Received: 29 July 2017 | Revised: 28 October 2017 | Accepted: 6 November 2017

DOI: 10.1111/1755-0998.12736

RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY RESOURCES

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

Andrea M. Quattrini[1] | Brant C. Faircloth[2] | Luisa F. Dueñas[3] | Tom C. L. Bridge[4,5] | Mercer R. Brugler[6,7] | Iván F. Calixto-Botía[3,8] | Danielle M. DeLeo[9,10] | Sylvain Forêt[11,†] | Santiago Herrera[12] | Simon M. Y. Lee[13] | David J. Miller[5] | Carlos Prada[14] | Gandhi Rádis-Baptista[15] | Catalina Ramírez-Portilla[3,8] | Juan A. Sánchez[3] | Estefanía Rodríguez[6] | Catherine S. McFadden[1]

[1]Department of Biology, Harvey Mudd College, Claremont, CA, USA

[2]Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, LA, USA

[3]Departamento de Ciencias Biológicas-Facultad de Ciencias, Laboratorio de Biología Molecular Marina (BIOMMAR), Universidad de los Andes, Bogotá, Colombia

[4]Queensland Museum Network, Townsville, QLD, Australia

[5]Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, Australia

[6]Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA

[7]Biological Sciences Department, NYC College of Technology, City University of New York, Brooklyn, NY, USA

[8]Department of Animal Ecology and Systematics, Justus Liebig Universität, Giessen, Germany

[9]Department of Biological Sciences, Florida International University, North Miami, FL, USA

[10]Biology Department, Temple University, Philadelphia, PA, USA

[11]Research School of Biology, Australian National University, Canberra, ACT, Australia

[12]Department of Biological Sciences, Lehigh University, Bethlehem, PA, USA

[13]State Key Laboratory of Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macao, China

[14]Department of Biological Sciences, University of Rhode Island, Kingston, RI, USA

[15]Institute for Marine Sciences, Federal University of Ceara, Fortaleza, CE, Brazil

[†]Deceased.

Correspondence
Andrea M. Quattrini, Department of Biology, Harvey Mudd College, Claremont, CA, USA.
Email: aquattrini@g.hmc.edu

Funding information
National Science Foundation, Grant/Award Number: 1457581, 1457817; The 2013 HIMB Pauley Program

Abstract

Anthozoans (e.g., corals, anemones) are an ecologically important and diverse group of marine metazoans that occur from shallow to deep waters worldwide. However, our understanding of the evolutionary relationships among the ~7,500 species within this class is hindered by the lack of phylogenetically informative markers that can be reliably sequenced across a diversity of taxa. We designed and tested 16,306 RNA baits to capture 720 ultraconserved element loci and 1,071 exon loci. Library preparation and target enrichment were performed on 33 taxa from all orders within the class Anthozoa. Following Illumina sequencing and Trinity assembly, we recovered 1,774 of 1,791 targeted loci. The mean number of loci recovered from each species was 638 ± 222, with more loci recovered from octocorals (783 ± 138 loci) than hexacorals (475 ± 187 loci). Parsimony informative sites

ranged from 26 to 49% for alignments at differing hierarchical taxonomic levels (e.g., Anthozoa, Octocorallia, Hexacorallia). The per cent of variable sites within each of three genera (*Acropora*, *Alcyonium*, and *Sinularia*) for which multiple species were sequenced ranged from 4.7% to 30%. Maximum-likelihood analyses recovered highly resolved trees with topologies matching those supported by other studies, including the monophyly of the order Scleractinia. Our results demonstrate the utility of this target-enrichment approach to resolve phylogenetic relationships from relatively old to recent divergences. Redesigning the baits with improved affinities to capture loci within each subclass will provide a valuable toolset to address systematic questions, further our understanding of the timing of diversifications and help resolve long-standing controversial relationships in the class Anthozoa.

KEYWORDS
coral, exon, phylogeny, target-capture, ultraconserved element, UCE

1 | INTRODUCTION

Anthozoan cnidarians play critical roles in many marine ecosystems. The class contains ~7,500 extant species (i.e., soft corals, sea fans, stony corals, black corals and anemones) that live worldwide in a variety of marine habitats—from tropical shallow waters to the cold, deep sea (Daly et al., 2007). Classification of Anthozoa has traditionally been based on morphological characters such as skeletal morphology, colony organization and soft-tissue anatomy of the polyps (Daly et al., 2007), including the arrangement of internal mesenteries (Fautin & Mariscal, 1991). Long-standing views have recognized the anthozoan subclasses Octocorallia and Hexacorallia as reciprocally monophyletic (Daly et al., 2007), a view also supported by recent phylogenomic analyses of 10s to 100s of genes (Pratlong, Rancurel, Pontarotti, & Aurelle, 2017; Zapata et al., 2015). Within each subclass, however, molecular phylogenetic studies have revealed widespread homoplasy in morphological characters and widespread polyphyly at the ordinal, subordinal, family and genus levels (e.g., Daly et al., 2017; Fukami et al., 2008; McFadden, Sánchez, & France, 2010; Rodríguez et al., 2014). Consequently, deep flaws exist in our understanding of the phylogenetic relationships among and within anthozoan orders. Attempts to resolve the deep phylogenetic relationships among anthozoans using molecular data have largely been unsuccessful due to relatively slow evolutionary rates of mitochondrial genomes (Forsman, Barshis, Hunter, & Toonen, 2009; Hellberg, 2006; Huang, Meier, Todd, & Chou, 2008; Shearer, Van Oppen, Romano, & Wörheide, 2002), lack of signal in rDNA (Berntson, Bayer, McArthur, & France, 2001; Daly, Fautin, & Cappola, 2003) and difficulty in identifying and developing PCR primers for single-copy nuclear genes that can be amplified across the entire class (McFadden et al., 2011).

Within most anthozoan orders, there is also a lack of phylogenetic resolution at the species level. This may be due to incomplete lineage sorting in gene trees, insufficient data due to the small number of currently available markers, hybridization and/or lack of morphological synapomorphies in taxonomy (Daly et al., 2017; Grajales & Rodríguez, 2016; McFadden et al., 2010, 2011, 2017; Prada et al., 2014; Rodríguez et al., 2014). Currently available markers are insufficient at resolving species boundaries for the majority of anthozoans. For octocorals, an extended mitochondrial barcode (*COI+igr1+mtMutS)* has proven useful for revealing cryptic species and delimiting species boundaries within some clades; however, the divergence criterion proposed (McFadden et al., 2011) to elucidate these boundaries is low (>0.5% *p*-distance) and often no genetic divergence is observed among congeneric species (Dueñas, Alderslade, & Sánchez, 2014; McFadden et al., 2011; Pante et al., 2015). The low genetic variability in the mitochondrial genome has been attributed to a unique mismatch repair enzyme (*mtMutS)* that potentially repairs mutations (Bilewitch & Degnan, 2011) thereby causing reduced mitochondrial sequence variation in octocorals when compared to other metazoans (Shearer et al., 2002). Mitochondrial sequence variation is also low in the hexacorals (Daly, Gusmão, Reft, & Rodríguez, 2010; Hellberg, 2006), creating difficulties in resolving species boundaries using traditional mitochondrial barcodes (i.e., COI, Hebert, Ratnasingham, & de Waard, 2003; Shearer & Coffroth, 2008). Although several studies have resolved species boundaries using a nuclear ITS marker (e.g., Medina, Weil, & Szmant, 1999; Pinzon & LaJeunesse, 2011), using ITS poses problems as it is not a single-locus marker (Vollmer & Palumbi, 2004) and there are often high levels of intraspecific variation (Van Oppen, Willis, Van Vugt, & Miller, 2000). Methods that allow for collecting and analysing numerous loci across shallow and deep levels of divergence are sorely needed.

NGS-based methods that have been developed to enable the capture of large numbers of homologous loci in large-scale phylogenetic studies include amplicon sequencing, restriction site-associated DNA (RADseq) methods, transcriptome sequencing and target enrichment of genomic DNA (see McCormack, Hird, Zellmer, Carstens, & Brumfield, 2013). Although RADSeq is an effective approach for species-level phylogenetics and species delimitation within anthozoan genera (e.g., Combosch & Vollmer, 2015; Herrera & Shank, 2016; Johnston et al., 2017; McFadden et al., 2017; Pante

et al., 2015), using RADseq to address deeper-level relationships is not feasible due to locus drop out (Althoff, Gitzendanner, & Segraves, 2007; McCormack, Hird, et al., 2013). Transcriptomic data have been used to reconstruct deep relationships within Cnidaria (Pratlong et al., 2017; Zapata et al., 2015), but the need for RNA limits the use of this method to taxa for which fresh material can be collected and preserved appropriately. Alternatively, target enrichment of ultraconserved elements (UCEs) (Faircloth et al., 2012) has proven robust in inferring species histories of both vertebrates [e.g., fishes (Faircloth, Sorenson, Santini, & Alfaro, 2013), birds (McCormack, Harvey, et al., 2013), reptiles (Crawford et al., 2012) and mammals (McCormack et al., 2012)] and invertebrates [e.g., arachnids (Starrett et al., 2016), hymenopterans (Branstetter, Longino, Ward, & Faircloth, 2017) and coleopterans (Baca, Alexander, Gustafson, & Short, 2017)] across shallow to deep timescales. UCEs occur in high numbers throughout genomes across the tree of life, including Cnidaria (Ryu, Seridi, & Ravasi, 2012), making them easy to identify and align among divergent species (Faircloth et al., 2012). As the name implies, UCEs are highly conserved regions of the genome, but the flanking regions surrounding UCEs are more variable and phylogenetically informative (Faircloth et al., 2012). Some advantages of using target enrichment of UCEs include that 100s to 1,000s of loci can be sequenced at a relatively low cost from a wide range of taxa (Faircloth et al., 2012); they can be generated from 100-year-old, formalin-preserved museum specimens and specimens with degraded DNA (McCormack, Tsai, & Faircloth, 2016; Ruane & Austin, 2017); and they have proven useful at resolving evolutionary questions across both shallow and deep timescales (Manthey, Campillo, Burns, & Moyle, 2016; McCormack, Harvey, et al., 2013; Smith, Harvey, Faircloth, Glenn, & Brumfield, 2014). Similar approaches using target enrichment of coding regions, or exon capturing (Bi et al., 2012; Hugall, O'Hara, Hunjan, Nilsen, & Moussalli, 2016; Ilves & López-Fernández, 2014), have also proven valuable in phylogenomics.

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

2 | MATERIALS AND METHODS

2.1 | Preparation of genomes and transcriptomes

Genomic and transcriptomic data were gathered from various sources for use in bait design and in silico testing (Table S1). All data were masked for repetitive regions, retroelements, small RNAs and transposons using Repeat Masker open-4.0 (Smit, Hubley, & Green, 2015). The N50 was calculated for each genome using stats.sh in the BBTOOLS package (Bushnell, 2015). We then constructed 2bit files for all genomes and transcriptomes (faToTwoBit, BLAT Suite, Kent, 2002) and simulated 100-bp paired reads from each genome and transcriptome using the program ART_ILLUMINA (Huang, Li, Myers, & Marth, 2012) in order to map simulated reads back to the genomes. All programs and parameters used for the entire workflow can be found in Appendix S1.

2.2 | Identification of UCE loci and bait design

We used the open-source program PHYLUCE (Faircloth, 2016) and followed the workflow in the online tutorial (http://phyluce.readthedocs.io/en/latest/tutorial-four.html), with a few modifications to identify conserved regions and design baits to target these regions for downstream next-generation sequencing (Faircloth, 2017). We first aligned an average of 34 million, 100-bp simulated reads from each of the four exemplar taxa, *Acropora digitifera*, *Exaiptasia pallida*, *Renilla muelleri* and *Pacifigorgia irene* to a base genome, *Nematostella vectensis*. *Nematostella vectensis* ("*nemve*") was chosen as the base genome for the primary bait design because it is one of the most well assembled and annotated anthozoan genomes. We used stampy v. 1 (Lunter & Goodson, 2011), with a substitution rate set at 0.05, to map conserved regions of each read-simulated genome to the base genome. Across all taxa, 0.6 to 1.8% of the reads mapped to the *nemve* genome. The resulting alignment file was transformed from SAM format into BAM format (samtools, Li et al., 2009) and then transformed into a BED formatted file (BEDtools, Quinlan & Hall, 2010). These BED files were sorted by scaffold/contig and then by position along that scaffold/contig. We then merged together the alignment positions in each file that were close (<100 bp) to one another using bedtools. In addition, sequences that included masked regions (>25%) or ambiguous (N or X) bases or were too short (<80 bp) were removed using phyluce_probe_strip_masked_loci_from_set. These steps resulted in BED files containing regions of conserved sequences shared between *nemve* and each of the exemplar taxa for further analysis. An SQLite table was created using phyluce_probe_get_multi_merge_table and included 70,312 loci that were shared between pairs of taxa.

We queried the SQLite table and output a list of 1,794 conserved regions found in *nemve* and the other four exemplar taxa using phyluce_probe_query_multi_merge_table. This list plus phyluce_probe_get_genome_sequences_from_bed was used to extract the conserved regions from the *nemve* genome. These regions were buffered to 160 bp by including an equal amount of 5′ and 3′ flanking sequence from the *nemve* genome. Another filter was performed at this stage to remove sequences <160 bp, sequences with >25% masked bases or sequences with ambiguous bases. A temporary set of sequence-capture baits was designed from the loci found in this final FASTA file. Using phyluce_probe_get_tiled_probes, we designed the bait set by tiling two 120-bp baits over each locus that overlapped in the middle by 40 bp (3× density). This temporary set of baits was screened to remove baits with >25% masked bases or high (>70%) or low (<30%) GC content. Any potential duplicates were also removed using phyluce_probe_easy_lastz and phyluce_probe_remove_duplicate_hits_from_probes_using_lastz. Bait sequences were

considered duplicates if they were ≥50% identical over ≥50% of their length.

The temporary bait set (2,131 baits, targeting 1,787 loci) was aligned back to *nemve* and the four exemplar taxa using phyluce_probe_run_multiple_lastzs_sqlite, with an identity value of 70% (the minimum sequence identity for which a bait could be an accepted match to the genome) and a minimum coverage of 83% (default value). From these alignments, baits that matched multiple loci were removed. We then extracted 180 bp of the sequences from the alignment files and input the data into FASTA files using phyluce_probe_slice_sequence_from_genomes. A list containing 710 loci found in at least three of the taxa was created. Based on this list of 710 loci, the anthozoan UCE bait set was redesigned to target these 710 loci using phyluce_probe_get_tiled_probe_from_multiple_inputs, *nemve* and the four exemplar genomes. Using this script, 120-bp baits were tiled (3× density, middle overlap) and screened for high (>70%) or low (<30%) GC content, masked bases (>25%) and duplicates. This bait set included a total of 5,459 nonduplicated baits targeting 710 anthozoan loci. All above methods were repeated to produce additional octocoral-specific baits and capture octocoral-specific loci. We repeated the above analyses using *R. muelleri* as the base genome and *P. irene*, *Paragorgia stephencairnsi* and *Antillogorgia bipinnata* as the exemplar taxa to add 1,317 baits targeting an additional 168 UCE loci to the data set.

2.3 | Identification of exon loci and bait design

To design baits to target exon regions, the above methods were repeated using available transcriptome data. An average of seven million reads from five exemplar transcriptome-enabled taxa (*A. digitifera*, Cerianthidae, *Edwardsiella lineata*, *Gorgonia ventalina* and *Paramuricea* sp.) were simulated and 1.1%–15.3% of these reads per species were aligned to the *nemve* transcriptome. After we converted the alignments to BED files, merged overlapping reads, and filtered data for short loci and repetitive regions, 44,215 conserved sequences were added to an SQLite database. We queried this database and selected 3,700 loci that were found in *nemve* and the additional five exemplar taxa. Following a second screening for masked regions, high/low GC content and duplicates, a temporary exon bait set (5,661 baits) targeting 3,633 exon loci was designed. The temporary baits were re-aligned to the transcriptomes of *nemve* and the additional five exemplar anthozoans to ensure we could locate the loci. A set of 906 loci that were found in *nemve* and the additional five exemplar anthozoans were added to an SQLite database. We redesigned the exon bait set to target these 906 exon loci using phyluce_probe_get_tiled_probe_from_multiple_inputs, *nemve* and the five exemplar transcriptomes. This bait set included a total of 8,080 nonduplicated baits targeting 906 loci across all anthozoans. To add more octocoral-specific baits and loci, we then repeated the above analyses with *Paramuricea* sp. as the base transcriptome and *Anthomastus* sp., *Corallium rubrum*, *Eunicea flexuosa*, *G. ventalina*, Keratoisidinae sp. and *Nepthyigorgia* sp. as the exemplar taxa to add 4,914 baits targeting an additional 407 loci to the data set.

2.4 | Final bait screening

All of the bait sets designed with various sets of data as described above (see Table S1) were screened against one another to remove redundant baits (≥50% identical over >50% of their length), allowing us to create a final nonduplicated Anthozoa bait set. We also screened these baits (70% identity, 70% coverage) against the *Symbiodinium minutum* genome using phyluce_probe_run_multiple_lastzs_sqlite and phyluce_probe_slice_sequence_from_genomes and removed loci that matched the symbiont. Bait names in the final bait FASTA file begin with "uce-" if designed using genomes to target UCEs and "trans-" if designed using transcriptomes to target exons.

2.5 | In silico test

In silico tests were performed to check how well the designed baits aligned to existing genomes and transcriptomes. First, phyluce_probe_run_multiple_lastzs_sqlite was used to align the UCE baits to the nine 2-bit formatted genomes and an outgroup genome (*Hydra magnipapillata)* and the exon baits to the 24 2-bit formatted transcriptomes (Table S1). An identity value of 50% was chosen for alignments (following the PHYLUCE tutorial). For each bait test, the matching FASTA data were sliced out of each genome or transcriptome, plus 200 bp of 5′ and 3′ flanking regions, using phyluce_probe_slice_sequence_from_genomes. This resulted in an average of 429 ± 178 *SD* (44 to 599 per species) UCE loci and 497 ± 230 *SD* (206 to 857) exon loci per anthozoan species (Table 1). To do a final screen for duplicates, loci were matched back to the baits using phyluce_assembly_match_contigs_to_probes, with a minimum coverage of 67% and minimum identity of 80% (default values following the PHYLUCE tutorial). Here, an average of 355 ± 166 *SD* (25 to 529 per species) nonduplicate UCE loci and 354 ± 210 *SD* (106 to 670) nonduplicate exon loci were recovered per anthozoan species (Table 1). Each locus was exported into a FASTA file and aligned with MAFFT (Katoh, Misawa, Kuma, & Miyata, 2002) using phyluce_align_seqcap_align with default parameters.

The resulting alignments were trimmed internally using GBlocks (Castresana, 2000; Talavera & Castresana, 2007) using phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed with default parameters. Two final data sets were then created using phyluce_align_get_only_loci_with_min_taxa, in which all locus alignments contained at least four of the ten taxa for the genome data and nine of the 24 taxa for the transcriptome data. We then concatenated the resulting alignments into separate supermatrices; one containing UCE loci from 10 genome-enabled taxa and the other containing exon loci from the 24 transcriptome-enabled taxa. Maximum-likelihood (ML) inference was conducted on each supermatrix using RAXML v8 (Stamatakis, 2014). This analysis was carried out using rapid bootstrapping, which allows for a complete analysis (20 ML searches and 200 bootstrap replicates) in one step. We also conducted a Bayesian inference (10 million generations, 35% burn-in) using EXABAYES (Aberer, Kobert, & Stamatakis, 2014). An extended majority rule

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

					# loci recovered	
Subclass	**Order**	**Species**	**# scaffolds**	**N50**	**Initial screen**	**Final screen**
Genomes						
Hexacorallia	Actiniaria	*Exaiptasia pallida*	4,312	442,145	518	417
Hexacorallia	Actiniaria	*Nematostella vectensis*	10,804	472,588	496	421
Hexacorallia	Actiniaria	*Stomphia* sp.	479,824	948	44	25
Hexacorallia	Scleractinia	*Acropora digitifera*	4,765	191,489	462	395
Hexacorallia	Scleractinia	*Acropora millepora*	12,559	181,771	511	414
Octocorallia	Alcyonacea	*Antillogorgia bipinnata*	426,978	3,212	230	134
Octocorallia	Alcyonacea	*Pacifigorgia irene*	183,211	2,323	547	491
Octocorallia	Alcyonacea	*Paragorgia stephencairnsi*	700,190	1,793	453	371
Octocorallia	Pennatulacea	*Renilla muelleri*	4,114	19,024	599	529
Hydrozoa	Aplanulata	*Hydra magnipapillata*	126,667	10,113	449	99
Transcriptomes						
Hexacorallia	Actiniaria	*Anemonia* sp.	14,279	703	235	106
Hexacorallia	Actiniaria	*Anthopleura elegantissima*	142,934	1,489	364	207
Hexacorallia	Actiniaria	*Edwardsia lineata*	90,440	1,035	841	623
Hexacorallia	Actiniaria	*Exaiptasia pallida*	60,101	2,159	553	264
Hexacorallia	Actiniaria	*Metridium* sp.	10,885	752	222	111
Hexacorallia	Actiniaria	*Nematostella vectensis*	27,273	1,524	836	614
Hexacorallia	Ceriantharia	Cerianthidae sp.	12,074	646	336	157
Hexacorallia	Scleractinia	*Acropora digitifera*	36,780	1,575	857	620
Hexacorallia	Scleractinia	*Acropora hyacinthus*	67,844	422	392	296
Hexacorallia	Scleractinia	*Fungia scutaria*	155,914	1,619	290	188
Hexacorallia	Scleractinia	*Montastraea cavernosa*	200,222	2,145	206	128
Hexacorallia	Scleractinia	*Orbicella faveolata*	32,463	1,736	408	194
Hexacorallia	Scleractinia	*Pocillopora damicornis*	70,786	976	242	152
Hexacorallia	Scleractinia	*Porites astreoides*	30,740	661	379	243
Hexacorallia	Scleractinia	*Platygyra daedalea*	51,200	684	483	284
Hexacorallia	Zoantharia	*Protopalythoa variabilis*	130,118	1,187	521	204
Octocorallia	Alcyonacea	*Anthomastus* sp.	9,368	610	339	272
Octocorallia	Alcyonacea	*Corallium rubrum*	48,074	2,470	734	606
Octocorallia	Alcyonacea	*Eunicea flexuosa*	165,709	1,095	580	507
Octocorallia	Alcyonacea	*Gorgonia ventalina*	90,230	1,149	731	670
Octocorallia	Alcyonacea	Keratoisidinae	12,385	702	541	429
Octocorallia	Alcyonacea	*Nephthyigorgia* sp.	14,677	762	698	619
Octocorallia	Alcyonacea	*Paramuricea* sp.	25,189	2,645	834	747
Octocorallia	Alcyonacea	*Scleronephthya* sp.	8,401	683	313	257

consensus tree was produced. A general-time reversible model of nucleotide substitution with a gamma-distributed rate variation (GTRGAMMA) was used in both ML and Bayesian analyses.

2.6 | In vitro test

Following the in silico test, the list of designed baits was sent to MYcroarray for synthesis. MYcroarray further screened and removed baits that either had repetitive elements or the potential to cross-hybridize (0.007% total baits removed). We then tested the bait set on 33 anthozoan specimens (Table 2), with both subclasses and all major orders and suborders (for Octocorallia) represented. DNA from these specimens included recent extractions from tissue that had been stored frozen (in liquid nitrogen) for 25 years or in 95% EtOH for up to 10 years, as well as extractions that had been stored frozen (−20°C) for 10 years (see Table S2).

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

Subclass	Order	Species	# contigs	Mean contig length (bp)	# UCEs	# exon loci	Total # loci
Hexacorallia	Actiniaria	*Actinostella* sp.	184,605	440	345	441	786
Hexacorallia	Actiniaria	*Bunodeopsis* sp.	82,100	413	285	257	364
Hexacorallia	Actiniaria	*Halcurias pilatus*[a,c]	89,449/**27,355**	379/**387**	254/**158**	258/**144**	512/**302**
Hexacorallia	Actiniaria	*Isosicyonis alba*[a,c]	88,159/**37,119**	368/**360**	210/**146**	184/**138**	394/**284**
Hexacorallia	Actiniaria	*Lebrunia danae*	187,114	403	340	368	708
Hexacorallia	Actiniaria	*Sicyonis* sp.[a,c]	50,490/**105,326**	402/**407**	174/**238**	287/**249**	461/**487**
Hexacorallia	Antipatharia	*Antipathes grandis*[a,b]	**57,950**	**323**	**185**	**197**	**382**
Hexacorallia	Antipatharia	*Myriopathes ulex*[a,b]	**96,476**	**356**	**248**	**267**	**515**
Hexacorallia	Ceriantharia	*Cerianthus membranaceus*[a,c]	146,327/**143,221**	397/**372**	206/**212**	231/**227**	437/**439**
Hexacorallia	Ceriantharia	*Pachycerianthus* sp.	101,786	426	188	198	386
Hexacorallia	Corallimorpharia	*Corynactis chilensis*[a,c]	15,433/**44,166**	362	95/**179**	77/**187**	172/**366**
Hexacorallia	Corallimorpharia	*Discosoma carlgreni*	37,499	353	223	260	483
Hexacorallia	Scleractinia	*Acropora muricata*	93,433	378	322	408	730
Hexacorallia	Scleractinia	*Pavona* sp.[a,b]	**57,223**	**340**	**232**	**251**	**483**
Hexacorallia	Scleractinia	*Pocillipora damicornis*	4,699	339	123	105	228
Hexacorallia	Scleractinia	*Stylophora pistillata*	162,597	394	297	311	606
Hexacorallia	Zoantharia	*Zoanthus* cf. *pulchellus*	164,870	373	209	195	542
Octocorallia	Alcyonacea	*Alcyonium acaule*	93,846	401	363	543	906
Octocorallia	Alcyonacea	*Alcyonium digitatum*	43,531	393	343	486	829
Octocorallia	Alcyonacea	*Alcyonium haddoni*	66,764	414	348	570	918
Octocorallia	Alcyonacea	*Chrysogorgia tricaulis*	111,571	413	235	331	566
Octocorallia	Alcyonacea	*Clavularia inflata*	84,673	352	247	325	572
Octocorallia	Alcyonacea	*Coelogorgia palmosa*	127,823	437	367	572	939
Octocorallia	Alcyonacea	*Cornularia pabloi*	107,331	371	292	359	651
Octocorallia	Alcyonacea	*Erythropodium caribaeorum*	119,210	398	316	417	733
Octocorallia	Alcyonacea	Keratoisidinae sp.	70,544	426	233	344	577
Octocorallia	Alcyonacea	*Parasphaerasclera valdiviae*	85,199	404	323	443	766
Octocorallia	Alcyonacea	*Plexaura kuna*	105,208	393	423	611	1034
Octocorallia	Alcyonacea	*Sinularia slieringsi*	75,970	377	321	516	837
Octocorallia	Alcyonacea	*Sinularia lochmodes*	58,759	386	314	514	828
Octocorallia	Alcyonacea	*Sinularia maxima*	42,099	366	304	528	832
Octocorallia	Alcyonacea	*Tubipora musica*	44,753	369	282	451	733
Octocorallia	Pennatulacea	*Virgularia schultzei*	49,954	381	269	509	777

[a]Kapa HyperPlus Kit Trial.
[b]Kapa Hyper Prep Kit Trial Library failed.
[c]Bait Concentration Trials.

DNA was extracted using a Qiagen DNeasy Blood & Tissue kit, Qiagen Gentra Kit, or a CTAB extraction protocol (McFadden, Alderslade, Van Ofwegen, Johnsen, & Rusmevichientong, 2006). DNA quality was assessed using a Nanodrop spectrophotometer, with 260/280 ratios ranging from 1.8 to 2.1 and 260/230 ratios ranging from 1.4 to 3.2. The initial concentration of each sample was measured with a Qubit 2.0 fluorometer. For the majority of samples, we then sheared approximately 600 ng DNA (10 ng per μl) to a target size range of 400–800 bp using sonication (Q800R QSonica Inc. Sonicator). For eight samples (Table 2), we sheared 35 μl (115–372 ng, average 217 ng) of EDTA-free DNA using enzymes from the Kapa HyperPlus (Kapa Biosystems) library preparation kit. These samples were mixed on ice with 5 μl of Kapa Frag buffer and 10 μl of the Kapa Frag enzyme and put on a precooled (4°C) thermocycler prior to incubation for 10–15 min at 37°C to achieve a target size range of 400–800 bp. After shearing, DNA was run out on a 1% agarose gel (120 V, 60 min). Small DNA fragments were removed from each sample (250 ng DNA) using a generic SPRI substitute (Glenn et al., 2016; Rohland & Reich, 2012) bead cleanup (3×). DNA was resuspended in 25 μl double-distilled water (ddH20).

Details of library preparation and target enrichment can be found in Appendix S2. Briefly, library preparation (Kapa Biosystems) was carried out on the majority of DNA samples (Table 2) using a Kapa Hyper Prep protocol. For the subset of the samples for which DNA was sheared using enzymes (Table 2), we followed the protocol in the Kapa Hyper Plus enzyme-shearing library preparation kit (Kapa Biosystems). Universal Y-yoke oligonucleotide adapters and custom iTru dual-indexed primers were used in library preparations (Glenn et al., 2016). For target enrichment, the MYcroarray MyBaits were diluted in 1/2 (250 ng) of the standard (500 ng) MyBaits reaction, using 2.5 μl of the baits and 2.5 μl of ddH20 for all samples. Different bait strengths were tested on a set of six samples (Table 2): full bait strength (500 ng), 1/2 bait strength (250 ng), 1/4 bait strength (125 ng) and 1/8 strength (63 ng). One combined pool of all enriched libraries was sent to Oklahoma Medical Research Facility for sequencing on 2/3 of a lane of Illumina HiSeq 3000 (150 bp PE reads).

2.7 | Postsequencing analyses

Demultiplexed Illumina reads were processed using PHYLUCE following the workflow in the online tutorial (http://phyluce.readthedocs.io/en/latest/tutorial-one.html/), with a few modifications (Appendix S1). The reads were first trimmed using the ILLUMIPROCESSOR WRAPPER program (see Faircloth et al., 2012) with default values and then assembled using Trinity v. 2.0 (Haas et al., 2013). We also assembled the data using Abyss 2.0 (Simpson et al., 2009) with a kmer value of 31. UCE and exon bait sequences were then separately matched to the assembled contigs (70% identity, 70% coverage) using phyluce_assembly_match_contigs_to_probes to locate the loci. Loci were then extracted using phyluce_assembly_get_match_counts and phyluce_assembly_get_fastas_from_match_counts, exported into separate FASTA files and aligned with default parameters using phyluce_align_seqcap_align, which uses MAFFT. Loci were internally trimmed with GBlocks using phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed with default parameters.

Data matrices of locus alignments were created using phyluce_align_get_only_loci_with_min_taxa, in which each locus had either 25% or 50% species occupancy. Concatenated locus alignments consisted of exon loci only, UCE loci only and all loci. The number of parsimony informative sites was calculated for each alignment across various taxonomic data sets. The script phyluce_align_get_informative_sites was used on the following taxonomic data sets: Anthozoa+genome+outgroup (33 taxa used in in vitro test, plus nine genome-enabled taxa and the outgroup *H. magnipapillata)*, Anthozoa (33 taxa used in in vitro test), Hexacorallia only (17 taxa used in in vitro test) and Octocorallia only (16 taxa used in in vitro test). The total number of variable sites, total number of parsimony informative sites and number of parsimony informative sites per locus were calculated. We also calculated the total number of variable sites and the number of variable sites per locus for alignments containing species in each of three genera: *Acropora* (*A. digitifera*, *A. millepora*, *A. muricata*), *Alcyonium* (*A. acaule*, *A. digitatum*, *A. haddoni*) and *Sinularia* (*S. slieringsi*, *S. lochmodes*, *S. maxima*). For the three *Acropora* species, we used loci from one target-capture enrichment sample and from the two *Acropora* genomes that were available.

ML inference was conducted on each alignment (exon loci only, UCE loci only and all loci) for the Anthozoa+genome+outgroup taxon set using RAxML v8. This analysis was carried out using rapid bootstrapping, which allows for a complete analysis (20 ML searches and 200 bootstrap replicates) in one step. We also conducted a Bayesian analysis (10 million generations, 35% burn-in) on the 25% and 50% all-loci data sets using ExaBayes (Aberer et al., 2014). An extended majority rule consensus tree was produced. A GTRGAMMA model was used in both ML and Bayesian analyses.

3 | RESULTS

3.1 | Identification of loci and bait design

A total of 16,306 baits were designed to capture 1,791 anthozoan loci with four to 10 baits targeting each locus. The principal UCE bait set included 5,513 baits designed to target 720 loci. The principal exon bait set included 10,793 baits to target 1,071 loci. Four loci that matched genomic regions in *Symbiodinium minutum* were removed from the data set. These loci, however, were also detected in azooxanthellate anthozoans, such as *Chrysogorgia tricaulis*.

3.2 | In silico test

We generated two alignment matrices, one consisting of the exon loci taken from the transcriptome-enabled taxa and the other one consisting of the UCE loci taken from the genome-enabled taxa. The alignment matrix generated with the UCE loci, which included the *H. magnipapillata* outgroup, had a total of 522 loci, with a trimmed mean locus length of 373 bp (95% CI: 8.4) and a total alignment length of 138,778 bp. The alignment matrix generated with the exon loci included 407 loci, with a trimmed mean locus length of 462 bp (95% CI: 5.8) and a total length of 220,139 bp. The ML phylogenies generated from these alignments were well-supported and recovered monophyletic subclasses and established ordinal relationships (Figure 1). The phylogeny generated with the UCE loci had 100% support at all the nodes (Figure 1a), whereas the phylogeny generated with the exon loci had complete support at the majority (86%) of the nodes (Figure 1b). Trees produced using Bayesian inference were congruent with ML results.

3.3 | In vitro test

The total number of reads obtained from Illumina sequencing ranged from 460,724 to 17,283,798 reads per sample (mean: 5,938,769 ± 3,407,199 *SD* reads) across all bait strengths and Kapa kits tested (Table S2). Quality and adapter trimming lead to the removal of 1.8%–10.5% reads from each sample, resulting in a mean of 5,486,800 ± 2,092,161 *SD* trimmed reads per sample (Tables S2

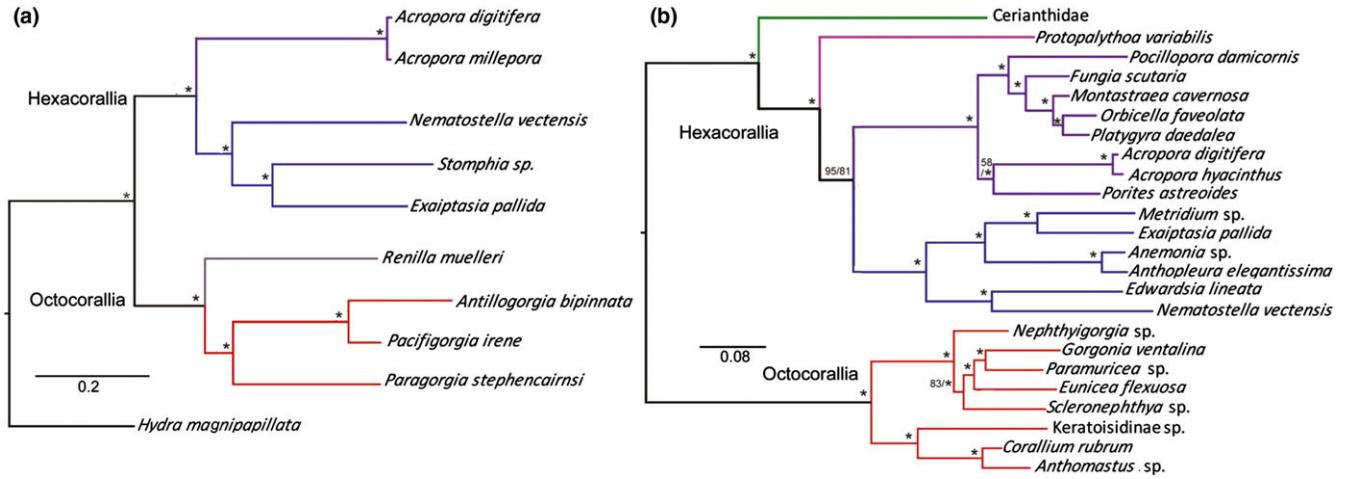

FIGURE 1 Maximum-likelihood phylogenies from in silico analyses. (a) Phylogeny constructed with a 138,778-bp concatenated genomic data set (522 loci) and rooted to *Hydra magnipapillata*. (b) Phylogeny constructed with 220,139-bp concatenated transcriptome data set (407 loci) with the Hexacorallia rooted to the Octocorallia. Bootstrap support (b.s.) values are followed by posterior probabilities (p.p.) from Bayesian analyses. * = 100% b.s. and 1.0 p.p. Branches are colour coded by order (green = Ceriantharia, pink = Zoantharia, purple = Scleractinia, blue = Actiniaria, red = Alcyonacea, grey = Pennatulacea)

and S3). Trimmed reads were assembled into 4,699 to 327,623 contigs per sample (mean: 92,076 ± 65,772 *SD* contigs) with a mean length of 384 ± 27 bp (range: 224 to 32,406 bp) using Trinity (Tables 2 and S3). Coverage averaged 2.5 to 9.9× per contig. No differences in numbers of contigs or reads were evident between libraries prepared using the two different Kapa kits (Hyper Prep or Hyper Plus) at 1/2 bait strength or between the different bait strengths used (1/8, 1/4, 1/2, full) (Fig. S1, Tables 2 and S3). Using Abyss, trimmed reads were assembled into 43,428 to 763,227 contigs per sample with a mean length of only 179 ± 24 bp. Because contig sizes were much smaller from Abyss than those assembled via Trinity, remaining analyses were conducted on the Trinity-assembled data.

A total of 713 UCE loci and 1,061 exon loci (1,774 total loci out of 1,791 targeted loci) were recovered from the assembled contigs. Mean length of UCE contigs was 598 ± 158 bp (range: 224 to 3,995 bp), and mean length of exon contigs was 593 ± 156 bp (range: 224–4,500 bp) (Table S2). No differences in numbers of loci were evident between the two different Kapa kits (Hyper Prep or Hyper Plus) at 1/2 bait strength or between the individuals subjected to the four different bait strengths used (Fig. S1, Tables 2 and S3). The number of loci recovered from each species using a Kapa Hyper prep kit with 1/2 bait strength was highly variable, ranging between 172 to 1,034 total loci per sample (mean: 638 ± 222 loci) (Tables 2 and S3), although few loci (172) were recovered from the sample with the fewest contigs (15,433). More loci were recovered from octocorals (mean: 783 ± 138 loci, range: 569–1,036 loci) compared to hexacorals (mean: 475 ± 187 loci, range: 172–786 loci), even after removing the sample with the fewest loci (498 ± 172 loci).

Alignment lengths, locus number and length, and the number of parsimony informative sites varied depending upon per cent (25 or 50%) of taxon occupancy per locus and type of taxonomic data set (Anthozoa+genome+outgroup, Anthozoa, Hexacorallia, Octocorallia) included in the GBlocks trimmed alignments (Table 3). The average percentage of parsimony informative sites across all alignments was 39%. For the comparisons within each of three genera (*Acropora, Alcyonium*, *Sinularia*), 382 to 426 loci were retained in the 100% alignment matrices (Table 4). Mean % variable sites per locus ranged from 4.7% to 30%, with the most variation found in the *Alcyonium* data set and the least found within *Acropora*. Per cent variation per locus ranged from 0 to 55%, with only one nonpolymorphic locus found in the *Acropora* data set.

Tree topologies were mostly congruent between the 25% and 50% Anthozoa+genome+outgroup data matrices using all loci and the Bayesian and ML analyses (Figure 2 and Fig. S2). Bootstrap support and posterior probabilities were higher overall in the 25% Anthozoa+genome+outgroup ML tree (Figure 2) compared to the 50% data set tree (Figure 2, Fig. S2). By rooting to the outgroup *H. magnipapillata*, monophyly for the currently established anthozoan subclasses and the hexacoral orders was recovered in all analyses except that the sister relationship of Ceriantharia to the rest of the hexacorals was not supported in the Bayesian analysis of the 25% data set. Only a few branches shifted between the ML trees produced with either of the data matrices. *Acropora digitifera* was sister to *A. muricata* in the 50% data set, but sister to *A. millepora* in the 25% data set. In Octocorallia, both *Cornularia pabloi* and *Erythropodium caribaeorum* shifted positions between 25% and 50% data sets. These two species and *Tubipora musica* also changed positions between Bayesian and ML analyses of the 50% data set.

Lower bootstrap support was found in ML trees created with only the exon loci (Fig. S3c,d) or the UCE loci (Fig. S3a,b), but tree topologies were congruent with the few exceptions noted above (Fig. S3). Cerianthids were also found to be sister to all other anthozoans in both 25% and 50% exon-locus data sets, but sister to hexacorals in the UCE-locus data sets. *Zoanthus* cf. *pulchellus* was sister

TABLE 3 Alignment matrix statistics for different taxonomic data sets. Matrix percentage equals the per cent occupancy of species per locus

Data set	% matrix	# loci	# loci (UCE/exon)	Alignment length	Mean locus length (± *SD* bp)	Locus length range (bp)	# PI sites	% PI sites
Anthozoa+genome+outgroup[a]	50	429	228/201	81,403	190 ± 89	23–549	40,041	49
	25	1,375	626/749	257,153	187 ± 91	23–601	119,117	46
Anthozoa	50	464	229/235	91,455	197 ± 93	50–667	43,501	48
	25	1,330	575/755	254,596	191 ± 99	19–823	109,930	43
Hexacorallia	50	438	223/215	89,757	205 ± 93	52–693	34,390	38
	25	1,052	529/523	248,476	236 ± 107	52–1362	63,968	26
Octocorallia	50	831	334/496	208,869	251 ± 127	51–967	70,369	34
	25	1,366	548/818	368,275	270 ± 132	51–1013	96,255	26

PI, parsimony informative sites.
[a]Includes 33 taxa used in test run, 9 genome-enabled taxa and the outgroup *Hydra magnipapillata*.

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

Data set	*N*	# loci	# loci (UCE/exon)	Alignment length	Mean locus length (± *SD* bp)	Locus length range (bp)	# variable sites	Range % variation per locus	Mean % variation per locus
Acropora	3	398	215/183	206,067	517 ± 73	229–670	9,474	0[a]–46.0	4.7 (4.3, 5.0)
Alcyonium	3	382	161/221	205,676	538 ± 250	129–1470	60,283	6.0–55.0	30 (28, 31)
Sinularia	3	426	162/264	248,264	583 ± 245	91–1423	14,231	0.3–27.0	5.5 (5.2, 5.6)

[a]Only one locus was not polymorphic.

to the actiniarians in the 25% exon-locus data set, but sister to a clade containing Actiniaria, Antipatharia, Corallimorpharia and Scleractinia in all other data sets (Fig. S3).

4 | DISCUSSION

Our results demonstrate the utility of the target-capture enrichment approach for inferring phylogenomic relationships in the class Anthozoa. To date, a few studies based on transcriptomic data have recovered well-supported phylogenomic relationships within Anthozoa, but these studies were based on only a handful (≤15) of taxa (Lin et al., 2016; Pratlong et al., 2017; Zapata et al., 2015) and were limited in scope. In general, phylogenomic studies based on transcriptomic data have provided well-supported and well-resolved phylogenies based on 100s to 1,000s of orthologs (Dunn et al., 2008; Kocot et al., 2011; Zapata et al., 2015). However, obtaining these types of sequencing data can be relatively expensive and requires high-quality RNA, two limitations that hinder the transcriptomic approach for large datasets. In addition, it is often not feasible to obtain RNA from rare taxa or taxa that have not been properly preserved for transcriptomics, such as museum specimens. In our study, we show that a sequence-capture approach for both UCEs and exons can be used to capture genome-wide data in anthozoans. To date, this approach has not been applied to anthozoans or to marine invertebrates more generally (except Hugall et al., 2016). We successfully designed a novel bait set based on existing transcriptomes and genomes and captured 1,774 loci from a diversity of anthozoans spanning >500 million years of divergence (Peterson et al., 2004). This target-enrichment approach has the capability to resolve evolutionary relationships at a wide range of divergence levels, from deep (orders, suborders) to shallow levels (species). This novel genomic resource can help to advance studies of systematics, divergence–time estimation and character evolution in the species-rich class Anthozoa.

4.1 | In vitro test results

The newly designed bait set successfully enriched 713 UCE loci and 1,061 exon loci across a diversity of anthozoans. These loci had an average of 39% parsimony informative sites, comparable to the arachnid (30% PI sites, Starrett et al., 2016) UCE data set, which targeted ~1,000 loci. The large range of loci recovered per anthozoan species (172–1036 loci) was also similar to the arachnid results (170–722 loci). We note that the number of loci recovered from octocorals was much higher than what was recovered from hexacorals. This result is perhaps because we added more octocoral-specific baits to the final bait set. And as we added more octocoral-specific baits, we removed baits that were potential paralogs; the majority of these were designed based on the hexacorals. As was made for the hymenopteran UCE bait set (Branstetter et al., 2017), we need to redesign the baitset and include additional octocoral-specific baits and hexacoral-specific baits to increase the success of locus capture. We will also design separate octocoral- and hexacoral-specific bait

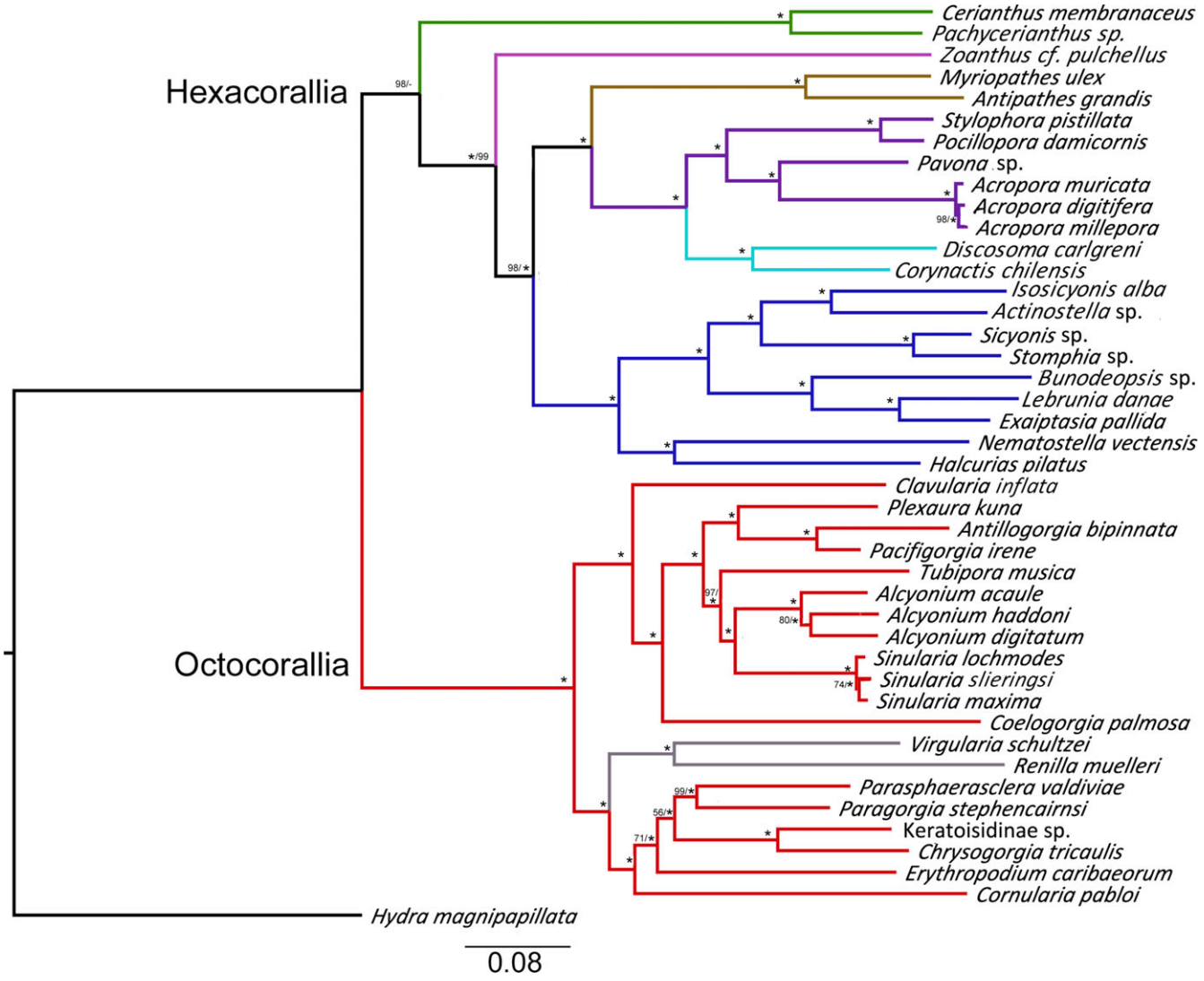

FIGURE 2 Maximum-likelihood phylogeny on the Anthozoa+genome+outgroup 25% matrix (257,728 bp, 1378 loci). The tree includes 33 taxa from the in vitro test, nine genome-enabled taxa and the outgroup *Hydra magnipapillata*. Bootstrap support (b.s.) values are followed by posterior probabilities (p.p) from Bayesian analyses * = 100% b.s. and 1.0 p.p.; - = not supported by Bayesian analysis. Branches are colour coded by order (green = Ceriantharia, pink = Zoantharia, brown = Antipatharia, purple = Scleractinia, lt. blue = Corallimorpharia, blue = Actiniaria, red = Alcyonacea, grey = Pennatulacea)

sets so that additional loci specific to each subclass can be targeted. Nevertheless, this first bait design and in vitro results from 33 taxa demonstrate the promising utility of the target-capture method for resolving anthozoan relationships across deep divergence levels.

The number of variable sites found at loci recovered from within three genera demonstrates that this is also a promising approach for species-level phylogenetics. Within all three genera examined, variable sites ranged up to 55% per locus, with a mean variation across all loci of 4.7, 5.5, and 30% in *Sinularia*, *Acropora* and *Alcyonium*, respectively. The high variation seen within *Alcyonium* is consistent with unpublished data (C. McFadden, unpubl. data) that suggest the three species are perhaps different genera. For *Sinularia*, average divergence estimates are also higher (~10×) than what has been demonstrated in other studies using mitochondrial barcoding markers (McFadden, Van Ofwegen, Beckman, Benayahu, & Alderslade, 2009). In fact, a 0.5% divergence level at an extended mitochondrial barcode (*mtMutS+igrl+COI*) was proposed as a conservative criterion for species delimitation (McFadden, Brown, Brayton, Hunt, & van Ofwegen, 2014; McFadden et al., 2011). Similarly, low divergence estimates at mitochondrial barcoding markers have been found among hexacoral congeners (Brugler, Opresko, & France, 2013; González-Muñoz et al., 2015; Shearer & Coffroth, 2008). Thus, these UCE and exon loci are promising for resolving species boundaries, although the level of intraspecific variation has yet to be determined. Our UCE and exon-locus data sets may serve as an alternative resource to RADseq to address species-boundary questions while simultaneously allowing for data to be combined and examined across deeper levels.

Because this was the first time the target-enrichment UCE approach had been tested on anthozoans, we compared different

concentrations of baits and different library preparation kits to determine whether or not particular methods would recover more loci. We found no differences in the number of loci recovered using different concentrations of baits in the hybridization and enrichment protocols. This bait-strength test suggested that the number of hybridizations obtained from one standard reaction could, at least, be doubled. We also found no differences between the two different Kapa kits used. The enzymatic DNA shearing that can be performed with the Kapa Hyper Plus kit may be useful for researchers who do not have access to a sonicator.

Following internal trimming with GBlocks and aligning of conserved loci, the mean locus length was much shorter (~190 bp) compared to the mean length of untrimmed loci (~600 bp). Therefore, some of the loci included in the ML analyses were relatively short (<100 bp), particularly in the Anthozoa+genome+outgroup data set. In alignments between highly divergent taxa (such as between hexacorals and octocorals), numerous poorly aligned positions and divergent positions were filtered with GBlocks. In contrast, the locus size was considerably higher within genera (~525 bp) because of fewer poorly aligned and divergent positions. Perhaps reperforming the GBlocks internal trimming with less stringent parameters would increase the size of loci in alignments of divergent taxa. Stringent alignment filtering, as carried out with GBlocks, can not only increase the proportion of unresolved branches, but can also lead to well-supported branches that are in fact incorrect (Tan et al., 2015). Different methods of aligning and filtering data will be explored in future work.

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

In general, the inferred phylogenetic relationships corresponded to those found in previous studies (Rodríguez et al., 2014; Zapata et al., 2015), although there were a few exceptions. One exception was the position of the stoloniferan octocoral *C. pabloi*. In all data sets, this species was nested within the clade containing sea pens (Pennatulacea) and calcaxonians (*C. tricaulis*, Keratoisidinae sp.), but this species has been previously found to be sister to the rest of the octocorals based on mitochondrial data (McFadden & van Ofwegen, 2012). The superfamily Actinostoloidea (*Sicyonis* sp., *Stomphia* sp.) was recovered as sister to superfamily Actinioidea (*Actinostella* sp., *Isosicyonis alba*) in all data sets. This result differed from the combined mitochondrial and nuclear rDNA data set of Rodríguez et al. (2014), which instead recovered Actinostoloidea as sister to both Actinioidea and Metridioidea (*Lebrunia danae*, *E. pallida*, *Bunodeopsis* sp.). Furthermore, trees in our study were rooted to *H. magnipapillata*, based on the results of Zapata et al. (2015); however, the unrooted trees indicated that *H. magnipapillata* was sister to the Octocorallia, a relationship (i.e., a paraphyletic Anthozoa) that has been noted by mitochondrial data (Kayal, Roure, Philippe, Collins, & Lavrov, 2013; Park et al., 2012), but not supported by phylogenomic analyses of transcriptomic data (Zapata et al., 2015). Zapata et al. (2015) also found that the position of the order Ceriantharia was phylogenetically unstable. Similarly, we found that the placement of Ceriantharia changed between the different exon and UCE data sets. The topologies resulting from exon data placed the ceriantharians as sister to all remaining anthozoans, a relationship also suggested by analysis of 16S and 18S ribosomal DNA (Stampar, Maronna, Kitahara, Reimer, & Morandini, 2014). Trees from UCE loci had ceriantharians as sister to hexacorals, a relationship supported by combined mitochondrial and nuclear rDNA data (Rodríguez et al., 2014). Future work must include different outgroup choices (i.e., sponges, bilateria, other cnidarians), while closely examining the distribution and strength of phylogenetic signal. This will help clarify the source of incongruence and resolve which loci strongly influence the resolution of a given "contentious" branch (Shen, Hittinger, & Rokas, 2017).

Whether or not scleractinians are monophyletic has been a controversial topic as a result of different phylogenetic analyses. In 2006, Medina, Collins, Takaoka, Kuehl, and Boore (2006) reported that scleractinians were polyphyletic with corallimorpharians. The "naked coral hypothesis" was thus proposed, suggesting that corallimorpharians arose from a scleractinian ancestor that had undergone skeletal loss during paleoclimate conditions when the oceans experienced increased CO_2 concentrations (Medina et al., 2006). Since that study, other studies based on transcriptomic data (Lin et al., 2016), rDNA (Fukami et al., 2008), and mitochondrial data (Fukami et al., 2008; Kayal et al., 2013; Kitahara et al., 2014; Park et al., 2012) recovered a monophyletic Scleractinia with corallimorpharians as the sister clade. Our results also recovered a monophyletic Scleractinia, thus supporting the conclusions of others that corallimorpharians are not naked corals. However, increased sampling of robust, complex and basal scleractinians is necessary to conclusively address this issue.

4.2 | Future research directions

The in silico and in vitro tests of the novel bait set demonstrate that the target-enrichment approach of UCEs and exons is a promising new genomic resource for inferring phylogenetic relationships among anthozoans. Using this bait set, target-capture enrichment of the

UCE and exon loci from at least 192 additional anthozoans is currently underway to further our understanding of character evolution and systematics of the clade. Adding more taxa will likely increase the accuracy of the phylogenetic inference. We also plan to use additional outgroup taxa, including medusozoan cnidarians and sponges, to help address whether or not octocorals are sister to hexacorals or medusozoans and resolve the position of ceriantharians. Finally, we plan to redesign the bait sets to create hexacoral- and octocoral-specific bait sets. We will include additional baits to increase the capture efficiency of loci that were targeted in this study, while adding more loci that are specific to each subclass. This target-enrichment approach provides a promising genomic resource to resolve phylogenetic relationships at deep to shallow levels of divergence, considerably advancing the current state of knowledge of anthozoan evolution.

ACKNOWLEDGEMENTS

Funding was provided by NSF-DEB #1457817 to CSM and #1457581 to ER. The 2013 HIMB Pauley Program also provided funds to AMQ for preliminary analyses. C. Oliveros and J. Salter helped during laboratory work and J. Bast helped during preliminary data analyses. F. Zapata and C. Dunn provided transcriptomic data sets. S. Lengyel, E. Cordes and R. Kulathinal aided DD with the *Paramuricea* transcriptome assembly, which was funded by a grant from Gulf of Mexico Research to support the "Ecosystem Impacts of Oil and Gas in the Gulf" (ECOGIG) research consortium. E. Bush provided computing support. Specimens from Irish waters were collected during cruise CE14004 on the RV Celtic Explorer led by L. Allcock, NUI Galway and supported by the Marine Institute's Shiptime Programme. Special thanks to Z. Forsman, R. Toonen and I. Knapp for organizing the 2013 HIMB Pauley program. Thanks to J. Ryan and three anonymous reviewers for constructive comments.

DATA ACCESSIBILITY

Tree and alignment files: Data Dryad Entry https://doi.org/10.5061/dryad.36n40; Raw Data: SRA GenBank SUB3122367, BioSample #SAMN07774920-4952; Anthozoan bait set: Data Dryad Entry https://doi.org/10.5061/dryad.36n40

AUTHOR CONTRIBUTION

A.M.Q., C.S.M., E.R., and B.C.F conceived and designed this study. A.M.Q. designed the baits; conducted library preparation, target enrichment and data analyses; and wrote the initial draft of the manuscript with significant contributions from C.S.M. B.C.F. developed protocols and guided A.M.Q. in laboratory and bioinformatic analyses. L.F.D. helped with preliminary analyses. M.B., E.R. and C.S.M. extracted DNA. I.C.B., D.M.D., S.F., S.H., S.L., D.J.M., C.P., G.R.B., C.R.P. and J.A.S. provided genomic or transcriptomic data for analysis. T.B. provided samples. All authors edited and approved the final version of this manuscript.

ORCID

Andrea M. Quattrini http://orcid.org/0000-0002-4247-3055
Brant C. Faircloth http://orcid.org/0000-0002-1943-0217
Luisa F. Dueñas http://orcid.org/0000-0002-0756-826X
Mercer R. Brugler http://orcid.org/0000-0003-3676-1226
Santiago Herrera http://orcid.org/0000-0001-7204-9434
David J. Miller http://orcid.org/0000-0003-0291-9531
Catalina Ramírez-Portilla http://orcid.org/0000-0002-5272-6060
Juan A. Sánchez http://orcid.org/0000-0001-7149-8369
Catherine S. McFadden http://orcid.org/0000-0002-8519-9762

REFERENCES

Aberer, A. J., Kobert, K., & Stamatakis, A. (2014). ExaBayes: Massively parallel Bayesian tree inference for the whole-genome era. *Molecular Biology and Evolution*, *31*(10), 2553–2556. https://doi.org/10.1093/molbev/msu236

Althoff, D. M., Gitzendanner, M. A., & Segraves, K. A. (2007). The utility of amplified fragment length polymorphisms in phylogenetics: A comparison of homology within and between genomes. *Systematic Biology*, *56*(3), 477–484. https://doi.org/10.1080/10635150701427077

Baca, S. M., Alexander, A., Gustafson, G. T., & Short, A. E. (2017). Ultraconserved elements show utility in phylogenetic inference of *Adephaga* (Coleoptera) and suggest paraphyly of *'Hydradephega'*. *Systematic Entomology*, *42*, 786–795. https://doi.org/10.1111/syen.12244

Berntson, E. A., Bayer, F. M., McArthur, A. G., & France, S. C. (2001). Phylogenetic relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA sequences. *Marine Biology*, *138*(2), 235–246. https://doi.org/10.1007/s002270000457

Bi, K., Vanderpool, D., Singhal, S., Linderoth, T., Moritz, C., & Good, J. M. (2012). Transcriptome-based exon capture enables highly cost-effective comparative genomic data collection at moderate evolutionary scales. *BMC Genomics*, *13*(1), 403. https://doi.org/10.1186/1471-2164-13-403

Bilewitch, J. P., & Degnan, S. M. (2011). A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. *BMC Evolutionary Biology*, *11*(1), 228. https://doi.org/10.1186/1471-2148-11-228

Branstetter, M. G., Longino, J. T., Ward, P. S., & Faircloth, B. C. (2017). Enriching the ant tree of life: Enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods in Ecology and Evolution*, *8*, 768–776. https://doi.org/10.1111/2041-210X.12742

Brugler, M. R., Opresko, D. M., & France, S. C. (2013). The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: Implications for black coral taxonomy and systematics. *Zoological Journal of the Linnean Society*, *169*(2), 312–361. https://doi.org/10.1111/zoj.12060

Bushnell, B. (2015). BBMap short-read aligner, and other bioinformatics tools. Retrieved from: sourceforge.net/projects/bbmap.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*(4), 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Combosch, D. J., & Vollmer, S. V. (2015). Trans-Pacific RAD-Seq population genomics confirms introgressive hybridization in Eastern Pacific *Pocillopora* corals. *Molecular Phylogenetics and Evolution*, *88*, 154–162. https://doi.org/10.1016/j.ympev.2015.03.022

Crawford, N. G., Faircloth, B. C., McCormack, J. E., Brumfield, R. T., Winker, K., & Glenn, T. C. (2012). More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biology Letters*, *8*(5), 783–786. https://doi.org/10.1098/rsbl.2012.0331

Daly, M., Brugler, M. R., Cartwright, P., Collins, A. G., Dawson, M. N., Fautin, D. G., … Romano, S. L. (2007). The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. *Zootaxa*, *1668*, 127–182.

Daly, M., Crowley, L. M., Larson, P., Rodríguez, E., Saucier, E. H., & Fautin, D. G. (2017). *Anthopleura* and the phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). *Organisms Diversity and Evolution*, *17*, 1–20.

Daly, M., Fautin, D. G., & Cappola, V. A. (2003). Systematics of the hexacorallia (Cnidaria: Anthozoa). *Zoological Journal of the Linnean Society*, *139*(3), 419–437. https://doi.org/10.1046/j.1096-3642.2003.00084.x

Daly, M., Gusmão, L., Reft, A., & Rodríguez, E. (2010). Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). *Integrative Comparative Biology*, *50*(3), 371–388. https://doi.org/10.1093/icb/icq081

Dueñas, L. F., Alderslade, P., & Sánchez, J. A. (2014). Molecular systematics of the deep-sea bamboo corals (Octocorallia: Isididae: Keratoisidinae) from New Zealand with descriptions of two new species of *Keratoisis*. *Molecular Phylogenetics and Evolution*, *74*, 15–28.

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., … Sørensen, M. V. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, *452*(7188), 745–749. https://doi.org/10.1038/nature06614

Faircloth, B. C. (2016). PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics*, *32*(5), 786–788. https://doi.org/10.1093/bioinformatics/btv646

Faircloth, B. C. (2017). Identifying conserved genomic elements and designing universal bait sets to enrich them. *Methods in Ecology and Evolution*, *8*, 1103–1112. https://doi.org/10.1111/2041-210X.12754

Faircloth, B. C., McCormack, J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, *61*(5), 717–726. https://doi.org/10.1093/sysbio/sys004

Faircloth, B. C., Sorenson, L., Santini, F., & Alfaro, M. E. (2013). A phylogenomic perspective on the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved elements (UCEs). *PLoS ONE*, *8*(6), e65923. https://doi.org/10.1111/2041-210X.12754

Fautin, D. G., & Mariscal, R. N. (1991). *Cnidaria: Anthozoa*, Vol. *2*, (pp. 267–358). New York, NY: Wiley-Liss.

Forsman, Z. H., Barshis, D. J., Hunter, C. L., & Toonen, R. J. (2009). Shape-shifting corals: Molecular markers show morphology is evolutionarily plastic in *Porites*. *BMC Evolutionary Biology*, *9*(1), 45. https://doi.org/10.1186/1471-2148-9-45

Fukami, H., Chen, C. A., Budd, A. F., Collins, A., Wallace, C., Chuang, Y. Y., … Knowlton, N. (2008). Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS ONE*, *3*(9), e3222. https://doi.org/10.1371/journal.pone.0003222

Glenn, T. C., Nilsen, R., Kieran, T. J., Finger, J. W., Pierson, T. W., Bentley, K. E., … Reed, K. (2016). Adapterama I: Universal stubs and primers for thousands of dual-indexed Illumina libraries (iTru & iNext). *BioRxiv*, https://doi.org/10.1101/049114

González-Muñoz, R., Simões, N., Mascaró, M., Tello-Musi, J. L., Brugler, M. R., & Rodríguez, E. (2015). Morphological and molecular variability of the sea anemone *Phymanthus crucifer* (Cnidaria, Anthozoa, Actiniaria, Actinoidea). *Journal of the Marine Biological Association of the United Kingdom*, *95*(1), 69–79. https://doi.org/10.1017/S0025315414000988

Grajales, A., & Rodríguez, E. (2016). Elucidating diversity within the Aiptasiidae, a widespread cnidarian-dinoflagellate model system (Cnidaria: Anthozoa: Actiniaria: Metridioidea). *Molecular Phylogenetics and Evolution*, *94*(A), 252–263. https://doi.org/10.1016/j.ympev.2015.09.004

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., … Regev, A. (2013). De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, *8*(8), 1494–1512. https://doi.org/10.1038/nprot.2013.084

Hebert, P. D., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*(Suppl 1), S96–S99. https://doi.org/10.1098/rsbl.2003.0025

Hellberg, M. E. (2006). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, *6*(1), 24. https://doi.org/10.1186/1471-2148-6-24

Herrera, S., & Shank, T. M. (2016). RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. *Molecular Phylogenetics and Evolution*, *100*, 70–79. https://doi.org/10.1016/j.ympev.2016.03.010

Huang, W., Li, L., Myers, J. R., & Marth, G. T. (2012). ART: A next-generation sequencing read simulator. *Bioinformatics*, *28*(4), 593–594. https://doi.org/10.1093/bioinformatics/btr708

Huang, D., Meier, R., Todd, P. A., & Chou, L. M. (2008). Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. *Journal of Molecular Evolution*, *66*(2), 167–174. https://doi.org/10.1007/s00239-008-9069-5

Hugall, A. F., O'Hara, T. D., Hunjan, S., Nilsen, R., & Moussalli, A. (2016). An exon-capture system for the entire class Ophiuroidea. *Molecular Biology and Evolution*, *33*(1), 281–294. https://doi.org/10.1093/molbev/msv216

Ilves, K. L., & López-Fernández, H. (2014). A targeted next-generation sequencing toolkit for exon-based cichlid phylogenomics. *Molecular Ecology Resources*, *14*(4), 802–811. https://doi.org/10.1111/1755-0998.12222

Jeffroy, O., Brinkmann, H., Delsuc, F., & Philippe, H. (2006). Phylogenomics: The beginning of incongruence? *Trends in Genetics*, *22*(4), 225–231. https://doi.org/10.1016/j.tig.2006.02.003

Johnston, E. C., Forsman, Z. H., Flot, J. F., Schmidt-Roach, S., Pinzón, J. H., Knapp, I. S. S., & Toonen, R. J. (2017). A genomic glance through the fog of plasticity and diversification in *Pocillopora*. *Scientific Reports*, *7*, 5991. https://doi.org/10.1038/s41598-017-06085-3

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Kayal, E., Roure, B., Philippe, H., Collins, A. G., & Lavrov, D. V. (2013). Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology*, *13*(1), 5. https://doi.org/10.1186/1471-2148-13-5

Kent, W. J. (2002). BLAT—the BLAST-like alignment tool. *Genome Research*, *12*(4), 656–664. https://doi.org/10.1101/gr.229202

Kitahara, M. V., Lin, M. F., Forêt, S., Huttley, G., Miller, D. J., & Chen, C. A. (2014). The "naked coral" hypothesis revisited–evidence for and against scleractinian monophyly. *PLoS ONE*, *9*(4), e94774. https://doi.org/10.1371/journal.pone.0094774

Kocot, K. M., Cannon, J. T., Todt, C., Citarella, M. R., Kohn, A. B., Meyer, A., … Halanych, K. M. (2011). Phylogenomics reveals deep molluscan relationships. *Nature*, *477*(7365), 452–456. https://doi.org/10.1038/nature10382

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., … Durbin, R. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lin, M. F., Chou, W. H., Kitahara, M. V., Chen, C. L. A., Miller, D. J., & Forêt, S. (2016). Corallimorpharians are not "naked corals": Insights into relationships between Scleractinia and Corallimorpharia from phylogenomic analyses. *PeerJ*, *4*, e2463. https://doi.org/10.7717/peerj.2463

Lunter, G., & Goodson, M. (2011). Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. *Genome Research*, *21*(6), 936–939. https://doi.org/10.1101/gr.111120.110

Manthey, J. D., Campillo, L. C., Burns, K. J., & Moyle, R. G. (2016). Comparison of target-capture and restriction-site associated DNA sequencing for phylogenomics: A test in cardinalid tanagers (Aves, Genus: *Piranga*). *Systematic Biology*, *65*(4), 640–650. https://doi.org/10.1093/sysbio/syw005

McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T., & Glenn, T. C. (2012). Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Research*, *22*(4), 746–754. https://doi.org/10.1101/gr.125864.111

McCormack, J. E., Harvey, M. G., Faircloth, B. C., Crawford, N. G., Glenn, T. C., & Brumfield, R. T. (2013). A phylogeny of birds based on over 1,500 loci collected by target enrichment and high-throughput sequencing. *PLoS ONE*, *8*(1), e54848. https://doi.org/10.1371/journal.pone.0054848

McCormack, J. E., Hird, S. M., Zellmer, A. J., Carstens, B. C., & Brumfield, R. T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*, *66*(2), 526–538. https://doi.org/10.1016/j.ympev.2011.12.007

McCormack, J. E., Tsai, W. L., & Faircloth, B. C. (2016). Sequence capture of ultraconserved elements from bird museum specimens. *Molecular Ecology Resources*, *16*(5), 1189–1203. https://doi.org/10.1111/1755-0998.12466

McFadden, C. S., Alderslade, P., Van Ofwegen, L. P., Johnsen, H., & Rusmevichientong, A. (2006). Phylogenetic relationships within the tropical soft coral genera *Sarcophyton* and *Lobophytum* (Anthozoa, Octocorallia). *Invertebrate Biology*, *125*(4), 288–305. https://doi.org/10.1111/j.1744-7410.2006.00070.x

McFadden, C. S., Benayahu, Y., Pante, E., Thoma, J. N., Nevarez, P. A., & France, S. C. (2011). Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources*, *11*(1), 19–31. https://doi.org/10.1111/j.1755-0998.2010.02875.x

McFadden, C. S., Brown, A. S., Brayton, C., Hunt, C. B., & van Ofwegen, L. P. (2014). Application of DNA barcoding in biodiversity studies of shallow-water octocorals: Molecular proxies agree with morphological estimates of species richness in Palau. *Coral Reefs*, *33*(2), 275–286.

McFadden, C. S., Haverkort-Yeh, R., Reynolds, A. M., Halàsz, A., Quattrini, A. M., Forsman, Z. H., … Toonen, R. J. (2017). Species boundaries in the absence of morphological, ecological or geographical differentiation in the Red Sea octocoral genus *Ovabunda* (Alcyonacea: Xeniidae). *Molecular Phylogenetics and Evolution*, *112*, 174–184. https://doi.org/10.1016/j.ympev.2017.04.025

McFadden, C. S., Sánchez, J. A., & France, S. C. (2010). Molecular phylogenetic insights into the evolution of Octocorallia: A review. *Integrative and Comparative Biology*, *50*, 389–410. https://doi.org/10.1093/icb/icq056

McFadden, C. S., & van Ofwegen, L. P. (2012). Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of *Cornularia* (Cornulariidae). *Invertebrate Systematics*, *26*(4), 331–356. https://doi.org/10.1071/IS12035

McFadden, C. S., Van Ofwegen, L. P., Beckman, E. J., Benayahu, Y., & Alderslade, P. (2009). Molecular systematics of the speciose Indo-Pacific soft coral genus, *Sinularia* (Anthozoa: Octocorallia). *Invertebrate Biology*, *128*(4), 303–323. https://doi.org/10.1111/j.1744-7410.2009.00179.x

Medina, M., Collins, A. G., Takaoka, T. L., Kuehl, J. V., & Boore, J. L. (2006). Naked corals: Skeleton loss in Scleractinia. *Proceedings of the National Academy of Sciences*, *103*(24), 9096–9100. https://doi.org/10.1073/pnas.0602444103

Medina, M., Weil, E., & Szmant, A. M. (1999). Examination of the *Montastraea annularis* species complex (Cnidaria: Scleractinia) using ITS and COI sequences. *Marine Biotechnology*, *1*(1), 89–97. https://doi.org/10.1007/PL00011756

Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S. C., Boisselier, M. C., & Samadi, S. (2015). Use of RAD sequencing for delimiting species. *Heredity*, *114*(5), 450–459. https://doi.org/10.1038/hdy.2014.105

Park, E., Hwang, D. S., Lee, J. S., Song, J. I., Seo, T. K., & Won, Y. J. (2012). Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. *Molecular Phylogenetics and Evolution*, *62*(1), 329–345. https://doi.org/10.1016/j.ympev.2011.10.008

Peterson, K. J., Lyons, J. B., Nowak, K. S., Takacs, C. M., Wargo, M. J., & McPeek, M. A. (2004). Estimating metazoan divergence times with a molecular clock. *Proceedings of the National Academy of Sciences*, *101*(17), 6536–6541. https://doi.org/10.1073/pnas.0401670101

Pinzon, J. H., & LaJeunesse, T. D. (2011). Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology*, *20*(2), 311–325. https://doi.org/10.1111/j.1365-294X.2010.04939.x

Prada, C., DeBiasse, M. B., Neigel, J. E., Yednock, B., Stake, J. L., Forsman, Z. H., … Hellberg, M. E. (2014). Genetic species delineation among branching Caribbean *Porites* corals. *Coral Reefs*, *33*(4), 1019–1030. https://doi.org/10.1007/s00338-014-1179-5

Pratlong, M., Rancurel, C., Pontarotti, P., & Aurelle, D. (2017). Monophyly of Anthozoa (Cnidaria): Why do nuclear and mitochondrial phylogenies disagree? *Zoologica Scripta*, *46*(3), 363–371. https://doi.org/10.1111/zsc.12208

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841–842. https://doi.org/10.1093/bioinformatics/btq033

Rodríguez, E., Barbeitos, M. S., Brugler, M. R., Crowley, L. M., Grajales, A., Gusmão, L., … Daly, M. (2014). Hidden among sea anemones: The first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. *PLoS ONE*, *9*(5), e96998. https://doi.org/10.1371/journal.pone.0096998

Rohland, N., & Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research*, *22*(5), 939–946. https://doi.org/10.1101/gr.128124.111

Ruane, S., & Austin, C. C. (2017). Phylogenomics using formalin-fixed and 100 + year-old intractable natural history specimens. *Molecular Ecology Resources*, *17*, 1003–1008. https://doi.org/10.1111/1755-0998.12655

Ryu, T., Seridi, L., & Ravasi, T. (2012). The evolution of ultraconserved elements with different phylogenetic origins. *BMC Evolutionary Biology*, *12*(1), 236. https://doi.org/10.1186/1471-2148-12-236

Shearer, T. L., & Coffroth, M. A. (2008). DNA BARCODING: Barcoding corals: Limited by interspecific divergence, not intraspecific variation. *Molecular Ecology Resources*, *8*(2), 247–255. https://doi.org/10.1111/j.1471-8286.2007.01996.x

Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, *11*(12), 2475–2487. https://doi.org/10.1046/j.1365-294X.2002.01652.x

Shen, X. X., Hittinger, C. T., & Rokas, A. (2017). Contentious relationships in phylogenomic studies can be driven by a handful of genes. *Nature Ecology and Evolution*, *1*, 0126. https://doi.org/10.1038/s41559-017-0126

Simpson, J. T., Wong, K., Jackman, S. D., Schein, J. E., Jones, S. J., & Birol, I. (2009). ABySS: A parallel assembler for short read sequence data. *Genome Research*, *19*(6), 1117–1123. https://doi.org/10.1101/gr.089532.108

Smit, A. F. A., Hubley, R., & Green, P. (2015). RepeatMasker Open-4.0. Retrieved from http://www.repeatmasker.org.

Smith, B. T., Harvey, M. G., Faircloth, B. C., Glenn, T. C., & Brumfield, R. T. (2014). Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Systematic Biology*, *63*(1), 83–95. https://doi.org/10.1093/sysbio/syt061

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stampar, S. N., Maronna, M. M., Kitahara, M. V., Reimer, J. D., & Morandini, A. C. (2014). Fast-evolving mitochondrial DNA in Ceriantharia: A reflection of hexacorallia paraphyly? *PLoS ONE*, *9*(1), e86612. https://doi.org/10.1371/journal.pone.0086612

Starrett, J., Derkarabetian, S., Hedin, M., Bryson, R. W., McCormack, J. E., & Faircloth, B. C. (2016). High phylogenetic utility of an ultraconserved element probe set designed for Arachnida. *Molecular Ecology Resources*, *17*(4), 812–823. https://doi.org/10.1111/1755-0998.12621

Streicher, J. W., Schulte, J. A., & Wiens, J. J. (2015). How should genes and taxa be sampled for phylogenomic analyses with missing data? An empirical study in iguanian lizards. *Systematic Biology*, *65*(1), 128–145.

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*(4), 564–577. https://doi.org/10.1080/10635150701472164

Tan, G., Muffato, M., Ledergerber, C., Herrero, J., Goldman, N., Gil, M., & Dessimoz, C. (2015). Current methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference. *Systematic Biology*, *64*(5), 778–791. https://doi.org/10.1093/sysbio/syv033

Van Oppen, M. J. H., Willis, B. L., Van Vugt, H. W. J. A., & Miller, D. J. (2000). Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. *Molecular Ecology*, *9*(9), 1363–1373. https://doi.org/10.1046/j.1365-294x.2000.01010.x

Vollmer, S. V., & Palumbi, S. R. (2004). Testing the utility of internally transcribed spacer sequences in coral phylogenetics. *Molecular Ecology*, *13*(9), 2763–2772. https://doi.org/10.1111/j.1365-294X.2004.02265.x

Wiens, J. J. (2005). Can incomplete taxa rescue phylogenetic analyses from long-branch attraction? *Systematic Biology*, *54*(5), 731–742. https://doi.org/10.1080/10635150500234583

Wiens, J. J., & Tiu, J. (2012). Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. *PLoS ONE*, *7*(8), e42925. https://doi.org/10.1371/journal.pone.0042925

Zapata, F., Goetz, F. E., Smith, S. A., Howison, M., Siebert, S., Church, S. H., … Daly, M. (2015). Phylogenomic analyses support traditional relationships within Cnidaria. *PLoS ONE*, *10*(10), e0139068. https://doi.org/10.1371/journal.pone.0139068

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Quattrini AM, Faircloth BC, Dueñas LF, et al. Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: New approaches to long-standing problems. *Mol Ecol Resour*. 2017;00:1–15. https://doi.org/10.1111/1755-0998.12736