomic analyses with missing data? An empirical study in iguanian lizards. *Systematic  
915 Biology*, 65(1), 128-145.  
916  
917 Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and  
918 ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, 56(4), 564-  
919 577.  
920  
921 Tan, G., Muffato, M., Ledergerber, C., Herrero, J., Goldman, N., Gil, M., & Dessimoz, C.  
922 (2015). Current methods for automated filtering of multiple sequence alignments frequently  
923 worsen single-gene phylogenetic inference. *Systematic Biology*, 64(5), 778-791.  
924  
925 Van Oppen, M. J. H., Willis, B. L., Van Vugt, H. W. J. A., & Miller, D. J. (2000). Examination  
926 of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear  
927 DNA sequence analyses. *Molecular Ecology*, 9(9), 1363-1373.  
928  
929 Vollmer, S. V., & Palumbi, S. R. (2004). Testing the utility of internally transcribed spacer  
930 sequences in coral phylogenetics. *Molecular Ecology*, 13(9), 2763-2772.  
931  
932 Wiens, J. J. (2005). Can incomplete taxa rescue phylogenetic analyses from long-branch  
933 attraction? *Systematic Biology*, 54(5), 731-742.  
934  
935 Wiens, J. J., & Tiu, J. (2012). Highly incomplete taxa can rescue phylogenetic analyses from the  
936 negative impacts of limited taxon sampling. *PloS one*, 7(8), e42925.  
937  
938 Zapata, F., Goetz, F. E., Smith, S. A., Howison, M., Siebert, S., Church, S. H., ... & Daly, M.  
939 (2015). Phylogenomic analyses support traditional relationships within Cnidaria. *PLoS  
940 One*, 10(10), e0139068.  
941  
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955 **Figure Captions**

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957 Figure 1. Maximum likelihood phylogenies from *in silico* analyses with bootstrap-support  
958 values. A) Phylogeny constructed with a 138,778 bp concatenated genomic dataset (522 loci)  
959 and rooted to *Hydra magnipapillata*. B) Phylogeny constructed with 219,339 bp concatenated  
960 transcriptome dataset (407 loci). The Hexacorallia clade was rooted to the Octocorallia clade.  
961 Nodes have 100% bootstrap support unless indicated. Branches are color coded by order  
962 (green=Ceriantharia, pink=Zoantharia, purple=Scleractinia, blue=Actiniaria, red=Alcyonacea,  
963 grey=Pennatulacea)

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965 Figure 2. Maximum likelihood phylogeny on the Anthozoa+genome+outgroup 25% matrix  
966 (257,728 bp, 1378 loci) with bootstrap-support values. The tree includes 33 taxa from the *in vitro*  
967 test, 9 genome-enabled taxa, and the outgroup *Hydra magnipapillata*. Nodes have 100%  
968 bootstrap support unless indicated. Branches are color coded by order (green=Ceriantharia,  
969 pink=Zoantharia, brown=Antipatharia, purple=Scleractinia, lt. blue=Corallimorpharia,  
970 blue=Actiniaria, red=Alcyonacea grey=Pennatulacea)

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971 Table 1. Number of loci recovered from *in silico* analyses after initial and final screens for potential paralogs. Also included are the  
 972 N50 and number of scaffolds for each genome/transcriptome used in analyses.

| Species                          | #Scaffolds | N50     | # Loci Recovered |              |
|----------------------------------|------------|---------|------------------|--------------|
|                                  |            |         | Initial Screen   | Final Screen |
| Genomes                          |            |         |                  |              |
| <i>Acropora digitifera</i>       | 4,765      | 191,489 | 462              | 395          |
| <i>Acropora millepora</i>        | 12,559     | 181,771 | 511              | 414          |
| <i>Antillogorgia bipinnata</i>   | 426,978    | 3,212   | 230              | 134          |
| <i>Exaiptasia pallida</i>        | 4,312      | 442,145 | 518              | 417          |
| <i>Nematostella vectensis</i>    | 10,804     | 472,588 | 496              | 421          |
| <i>Pacifigorgia irene</i>        | 183,211    | 2,323   | 547              | 491          |
| <i>Paragorgia stephencairnsi</i> | 700,190    | 1,793   | 453              | 371          |
| <i>Renilla muelleri</i>          | 4,114      | 19,024  | 599              | 529          |
| <i>Stomphia</i> sp.              | 479,824    | 948     | 44               | 25           |
| <i>Hydra magnipapillata</i>      | 126,667    | 10,113  | 449              | 99           |
| Transcriptomes                   |            |         |                  |              |
| <i>Acropora digitifera</i>       | 36,780     | 1,575   | 857              | 620          |
| <i>Acropora hyacinthus</i>       | 67,844     | 422     | 392              | 296          |
| <i>Anemonia</i> sp.              | 14,279     | 703     | 235              | 106          |
| <i>Anthomastus</i> sp.           | 9,368      | 610     | 339              | 272          |
| <i>Anthopleura elegantissima</i> | 142,934    | 1,489   | 364              | 207          |
| Cerianthidae sp.                 | 12,074     | 646     | 336              | 157          |
| <i>Corallium rubrum</i>          | 48,074     | 2,470   | 734              | 606          |
| <i>Edwardsia lineata</i>         | 90,440     | 1,035   | 841              | 623          |
| <i>Eunicea flexuosa</i>          | 165,709    | 1,095   | 580              | 507          |
| <i>Exaiptasia pallida</i>        | 60,101     | 2,159   | 553              | 264          |
| <i>Fungia scutaria</i>           | 155,914    | 1,619   | 290              | 188          |
| <i>Gorgonia ventalina</i>        | 90,230     | 1,149   | 731              | 670          |
| Keratoisidinae                   | 12,385     | 702     | 541              | 429          |
| <i>Metridium</i> sp.             | 10,885     | 752     | 222              | 111          |
| <i>Montastraea cavernosa</i>     | 200,222    | 2,145   | 206              | 128          |

|                                 |         |       |     |     |
|---------------------------------|---------|-------|-----|-----|
| <i>Nematostella vectensis</i>   | 27,273  | 1,524 | 836 | 614 |
| <i>Nephytigorgia</i> sp.        | 14,677  | 762   | 698 | 619 |
| <i>Orbicella faveolata</i>      | 32,463  | 1,736 | 408 | 194 |
| <i>Paramuricea</i> sp.          | 25,189  | 2,645 | 834 | 747 |
| <i>Pocillopora damicornis</i>   | 70,786  | 976   | 242 | 152 |
| <i>Porites astreoides</i>       | 30,740  | 661   | 379 | 243 |
| <i>Protopalythoa variabilis</i> | 130,118 | 1,187 | 521 | 204 |
| <i>Scleronephthya</i> sp.       | 8,401   | 683   | 313 | 257 |
| <i>Platygyra daedalea</i>       | 51,200  | 684   | 483 | 284 |

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Table 2. List of species used in the *in vitro* test of designed baits with assembly summary statistics. Results are from the Kapa Hyper Prep and Hyper Plus (in bold) library preparation kits with target enrichments performed using 250 ng of baits.

| Sub-Class    | Order            | Species                           | Total # Reads               | # Contigs               | Mean Contig Length (bp) | # UCEs          | # Exon Loci     | Total # Loci    |
|--------------|------------------|-----------------------------------|-----------------------------|-------------------------|-------------------------|-----------------|-----------------|-----------------|
| Hexacorallia | Actiniaria       | <i>Actinostella</i> sp.           | 8,984,265                   | 184,605                 | 440                     | 345             | 441             | 786             |
| Hexacorallia | Actiniaria       | <i>Bunodeopsis</i> sp.            | 4,275,127                   | 82,100                  | 413                     | 285             | 257             | 364             |
| Hexacorallia | Actiniaria       | <i>Halcurias pilatus</i> *^       | 6,269,786/ <b>5,978,750</b> | 89,449/ <b>27,355</b>   | 379/ <b>387</b>         | 254/ <b>158</b> | 258/ <b>144</b> | 512/ <b>302</b> |
| Hexacorallia | Actiniaria       | <i>Isosicyonis alba</i> *^        | 7,568,471/ <b>4,138,229</b> | 88,159/ <b>37,119</b>   | 368/ <b>360</b>         | 210/ <b>146</b> | 184/ <b>138</b> | 394/ <b>284</b> |
| Hexacorallia | Actiniaria       | <i>Lebrunia danae</i>             | 9,149,474                   | 187,114                 | 403                     | 340             | 368             | 708             |
| Hexacorallia | Actiniaria       | <i>Sicyonis</i> sp.^*             | 3,215,639/ <b>5,435,226</b> | 50,490/ <b>105,326</b>  | 402/ <b>407</b>         | 174/ <b>238</b> | 287/ <b>249</b> | 461/ <b>487</b> |
| Hexacorallia | Antipatharia     | <i>Antipathes grandis</i> ^%      | <b>319,7830</b>             | <b>57,950</b>           | <b>323</b>              | <b>185</b>      | <b>197</b>      | <b>382</b>      |
| Hexacorallia | Antipatharia     | <i>Myriopathes ulex</i> ^%        | <b>416,0743</b>             | <b>96,476</b>           | <b>356</b>              | <b>248</b>      | <b>267</b>      | <b>515</b>      |
| Hexacorallia | Ceriantharia     | <i>Cerianthus membranaceus</i> ^* | 7,761,662/ <b>7,110,970</b> | 146,327/ <b>143,221</b> | 397/ <b>372</b>         | 206/ <b>212</b> | 231/ <b>227</b> | 437/ <b>439</b> |
| Hexacorallia | Ceriantharia     | <i>Pachycerianthus</i> sp.        | 6,149,439                   | 101,786                 | 426                     | 188             | 198             | 386             |
| Hexacorallia | Corallimorpharia | <i>Corynactis chilensis</i> ^*    | 1,375,885/ <b>3,426,667</b> | 15,433/ <b>44,166</b>   | 362                     | 95/ <b>179</b>  | 77/ <b>187</b>  | 172/ <b>366</b> |
| Hexacorallia | Corallimorpharia | <i>Discosoma carlgreni</i>        | 2,604,601                   | 37,499                  | 353                     | 223             | 260             | 483             |
| Hexacorallia | Scleractinia     | <i>Acropora muricata</i>          | 539,8947                    | 93,433                  | 378                     | 322             | 408             | 730             |
| Hexacorallia | Scleractinia     | <i>Pavona</i> sp.^%               | <b>3,623,661</b>            | <b>57,223</b>           | <b>340</b>              | <b>232</b>      | <b>251</b>      | <b>483</b>      |

|              |              |                                    |           |         |     |     |     |      |
|--------------|--------------|------------------------------------|-----------|---------|-----|-----|-----|------|
| Hexacorallia | Scleractinia | <i>Pocillipora damicornis</i>      | 412,075   | 4,699   | 339 | 123 | 105 | 228  |
| Hexacorallia | Scleractinia | <i>Stylophora pistillata</i>       | 8,439,334 | 162,597 | 394 | 297 | 311 | 606  |
| Hexacorallia | Zoantharia   | <i>Zoanthus cf. pulchellus</i>     | 9,990,392 | 164,870 | 373 | 209 | 195 | 542  |
| Octocorallia | Alcyonacea   | <i>Alcyonium acaule</i>            | 5,456,059 | 93,846  | 401 | 363 | 543 | 906  |
| Octocorallia | Alcyonacea   | <i>Alcyonium digitatum</i>         | 3,071,709 | 43,531  | 393 | 343 | 486 | 829  |
| Octocorallia | Alcyonacea   | <i>Alcyonium haddoni</i>           | 4,631,218 | 66,764  | 414 | 348 | 570 | 918  |
| Octocorallia | Alcyonacea   | <i>Chrysogorgia tricaulis</i>      | 7,439,249 | 111,571 | 413 | 235 | 331 | 566  |
| Octocorallia | Alcyonacea   | <i>Clavularia inflata</i>          | 5,264,312 | 84,673  | 352 | 247 | 325 | 572  |
| Octocorallia | Alcyonacea   | <i>Coelogorgia palmosa</i>         | 4,946,043 | 127,823 | 437 | 367 | 572 | 939  |
| Octocorallia | Alcyonacea   | <i>Cornularia pabloi</i>           | 5,726,156 | 107,331 | 371 | 292 | 359 | 651  |
| Octocorallia | Alcyonacea   | <i>Erythropodium caribaeorum</i>   | 6,027,862 | 119,210 | 398 | 316 | 417 | 733  |
| Octocorallia | Alcyonacea   | Keratoisidinae sp.                 | 4,547,638 | 70,544  | 426 | 233 | 344 | 577  |
| Octocorallia | Alcyonacea   | <i>Parasphaerasclera valdiviae</i> | 4,456,509 | 85,199  | 404 | 323 | 443 | 766  |
| Octocorallia | Alcyonacea   | <i>Plexaura kuna</i>               | 5,542,659 | 105,208 | 393 | 423 | 611 | 1034 |
| Octocorallia | Alcyonacea   | <i>Sinularia slieringsi</i>        | 4,406,351 | 75,970  | 377 | 321 | 516 | 837  |
| Octocorallia | Alcyonacea   | <i>Sinularia lochmodes</i>         | 3,62,2360 | 58,759  | 386 | 314 | 514 | 828  |
| Octocorallia | Alcyonacea   | <i>Sinularia maxima</i>            | 2,563,304 | 42,099  | 366 | 304 | 528 | 832  |
| Octocorallia | Alcyonacea   | <i>Tubipora musica</i>             | 2,891,339 | 44,753  | 369 | 282 | 451 | 733  |
| Octocorallia | Pennatulacea | <i>Virgularia schultzei</i>        | 3,230,517 | 49,954  | 381 | 269 | 509 | 777  |

978 ^ Kapa HyperPlus Kit Trial  
979 %Kapa Hyper Prep Kit Trial failed  
980 \* Probe Concentration Trials  
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991 Table 3. Alignment matrix statistics for different taxonomic datasets. Matrix percentage equals the percent occupancy of species per  
 992 locus. PI=parsimony informative sites.

| Dataset                   | % Matrix | # Loci | # Loci (UCE, exon) | Alignment Length | Mean Locus Length ( $\pm$ SD bp) | Locus Length Range (bp) | # PI Sites | %PI Sites |
|---------------------------|----------|--------|--------------------|------------------|----------------------------------|-------------------------|------------|-----------|
| Anthozoa+genome+outgroup* | 50       | 429    | 228, 201           | 81,403           | 190 $\pm$ 89                     | 23-549                  | 40,041     | 49        |
|                           | 25       | 1375   | 626, 749           | 257,153          | 187 $\pm$ 91                     | 23-601                  | 119,117    | 46        |
| Anthozoa                  | 50       | 464    | 229, 235           | 91,455           | 197 $\pm$ 93                     | 50-667                  | 43,501     | 48        |
|                           | 25       | 1330   | 575, 755           | 254,596          | 191 $\pm$ 99                     | 19-823                  | 109,930    | 43        |
| Hexacorallia              | 50       | 438    | 223, 215           | 89,757           | 205 $\pm$ 93                     | 52-693                  | 34,390     | 38        |
|                           | 25       | 1052   | 529, 523           | 248,476          | 236 $\pm$ 107                    | 52-1362                 | 63,968     | 26        |
| Octocorallia              | 50       | 831    | 334, 496           | 208,869          | 251 $\pm$ 127                    | 51-967                  | 70,369     | 34        |
|                           | 25       | 1366   | 548, 818           | 368,275          | 270 $\pm$ 132                    | 51-1013                 | 96,255     | 26        |

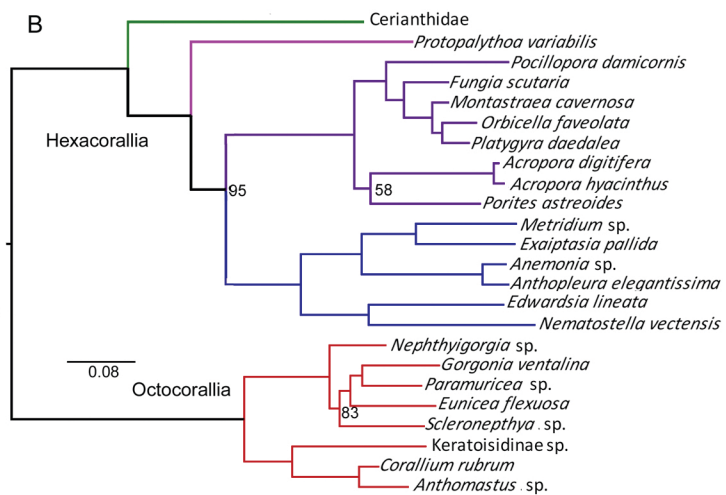
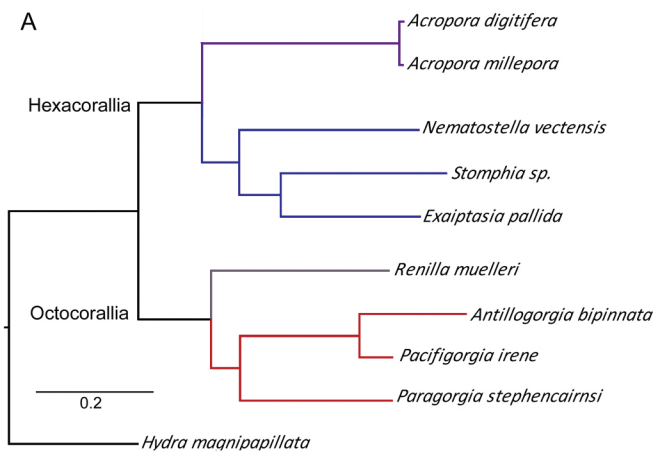
993 \* includes 33 taxa used in test run, 9 genome-enabled taxa, and the outgroup *Hydra magnipapillata*

994  
 995 Table 4. Summary statistics for congeneric species alignments. Mean % variation per locus is also included for UCE loci and exon  
 996 loci, respectively (in parentheses).

| Dataset          | n | # Loci | # Loci (UCE, Exon) | Alignment Length | Mean Locus Length ( $\pm$ SD bp) | Locus Length Range (bp) | # Variable Sites | Range % Variation per Locus | Mean % Variation per Locus |
|------------------|---|--------|--------------------|------------------|----------------------------------|-------------------------|------------------|-----------------------------|----------------------------|
| <i>Acropora</i>  | 3 | 398    | 215, 183           | 206,067          | 517 $\pm$ 73                     | 229-670                 | 9,474            | 0*-46.0                     | 4.7 (4.3, 5.0)             |
| <i>Alcyonium</i> | 3 | 382    | 161, 221           | 205,676          | 538 $\pm$ 250                    | 129-1470                | 60,283           | 6.0-55.0                    | 30 (28, 31)                |
| <i>Simularia</i> | 3 | 426    | 162, 264           | 248,264          | 583 $\pm$ 245                    | 91-1423                 | 14,231           | 0.3-27.0                    | 5.5 (5.2, 5.6)             |

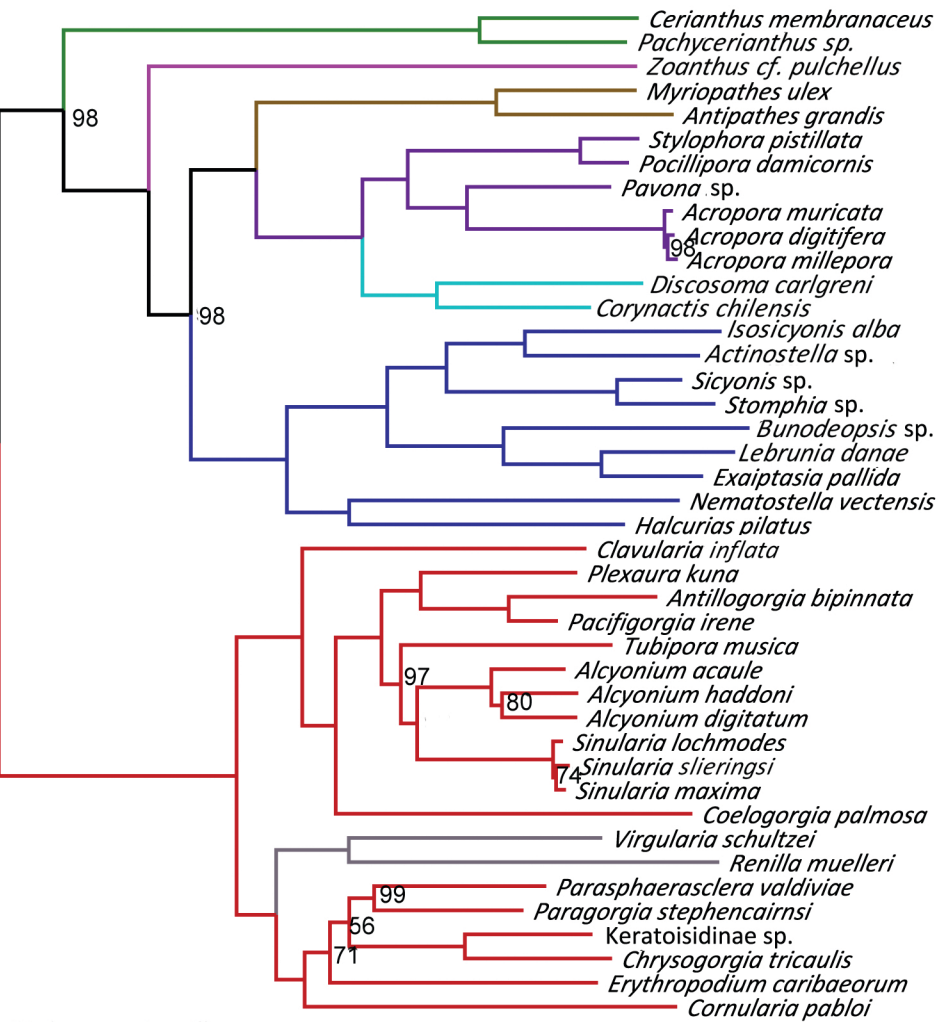
998 \* Only one locus was not polymorphic

999



Hexacorallia

Octocorallia



*Hydra magnipapillata*

0.08