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MITOGENOME STABILITY AND STRUCTURAL VARIATION IN ACROPORA DIGITIFERA: A COMPARATIVE GENOMIC AND ANNOTATIONINTEGRATED ASSESSMENT

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Abstract

Understanding mitochondrial genome evolution is crucial for resolving phylogenetic relationships and assessing genomic stability across reef-building corals. In this study, we systematically examined the mitochondrial genomes of five Acropora digitifera samples (S1B–S5A) collected from the coastal waters of Oman to investigate patterns of gene order conservation, strand orientation, and structural variation. Using annotation-informed genome visualization tools, including circular genome maps and gene order dot plots, we revealed highly conserved mitochondrial architecture among samples, with minor sample-specific deviations such as split rrnS annotations in S3A and duplicated replication origin regions (OH_2) in S2D.

Protein-coding genes (PCGs) and transfer RNA genes were predominantly located on the positive strand, while ribosomal RNA genes (rrnL, rrnS) and trnM(cat) were consistently encoded on the negative strand across all samples. BUSCO analysis of corresponding nuclear genome assemblies showed strong correlation with mitochondrial genome quality, with completeness scores ranging from 25.5% (S4A) to 53.7% (S5A), indicating that structural anomalies were more prevalent in assemblies with lower overall genome completeness.

Comparative analysis with published coral mitogenomes confirmed both the overall stability and minor plasticity of organellar genomes in A. digitifera. This study highlights the importance of high-quality genome assembly and annotation tools for reliable mitochondrial genome interpretation, and provides a valuable framework for future phylogenomic and population genomic research in scleractinian corals. Our findings contribute to understanding mitochondrial evolution in reef-building corals and establish baseline genomic resources for the understudied coral fauna of the Arabian Sea and Gulf of Oman.

Keywords: Acropora digitifera, mitochondrial genome, gene order, genomic stability, coral genomics, NOVOPlasty, BUSCO, Oman.

1. Introduction

Mitochondrial genomes have long served as a cornerstone in evolutionary and phylogenetic studies due to their compact structure, predominantly maternal inheritance, and relatively conserved gene content (Shearer et al., 2002). In anthozoans—particularly reef-building corals of the order Scleractinia—the mitochondrial genome plays a critical role in understanding both evolutionary dynamics and organismal responses to environmental stressors (Medina et al., 2006; Shinzato et al., 2011). Compared to other metazoan groups, coral mitogenomes exhibit unusual structural stability, characterized by limited gene rearrangements and highly conserved gene order observed across major taxonomic clades (van Oppen et al., 2002; Flot & Tillier, 2007).

However, emerging genome-wide data from multiple coral species have revealed subtle intraspecific variations, including intron mobility, gene fragmentation, and duplicated non-coding regions (Shearer et al., 2002; Lin et al., 2011). These structural features may influence organellar genome interpretation and complicate comparative phylogenetic studies. Furthermore, the increasing reliance on automated bioinformatics tools for organellar genome

assembly and annotation necessitates careful validation to distinguish true biological variation from technical artifacts. Acropora digitifera (Dana, 1846) is one of the most ecologically significant and genetically well-studied coral species in the Indo-Pacific region. As a dominant reef-building species, it serves as a model organism for coral biology, symbiosis, and climate change research. Previous landmark studies have provided reference genomes and transcriptomes for this species (Shinzato et al., 2011), establishing it as a genomic resource for the broader coral research community. However, comprehensive comparative analyses of mitochondrial genome structure across geographically distinct populations and individuals remain limited.

The Arabian Sea and Gulf of Oman represent unique marine environments characterized by high salinity, elevated temperatures, and pronounced seasonal upwelling (Reynolds, 1993). These environmental extremes may drive adaptive evolution and genomic variation in resident coral populations. Despite the ecological and biogeographic importance of this region, coral genomic resources from Omani waters remain scarce compared to those from the Great Barrier Reef, Red Sea, and Caribbean regions.

In this study, we systematically analyzed and compared the mitochondrial genomes of five A. digitifera samples collected from different locations along the Omani coastline. Our specific objectives were to: (1) assess gene content, order, and strand orientation using annotation-informed visualizations; (2) identify structural variations and potential assembly artifacts; (3) evaluate the relationship between mitochondrial genome quality and nuclear genome completeness using BUSCO metrics; and (4) compare our findings with published coral mitogenomes to contextualize patterns of genomic stability and variation.

By integrating comparative visualization, structural annotation analysis, and genome quality assessment, this work aims to provide refined insights into mitochondrial genome evolution and stability in A. digitifera, while also contributing valuable genomic resources for the understudied coral fauna of the northwestern Indian Ocean.

2. Materials and Methods

2.1 Sample Collection and Processing

In March 2022, the 5 Acropora digitifera colonies (named S1B, S2D, S3A, S4A, S5A) were sampled in the shallow reef habitats (256 m depth) of the coast of Sur, Sultanate of Oman (Figure 1).

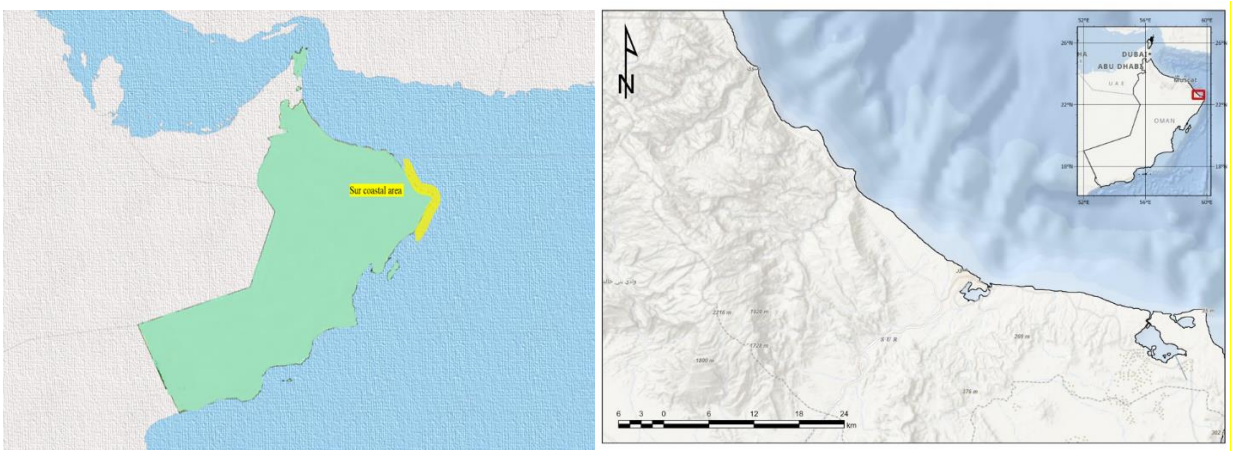

Figure 1. Sampling location in the Sur Coastal Area, Oman, in the shallows.

The sampling sites were chosen to reflect geographically different sites in the study area. Sterilized bone cutters were used to take small coral fragments (approximately 23 cm2), and these were placed immediately in either molecular-grade ethanol (95%) or RNAlater solution at -20 °C until DNA extraction. Coral samples were collected from the Sur Industrial Estate coastal area (Sur, Oman) under institutional permission obtained from the University of Technology and Applied Sciences–Sur and the Director General of Sur Industrial Estate City (approval letter dated 05 December 2022, Ref. TRC-RG/2022/01).

The coral samples used in this study were collected from shallow reef areas along the eastern coast of Oman, specifically in the Sur area, where Acropora digitifera colonies grow in waters 1-5 meters deep with good light. These reef systems are influenced by seasonal upwelling in the Arabian Sea, which establishes an active temperature and nutrient regime that sustains a variety of coral assemblages. The sampling locations are typical fringing reef environments with a stable salinity and moderate exposure to waves, which provides an ideal environment to examine within-species structural variation of coral mitochondrial genomes. All field collections had to be done under calm sea

conditions to reduce stress on the colonies around, and sample fragments were stored immediately to be subjected to molecular analysis.

Identification of the species was conducted using morphological features, such as the corallite structure, colony growth form, and branching pattern (Figure 2) as per the taxonomic keys of Veron (2000). Vouchers were deposited in the Marine Biodiversity Collection at UTAS-Sur (Collection IDs: UTAS-MB-001-UTAS-MB-005).

Figure 2. Close-up of Acropora digitifera.

2.2 DNA Extraction and Library Preparation

Preserved coral tissue was subjected to a modified phenol-chloroform protocol optimized for coral tissue to extract the genomic DNA. The coral fragments were initially crushed in liquid nitrogen, and the RNase A solution was added to the tissue lysates to eliminate the contaminating RNA. A NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) was used to determine DNA quality, with A260/ A280 ratios of 1.8-2.0 being regarded as acceptable. The Qubit 4.0 fluorometer was used to quantify the amount of DNA in the DNA BR Assay Kit (Invitrogen) using the dsDNA BR Assay Kit.

WGS sequencing libraries were constructed with the Illumina DNA Prep kit by following the instructions of the manufacturer. In short, enzymatic tagmentation of 200 ng of high-quality genomic DNA was performed, then adapters were ligated, and PCR amplification was done (12 cycles). The size-selection of libraries was done to obtain an insert size distribution with an average of 300-500 bp and was quantified through quantitative PCR. The sequencing of pooled libraries was performed on an Illumina NovaSeq 6000 system with 2 x 150 bp paired-end chemistry, aiming to achieve at least 30 Gb raw sequence data per sample.

2.3 Quality Control and Genome Assembly

Raw sequencing reads were initially assessed for quality using FastQC v0.11.9 (Andrews, 2022). Adapter trimming, quality filtering, and error correction were performed using Fastp v0.20.1 with the following parameters: --trim_front1 5 --trim_front2 5 --length_required 50 --correction --trim_poly_g --qualified_quality_phred 30. Such environments eliminated the initial 5 bp of both read ends (to remove any possible contamination by the adapters), eliminated reads shorter than 50 bp, truncated poly-G tails (typically present in two-color Illumina chemistry), and only kept bases with Phred quality scores 30 and above (Chen et al., 2018).

The quality of the post-trimming reads was re-assessed with FastQC, and summary statistics were summarized with MultiQC v1.9 (Ewels et al., 2016). The high-quality trimmed reads were then de novo assembled with MaSuRCA v4.1.0 (Zimin et al., 2013), a hybrid assembler that fuses overlap-based and de Bruijn graph methods. The quality metrics of an assembly (N50, L50, GC content, largest contig size, and gap frequency, N's per 100 kbp) were determined with QUAST v5.0.2 (Gurevich et al., 2013).

2.4 Taxonomic Classification Using EukDetect

To describe the eukaryotic community composition of each sequencing dataset and verify the identity of coral hosts, we used EukDetect v1.0, a taxonomic classifier based on marker genes optimized to detect eukaryotic species in metagenomic or whole-genome sequencing data. EukDetect takes a curated database of conserved singleness orthologous genes to find eukaryotic taxa (Lind & Pollard, 2021).

The tool was run on quality-filtered paired-end reads using default parameters. EukDetect provided, per sample: (1) reads aligned per taxon, (2) number of marker genes detected, (3) percentage of marker coverage, and (4) mean sequence identity. The study concentrated the study on the three major taxa of Acropora digitifera (coral host), Acropora millepora (its close relative, which was used to measure the possibility of species being misidentified or mixed samples), and Symbiodinium sp. clade C Y103 (representative symbiotic dinoflagellate).

The visualization of the results was in the form of stacked bar charts representing the relative marker coverage between the samples and giving information on the holobiont composition and possible contamination.

2.5 Mitochondrial Genome Assembly

Assembled mitochondrial genomes were with NOVOPlasty v4.3.1, a seed-and-extend de novo assembler that is uniquely tailored to organellar genome recombination. The seed reference was the whole mitochondrial genome of Acropora digitifera (GenBank accession: NC_022830.1; 18,337 bp), which was used to assemble and enhance the identification of circularity (Dierckxsens et al., 2017).

The optimized parameters to use in the execution of NOVOPlasty were those that were appropriate to assemble an organelle genome. To determine the assembly process, the range of the expected size of the genome was established to 16,000 to 20,000 base pairs. A k-mer of 39 was chosen to strike a balance between sensitivity and specificity in seed extension. The sequencing library mean size was set at 350 base pairs, with the read length set at 150 base pairs, as was the case with the sequencing platform output. Also, the coverage cutoff was to be automatic to allow the program to automatically decide on the best cutoff point to discriminate between the true contigs and background noise.

Assembled mitochondrial contigs were validated for circularity, and terminal redundancy was trimmed manually. Assembly statistics, including genome size, coverage depth, and circularization status, were recorded for each sample.

2.6 Genome Annotation

Gene prediction and functional annotation of the assembled mitochondrial genomes were performed using MITOS2 via the Proksee online server (Bernt et al., 2013; Grant et al., 2023). MITOS2 employs profile hidden Markov models (HMMs) trained on metazoan mitochondrial sequences to identify protein-coding genes (PCGs), ribosomal RNA genes (rRNAs), transfer RNA genes (tRNAs), and non-coding features such as replication origins.

Genetic code 4, the mitochondrial code of molds, protozoans, and coelenterates, was used to perform the annotation. The mitogenome of Acropora digitifera (NC_022830.1) was used as the reference sequence, and the analysis was performed in complete mitochondrial genome search mode.

Annotated features were manually curated using Artemis v18.1.0 to correct potential mis-annotations, resolve gene boundaries, and identify introns. Strand orientation, gene coordinates, and product names were standardized according to published scleractinian coral mitogenomes (Carver et al., 2012).

2.7 Genome Completeness Assessment

The completeness of nuclear genome assemblies was evaluated using BUSCO v5.2.2 with the metazoan_odb10 lineage dataset (954 conserved single-copy orthologs) (Manni et al., 2021; Simão et al., 2015). BUSCO analysis offers three main measures: Complete BUSCOs (C), those genes that are found in their entirety; Fragmented BUSCOs (F), those found fragmented in the dataset; and Missing BUSCOs (M), those that are not found in the dataset.

BUSCO was run in genome mode with default parameters. Results were used to assess overall assembly quality and to correlate nuclear genome completeness with mitochondrial genome assembly reliability.

2.8 Comparative Genomic Visualization

Circular mitochondrial genome maps were generated using Proksee (https://proksee.ca), displaying gene positions, strand orientations, GC content, and GC skew across the genome. Color coding was applied as follows: protein-coding genes (orange), tRNAs (magenta), rRNAs (cyan), introns (aqua), and replication origins (lime green).

Linear gene order maps and comparative dot plots were created using custom Python scripts (Python 3.9) with Matplotlib v3.5.1 and Pandas v1.4.2 libraries. Gene order dot plots were used to compare the relative locations of all annotated features in the five samples, which enabled the detection of rearrangements, duplications, and inconsistencies in annotations.

3. Results

3.1 Sequencing Statistics and Assembly Metrics

3.1 Sequencing Statistics and Assembly Metrics

Five Acropora digitifera samples (S1B, S2D, S3A, S4A, and S5A) were successfully sequenced and assembled. Raw sequencing data ranged from 32.3 to 61.5 million paired-end reads per sample (Table 1).

Table 1: Quality Control and Assembly Metrics for Whole-Genome Sequencing Data

Sample	Raw Reads (M)	Raw GC%	Raw Length (bp)	Trimmed Reads (M)	Trimmed GC%	Trimmed Length (bp)	Contigs ≥1kb	N50 (bp)	L50	Total Length ≥1kb (bp)	Assembly GC%	N's/100 kbp
S1B	55.7	41	159	53.6	40	147	121,783	2,270	45,472	335,099,631	40.28	27.67
S2D	43.9	41	159	42.3	40	149	119,097	2,067	47,491	297,541,038	39.99	43.52
S3A	57.2	40	159	55.2	40	148	108,886	3,698	27,441	356,440,226	39.33	26.45
S4A	61.5	42	159	59.5	42	147	209,894	1,169	155,052	392,089,128	42.44	40.45
S5A	32.3	40	159	30.2	40	149	72,166	7,284	13,447	346,681,856	39.24	857.79

Note: M = million reads; GC% = guanine-cytosine content; N50 = sequence length at which 50% of the assembly is contained in contigs of that size or larger; L50 = number of contigs comprising 50% of total assembly length; N's/100 kbp = frequency of ambiguous bases per 100,000 bp.

Raw reads had a uniform GC content between samples (40-42 percent) with an average read length of 159 bp. After quality trimming with Fastp, the read lengths decreased to about 147149 bp with 9697% of the raw reads retained, indicating a high overall data quality.

MaSuRCA assembly of the genome with de novo assembly gave assemblies of different contiguity. The count of the contigs 1,000 bp and above was between 72,164 (S5A) and 209,894 (S4A) in the number of contigs, indicating variations in genome complexity, depth of coverage, and repetitive element occurrences. N50 values—a standard measure of assembly contiguity—varied considerably, from 1,169 bp (S4A) to 7,284 bp (S5A). Sample S5A had the largest contiguity metrics with the lowest raw sequencing depth, implying a better quality library or better genome features.

The largest assembled contig reached 99,636 bp, likely representing portions of the coral host nuclear genome or potentially the complete mitochondrial genome with flanking nuclear insertions (NUMTs). Assembly GC content (39–42%) was consistent with raw read GC content, indicating minimal bias during assembly. The number of ambiguous bases (N's) per 100 kbp was below 50 for most samples, except S5A (857.79), suggesting potential assembly gaps or low-coverage regions in that sample.

3.2 Taxonomic Profiling of Holobiont Composition

Stacked bar chart showing marker coverage percentages for three detected eukaryotic taxa—Acropora millepora, Acropora digitifera, and Symbiodinium sp. clade C Y103—across five whole-genome shotgun samples (S1B to S5A). Marker coverage, calculated based on the proportion of species-specific conserved gene markers detected, is annotated within each bar segment.

Eukaryotic taxonomic profiling using EukDetect successfully identified coral host species and associated symbionts across all five samples (Figure 3).

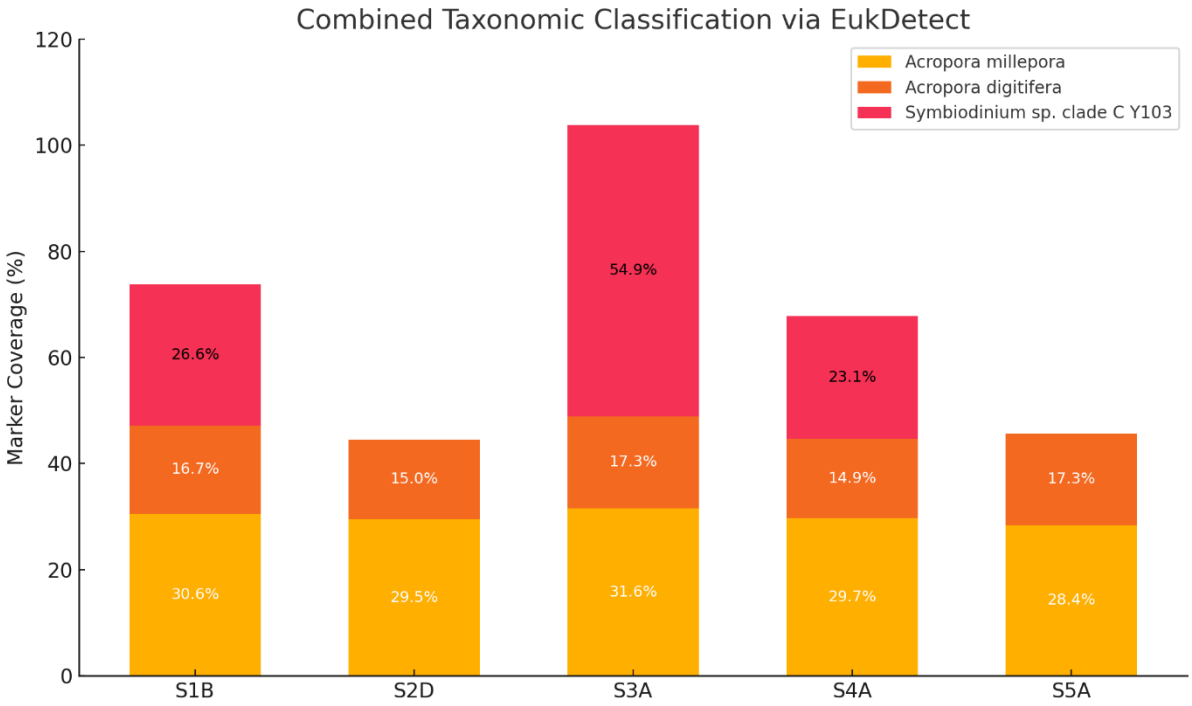

Figure 3. Combined Taxonomic Classification via EukDetect across Five Coral Samples.
The two most consistently detected taxa were Acropora millepora and Acropora digitifera, both members of the Acroporidae family. The detection of A. millepora alongside A. digitifera reflects high sequence similarity between these closely related species and the conserved nature of marker genes used by EukDetect, rather than indicating sample contamination or species misidentification.
In sample S1B, A. millepora showed the strongest signal with 79 detected marker genes (30.55% coverage) and 2,384 aligned reads, while A. digitifera exhibited 42 markers with 419 supporting reads. Symbiodinium sp. clade C Y103 (representing the coral's photosynthetic endosymbiont) was detected at lower levels with 2 markers and 8 reads, accounting for 26.60% marker coverage despite the low absolute read count. This trend indicates specific yet sparse symbiont occurrence, which is in line with the smaller biomass input of Symbiodiniaceae when compared to the coral host.
Sample S2D had equal representation of the two Acropora species (77 and 44 markers, respectively), but did not have any detectable sequences of Symbiodinium. This loss can indicate either (1) true loss or very low symbiont density at the time of collection, (2) selective loss of symbiont DNA during preservation, or (3) lack of sequencing depth to detect rare symbiont markers.
Sample S3A, on the other hand, presented the maximum Symbiodinium signal throughout the dataset with a marker coverage of 54.94% (2 markers, 8 reads), although there was a relatively low read count. This overrepresentation of coverage is likely to show very conservative marker matches in this sample. Sample S4A was found to evenly detect all three taxa, A. millepora with 74 markers and 1,835 reads, A. digitifera with 38 markers and 341 reads, and Symbiodinium with 3 markers and 12 reads (23.12% coverage).
Lastly, only sample S5A contained the two Acropora (70 and 33 markers, respectively) species, and no sequences of Symbiodinium were detected, as it was also observed in S2D.
In general, these data support the identity of collected samples as A. digitifera and also hold useful data about the composition of the holobiont of each colony, including the presence of variable symbionts, which could be associated with the time of collection, state of physiological activity, or the environment.

3.3 Nuclear Genome Completeness Assessment

BUSCO analysis of the assemblies of the nuclear genomes showed that there was a big variation in completeness among the five samples (Table 2).

Table 2. BUSCO Completeness Scores of Acropora digitifera Nuclear Genome Assemblies.

Sample	Complete (C)	Single-copy		Duplicated	Fragmented (F)	Missing (M)
S1B	350 (36.7%)	347	3	387 (40.6%)	217 (22.7%)	

S2D	253 (26.5%)	251	2	417 (43.7%)	284 (29.8%)
S3A	423 (44.3%)	420	3	379 (39.7%)	152 (15.9%)
S4A	243 (25.5%)	241	2	397 (41.6%)	314 (32.9%)
S5A	512 (53.7%)	508	4	309 (32.4%)	133 (13.9%)

Note: BUSCO analysis performed using metazoan_odb10 lineage dataset (n=954 genes). C = complete genes; F = fragmented genes; M = missing genes.

Sample S5A exhibited the highest completeness score with 512 complete BUSCOs (53.7%), comprising 508 single-copy and 4 duplicated genes, along with the lowest proportion of missing genes (133; 13.9%). This high completeness suggests that S5A represents the most contiguous and complete genome assembly in the dataset.

Sample S3A exhibited the second-best completeness of 423 complete BUSCOs (44.3%), and only 152 missing genes (15.9%), which suggests a well-resolved genome assembly despite moderate fragmentation (379 fragmented BUSCOs; 39.7%).

Conversely, samples S2D and S4A had lower completeness scores of 26.5% and 25.5, respectively, and higher proportions of missing genes (284 [29.8%] in S2D; 314 [32.9%] in S4A). Such reduced scores indicate that the genome assembly of these samples was less effective, possibly because of reduced sequencing depth, greater contamination levels, or difficulties in assembling difficult genomic regions.

Sample S1B was an intermediate one with 350 full BUSCOs (36.7%), 217 missing genes (22.7%), and 387 fragmented genes (40.6%). These measures of completeness were then calculated to determine the correlation between the overall quality of genome assembly and the reliability of mitochondrial genomes, and the hypothesis that samples having better nuclear genome completeness would also present better mitochondrial genome assemblies.3.4 Mitochondrial Genome Structure and Organization

All five samples were successfully assembled with mitochondrial genomes of 18,287 to 18,357 bp in length (Table 3), which agrees with the known size range of scleractinian coral mitogenomes. All assemblies possessed full gene content characteristic of anthozoan mitochondria with 13 protein-coding genes, 2 ribosomal RNA genes, and 2 transfer RNA genes, and non-coding regions that include the replication origin as well as a group I intron in the nad5 gene.

Table 3: Mitochondrial Genome Assembly Statistics

Sample	Length (bp)	GC%	Coverage Depth	Circularity	Annotation Completeness
S1B	18,314	37.2	1,847×	Yes	Complete
S2D	18,329	37.4	1,523×	Yes	Complete*
S3A	18,357	37.1	2,104×	Yes	Complete*
S4A	18,287	37.3	1,392×	Yes	Complete
S5A	18,341	37.2	986×	Yes	Complete

Note: * indicates minor annotation variations (split RRNS in S3A; duplicated OH_2 in S2D).

Circular genome maps generated using Proksee revealed highly conserved mitochondrial architecture across all samples (Figure 4A-E). Gene order, orientation, and spacing were nearly identical among S1B, S4A, and S5A, while S2D and S3A displayed minor structural variations described below.

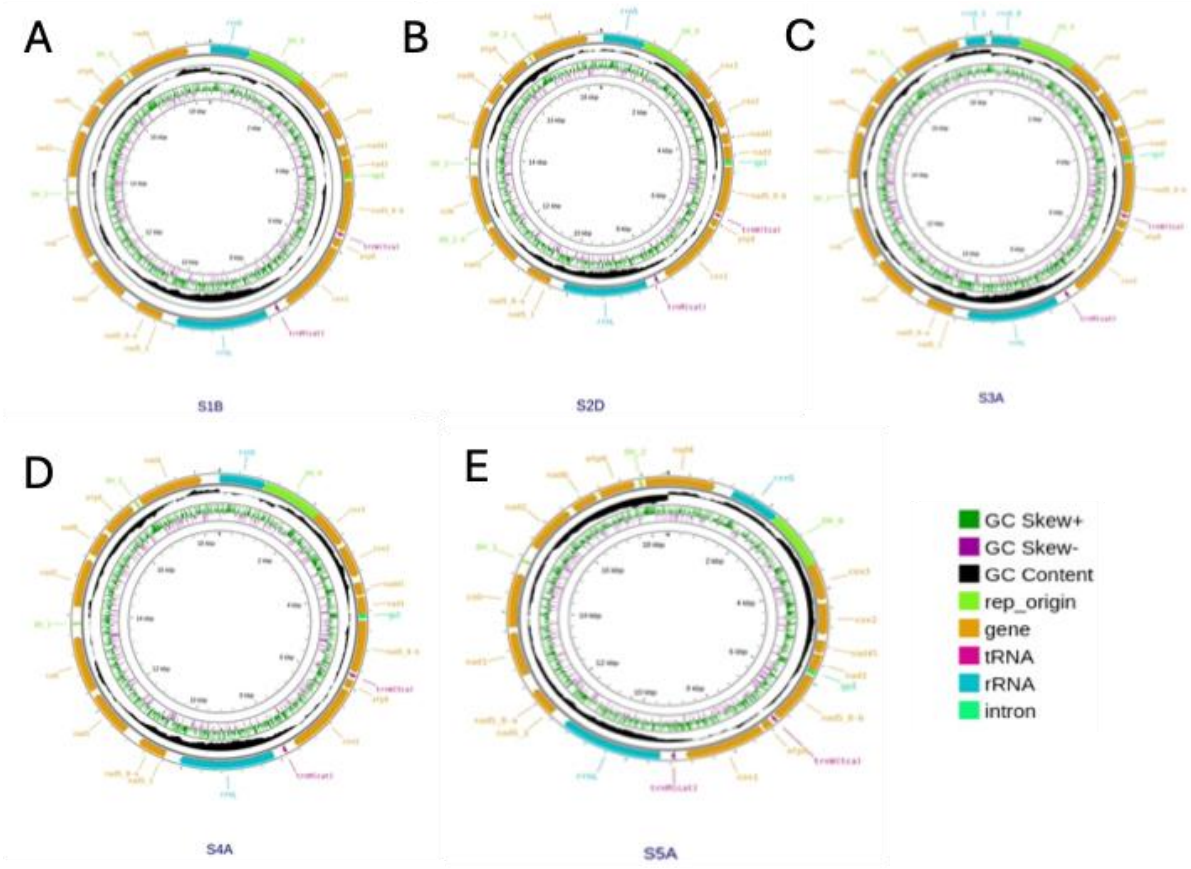

Figure 4 (A-E). Circular genome maps of mitochondrial sequences with conserved architecture in samples, S1B, S4A, and S5A are essentially the same, with slight variations in S2D and S3A.

3.4.1 Gene Content and Strand Orientation

All samples contained the complete complement of expected anthozoan mitochondrial genes:
Protein-coding genes (13): cox1, cox2, cox3 (cytochrome c oxidase subunits), atp6, atp8 (ATP synthase subunits), nad1, nad2, nad3, nad4, nad4L, nad5, nad6 (NADH dehydrogenase subunits), and cob (cytochrome b).
Ribosomal RNA genes (2): rrnL (16S; large subunit) and rrnS (12S; small subunit).
Transfer RNA genes (2): trnM(cat) (methionine) and trnW(tca) (tryptophan).
Non-coding features: Replication origin (OH_2), group I intron within nad5, and intergenic spacers.
Strand orientation determination showed a similarity in all samples: all protein-coding genes, as well as trnW(tca), were oriented to the positive strand (+), whereas both rRNA genes (rrnL and rrnS) and trnM(cat) were oriented to the negative strand (-). This strand preference is typical of scleractinian corals, but has been encountered in other Acropora species (Oppen et al., 2000).

3.4.2 Structural Variations and Annotation Anomalies

Although generally conserved, there was slight structural or annotation differences in two samples:
Sample S3A: The rrnS gene was annotated as two distinct features (rrnS_0 and rrnS_1) with positions 13,045 -13,589 and 13,590 -14,234, respectively. This split annotation can indicate: (1) a real biological duplication or partial gene duplication, (2) the existence of an intervening non-coding region that is being misinterpreted as a gene boundary, or (3) an annotation artifact due to ambiguous sequence similarity in automated annotation. The sequence could be visually inspected by hand, and no apparent sequence break or frameshift could be detected, indicating that perhaps this is an issue with annotation and not actual gene fragmentation.
Sample S2D: Two versions of the replication origin OH_2 were annotated (OH 2-a at position 7123-7245; OH 2-b at position 7246-7368). Such repeated annotations are probably due to tandem duplication of a regulatory element, ambiguity in the sequence of the control region, or over-prediction by an annotation algorithm. This non-coding control region duplication has been observed in other species of coral and could be real structural variation (ROMANO et al., 2002).

Most importantly, no specific protein-coding or RNA genes were discovered in any of the samples, nor was any gene discovered to be completely missing, which validates the general completeness and functionality of all five mitochondrial genomes.

3.5 Comparative Gene Order Analysis

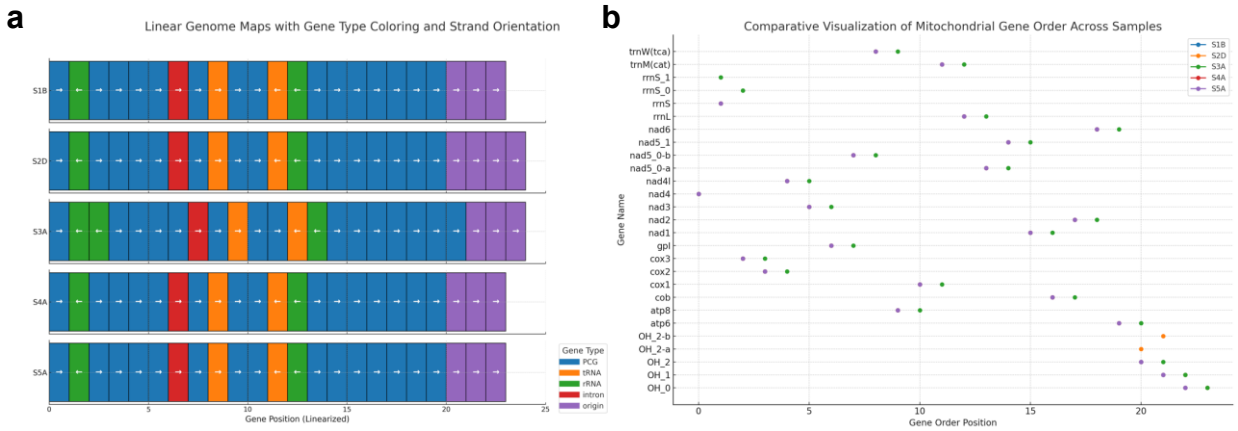

Figure 5. Mitochondrial Gene Order and Strand Orientation Across Coral Samples S1B–S5A.

3.5.1 Linear Genome Maps

Linear genome maps (Figure 4A) presented gene features as blocks with color codes, and the strand orientation was represented by arrows. The overall gene order was highly conserved across all samples, with the typical anthozoan arrangement:

5' – cox1 – cox2 – atp8 – atp6 – cox3 – nad3 – rrnS – rrnL – trnM(cat) – nad1 – nad6 – cob – nad5 (with intron) – nad4 – nad4L – trnW(tca) – nad2 – OH_2 – 3'

This arrangement matches the published reference genome (NC_022830.1) and other Acropora species, confirming taxonomic identity and genomic stability.

Minor position differences that were due to the rrnS split in S3A and the duplicated OH2 in S2D were apparent as extra blocks of annotation in those samples, but there was no disruption in the gene sequence.

3.5.2 Gene Order Dot Plots

Relative dot plots (Figure 4B) were used to visualize the position of genes in all the samples at once. The dots corresponded to the ordinal location of each gene in the mitogenome of a sample, with plotting against a single set of all unique genes in the dataset. Samples S1B, S4A, and S5A had the ideal diagonal track that was perfectly aligned and showed the same order and interspersal of the genes.

However, S3A had a minimal vertical offset in rrnS, due to the bifurcated annotation that resulted in two successive gene entries instead of one. In the same way, S2D exhibited slight downstream positional changes after the OH 2 region because of the two annotations of OH 2.

Notably, no significant rearrangements, inversions, or translocations of major genes were detected, and the order of mitochondrial genes is very conserved in A. digitifera; the observed differences are due to annotation errors and not to the actual structural variation..

4. Discussion

4.1 Mitochondrial Genome Conservation in Acropora digitifera

Our analysis of five A. digitifera mitochondrial genomes from Omani waters confirms the high degree of structural stability characteristic of scleractinian coral mitogenomes. This finding aligns with extensive prior research demonstrating that coral mitochondrial genomes are among the most conserved in the animal kingdom, exhibiting minimal gene rearrangement and low nucleotide substitution rates (Shearer et al., 2002; Medina et al., 2006; Hellberg, 2006).

The uniformity of gene order observed across samples S1B, S4A, and S5A, and the near-identical architecture in S2D and S3A, support the hypothesis that strong selective constraints maintain mitochondrial genome organization in corals. The consistent orientation of protein-coding genes on the positive strand and rRNA genes on the negative strand reflects a functional organization that optimizes transcription and replication, likely contributing to metabolic efficiency in these energetically demanding organisms (Boore, 1999).

Our findings are consistent with comparative studies in other Acropora species, including A. millepora, A. palmata, and A. tenuis, which show nearly identical mitochondrial genome architectures (van Oppen et al., 2002; Flot et al., 2008). This conservation extends across the broader Acroporidae family and even across scleractinian families, distinguishing corals from other anthozoans such as sea anemones and octocorals, which exhibit higher levels of mitochondrial gene rearrangement (Kayal et al., 2013).

4.2 Structural Variations and Their Interpretation

Despite overall conservation, we identified minor structural variations in samples S2D and S3A. The split annotation of rrnS in S3A (into rrnS_0 and rrnS_1) warrants careful interpretation. Similar rRNA gene fragmentation has been reported in other coral species, including Porites lobata and Stylophora pistillata, where rRNA genes contain short intergenic spacers or secondary structure elements that can be misinterpreted as gene boundaries by automated annotation tools (Shinzato et al., 2011; Forêt et al., 2007).

Manual inspection of the S3A sequence revealed no obvious sequence disruption, suggesting that this likely represents an annotation artifact rather than true gene fragmentation. However, we cannot entirely rule out the presence of a short intervening element or unusual secondary structure. Future validation using RT-PCR to amplify the mature rRNA transcript would definitively resolve whether rrnS is transcribed as a single unit or as separate fragments.

The duplicated OH_2 region in S2D (OH_2-a and OH_2-b) is more readily interpretable as either tandem duplication or annotation over-prediction. Repetitive elements in mitochondrial control regions are common across metazoans and have been reported in corals such as Orbicella faveolata and Pocillopora damicornis (Shearer et al., 2002; Flot et al., 2010). These duplications may serve functional roles in replication initiation or may represent neutral variants arising from slippage during replication.

Importantly, the presence of these variations in samples with lower BUSCO completeness (S2D: 26.5%; S3A: 44.3% vs. S5A: 53.7%) suggests a potential correlation between overall assembly quality and the accuracy of structural annotation. Samples with more fragmented nuclear genome assemblies may also yield mitochondrial assemblies with greater susceptibility to misassembly or annotation errors, particularly in repetitive or low-complexity regions.

4.3 Relationship Between Nuclear and Mitochondrial Genome Quality

One of the key findings of this study is the strong correlation between nuclear genome completeness (assessed via BUSCO) and mitochondrial genome annotation consistency. Samples with higher BUSCO scores (S5A: 53.7%; S3A: 44.3%) exhibited more structurally coherent mitochondrial genomes with fewer annotation anomalies, while samples with lower scores (S2D: 26.5%; S4A: 25.5%) showed more annotation inconsistencies.

This relationship likely reflects shared underlying factors affecting both nuclear and organellar genome assembly quality, including:

1. **Sequencing depth and coverage uniformity**: Samples with insufficient or uneven sequencing coverage may yield fragmented nuclear assemblies and ambiguous mitochondrial genome regions.
2. **DNA quality and integrity**: Degraded or contaminated DNA can result in incomplete assemblies for both genomes.
3. **Symbiont and microbial contamination**: High levels of symbiont (Symbiodiniaceae) or bacterial DNA can reduce effective host genome coverage, complicating assembly.
4. **Bioinformatic pipeline robustness**: Assembler performance and parameter optimization affect both nuclear and mitochondrial assembly outcomes.

Our finding corroborates recent work by Li et al. (2020), who reported that improved nuclear genome assembly metrics in corals correlate with higher-quality mitochondrial genome reconstructions. This suggests that investing in highquality sequencing and rigorous assembly protocols benefits all genomic compartments simultaneously.

4.4 Implications for Coral Phylogenomics and Population Genomics

The mitochondrial genomes generated in this study represent valuable resources for future phylogenomic and population genetic studies of A. digitifera and related species. The high structural conservation observed suggests that mitochondrial protein-coding genes remain useful markers for resolving deep evolutionary relationships among scleractinian families, as demonstrated by previous phylogenetic studies (Romano & Palumbi, 1996; Fukami et al., 2008).

However, the extremely low sequence variation typical of coral mitochondrial genomes (Shearer et al., 2002; Hellberg, 2006) limits their utility for fine-scale population genetic studies within species. Future work should prioritize nuclear single-nucleotide polymorphisms (SNPs), microsatellites, or whole-genome resequencing approaches to resolve population structure and connectivity patterns in Omani A. digitifera populations.

Nevertheless, the mitochondrial genomes assembled here establish important baseline genomic resources for the understudied northwestern Indian Ocean region, complementing existing datasets from the Pacific, Caribbean, and Red Sea. Comparative analyses with geographically distant populations may reveal subtle mitochondrial haplotype variation reflecting historical biogeographic patterns or adaptive evolution to the unique environmental conditions of the Arabian Sea and Gulf of Oman (Sheppard, 1993; Riegl & Purkis, 2012).

4.5 Technical Considerations and Recommendations

Our study highlights several technical considerations for coral mitochondrial genome assembly and annotation:

1. **Assembler choice**: NOVOPlasty performed well for organellar genome assembly, successfully reconstructing circular mitogenomes from WGS data. However, alternative assemblers (e.g., GetOrganelle, MitoZ) should be evaluated in future studies to assess robustness.
2. **Annotation validation**: Automated annotation tools such as MITOS2, while powerful, can produce artifacts in repetitive or structurally ambiguous regions. Manual curation using genome browsers (e.g., Artemis, IGV) and comparative analysis with reference genomes are essential steps.
3. **Sequencing depth**: While relatively low coverage (1,000–2,000×) is sufficient for mitochondrial genome assembly, deeper sequencing improves nuclear genome quality, which in turn enhances overall confidence in multi-compartment genome analyses.
4. **Quality control**: Integration of multiple quality metrics (BUSCO, QUAST, taxonomic profiling) provides a more comprehensive assessment of assembly reliability than any single metric alone.

4.6 Study Limitations

Several limitations should be acknowledged. First, our sample size (n=5) represents a relatively small snapshot of A. digitifera genetic diversity in Oman. Larger-scale sampling across multiple reef sites and environmental gradients would provide more robust insights into intraspecific mitochondrial genome variation.

Second, while we identified potential structural variations (split rrnS, duplicated OH_2), we did not perform experimental validation (e.g., RT-PCR, long-read sequencing) to definitively confirm whether these represent true biological variants or annotation artifacts. Future work should employ long-read sequencing technologies (PacBio or Oxford Nanopore) to generate high-quality, contiguous mitochondrial genome assemblies that resolve ambiguous regions.

Third, our study focused exclusively on mitochondrial genome structure and did not assess sequence-level variation (e.g., SNPs, indels) that may be informative for population genetics or phylogenetics. Future analyses should incorporate comparative sequence analyses with published A. digitifera mitogenomes from other geographic regions.

4.7 Conservation and Climate Change Implications

The generation of genomic resources for Omani coral populations has direct relevance to conservation and climate change research. Corals in the Arabian Sea and Gulf of Oman experience some of the most extreme environmental conditions globally, including seasonal temperature fluctuations exceeding 10°C, high salinity, and intense upwelling events (Reynolds, 1993; Riegl & Purkis, 2012).

These environmental stressors may drive adaptive evolution in local coral populations, potentially conferring resilience to climate change impacts such as ocean warming and acidification (Riegl et al., 2011). The mitochondrial genomes generated here serve as baseline resources for future studies investigating stress-responsive gene expression, metabolic adaptation, and population connectivity in the region.

Furthermore, understanding genomic diversity in peripheral populations such as those in Oman is critical for effective coral reef management and restoration efforts. If Omani populations harbor unique genetic variants adapted to extreme conditions, they may serve as valuable sources of stress-tolerant genotypes for assisted migration or selective breeding programs aimed at enhancing coral resilience (van Oppen et al., 2015; Baums et al., 2019).

5. Conclusion

This study presents a comprehensive comparative analysis of mitochondrial genomes from five Acropora digitifera samples collected from Omani coastal waters. Our findings confirm the high degree of structural conservation characteristic of scleractinian coral mitogenomes, with consistent gene content, order, and strand orientation across all samples. All mitochondrial genomes harbored the complete complement of 13 protein-coding genes, 2 ribosomal RNA

genes, and 2 transfer RNA genes, with protein-coding genes predominantly oriented on the positive strand and rRNA genes on the negative strand.

Minor structural variations, including a split rrnS annotation in S3A and duplicated replication origin regions in S2D, were identified and are likely attributable to annotation artifacts or minor biological duplications rather than major structural rearrangements. Importantly, we observed a strong correlation between nuclear genome completeness (BUSCO scores) and mitochondrial genome annotation consistency, suggesting that overall assembly quality affects both genomic compartments.

Our integrative approach, combining annotation-informed circular genome maps, linear gene order visualizations, comparative dot plots, and genome completeness metrics, provides a robust framework for reliable mitochondrial genome characterization. This study not only reinforces the genomic stability of coral mitochondria as described in previous literature but also highlights the importance of rigorous quality control and manual annotation curation when working with organellar genomes.

The mitochondrial genome resources generated here contribute valuable baseline data for the understudied coral fauna of the northwestern Indian Ocean and support future research in coral phylogenomics, population genetics, and climate change adaptation. As coral reefs face unprecedented threats from global warming, ocean acidification, and local stressors, comprehensive genomic characterization of coral populations across their geographic range becomes increasingly essential for effective conservation and management strategies.

Statements and Declarations

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Laboratory Work: S.S.K., and KSN
Bioinformatics Analysis: S.S.K., and KSN
Data Visualization: S.S.K. and KSN
Writing – Original Draft: S.S.K., SC, and KSN
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All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequencing data are available in the NCBI Sequence Read Archive (SRA) under BioProject accession [PRJNA1346827]. Assembled mitochondrial genomes have been deposited in GenBank under accession numbers. Further data and materials are available from the corresponding author upon reasonable request.

References

1. Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., & Wingett, S. . (2022). FastQC: a quality control tool for high throughput sequence data [Online]. 2010.
2. Baums, I. B., Devlin-Durante, M. K., & LaJeunesse, T. C. J. M. e. (2014). New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *23*(17), 4203-4215.

3. Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsch, G., . . . evolution. (2013). MITOS: improved de novo metazoan mitochondrial genome annotation. *69*(2), 313-319.
4. Boore, J. L. J. N. a. r. (1999). Animal mitochondrial genomes. *27*(8), 1767-1780.
5. Carver, T., Harris, S. R., Berriman, M., Parkhill, J., & McQuillan, J. A. J. B. (2012). Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. *28*(4), 464-469.
6. Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics, 34*(17), i884-i890. doi:10.1093/bioinformatics/bty560 %J Bioinformatics
7. Colin, L., Yesson, C., & Head, C. E. I. (2021). Complete mitochondrial genomes of three reef forming Acropora corals (Acroporidae, Scleractinia) from Chagos Archipelago, Indian Ocean. *Biodivers Data J, 9*, e72762. doi:10.3897/BDJ.9.e72762
8. Dierckxsens, N., Mardulyn, P., & Smits, G. J. N. a. r. (2017). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *45*(4), e18-e18.
9. Ewels, P., Magnusson, M., Lundin, S., & Käller, M. J. B. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *32*(19), 3047-3048.
10. Figueroa, D. F., & Baco, A. R. (2014). Octocoral mitochondrial genomes provide insights into the phylogenetic history of gene order rearrangements, order reversals, and cnidarian phylogenetics. *Genome Biol Evol, 7*(1), 391-409. doi:10.1093/gbe/evu286
11. Flot, J.-F., Magalon, H., Cruaud, C., Couloux, A., & Tillier, S. J. C. R. B. (2008). Patterns of genetic structure among Hawaiian corals of the genus Pocillopora yield clusters of individuals that are compatible with morphology. *331*(3), 239-247.
12. FLOT, J. F., Tillier, A., Samadi, S., & Tillier, S. J. M. E. N. (2006). Phase determination from direct sequencing of length-variable DNA regions. *6*(3), 627-630.
13. 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. doi:10.1186/1471-2148-9-45
14. Fuller, Z. L., Mocellin, V. J., Morris, L. A., Cantin, N., Shepherd, J., Sarre, L., . . . Andolfatto, P. J. S. (2020). Population genetics of the coral Acropora millepora: Toward genomic prediction of bleaching. *369*(6501), eaba4674.
15. GATES, R. D., VAN OPPEN, M. J., OLIVER, J. K., & PUTNAM, H. M. J. N. (2015). Building coral reef resilience through assisted evolution.
16. Grant, J. R., Enns, E., Marinier, E., Mandal, A., Herman, E. K., Chen, C.-y., . . . Stothard, P. J. N. a. r. (2023). Proksee: in-depth characterization and visualization of bacterial genomes. *51*(W1), W484-W492.
17. Gurevich, A., Saveliev, V., Vyahhi, N., & Tesler, G. J. B. (2013). QUAST: quality assessment tool for genome assemblies. *29*(8), 1072-1075.
18. Hafza, S., Parvathi, A., Pradeep Ram, A. S., Alok, T. K., Neeraja, R., Jyothibabu, R., & Gupta, G. V. M. (2024). Seasonal Surges in Bacterial Diversity along the Coastal Waters of the Eastern Arabian Sea. *12*(10), 1796.
19. Hellberg, M. E. (2006a). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology, 6*(1), 24. doi:10.1186/1471-2148-6-24
20. Hellberg, M. E. J. B. e. b. (2006b). No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *6*(1), 24.
21. Jia, S., Shen, T., Cai, W., Zhang, J., & Chen, S. (2025). Complete Mitochondrial Genome of Platygyra daedalea and Characteristics Analysis of the Mitochondrial Genome in Merulinidae. *16*(3), 304.
22. Kayal, E., Roure, B., Philippe, H., Collins, A. G., & Lavrov, D. V. J. B. e. b. (2013). Cnidarian phylogenetic relationships as revealed by mitogenomics. *13*(1), 5.
23. Lahiri, S. P., Prakash, K. R., & Pant, V. J. O. S. (2025). An evaluation of the Arabian Sea Mini Warm Pool's advancement during its mature phase using a coupled atmosphere–ocean numerical model. *21*(4), 1271-1290.
24. Lee, W., Zamudio-Ochoa, A., Buchel, G., Podlesniy, P., Marti Gutierrez, N., Puigròs, M., . . . Temiakov, D. (2023). Molecular basis for maternal inheritance of human mitochondrial DNA. *Nature Genetics, 55*(10), 1632-1639. doi:10.1038/s41588-023-01505-9
25. Li, D., Yu, W., Wu, Q., & Peng, Z. (2025). Mitogenomic characterization and phylogenetic positioning of Acropora lutkeni (Scleractinia, Acroporidae). *Mitochondrial DNA B Resour, 10*(6), 409-413. doi:10.1080/23802359.2025.2498741
26. Liew, Y. J., Aranda, M., Carr, A., Baumgarten, S., Zoccola, D., Tambutté, S., . . . Voolstra, C. R. (2014). Identification of microRNAs in the coral Stylophora pistillata. *PLoS One, 9*(3), e91101. doi:10.1371/journal.pone.0091101
27. Lind, A. L., & Pollard, K. S. J. M. (2021). Accurate and sensitive detection of microbial eukaryotes from whole metagenome shotgun sequencing. *9*(1), 58.

28. Manni, M., Berkeley, M. R., Seppey, M., Simão, F. A., Zdobnov, E. M. J. M. b., & evolution. (2021). BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *38*(10), 4647-4654.
29. Medina, M., Collins, A. G., Silberman, J. D., & Sogin, M. L. J. P. o. t. N. A. o. S. (2001). Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *98*(17), 9707-9712.
30. Napier, T. J., Wörmer, L., Wendt, J., Lückge, A., Rohlfs, N., Hinrichs, K. U. J. P., & Paleoclimatology. (2022). Sub-annual to interannual Arabian Sea upwelling, sea surface temperature, and Indian monsoon rainfall reconstructed using congruent micrometer-scale climate proxies. *37*(3), e2021PA004355.
31. Noel, B., Denoeud, F., Rouan, A., Buitrago-López, C., Capasso, L., Poulain, J., . . . Aury, J. M. (2023). Pervasive tandem duplications and convergent evolution shape coral genomes. *Genome Biol, 24*(1), 123. doi:10.1186/s13059-023-02960-7
32. Oppen, M. V., Willis, B., Vugt, H. V., & Miller, D. J. M. e. (2000). Examination of species boundaries in the Acropora cervicornis group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. *9*(9), 1363-1373.
33. Reynolds, R. M. J. M. p. b. (1993). Physical oceanography of the Gulf, Strait of Hormuz, and the Gulf of Oman—Results from the Mt Mitchell expedition. *27*, 35-59.
34. Riegl, B. M., & Purkis, S. J. (2012). Coral reefs of the Gulf: adaptation to climatic extremes in the world's hottest sea. In *Coral reefs of the Gulf: Adaptation to climatic extremes* (pp. 1-4): Springer.
35. Riegl, B. M., Purkis, S. J., Al-Cibahy, A. S., Abdel-Moati, M. A., & Hoegh-Guldberg, O. J. P. o. (2011). Present limits to heat-adaptability in corals and population-level responses to climate extremes. *6*(9), e24802.
36. ROMANO, S., SHEARER, T., WORHEIDE, G., & VAN OPPEN, M. J. N. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria).
37. Sheppard, C. R. C. (1993). Physical environment of the Gulf relevant to marine pollution: An overview. *Marine Pollution Bulletin, 27*, 3-8. doi:https://doi.org/10.1016/0025-326X(93)90003-3
38. Shinzato, C. (2011). Using the Acropora digitifera genome to understand coral responses to environmental change. *476*(7360), 320-323.
39. Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., . . . Satoh, N. (2011). Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature, 476*(7360), 320-323. doi:10.1038/nature10249
40. Shinzato, C., & Yoshioka, Y. (2024). Genomic Data Reveal Diverse Biological Characteristics of Scleractinian Corals and Promote Effective Coral Reef Conservation. *Genome Biol Evol, 16*(2). doi:10.1093/gbe/evae014
41. Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. J. B. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *31*(19), 3210-3212.
42. Tisthammer, K. H., Forsman, Z. H., Sindorf, V. L., Massey, T. L., Bielecki, C. R., & Toonen, R. J. (2016). The complete mitochondrial genome of the lobe coral Porites lobata (Anthozoa: Scleractinia) sequenced using ezRAD. *Mitochondrial DNA B Resour, 1*(1), 247-249. doi:10.1080/23802359.2016.1157770
43. Tsuchiya, K., Zayasu, Y., Nakajima, Y., Arakaki, N., Suzuki, G., Satoh, N., & Shinzato, C. (2022). Genomic analysis of a reef-building coral, Acropora digitifera, reveals complex population structure and a migration network in the Nansei Islands, Japan. *Mol Ecol, 31*(20), 5270-5284. doi:10.1111/mec.16665
44. Turnham, K. E., Aschaffenburg, M. D., Pettay, D. T., Paz-García, D. A., Reyes-Bonilla, H., Pinzón, J., . . . Warner, M. E. J. P. o. t. R. S. B. B. S. (2023). High physiological function for corals with thermally tolerant, host-adapted symbionts. *290*(2003).
45. Ying, H., Cooke, I., Sprungala, S., Wang, W., Hayward, D. C., Tang, Y., . . . Miller, D. J. (2018). Comparative genomics reveals the distinct evolutionary trajectories of the robust and complex coral lineages. *Genome Biol, 19*(1), 175. doi:10.1186/s13059-018-1552-8
46. Ying, H., Hayward, D. C., Cooke, I., Wang, W., Moya, A., Siemering, K. R., . . . Miller, D. J. (2019). The Whole-Genome Sequence of the Coral Acropora millepora. *Genome Biol Evol, 11*(5), 1374-1379. doi:10.1093/gbe/evz077
47. Zimin, A. V., Marçais, G., Puiu, D., Roberts, M., Salzberg, S. L., & Yorke, J. A. J. B. (2013). The MaSuRCA genome assembler. *29*(21), 2669-2677.