An enhanced target-enrichment bait set for Hexacorallia provides phylogenomic resolution of the staghorn corals (Acroporidae) and close relatives.

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Abstract: The phylogenetic utility of targeted enrichment methods has been demonstrated in taxa that often have a history of single gene marker development. These genomic capture methods are now being applied to resolve evolutionary relationships from deep to shallow timescales in clades that were previously deficient in molecular marker development and lacking robust morphological characters that reflect evolutionary relationships. Effectively capturing 1000s of loci, however, in a diverse group across a broad time scale requires a bait set that incorporates multiple baits per locus. We redesigned a custom bait set for the cnidarian class Anthozoa to target 1,436 UCE loci and 1,572 exon regions within the subclass Hexacorallia. We test this redesigned bait set on 99 specimens of hard corals (Scleractinia) spanning both the "complex" (Acroporidae, Agariciidae) and "robust" (Fungiidae) clades. With focused sampling in the staghorn coral genus *Acropora* we explore the ability of capture data to inform the taxonomy of a clade deficient in molecular resolution. A mean of 1850 (± 298) loci were captured per taxon (955 UCEs, 894 exons). A 75% complete concatenated alignment included 1792 loci (991 UCE, 801 exons) and ~1.87 million base pairs. Parsimony informative sites varied from 48% for alignments including all three families, to 1.5% among samples within a single *Acropora* species. Maximum likelihood and Bayesian analyses recover highly resolved topologies and robust molecular relationships not previously found with traditional markers within the Acroporidae. Species level relationships within the *Acropora* genus do not support traditional morphological groups or morphological phylogenies. Both UCE and exon datasets delineated six well-supported clades within *Acropora.* The enhanced bait set for Hexacorallia will allow researchers to survey the evolutionary history of important groups of reef building corals where previous molecular marker development has been unsuccessful.

1. Introduction

Molecular systematics has progressed at an uneven pace across the tree of life. Several plant and animal branches on the tree of life have benefited the most from the development of single gene mitochondrial and nuclear markers resulting in large scale phylogenies (Jetz et al., 2012; Rabosky et al., 2018; Tonini et al., 2016; Upham et al., 2019) leveraging decades worth of molecular data from public resources (https://www.ncbi.nlm.nih.gov/). These large-scale phylogenies have provided a deep time perspective, systematic resolution, and often very complete biodiversity inventories of taxa. While few if any higher-level taxa can be considered complete from a species level molecular perspective, the enhanced taxonomic framework provided by these 'Tree of Life' projects has allowed researchers to complete shallow clades with species inventories using taxonomy-based grafting and polytomy resolution methods (Rabosky et al., 2018; Thomas et al., 2013) to incorporate species not yet sampled by genetic methods. These new methods have enabled testing of broad evolutionary and ecological hypotheses across broad taxonomic groups (Jetz et al., 2012; Pyron et al., 2013; Rabosky et al., 2018). While these approaches come with their own hurdles (Rabosky, 2015), new phylogenomic tools are facilitating a shift from the 'top-down' approach of sampling higher taxonomic ranks to a 'bottom-up' approach attempting to sample complete clades of species and even populations on shallow evolutionary time scales, leveraging high throughput sequencing technologies (Derkarabetian et al., 2018; Manthey et al., 2016; Smith et al., 2014).

Two popular phylogenomic methodologies are (i) Restriction site-associated DNA sequencing (e.g. RADseq), and (ii) Targeted sequence capture of conserved loci (Faircloth et al., 2012; Lemmon et al., 2012). Both methodologies use high-throughput sequencing, genome reduction and sample multiplexing to generate genomic datasets for hundreds of samples in substantially less time than traditional Sanger sequencing approaches (Branstetter

et al., 2017). While targeted capture and RADseq data produce similar topologies (Collins and Hrbek, 2018), targeted capture of ultraconserved elements (UCEs) or exons has grown in popularity due to their comparability across taxa, tolerance of lower quality DNA templates and ability to resolve relationships at deep and shallow time scales (Faircloth et al., 2012; Harvey et al., 2016).

Targeted capture techniques were recently used for phylogenomic reconstruction across the anthozoan tree of life (Quattrini et al., 2018), with available anthozoan genomes and transcriptomes used to design a bait set to capture both UCEs and conserved exons. An average of 638 ± 222 UCE/exon loci were captured per sample, but capture rates differed greatly between Hexacorallia and Octocorallia, two major subclasses of Anthozoa, with higher recovery from the Octocorallia (soft corals, sea fans) compared with the Hexacorallia (stony corals, black corals, anemones, zoanthids). This difference in target efficiency is likely the result of the addition of octocoral-specific baits and the removal of paralogous (mostly hexacoral-specific) baits in the bait design process (Quattrini et al., 2018). In this case, separate octocoral and hexacoral-specific bait sets will increase capture efficiency and thus phylogenetic resolution across evolutionary time scales.

Within the Hexacorallia, the order Scleractinia (stony corals) has historically received significant research interest due to their role as the key ecosystem engineers on coral reefs, which host an estimated 830,000 multicellular species (Fisher et al., 2015). Hermatypic (reef-building) Scleractinia are generally colonial and represent approximately half of all scleractinian species. The capacity of stony corals to build reefs is attributed to their photosymbiotic relationship with dinoflagellates of the family Symbiodiniaceae, which mainly restricts both hermatypic corals and coral reefs to shallow tropical and sub-tropical regions (Kleypas et al., 1999). In recent years, molecular phylogenetics has fundamentally altered our understanding of the systematics and evolution of the Scleractinia, revealing that

most morphological characters traditionally used to identify families, genera and species do not reflect their evolutionary history (Fukami et al., 2008, 2004; Romano and Palumbi, 1996). This has led to taxonomic revisions of the Scleractinia at every taxonomic level (Kitahara et al., 2016).

Within Scleractinia, the family Acroporidae is the most speciose family, accounting for approximately one-third of all reef-building coral species (Madin et al., 2016). Despite their ecological importance and use as a model system to understand coral biology and symbioses, there is, as yet, no well-resolved species-level molecular phylogeny for the family Acroporidae. Like plants, corals and other Anthozoans have mitochondrial substitution rates that are slower than rates of substitution across the nuclear genome (Fukami et al., 2000; Hellberg, 2006; Shearer et al., 2002; Van Oppen et al., 1999) limiting the utility of mitochondrial makers in DNA barcoding, phylogeography and shallow species-level phylogenetics in corals (Chen et al., 2009; Hellberg, 2006; Huang et al., 2008; McFadden et al., 2011). In particular, the diverse and ecologically dominant genus *Acropora* (staghorn corals) is notorious for its lack of systematic resolution with traditional mitochondrial and nuclear marker development (Chen et al., 2009). The relatively recent species level divergence and population level expansion in *Acropora* (Bellwood et al., 2017; Renema et al., 2016), means traditional mitochondrial markers offer little information to define species boundaries and robust systematic relationships. The lack of reliable molecular markers for the genus also means that the rampant synonymization of nominal species is based entirely on qualitative morphological characters (Veron and Wallace, 1984; Wallace, 1999; Wallace et al., 2012), despite their proven unreliability in determining evolutionary relationships across numerous taxonomic levels. As a result, while the genus *Acropora* contains 413 nominal species (Hoeksema and Cairns, 2019), the most recent taxonomic revision of the genus, based on morphology, recognises only 122 species (Wallace et al., 2012). Targeted sequence

capture offers a new molecular tool for disentangling the relationships in hexacorals, from deep relationship among families and genera to shallow level species relationships. The family Acroporidae offers an extreme test case for the utility of a universal Hexacoral bait set for targeted sequence capture.

The aim of this study is to redesign the previously developed anthozoan UCE/exon bait set (Quattrini et al., 2018) in order to capture additional loci and enhance the capture efficiency of loci within the subclass Hexacorallia. We highlight a method for redesigning a class-level custom bait set to increase the number of captured loci within shallower clades. We use these methods to provide an enhanced RNA bait set for the anthozoan subclass Hexacorallia that targets both ultraconserved loci and exonic regions for phylogenomic reconstruction. To test the efficiency of this bait set we focus on the phylogenetically unresolved staghorn coral family Acroporidae. We include specimens from several defined morphological groups (Wallace, 1999) sampled across the Indo-Pacific with taxonomic identity determined by morphological comparisons with type materials and the original descriptions of all nominal species. We highlight the utility of targeted capture approaches for taxa that have not benefited from decades of single gene marker development. Our results demonstrate the utility of targeted capture approaches in unravelling relationships in what have been phylogenetically challenging taxa.

2. Materials and Methods

2.1. Bait Design

We re-designed a bait set, originally aimed for target-capturing UCE and exon loci in anthozoans (anthozoa-v1, Quattrini et al., 2018), to have higher specificity for and target additional loci in hexacorals. Results generated from target-capture of UCE and exon loci in 235 anthozoans (Quattrini and McFadden, unpubl. data) with the anthozoa-v1 bait set were

screened to remove baits that performed poorly in hexacorals or in anthozoans more generally. Thus, 5844 baits targeting 1,123 loci (553 UCE and 570 exon loci) were retained from the anthozoa-v1 bait set. Using the program Phyluce (Faircloth, 2016) and following methods in Quattrini et al. (2018), we added additional hexacoral-specific baits to 1) improve target-capture performance of the anthozoa-v1 loci and 2) target additional hexacoral loci not included in the anthozoa-v1 bait set. Methods are briefly outlined below, however, for more details see both Quattrini et al. (2018) and the Phyluce documentation (https://phyluce.readthedocs.io/en/latest/tutorial-four.html).

To redesign baits targeting UCE loci, we first mapped 100 bp simulated-reads from the genomes of four exemplar taxa, *Acropora tenuis, Montastraea cavernosa, Amplexidiscus fenestrafer* and *Discosoma sp.*, to a masked *Nematostella vectensis (nemve)* genome (Suppl Table S1). Reads were mapped, with 0.05 substitution rate, using stampy v. 1 (Lunter and Goodson, 2011), resulting in 0.8 to 1.0% of reads aligning to the *nemve* genome. Any alignments that included masked regions (>25%) or ambiguous bases (N or X) or were too short (<80 bp) were removed using *phyluce_probe_strip_masked_loci_from_set*. An SQLite table that included regions of conserved sequences shared between *nemve* and each of the exemplar taxa was created using *phyluce_probe_get_multi_merge_table*. This table was queried using *phyluce_probe_query_multi_merge_table* to output a file containing conserved loci found in *nemve* and all other exemplar taxa.

Phyluce_probe_get_genome_sequences_from_bed was used to extract these conserved regions from the *nemve* genome. Regions were then buffered to 160 bp by including an equal amount of 5' and 3' flanking sequence from the *nemve* genome. A temporary set of target capture baits was then designed using *phyluce_probe_get_tiled_probes*; two 120 bp baits were tiled over each locus and overlapped in the middle by 40 bp (3X density). This temporary set of baits was screened to remove baits with >25% masked bases or high (>70%)

or low (<30%) GC content. At this stage, we concatenated the temporary baits with the baits retained from the anthozoa-v1 bait set and then removed any potential duplicates 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.

This new temporary bait set was aligned (with an identity value of 70% and a minimum coverage of 83%) to the genomes of *A. digitifera*, *Exaiptasia pallida*, *Discosoma* sp., *M. cavernosa*, and *N. vectensis* (Suppl. Table S1), and UCE loci of *Cerianthus membranaceus*, *Zoanthus* cf. *pulchellus*, and *Myriopathes ulex* (Quattrini et al., 2018) using *phyluce_probe_run_multiple_lastzs_sqlite*. 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 loci found in at least five of the taxa was created. The anthozoan UCE bait set was re-designed to target these loci using *phyluce_probe_get_tiled_probe_from_multiple_inputs*. Using this script, 120-bp baits were tiled (3X density, middle overlap) and screened for high (>70%) or low (<30%) GC content, masked bases (>25%), and duplicates as described above. Finally, the baitset was screened against the *Symbiodinium minutum* (Suppl. Table S1) genome to look for any potential symbiont loci using the scripts *phyluce_probe_run_multiple_lastzs_sqlite* and *phyluce_probe_slice_sequence_from_genomes*, with a minimum coverage of 70% and minimum identity of 60%. This UCE bait set included a total of 15,226 non-duplicated baits targeting 1,436 loci.

All of the above methods were repeated using transcriptome data to re-design the baits for target-capture of exons. We mapped 100 bp simulated-reads from the transcriptomes of three exemplar taxa, *A. digitifera*, Cerianthidae, and *Edwardsiella lineata*, to the *nemve*

transcriptome (Suppl. Table S1), resulting in 4.5 to 15.3% of reads for each alignment. From these alignments, conserved sequences were added to an SQLite database. We queried this database to select loci found in *nemve* and the three exemplar taxa. Following a screening for masked regions, high/low GC content, and duplicates, a temporary exon bait set was designed. At this stage, we concatenated the temporary baits with the baits retained from the anthozoa-v1 bait set and then removed any potential duplicates 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 baits were re-aligned to the transcriptomes of *A. digitifera*, Cerianthidae, *Protopalythoa variabilis*, *Orbicella faveolata*, *Pocillopora damicornis*, *E. lineata*, *N. vectensis* (Suppl. Table S1), and exon loci of *Lebrunia danae* and *Myriopathes ulex* (Quattrini et al., 2018) to ensure we could capture the loci with this bait set. We then re-designed the bait set to target these exon loci using *phyluce_probe_get_tiled_probe_from_multiple_inputs*. Using this script, 120-bp baits were tiled (3X density, middle overlap) and screened for high (>70%) or low (<30%) GC content, masked bases (>25%), and duplicates as described above. Finally, this bait set was also screened against the *S. minutum* genome to look for any potential symbiont loci using the scripts *phyluce_probe_run_multiple_lastzs_sqlite* and *phyluce_probe_slice_sequence_from_genomes*, with a minimum coverage of 70% and minimum identity of 60%. This exon bait set included a total of 15,750 non-duplicated baits targeting 1,572 loci.

The exon and UCE bait sets were concatenated and then screened to remove redundant baits (≥50% identical over >50% of their length), allowing us to create a final non-duplicated hexacoral-v2 bait set. For this study, we subset this hexacoral bait set to include

baits designed against all scleractinians as well as the corallimorpharians, the antipatharian, and *N. vectensis*. This reduced the cost of the bait synthesis, while still allowing us to target a maximum number of loci. Baits were synthesized by Arbor BioSciences (Ann Arbor, MI).

2.2. Sample collection and morphology assessment

We tested the efficiency of this enhanced bait set on capturing loci from 99 specimens from the families Acroporidae (n=86), Agariciidae (n=5), and Fungiidae (n=9). Specimens were collected on snorkel and scuba using chisel and hammer from 2015 to 2017. Specimens spanning five of the six recognised genera of Acroporidae (*Alveopora* is unsampled) were sampled from locations across the Indo-Pacific (Suppl Table S2). The outgroup species chosen include specimens from the Agariciidae (*Leptoseris*), a close sister family of Acroporidae, and species from the more distantly related Fungiidae. Both Acroporidae and Agariciidae are members of the "complex" clade within Scleractinia, while Fungiidae is from the "robust" clade (Romano and Palumbi, 1996). This taxon sampling enables testing of the capture efficiency of the bait set at both deep (among major scleractinian clades and families) and shallow (among acroporid genera and species) evolutionary time scales.

Acroporid specimens were identified by comparing skeletons to the 'type' material and the original descriptions of all nominal species. Uncertainties in the identifications are indicated by the use of a series of open nomenclature (ON) qualifiers (Bengtson, 1988; Sigovini et al., 2016) which provides flexibility to assign specimens to nominal species with varying degrees of certainty. Specimens with skeletons that closely resemble the original type specimen and were collected from the type location (e.g. *Acropora pichoni;* Fig. 1, Suppl Table S2) were designated as 'topotypes' and are given the nominal species name with no qualifier. Specimens that closely resemble the type of a nominal species but were not sampled from the type locality are given the qualifier *cf.* (e.g. *Acropora cf. plumosa;* Fig. 1,

Suppl Table S2). Specimens that have morphological affinities to a nominal species are given the qualifier *aff.* (e.g. *Acropora aff. turaki;* Fig. 1, Suppl Table S2). These specimens may represent geographical variants of species with high morphological plasticity or undescribed species. Species that could not be matched with the type material of any nominal species were labelled as *sp.* in addition to the voucher number in its specific epithet (e.g. *Acropora sp. 69-TER 35;* Fig. 1, Suppl Table S2); these specimens are most probably undescribed species. In addition to identifying specimens with comparisons to nominal type material, we also categorized specimens into morphological grouping ("morpho-groups") delineated by Wallace (1999) in a cladistic analysis of morphological traits. Wallace (1999) placed species into morpho-groups base on a phylogeny reconstructed from qualitative trait data. For example, the species *A. walindii* was placed in the '*elegans group'.* By categorizing our *Acropora* and *Isopora* specimens in this manner we can assess the molecular support for these morphological groups. Our sampling contains representatives of 16 of the 25 morpho-groups (including *Isopora)* delineated by Wallace (1999).

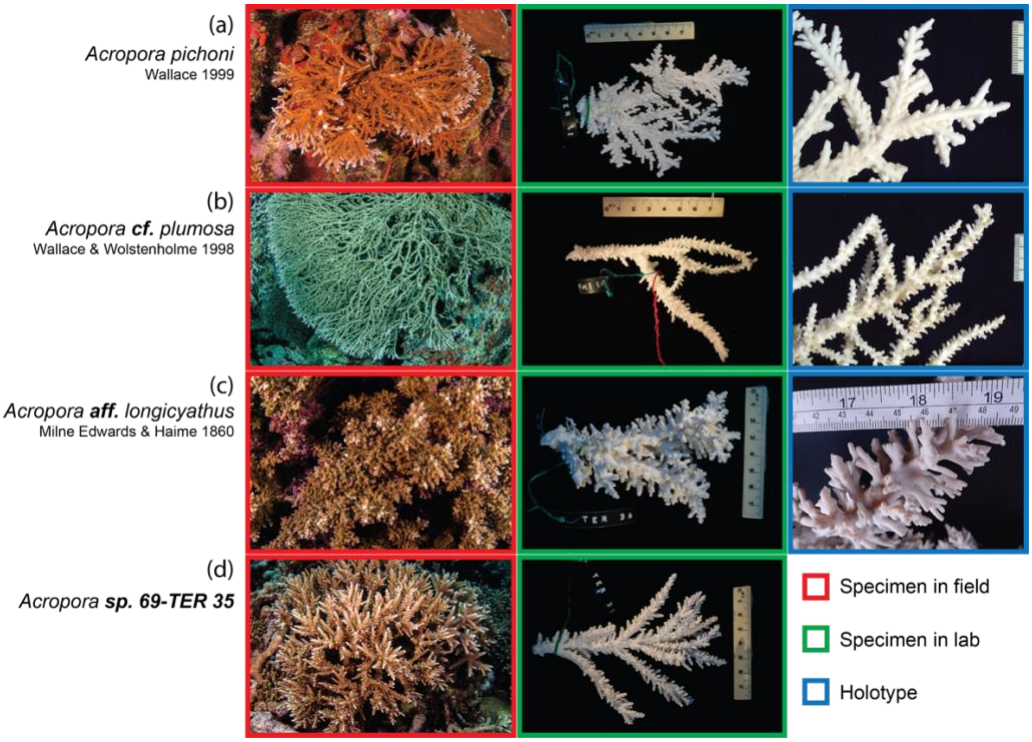


Figure 1. Coral specimens were identified by comparison to type material when available (in situ examination at MTQ; images and original descriptions). Based on these comparisons specimens were (a) identified to nominal species if they were considered a topotype sample; (b) assigned the ON qualifier "cf" if they resembled the type material but were not collected from the type location; (c) assigned the ON qualifier "aff." if they had morphological affinities to a nominal species but could not be reliably identified using the information available; and (d) assigned the ON qualifier "sp." if they showed little resemblance to any nominal species type specimen.

2.3. DNA extraction and target enrichment

DNA was extracted using a Qiagen DNeasy Blood & Tissue kit or a CTAB extraction protocol. DNA quality was assessed using a Nanodrop spectrophotometer, with 260/280 ratios ranging from 1.8-2.1 and 260/230 ratios ranging from 1.4-3.2. The initial concentration of each sample was measured with a Qubit 2.0 fluorometer and sent to Arbor Biosciences (Ann Arbor, MI) for library preparation.

Library preparation was performed by Arbor Biosciences following details in Quattrini et al., 2018). A total of 600 ng DNA (10 ng per μL) was sheared to a target size range of 400-800 bp using sonication, and the Kapa Hyper Prep (Kapa Biosystems) protocol was used. Universal Y-yoke oligonucleotide adapters and custom iTru dual-indexed primers as described by Glenn et al., (2019) were used. For target enrichment, the Arbor Biosciences MyBaits v. IV protocol was followed. Target enrichment was performed on pools of up to eight samples. Following target-capture enrichment, target-enriched libraries were sequenced on one lane of Illumina HiSeq 3000 (150bp PE reads). Library preparation and target enrichment were conducted by Arbor Biosciences.

2.4. Post-sequencing analyses

De-multiplexed Illumina reads were processed using *Phyluce* (Faircloth, 2016; http://phyluce.readthedocs.io/en/latest/tutorial-one.html/), with a few modifications. Reads were trimmed using the Illumiprocessor wrapper program (Faircloth et al., 2012) for *trimmomatic* (Bolger et al., 2014) with default values and then assembled using Spades v. 3.10 (Bankevich et al., 2012; Nurk et al., 2013). UCE and exon bait sequences were then separately matched to the assembled contigs at 70% identity and 70% coverage using *phyluce_assembly_match_contigs_to_probes*. *Phyluce_assembly_get_match_counts* and *phyluce_assembly_get_fastas_from_match_counts* were used to extract loci into FASTA files. Locus coverage was estimated using *phyluce_assembly_get_trinity_coverage* and *phyluce_assembly_get_trinity_coverage_for_uce_loci*. *Phyluce_align_seqcap_align* was used to align (with MAFFT; Katoh et al., 2002) and edge trim the loci across individuals, with default settings. Alignment matrices were created in which each locus was represented by either 75% or 95% species occupancy. Concatenated locus alignments consisted of exon loci only, UCE loci only, and all loci. The total number of variable sites, total number of

parsimony informative sites, and number of parsimony informative sites per locus were calculated (using *phyluce_align_get_informative_sites*) for alignments across the following taxonomic datasets: all taxa (n=96), Acroporidae (n=83), *Acropora* (n=65), and *Acropora walindii* (n=3).

Maximum likelihood inference was conducted on each alignment (exon loci only, UCE loci only, and all loci) for both 75% and 95% data matrices (six alignment sets) 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 analysis (100 million generations, 35% burnin) on both taxon occupancy matrices, for all loci only, 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. Bait design

The hexa-v2 bait set for all hexacorals included 25,514 baits targeting 2,499 loci (1,132 UCE and 1,367 exon loci). The bait set subset for scleractinians included 16,263 baits designed to target a total of 2,497 loci. The UCE specific bait set consisted of 8,880 baits that targeted 1,132 loci. The exon specific bait consisted of 7,383 baits that targeted 1,365 loci. In screening the combined baits set for potential hits to the *Symbiodinium* genome, 141 loci were removed. Bait sets are included as supplemental files 1 (hexa-v2) and 2 (hexa-v2-scleractinia); baits designed against transcriptomes have “design: hexatrans” in the .fasta headers.

3.2. Enrichment statistics and matrix results

342 The total number of reads obtained from Illumina sequencing ranged from 3,206 to
343 10,357,494 reads per sample (Suppl Table S3). Following removal of one sample (*Acropora*
344 *cf. tenella*) that failed sequencing (<4K reads), and quality and adapter trimming, a mean of
345 3,766,889 ± 1,516,262 SD trimmed reads per sample was retained (Tables 1). Trimmed reads
346 were assembled into a mean of 11,858 ± 9,682 SD contigs per sample (range: 1,498 to
347 53,766) with a mean length of 506 ± 75 bp using SPAdes (Tables 1; Table S3). Read
348 coverage per contig ranged from 0.3 to 75X.
349 A total of 1131 UCE loci and 1332 exon loci (2463 total loci out of 2,497 targeted
350 loci) were recovered from the assembled contigs (Fig. 2, Table 1). Following the removal of
351 two samples (*Montipora cf. aequituberculata*, *Montipora cf. capitata*) due to relatively few
352 loci recovered (Fig. 2, <1000 loci), mean number of loci was 1,900 ± 140 SD per sample
353 (range: 1,178 to 2,051). We recovered slightly higher numbers of loci from acroporids (1,930
354 ± 116 SD) than from agariciids (1,632 ± 182) and fungiids (1,773 ± 73 SD). The total
355 number of UCE loci recovered was 983 ± 63 SD per sample (range: 657 to 1051) with a
356 mean length of 933 ± 139 bp (range: 297 to 1,099 bp). The total number of exon loci
357 recovered was 917 ± 82 (range: 521 to 1009) with a mean length of 976 ± 168 bp (range: 287
358 to 1,192 bp) (Suppl Table S3). Read coverage per locus ranged from 1 to 337X for UCE loci
359 and 0.5 to 270X for exon loci.
360

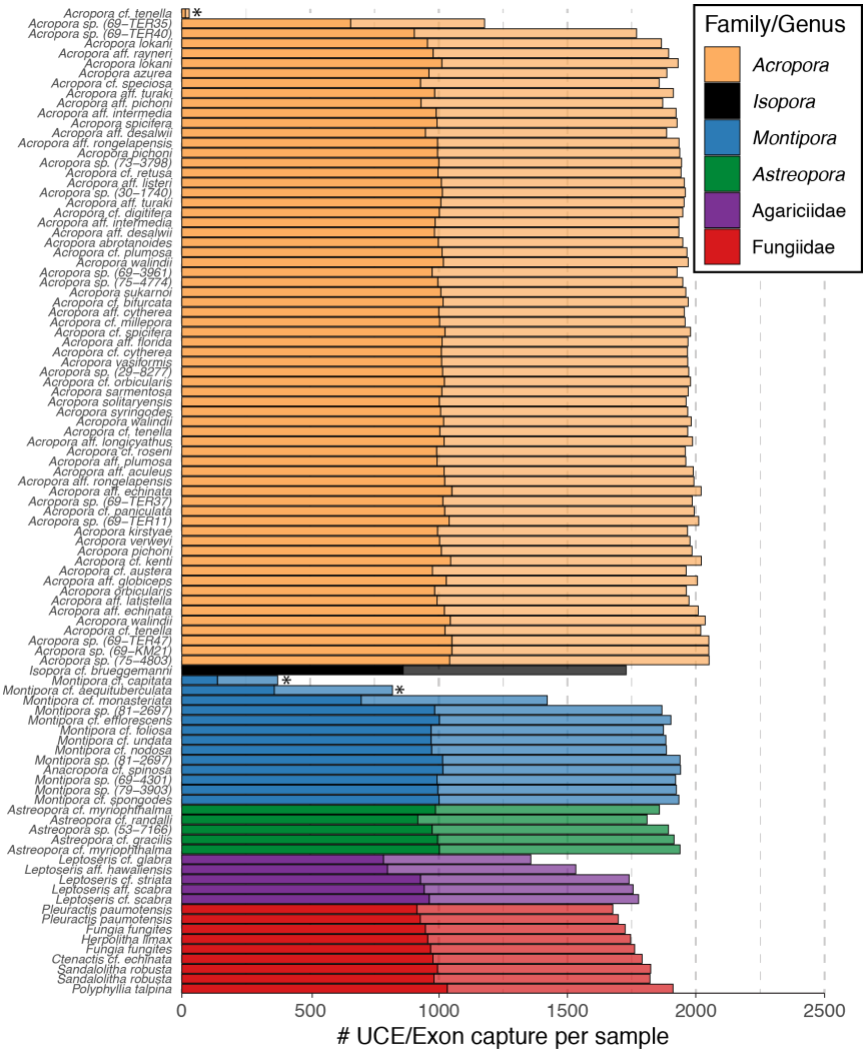


Figure 2. Number of UCE (solid) and exon (transparent) loci captured per taxa. Colours reflect family/genus membership. * denotes samples that were subsequently removed due to low capture rate.

Extracted loci were aligned and pruned to form 75% and 95% occupancy matrices for UCEs only and exons only. The final UCE only alignments consisted of 991 and 470 loci (75% and 95% matrices respectively) for 96 samples that passed quality assessment. The final exon only alignments contained 801 and 148 loci (75% and 95% matrices respectively) for the 96 samples. The final combined concatenated alignments contained 1792 UCE/exon loci in the 75% matrix and 618 in the 95% matrix (Table 2). The number of parsimony informative sites varied from 48% in alignments containing all three families (Acroporidae, Agariciidae, Fungiidae), to 1.5% among samples within a single *Acropora* species (*A.*

374 *walindii*). Within the family Acroporidae parsimony informative sites accounted for 38%
375 among genera, and 18% within the genus *Acropora* (Table 2).
376
377 *3.4. Coral phylogenomics*
378 All six alignment matrices produced robust phylogenies separating the three families and the
379 deep split between "complex" (Acroporidae + Agariciidae) and "robust" (Fungiidae) clades
380 within the Scleractinia (Fig. 3; Suppl Figs S1-3). Given the congruent topologies across
381 alignment sets, we discuss all systematic relationships below referring to the combined
382 UCE/exon data alignments with 75% completeness (Figs 3-4).
383 Both maximum likelihood (ML) and Bayesian analyses resulted in high support for
384 nodes in resulting consensus topologies (Fig. 3, Suppl Fig. S4). In the ML tree 83% of
385 internal nodes are resolved with RAxML bootstrap values of 100 (92% ≥ 70), and in the
386 Bayesian analyses 94% of nodes received posterior probabilities of 1 (96% ≥ 0.7; Suppl Fig.
387 S4). Within the Acroporidae, the genus *Astreopora* is the first lineage to branch off in the
388 family, followed by *Montipora* which contains *Anacropora* (Fig. 4). *Isopora* and *Acropora*
389 are recovered as sister clades but independent genera. Within *Acropora*, all phylogenetic
390 reconstructions highlight six major clades with high resolution (Fig. 4). Our molecular
391 phylogeny indicates that most of the morphological species groups of Wallace (1999) are
392 paraphyletic (9 of the 16 groups represented) (Fig. 4 inset). For example, species assigned to
393 the *robusta* group appear in clades VI, V and III in the phylogeny (Fig. 4). Similarly,
394 representatives of the *echinata*, *aspera* and *divaricata* groups are found across multiple
395 clades. Representatives of some morpho-groups (e.g. *elegans* and *hyacinthus* groups*)* occur in
396 the same molecular clade, but are paraphyletic at the species level, and do not reflect
397 morphological relationships among member species (Fig. 4).
398

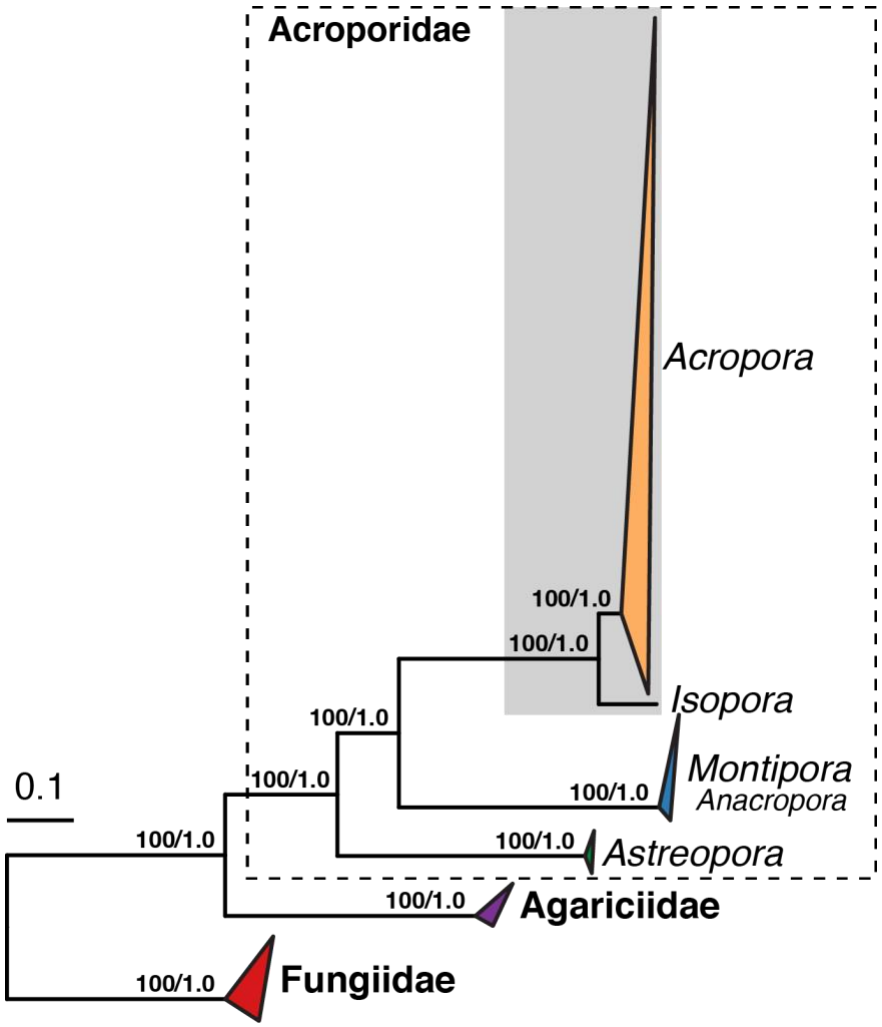
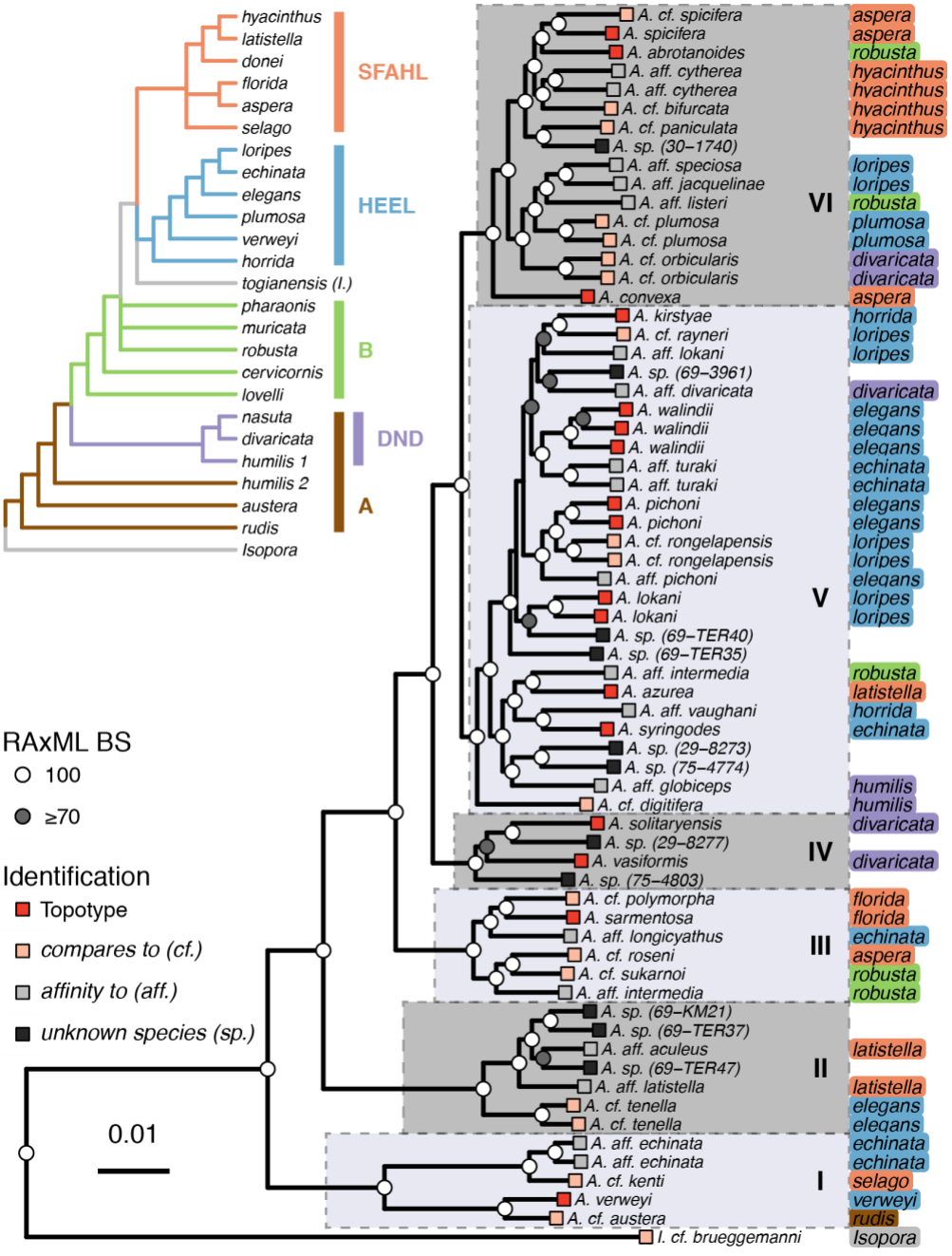


Figure 3. RAxML phylogeny based on 75% UCE/exon complete matrix. Phylogenetic relatiohsip are delineated between Acroporidae genera (dashed outline) and outgroup families Agariciidae and Fungiidae. Acroporidae and Agariciidae are both from the complex scleractinian clade, while Fungiidae is from the robust clade (Romano & Palumbi, 1996). Node values show RAxML bootstrap values and posterior probabilities from ExaBayes analysis. Species level relationships within the Acropora/Isopora clade (in grey box) are shown in Figure 4.



408

409 **Figure 4.** Phylogeny of Acropora and Isopora extracted from the ML analysis of the
410 combined UCE/exon 75% complete matrix. Inset cladogram depicts the systematic
411 relationships of morpho-groups from Wallace (1999) with branch colours highlighting the
412 major named morphological clades: Clade A (rudis, austera and humilis 2 groups) which
413 contains the DND clade (digitifera-nasuta-divaricata and humilis group 1); clade B (lovelli,
414 cervicornis, robusta, muricata, pharonis); the HEEL clade (horrida-elegans-echinata-loripes);
415 the SFAHL clade (selago-florida-aspera-hyacinthus-latistella). Specimen are labels indicate
416 morpho-group designation coloured by morphological clade (inset). Squares at tips reflect the
417 ON identification for each specimen (see legend). Bootstrap values of either 100% (white) or

418 greater than 70% (grey) are indicating on internal nodes. Robust molecular clades are
419 delineated I-VI.
420
421 Of the 65 *Acropora* specimens, 17 were classified as topotypes and 18 designated as
422 *cf.* In combination, these 35 specimens represent 26 nominal species of *Acropora*. In
423 addition, 20 specimens had affinities (*aff.*) to nominal species and 12 samples could not be
424 matched to any of the 414 nominal species (Suppl Table S1). In general, specimens given the
425 same open nomenclature clustered together with high bootstrap support (e.g. *A. walindii, A.*
426 *aff cytherea, A. cf plumosa*), as did the specimens identified as topotypes and those that
427 matched the type from another region (e.g. *A. spicifera and A. cf spicifera*) (Fig. 4). However,
428 not surprisingly, specimens with an affinity to a given nominal species often occurred in
429 different clades (e.g *A. aff. pichoni, A. aff. intermedia,* Fig. 4) suggesting that the
430 morphological characters on which these affinities were qualitatively assessed (gross
431 morphology and radial corallite shape) are not phylogenetically informative.
432
433 **4. Discussion**
434 *4.1 An enhanaced target-enrichment baits set for Hexacorallia*
435 Here, we provide a pipeline to redesign a class level UCE/exon bait set (Anthozoa; (Quattrini
436 et al., 2018) to improve the capture efficiency of loci from a variety of taxa within a lower
437 taxonomic rank (subclass Hexacorallia). Our new bait set, however, also includes a subset of
438 the originally targeted loci (anthozoa-v1), which will allow for future comparisons and
439 integration with the original probe set. We tested our enhanced hexacoral UCE/exon probe
440 design by focusing on a phylogenomic reconstruction of the staghorn corals (Acroporidae),
441 while also including families from both complex (Agariciidae) and robust (Fungiidae) clades
442 within the Scleractinia. This new enhanced probe set increases the capture efficiency of
443 UCE/exons for scleractinians when compared to the class-level design (Quattrini et al., 2018)

and results in higher phylogenetic resolution within an important group of reef building corals.

Our new bait design greatly increases the number of UCE and exon loci captured within the Hexacorallia (Fig. 2; Table 1, 2). Overall, there is an almost four-fold increase in the number of loci captured with the hexa-v2 bait set when compared to the original antho-v1 bait set. Resulting alignments retain higher numbers of loci for phylogenomic reconstruction within hexacorals in more complete alignment matrices: 1792 and 618 UCE/exon loci in 75% and 95% complete matrices respectively, compared to 438 UCE/exon loci in a 50% complete matrix (Quattrini et al., 2018). In addition, individual locus alignments are longer (1042 ± 529 bp; Table 2) when compared to those captured from the antho-v1 probe design (205 ± 93 bp; Quattrini et al., 2018), with a greater number of parsimony informative sites (46%; Table 2). Fewer numbers of reads and contigs were recovered in this study compared to Quattrini et al., (2018) therefore, it is possible that more on-target reads were obtained using this new bait set and/or the MyBaits v IV protocol, which was not used in Quattrini et al., (2018). In parallel to our redesign approach of the Anthozoa probe set, Quek et al., (2020) recently released a transcriptome-based targeted-enrichment bait set focused on Scleractinia targeting 1,139 exon regions. This Scleractinia focused exon bait set appears to have no overlap with the targeted exons of the Antho-v1 bait design (Quattrini et al., 2018; Quek et al., 2020) and only overlap in 12 exon loci, at a 50% similarity level, in our enhanced hexa-v2 scleractinia bait set (AMQ). While the dissimilarity are like due to the different taxonomic focus (Quek et al., 2020), future combinations of these independent bait designs will improve and expand the phylogenomic resolution of corals.

4.2 Coral systematics and taxonomy

The redesigned bait set provides a high throughput tool for phylogenetic inference in a systematically challenging group of corals. Initial molecular assessment of numerous groups within the Scleractinia have highlighted the fact that traditional morphological taxonomic schemes do not reflect systematic relationships or the evolutionary history revealed by molecular phylogenetics (Fukami et al., 2004; Huang et al., 2014; Kitahara et al., 2016; Romano and Palumbi, 1996). Similarly, our targeted capture data identifies many problems with the current systematics of the Acroporidae (Fig. 4), in particular, the incongruence between morphological and molecular based phylogenies (see also Richards et al., 2013). Unlike many other groups that have gained systematic resolution through the sequencing of single gene mitochondrial and nuclear markers, such markers provided limited phylogenetic resolution in the Scleractinia, particularly the hyperdiverse Acroporidae. However, a handful of informative markers have been exploited in an opportunistic way for phylogenetics in some clades. For example, the mitochondrial spacer region between COI and 16S rRNA has been used to resolve species boundaries in agariciid genera *Leptoseris* and *Pavona* from Hawaii (Luck et al., 2013; Pochon et al., 2015; Terraneo et al., 2017). Similarly, the putative mitochondrial control region and an open reading frame located between the mitochondrial ATP6 and NAD4 genes have provided resolution among genera in the family Pocilloporidae (Flot et al., 2011; Pinzón et al., 2013; Schmidt-Roach et al., 2013). However, the utility of these and other markers are not universal across coral taxa and sampling has not been uniform across clades or genes.

Obtaining informative phylogenetic markers capable of delineating species has been particularly problematic in the Acroporidae (but see Van Oppen et al., 2000; Márquez, Van Oppen, Willis, Reyes, & Miller, 2002). A recent phylogenetic reconstruction for the family Acroporidae (Huang et al., 2018) which mined two decades of molecular data resulted in an alignment representing a total 119 accepted species across seven mitochondrial markers and

two nuclear markers (ITS1 and 2, Pax-C). However, species sampling varied greatly among gene datasets (n = 17-73 species), resulting in a concatenated matrix that was 35% complete and where 25% of sampled species were represented by a single gene (Huang et al., 2018). Our testing of the enhanced bait set resulted in a 75% complete matrix consisting of 1792 UCE/exon loci for specimens linked to 26 nominal and 32 potentially undescribed species, where the lowest number of loci for a single species in the alignment was 976. Phylogenomic reconstructions of our UCE/exon dataset agree with other molecular phylogenetic studies of Scleractinia with a deep split between "complex" and "robust" corals (Romano and Palumbi, 1996; Ying et al., 2018) and the relationships among acroporid genera (Fig. 3; Fukami et al., 2000). At a shallower scale, this enhanced bait design provided a level of resolution within the genus *Acropora* that has not been achieved with available single markers or morphological analysis.

4.3. Phylogenomic resolution of staghorn coral

Phylogenomic reconstruction, using both ML and Bayesian methods, consistently resolved six molecular clades within *Acropora* with high support (bootstrap = 100; posterior =1), regardless of alignment type (Fig. 4; Suppl Figs S1-S4). While some traditional morphological characters seem to distinguish nominal species lineages they appear to offer little congruence with the molecular systematic relationships reconstructed here (Fig. 4) and in previous single gene reconstructions within *Acropora* (Huang et al., 2018; Richards et al., 2016, 2013). Richards et al. (2013) found 6-7 clades within *Acropora*, although the phylogenetic resolution was too low to support relationships among and within the clades. Despite little overlap in the species sampled, deep splits in the single gene tree of Richards et al. (2013) show some concordance with the arrangement of Clade I and II in our reconstruction (Fig. 4.), however, other clades differ considerably. Our sampling only

518 presents a small fraction of the diversity of *Acropora* and so increased sampling effort could
519 reveal further differentiation within and among the molecular clades highlighted here. Given
520 our sampling includes a broad range of traditional morpho-groupings (Fig. 4) from across the
521 Indo-Pacific (Table S2), it is probable that the genus *Acropora* is broadly represented by the
522 six molecular clades found here.
523

524 **5. Conclusions**

525 Our enhanced hexacoral bait set has the ability to generate new phylogenomic datasets to
526 resolve deep to shallow-level evolutionary relationships among reef building corals and their
527 relatives. This new bait set improved on the capture efficiency of the previous anthozoan bait
528 design resulting in higher numbers of UCE and exons in more complete and longer
529 alignments. Our subsequent phylogenomic analyses demonstrated that the
530 macromorphological characters traditionally used for taxonomic identification in corals do
531 not reflect evolutionary relationships. As climate change impacts coral reefs around the
532 world, conservation efforts rely on a robust taxonomy (Thomson et al., 2018) and a
533 phylogenetic framework in which to assess extinction risk (Huang, 2012). Importantly, over
534 50 % of our specimens cannot readily be assigned to any of the 414 nominal species of
535 *Acropora*, suggesting the true diversity of this genus has been seriously underestimated in
536 recent revisions (Wallace et al., 2012). Our new bait set, in conjunction with wider
537 geographic sampling of species and a close examination of the type material, will provide a
538 renewed taxonomic focus for reef building corals.
539

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553

554 **Author Contributions**

555 PFC designed the project with help from AMQ and CSM. Sample collection was conducted
556 by AB, TB, TER and MG. Morphological assessment of acroporids was conducted by TB
557 and AB. The hexa-2 bait set was designed by AMQ. AMQ assembled contigs and performed
558 phylogenomic analyses. Initial draft of paper was written by PFC and AMQ with input from
559 TB. All authors contributed to subsequent drafts.

560

561 **Data Accessibility**

562 Tree and alignment files: Data Dryad Entry https://doi.org/10.5061/dryad.9p8cz8wc8;
563 Raw Data: SRA GenBank SUB6852542, BioSample #SAMN13871686-1781;
564 Hexacoral bait set: Supplemental Files 2 and 3, Data Dryad Entry
565 https://doi.org/10.5061/dryad.9p8cz8wc8

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807

808 **Figure Captions**

809 **Figure 1.** Coral specimens were identified by comparison to type material when available (*in*
810 *situ* examination at MTQ; images and original descriptions). Based on these comparisons
811 specimens were (a) identified to nominal species if they were considered a topotype sample;
812 (b) assigned the ON qualifier *"cf"* if they resembled the type material but were not collected
813 from the type location; (c) assigned the ON qualifier *"aff."* if they had morphological
814 affinities to a nominal species but could not be reliably identified using the information
815 available; and (d) assigned the ON qualifier *"sp."* if they showed little resemblance to any
816 nominal species type specimen.

817 **Figure 2.** Number of UCE (solid) and exon (transparent) loci captured per taxa. Colours
818 reflect family/genus membership. * denotes samples that were subsequently removed due to
819 low capture rate.
820 **Figure 3.** RAxML phylogeny based on 75% UCE/exon complete matrix. Phylogenetic
821 relatiohsip are delineated between Acroporidae genera (dashed outline) and outgroup families
822 Agariciidae and Fungiidae. Acroporidae and Agariciidae are both from the complex
823 scleractinian clade, while Fungiidae is from the robust clade (Romano & Palumbi, 1996).
824 Node values show RAxML bootstrap values and posterior probabilities from ExaBayes
825 analysis. Species level relationships within the Acropora/Isopora clade (in grey box) are
826 shown in Figure 4.
827 **Figure 4.** Phylogeny of *Acropora* and *Isopora* extracted from the ML analysis of the
828 combined UCE/exon 75% complete matrix. Inset cladogram depicts the systematic
829 relationships of morpho-groups from Wallace (1999) with branch colours highlighting the
830 major named morphological clades: Clade A (*rudis, austera* and *humilis 2* groups) which
831 contains the DND clade (*digitifera-nasuta-divaricata* and *humilis* group 1); clade B (*lovelli,*
832 *cervicornis, robusta, muricata, pharonis*); the HEEL clade (*horrida-elegans-echinata-*
833 *loripes*); the SFAHL clade (*selago-florida-aspera-hyacinthus-latistella*). Specimen are labels
834 indicate morpho-group designation coloured by morphological clade (inset). Squares at tips
835 reflect the ON identification for each specimen (see legend). Bootstrap values of either 100%
836 (white) or greater than 70% (grey) are indicating on internal nodes. Robust molecular clades
837 are delineated I-VI.

838 **Table 1.** List of specimens used in the in vitro test of enhanced Hexacoral bait set with assembly summary statistics. Greyed out samples were
839 removed from further analyses due to low contig number and/or matches to loci. Open nomenclature qualifiers (*cf.*, *aff.*, *sp.*) are used in species
840 names to denote confidence in specimen identification to nominal species.

Family	Species	Specimen No.	# Contigs	# UCE	UCE Mean Length (bp)	# Exon	Exon Mean Length (bp)	Total # Loci
Acroporidae	*Acropora abrotanoides*	16-5870	10335	998	980	951	1075	1949
Acroporidae	*Acropora aff. aculeus*	PN47	7971	1021	988	969	1069	1990
Acroporidae	*Acropora aff. cytherea*	74-4537	7393	1010	1001	957	1098	1967
Acroporidae	*Acropora aff. cytherea*	30-5227	11194	1000	1002	955	1095	1955
Acroporidae	*Acropora aff. divaricata*	73-3798	7680	1000	1041	944	1136	1944
Acroporidae	*Acropora aff. echinata*	KM27	8079	1051	844	969	893	2020
Acroporidae	*Acropora aff. echinata*	KM32	7965	1022	949	987	1022	2009
Acroporidae	*Acropora aff. globiceps*	17-6445	5767	1029	997	977	1082	2006
Acroporidae	*Acropora aff. intermedia*	30-5271	7164	986	1083	948	1167	1934
Acroporidae	*Acropora aff. intermedia*	B6	7762	990	1003	933	1104	1923
Acroporidae	*Acropora aff. jacquelinae*	PN54	6531	983	1056	951	1143	1934
Acroporidae	*Acropora aff. latistella*	75-4779	9676	993	1035	980	1112	1973
Acroporidae	*Acropora aff. listeri*	75-4879	10598	1010	819	945	866	1955
Acroporidae	*Acropora aff. lokani*	PN51	10063	978	1025	916	1105	1894
Acroporidae	*Acropora aff. longicyathus*	TER_36	9322	1021	782	966	830	1987
Acroporidae	*Acropora aff. pichoni*	TER_20	9475	931	981	940	1095	1871
Acroporidae	*Acropora aff. speciosa*	PN62	8978	948	1065	938	1153	1886
Acroporidae	*Acropora aff. turaki*	KM35	8492	984	1099	928	1170	1912
Acroporidae	*Acropora aff. turaki*	KM50	8194	1008	933	946	1013	1954
Acroporidae	*Acropora azurea*	75-4857	8498	962	1055	925	1154	1887
Acroporidae	*Acropora cf. austera*	75-4732	8289	975	1090	987	1183	1962

Acroporidae	*Acropora cf. bifurcata*	29-8193	9438	1017	967	953	1053	1970
Acroporidae	*Acropora cf. digitifera*	81-2705	6123	1002	940	947	1014	1949
Acroporidae	*Acropora cf. kenti*	75-4865	5133	1046	722	975	747	2021
Acroporidae	*Acropora cf. orbicularis*	16-5974	13383	1022	885	957	965	1979
Acroporidae	*Acropora cf. orbicularis*	29-8212	14180	984	1043	979	1102	1963
Acroporidae	*Acropora cf. paniculata*	75-4686	5743	1023	920	970	1005	1993
Acroporidae	*Acropora cf. plumosa*	KM51	12294	1013	974	952	1067	1965
Acroporidae	*Acropora cf. plumosa*	69-4038	6009	993	997	968	1091	1961
Acroporidae	*Acropora cf. polymorpha*	B4	6351	1013	953	956	1042	1969
Acroporidae	*Acropora cf. rayneri*	TER_44	10527	929	957	928	1046	1857
Acroporidae	*Acropora cf. rongelapensis*	PN44	7277	995	1059	939	1181	1934
Acroporidae	*Acropora cf. rongelapensis*	PN45	8232	1023	968	969	1050	1992
Acroporidae	*Acropora cf. roseni*	29-8208	10316	992	1078	967	1192	1959
Acroporidae	*Acropora cf. spicifera*	29-8257	13415	1024	943	955	1042	1979
Acroporidae	*Acropora cf. sukarnoi*	29-8294	6943	1008	984	953	1079	1961
Acroporidae	*Acropora cf. tenella*	69-3957	6206	1004	1060	964	1168	1968
Acroporidae	*Acropora cf. tenella*	PN43	6889	1024	923	995	1008	2019
Acroporidae	*Acropora cf. tenella*	PN52	47	14	247	15	257	29
Acroporidae	*Acropora convexa*	30-5251	7817	1003	993	955	1081	1958
Acroporidae	*Acropora kirstyae*	74-4386	6885	995	1059	972	1159	1967
Acroporidae	*Acropora lokani*	TER_8	12433	956	1036	910	1120	1866
Acroporidae	*Acropora lokani*	69-4073	14983	1012	948	919	1007	1931
Acroporidae	*Acropora pichoni*	69-3948	7471	1010	977	975	1063	1985
Acroporidae	*Acropora pichoni*	TER_42	13089	995	981	942	1055	1937
Acroporidae	*Acropora sarmentosa*	74-4500	6377	1012	974	958	1041	1970
Acroporidae	*Acropora solitaryensis*	79-3886	10498	1002	920	960	1009	1962
Acroporidae	*Acropora sp. (29-8273)*	29-8273	7395	997	930	945	1024	1942
Acroporidae	*Acropora sp. (29-8277)*	29-8277	16145	1015	966	957	1052	1972

Acroporidae	*Acropora sp. (30-1740)*	30-1740	10037	1014	903	945	965	1959
Acroporidae	*Acropora sp. (69-3961)*	69-3961	7763	974	1034	953	1144	1927
Acroporidae	*Acropora sp. (69-KM21)*	KM21	3193	1051	541	999	528	2050
Acroporidae	*Acropora sp. (69-TER11)*	TER_11	14622	1040	698	971	733	2011
Acroporidae	*Acropora sp. (69-TER35)*	TER_35	1498	657	297	521	287	1178
Acroporidae	*Acropora sp. (69-TER37)*	TER_37	3041	1016	533	970	518	1986
Acroporidae	*Acropora sp. (69-TER40)*	TER_40	11472	905	832	864	893	1769
Acroporidae	*Acropora sp. (69-TER47)*	TER_47	4805	1051	833	999	887	2050
Acroporidae	*Acropora sp. (75-4774)*	75-4774	9197	996	980	953	1080	1949
Acroporidae	*Acropora sp. (75-4803)*	75-4803	3251	1042	614	1009	612	2051
Acroporidae	*Acropora spicifera*	30-5267	10635	993	1047	934	1155	1927
Acroporidae	*Acropora syringodes*	74-4532	11115	1007	1019	961	1107	1968
Acroporidae	*Acropora vasiformis*	16-5867	7941	1011	923	957	1021	1968
Acroporidae	*Acropora verweyi*	75-4990	9515	1003	1032	974	1114	1977
Acroporidae	*Acropora walindii*	KM30	8091	1019	989	963	1080	1982
Acroporidae	*Acropora walindii*	69-4031	8058	1018	985	952	1077	1970
Acroporidae	*Acropora walindii*	TER_13	3883	1045	817	991	880	2036
Acroporidae	*Anacropora cf. spinosa*	53-7072	43708	1016	955	924	903	1940
Acroporidae	*Astreopora cf. gracilis*	53-6964	48967	995	1011	920	984	1915
Acroporidae	*Astreopora cf. myriophthalma*	53-6863	20538	987	931	871	907	1858
Acroporidae	*Astreopora cf. myriophthalma*	53-7112	53608	1002	1034	936	1003	1938
Acroporidae	*Astreopora cf. randalli*	53-7115	49989	918	1017	892	1021	1810
Acroporidae	*Astreopora sp. (53-7166)*	53-7166	53766	974	1022	919	1027	1893
Acroporidae	*Isopora cf. brueggemanni*	53-7063	9041	861	987	868	1050	1729
Acroporidae	*Montipora cf. aequituberculata*	81-2751	11092	360	739	460	637	820
Acroporidae	*Montipora cf. capitata*	TER_9	18227	139	805	234	722	373
Acroporidae	*Montipora cf. efflorescens*	81-2884	9249	1002	762	901	749	1903
Acroporidae	*Montipora cf. foliosa*	74-4576	13959	970	864	904	816	1874

Acroporidae	*Montipora cf. monasteriata*	TER_46	15035	698	949	724	917	1422
Acroporidae	*Montipora cf. nodosa*	75-5006	14672	972	1001	913	963	1885
Acroporidae	*Montipora cf. spongodes*	81-2847	5295	1001	646	933	633	1934
Acroporidae	*Montipora cf. undata*	79-3890	11248	970	888	913	871	1883
Acroporidae	*Montipora sp. (69-4301)*	69-4301	15035	993	874	928	866	1921
Acroporidae	*Montipora sp. (79-3903)*	79-3903	6283	996	722	928	700	1924
Acroporidae	*Montipora sp. (81-2697)*	81-2791	7488	1015	741	923	723	1938
Acroporidae	*Montipora sp. (81-2697)*	81-2697	14906	984	846	884	837	1868
Agariciidae	*Leptoseris aff. hawaiiensis*	PN36	13524	800	907	733	867	1533
Agariciidae	*Leptoseris aff. incrustans*	PN25	14186	943	981	813	949	1756
Agariciidae	*Leptoseris cf. fragilis*	PN12	15120	929	1038	811	983	1740
Agariciidae	*Leptoseris cf. glabra*	PN09	2159	785	515	573	470	1358
Agariciidae	*Leptoseris cf. scabra*	PN26	9257	963	994	814	967	1777
Fungiidae	*Ctenactis cf. echinata*	Cf	12759	977	987	814	926	1791
Fungiidae	*Fungia fungites*	60f	15389	948	1019	777	950	1725
Fungiidae	*Fungia fungites*	903f	12924	968	969	794	925	1762
Fungiidae	*Herpolitha limax*	4f	15865	957	1056	789	994	1746
Fungiidae	*Pleuractis paumotensis*	3f	18010	915	1038	762	975	1677
Fungiidae	*Pleuractis paumotensis*	11f	14016	928	997	770	945	1698
Fungiidae	*Polyphyllia talpina*	7f	15708	1033	960	878	921	1911
Fungiidae	*Sandalolitha robusta*	10f	8447	995	892	829	833	1824
Fungiidae	*Sandalolitha robusta*	6f	13475	981	963	840	906	1821

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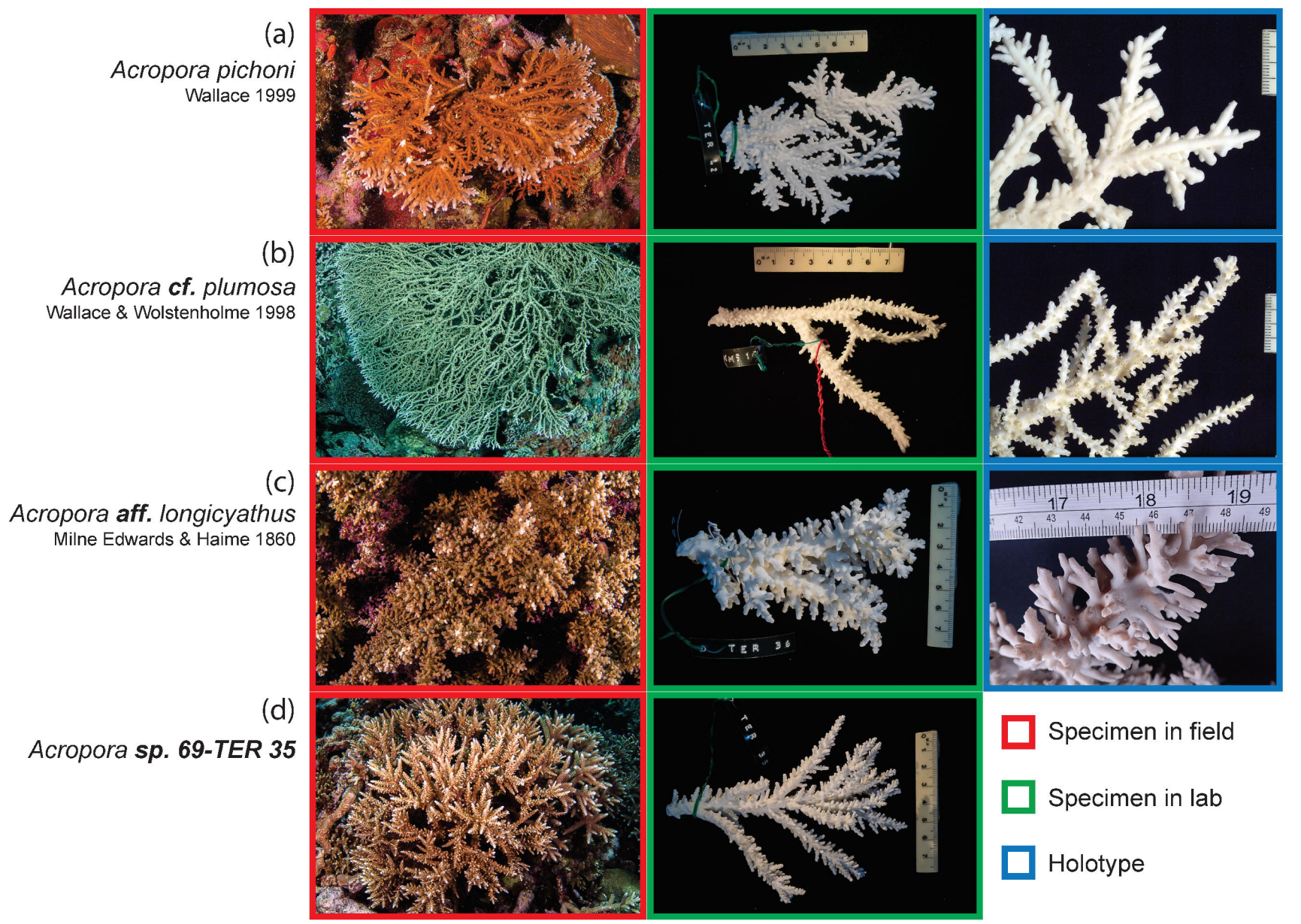
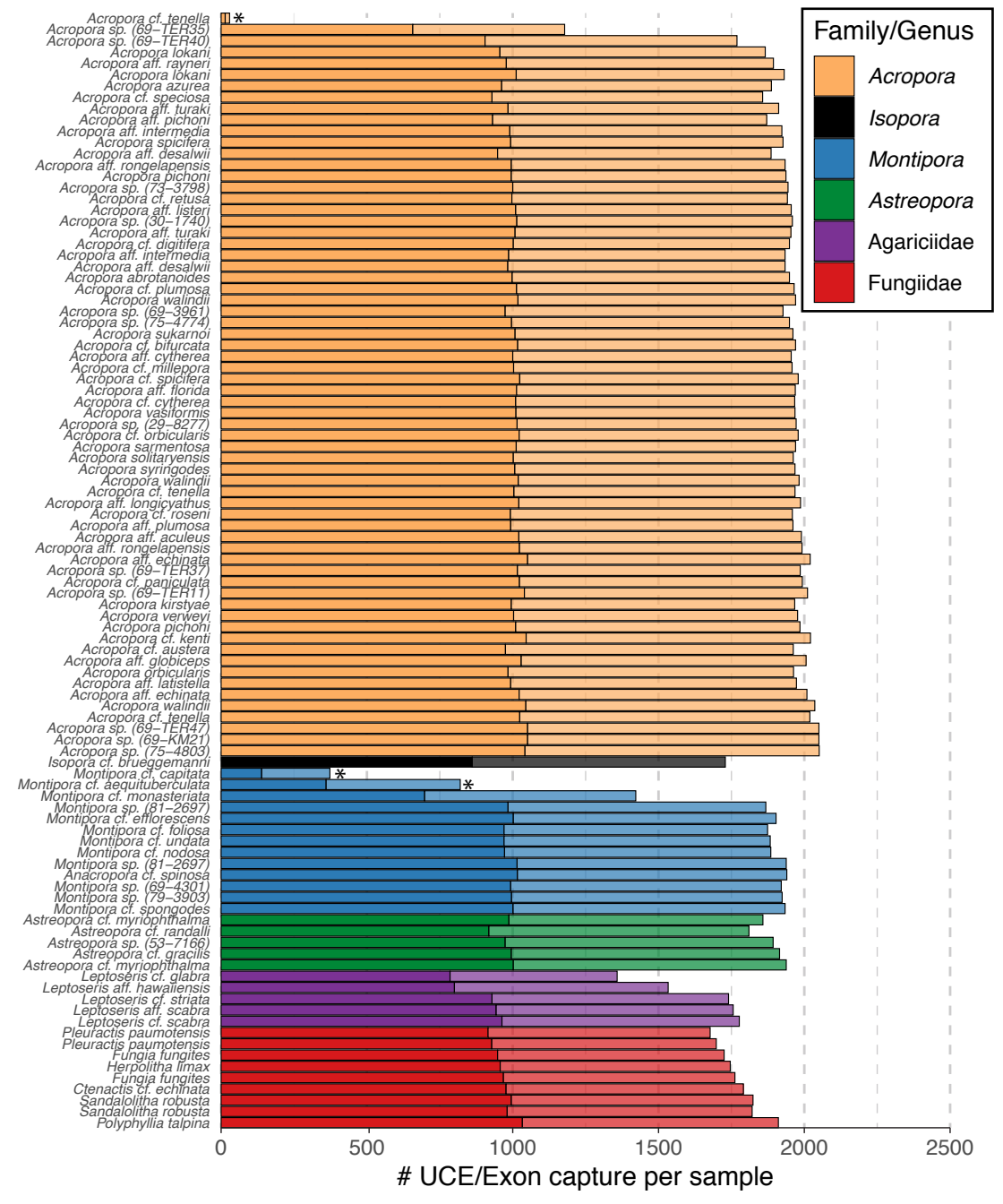
845 **Table 2.** Alignment matrix statistics for different taxonomic datasets. Matrix percentage equals the percent occupancy of species per locus. PI =

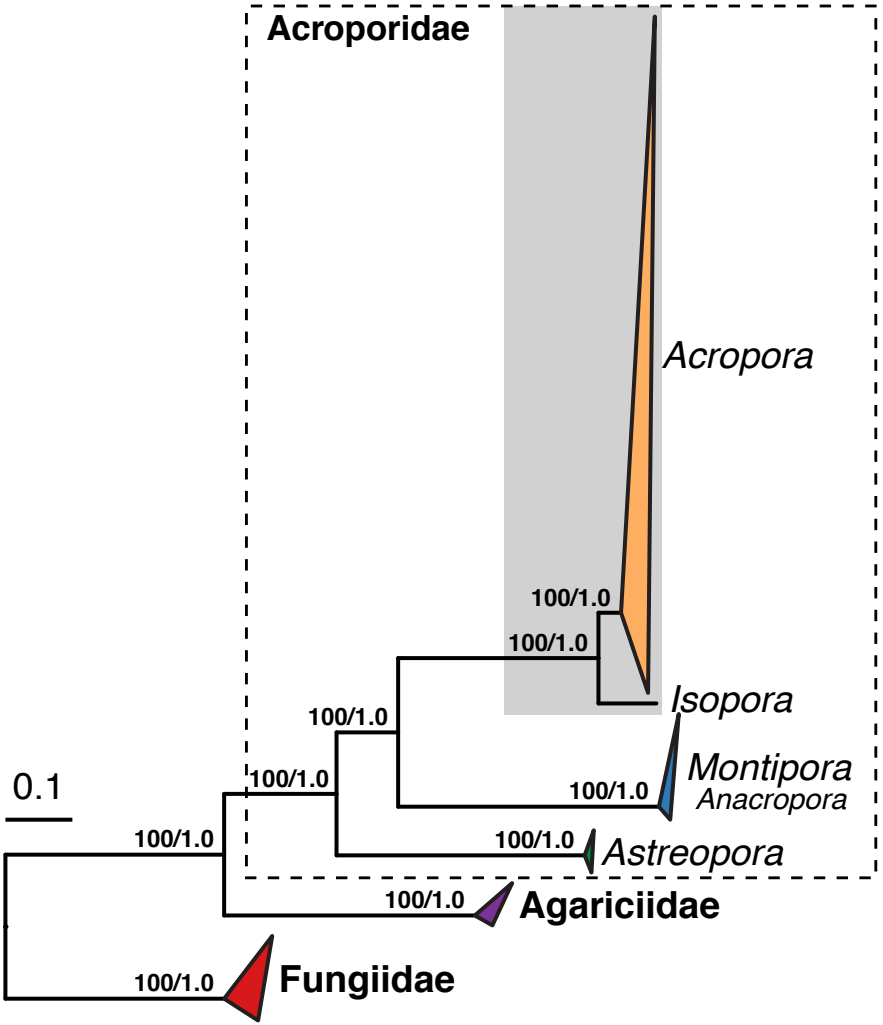
846 parsimony informative sites as calculated in Phyluce.

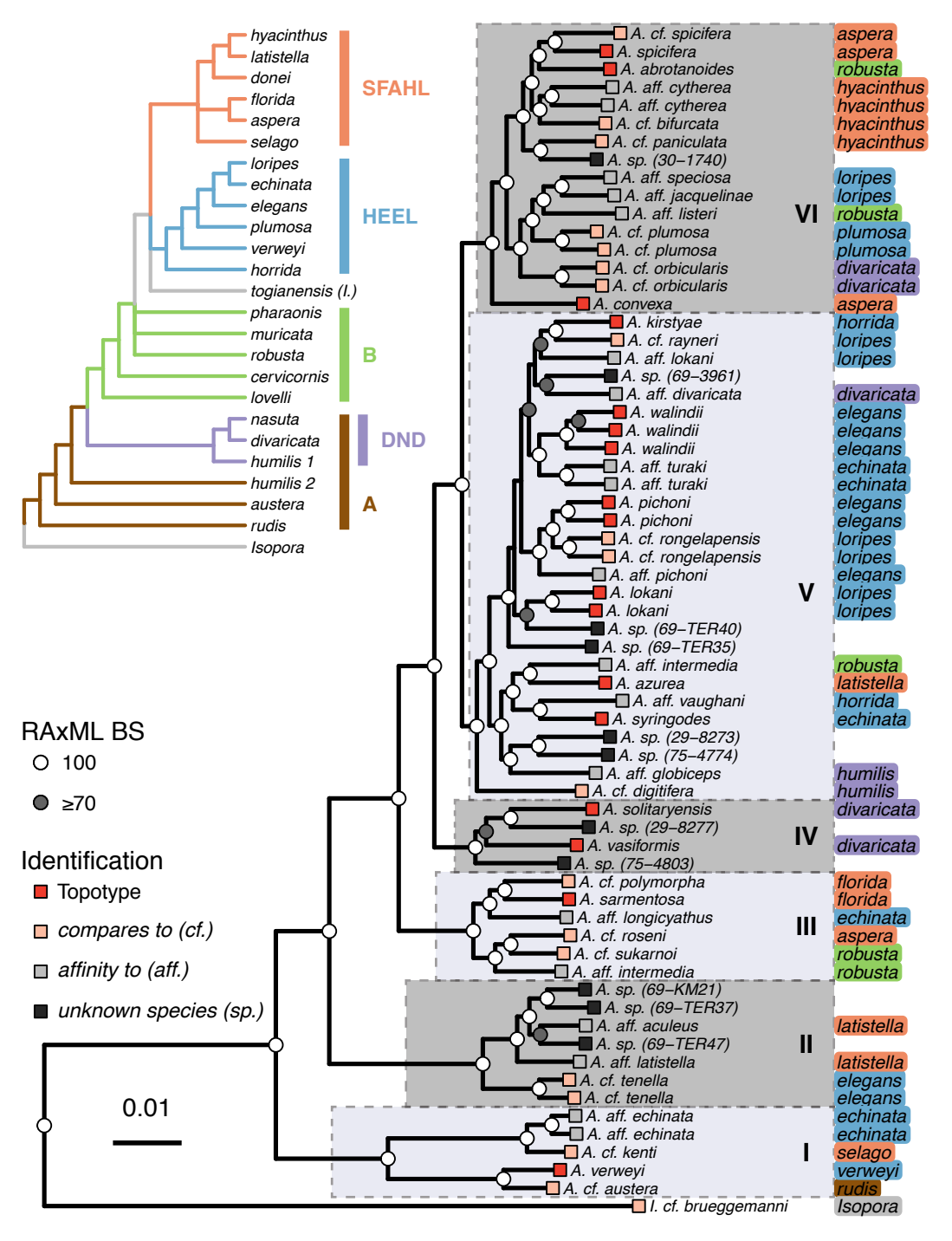
Dataset	# Taxa	% Matrix	Total # Loci	# UCE/exon Loci	Alignment Length	Mean Aligned Locus Length (± SD bp)	Aligned Locus Length Range (bp)	# PI Sites	% PI Sites[a]
All Taxa	96	75	1792	991/801	1,868,953	1042 ± 529	106-4220	697,843	46
	96	95	618	470/148	611,631	989 ± 413	222-3653	238,569	45
Acroporidae	83	75	1852	992/860	2,236,868	1208 ± 532	267-4316	671,600	38
	83	95	899	596/303	1,055,570	1174 ± 484	423-4213	325,959	38
Acropora	64	75	1916	993/923	2,280,802	1190 ± 456	342-3837	327,541	18
	64	95	1227	739/488	1,410,701	1157 ± 402	494-3365	189,608	16
A. walindii	3	100	1781	949/832	1,746,225	980 ± 256	338-3238	27,248[b]	1.5

847 [a] calculated by dividing # PI sites by # sites checked for differences
848 [b] calculated as number of variable sites present in all loci
849
850


(a)
Acropora pichoni
Wallace 1999
(b)
Acropora cf. plumosa
Wallace & Wolstenholme 1998
(c)
Acropora aff. longicyathus
Milne Edwards & Haime 1860
(d)
Acropora sp. 69-TER 35
Specimen in field
Specimen in lab
Holotype


Family/Genus
Acropora
Isopora
Montipora
Astreopora
Agariciidae
Fungiidae
Acropora cf. tenella
Acropora sp. (69–TER35)
Acropora sp. (69–TER40)
Acropora lokani
Acropora aff. rayneri
Acropora lokani
Acropora azurea
Acropora cf. speciosa
Acropora aff. turaki
Acropora aff. pichoni
Acropora aff. intermedia
Acropora spicifera
Acropora aff. desalwii
Acropora aff. rongelapensis
Acropora pichoni
Acropora sp. (73–3798)
Acropora cf. retusa
Acropora aff. listeri
Acropora sp. (30–1740)
Acropora aff. turaki
Acropora cf. digitifera
Acropora aff. intermedia
Acropora aff. desalwii
Acropora abrotanoides
Acropora cf. plumosa
Acropora walindii
Acropora sp. (69–3961)
Acropora sp. (75–4774)
Acropora sukarnoi
Acropora cf. bifurcata
Acropora aff. cytherea
Acropora cf. millepora
Acropora cf. spicifera
Acropora aff. florida
Acropora cf. cytherea
Acropora vasiformis
Acropora sp. (29–8277)
Acropora cf. orbicularis
Acropora sarmentosa
Acropora solitaryensis
Acropora syringodes
Acropora walindii
Acropora cf. tenella
Acropora aff. longicyathus
Acropora cf. roseni
Acropora aff. plumosa
Acropora aff. aculeus
Acropora aff. rongelapensis
Acropora aff. echinata
Acropora sp. (69–TER37)
Acropora cf. paniculata
Acropora sp. (69–TER11)
Acropora kirstyae
Acropora verweyi
Acropora pichoni
Acropora cf. kenti
Acropora cf. austera
Acropora aff. globiceps
Acropora orbicularis
Acropora aff. latistella
Acropora aff. echinata
Acropora walindii
Acropora cf. tenella
Acropora sp. (69–TER47)
Acropora sp. (69–KM21)
Acropora sp. (75–4803)
Isopora cf. brueggemanni
Montipora cf. capitata
Montipora cf. aequituberculata
Montipora cf. monasteriata
Montipora sp. (81–2697)
Montipora cf. efflorescens
Montipora cf. foliosa
Montipora cf. undata
Montipora cf. nodosa
Montipora sp. (81–2697)
Anacropora cf. spinosa
Montipora sp. (69–4301)
Montipora sp. (79–3903)
Montipora cf. spongodes
Astreopora cf. myriophthalma
Astreopora cf. randalli
Astreopora sp. (53–7166)
Astreopora cf. gracilis
Astreopora cf. myriophthalma
Leptoseris cf. glabra
Leptoseris aff. hawaiiensis
Leptoseris cf. striata
Leptoseris aff. scabra
Leptoseris cf. scabra
Pleuractis paumotensis
Pleuractis paumotensis
Fungia fungites
Herpolitha limax
Fungia fungites
Ctenactis cf. echinata
Sandalolitha robusta
Sandalolitha robusta
Polyphyllia talpina
0
500
1000
1500
2000
2500
UCE/Exon capture per sample


Acroporidae
Acropora
100/1.0
100/1.0
Isopora
100/1.0
Montipora
Anacropora
100/1.0
100/1.0
0.1
100/1.0
100/1.0
Astreopora
100/1.0
Agariciidae
100/1.0
Fungiidae


hyacinthus
latistella
donei
florida
aspera
selago
SFAHL
loripes
echinata
elegans
plumosa
verweyi
horrida
HEEL
togianensis (I.)
pharaonis
muricata
robusta
cervicornis
lovelli
B
nasuta
divaricata
humilis 1
DND
humilis 2
austera
rudis
A
Isopora
A. cf. spicifera
A. spicifera
A. abrotanoides
A. aff. cytherea
A. aff. cytherea
A. cf. bifurcata
A. cf. paniculata
A. sp. (30–1740)
A. aff. speciosa
A. aff. jacquelinae
A. aff. listeri
A. cf. plumosa
A. cf. plumosa
A. cf. orbicularis
A. cf. orbicularis
A. convexa
VI
aspera
aspera
robusta
hyacinthus
hyacinthus
hyacinthus
hyacinthus
loripes
loripes
robusta
plumosa
plumosa
divaricata
divaricata
aspera
A. kirstyae
A. cf. rayneri
A. aff. lokani
A. sp. (69–3961)
A. aff. divaricata
A. walindii
A. walindii
A. walindii
A. aff. turaki
A. aff. turaki
A. pichoni
A. pichoni
A. cf. rongelapensis
A. cf. rongelapensis
A. aff. pichoni
A. lokani
A. lokani
A. sp. (69–TER40)
A. sp. (69–TER35)
A. aff. intermedia
A. azurea
A. aff. vaughani
A. syringodes
A. sp. (29–8273)
A. sp. (75–4774)
A. aff. globiceps
A. cf. digitifera
V
horrida
loripes
loripes
divaricata
elegans
elegans
elegans
echinata
echinata
elegans
elegans
loripes
loripes
elegans
loripes
loripes
robusta
latistella
horrida
echinata
humilis
humilis
A. solitaryensis
A. sp. (29–8277)
A. vasiformis
A. sp. (75–4803)
IV
divaricata
divaricata
A. cf. polymorpha
A. sarmentosa
A. aff. longicyathus
A. cf. roseni
A. cf. sukarnoi
A. aff. intermedia
III
florida
florida
echinata
aspera
robusta
robusta
A. sp. (69–KM21)
A. sp. (69–TER37)
A. aff. aculeus
A. sp. (69–TER47)
A. aff. latistella
A. cf. tenella
A. cf. tenella
II
latistella
latistella
elegans
elegans
A. aff. echinata
A. aff. echinata
A. cf. kenti
A. verweyi
A. cf. austera
I
echinata
echinata
selago
verweyi
rudis
I. cf. brueggemanni
Isopora
RAxML BS
100
≥70
Identification
Topotype
compares to (cf.)
affinity to (aff.)
unknown species (sp.)
0.01