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1 **Skimming genomes for systematics and DNA barcodes of corals**

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40
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43 manuscript. LJM, HB, JH, KM, and EEE conducted genomic analyses. SH, CSM, JH, and EEE
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46 **Abstract**
47
48 1: Numerous genomic methods developed over the past two decades have enabled the discovery
49 and extraction of orthologous loci to help resolve phylogenetic relationships across various taxa
50 and scales. Genome skimming (or low-coverage whole genome sequencing) remains a low-cost,
51 promising method to not only extract high-copy loci, but also 100s to 1000s of phylogenetically
52 informative single-copy nuclear loci (e.g., ultraconserved elements [UCEs] and exons) from
53 contemporary and historical museum samples. The subphylum Anthozoa, which includes
54 important ecosystem engineers (e.g., stony corals, black corals, anemones and octocorals) in the
55 marine environment, is in critical need of phylogenetic resolution and thus might benefit from a
56 genome-skimming approach.

57 2: Genome skimming was conducted on 242 hexacorals and octocorals collected from 1890 to
58 2022. Using previously developed target-capture baitsets, we bioinformatically obtained UCEs
59 and exons from the genome-skimming data and incorporated them with data from previously
60 published target-capture studies. We also extracted partial to whole mitogenomes and nuclear
61 rRNA genes from the skim data.

62 3: The mean number of UCE and exon loci extracted from the genome skimming data was 1,837
63 ± 662 SD for octocorals and 1,422 ± 720 loci for hexacorals; phylogenetic relationships were
64 well resolved within each class. A mean of 1,422 ± 720 loci were obtained from the historical
65 museum specimens, with 1,253 loci recovered from the oldest specimen collected in 1886 and
66 1,336 loci recovered from a holotype. The nuclear *rRNA* genes and the majority of mitochondrial
67 genes were successfully obtained from >95% of samples. Out of 99 circularized mitogenomes,
68 88% were recovered in samples from which we obtained >15M paired-end (PE) reads (>30M

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69 total reads); there was more variability in whether mitogenomes were circularized or not in
70 samples with <15M PE reads.

71 4: Bioinformatically pulling UCEs, exons, mitochondrial genomes, and nuclear rRNA genes
72 from genome skimming is a viable and low-cost option for phylogenetic studies. This approach
73 can be used to review and support taxonomic revisions and reconstruct evolutionary histories,
74 including historical museum and type specimens.

75

76 Introduction

77 The advent of novel genomic methods and analyses has revolutionized our ability to
78 resolve phylogenetic relationships across the tree of life. Numerous genomic methods (e.g.,
79 whole genome sequencing, transcriptomics, restriction-site associated sequencing, target-
80 capture) developed over the past two decades have enabled the discovery and extraction of
81 orthologous loci across multiple phyla. While high-quality whole genomes or transcriptomes are
82 ideal in many situations, obtaining this genetic information from most animal taxa is still not
83 technically feasible. But over the past decade, the average cost of high-throughput sequencing
84 has rapidly decreased (Park & Kim, 2016). Now, we can multiplex many more taxa and obtain
85 more genomic data (i.e., base pairs) per sample at a much lower cost than ever before. Therefore,
86 genome skimming, or low-coverage whole genome sequencing (WGS), could be used to readily
87 obtain enough orthologous loci, including conventional DNA barcodes, at a relatively low cost
88 for phylogenomic studies (Trevisan et al., 2019; Liu et al., 2021).

89 Genome skimming has been used in prior studies to obtain whole mitochondrial genomes
90 and nuclear DNA loci for phylogenetic studies (e.g., Malé et al., 2014; Liu et al., 2021;
91 GoLightly et al., 2022; Taite et al., 2023). In addition, genome skimming has increasingly been
92 used to help build DNA barcode reference databases for applications such as environmental

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93 DNA (eDNA) sequencing (Zeng et al., 2018; Hoban et al., 2022; Zhang et al., 2023). This
94 method's potential, however, for other applications remains unrealized, as typically more than
95 99% of the sequence data produced by skimming is not used (Bohmann et al., 2020). Low-
96 coverage genome skims could readily be used to bioinformatically pull out ultraconserved
97 elements (UCEs), exons, and other genes of interest. And because this method does not
98 necessarily need high-quality DNA such as other methods (i.e., RAD Sequencing), genome
99 skimming might be useful for historical samples that are housed in natural history museums
100 across the globe (see Tin et al., 2014; Yeates et al., 2016; Bakker 2017; Liu et al., 2021; Hoban
101 et al., 2022). Thus, this method should be more thoroughly explored for various applications
102 across different qualities and quantities of genomic DNA.

103 Phylogenomic studies of marine invertebrates might benefit from a genome-skimming
104 approach. In particular, the subphylum Anthozoa (*sensu* McFadden et al., 2022; phylum
105 Cnidaria) is in critical need of taxonomic revision and resolution across family, genus, and
106 species levels that will ultimately help in discriminating species and improving estimates of
107 species diversity and distribution. Anthozoans are a diverse group of marine invertebrates,
108 including sea anemones and corals, which are essential in building marine ecosystems from polar
109 to tropical regions and the coasts to the abyss. Anthozoans currently comprise ~7500 valid
110 species (Daly et al., 2007) in two classes (Hexacorallia and Octocorallia, McFadden et al., 2022),
111 but this number might be grossly underestimated (Plaisance et al., 2011; Bridge et al., 2023).
112 Recently (i.e., in the past five years), the number of phylogenomic studies on anthozoans has
113 grown rapidly. These studies have used a variety of methods, such as restriction-site associated
114 sequencing (RADSeq, Reitzel et al., 2013; Herrera & Shank, 2016, Quattrini et al., 2019;
115 Arrigoni et al., 2020), transcriptomics (Zapata et al., 2015), and target-capture genomics (e.g.,

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116 Quattrini et al., 2020; Untiedt et al., 2019; Glon et al., 2021; McFadden et al., 2021, 2022; Bridge
117 et al., 2023) to resolve questions at a range of scales. Target-capture of UCEs and exons, in
118 particular, has shown much promise in resolving phylogenetic relationships of anthozoans across
119 deep (i.e., orders, Quattrini et al., 2020; McFadden et al., 2021, 2022) to shallow (i.e., closely
120 related species, Erickson et al., 2021; Glon et al., 2023; Bridge et al., 2023) time scales.

121 The original Anthozoa UCE and exons baitset was designed by Quattrini et al. (2018) and
122 redesigned by Erickson et al. (2021) for Octocorallia and Cowman et al. (2021) for Hexacorallia.
123 These baitsets target 1000s of loci, but do not include baits for mitochondrial genes or the
124 nuclear ribosomal RNA (*rRNA*) genes. Although using mitochondrial genes and *rRNA* genes for
125 phylogenomic studies of Anthozoa is cautioned (Figueroa & Baco 2015; Herrera & Shank, 2016;
126 Quattrini et al., 2023), the utility of these markers goes beyond phylogenomic analyses. For
127 example, mitogenome evolution across Anthozoa is intriguing as they exhibit a range of
128 properties unique among metazoans, including gene order rearrangements (Brockman &
129 McFadden, 2012; Lin et al., 2014; Figueroa & Baco, 2015; Seiblitz et al., 2022), a mismatch
130 repair enzyme in Octocorallia (*mtMutS*, Bilewitch & Degnan, 2011), gene introns in the
131 Hexacorallia (e.g., a homing endonuclease, Fukami et al., 2007, Barrett et al., 2020), and
132 bipartite mitogenomes (Hogan et al., 2019). In some cases, mitogenomes have been used as
133 taxonomic characters, as certain gene orders in mitogenomes appear to be restricted to particular
134 families (see Seiblitz et al., 2022). Finally, with the recent efforts to monitor coral ecosystems
135 with environmental DNA, there is a need to increase the number of taxa and loci in reference
136 databases (McCartin et al., in review). Because genome skimming enables the production of low-
137 coverage yet highly fragmented genomes, this method, followed by bioinformatic analyses, holds
138 promise in not only obtaining whole mitogenomes and nuclear *rRNA* genes, but also UCEs and

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139 exons, as well as other genes of potential interest, from a range of DNA sample types (i.e.,
140 contemporary to historical samples) for a relatively low cost.
141 Here, we tested the utility of using genome-skimming data to bioinformatically obtain
142 whole mitogenomes, nuclear *rRNA* genes, UCEs, and exons from hexacorals and octocorals.
143 Although most of our efforts were focused on recently collected (< 20 years) specimens
144 preserved specifically for genetic purposes, we also tested the utility of this approach to obtain
145 UCEs, exons, and mitogenomes from historical material collected more than 100 years ago.
146

147 **Methods**

148 *Collections*

149 Octocorals (n=177) and hexacorals (n=32, including 30 antipatharians or black corals,
150 one scleractinian [*Javania*], and one zoanthid [*Umimayanthus*]) were collected from the Gulf of
151 Mexico, Caribbean Sea, and off the southeastern US coast from 2006 to 2019 on various
152 expeditions. Specimens were collected with both Remotely Operated Vehicles (ROV) and
153 SCUBA. Tissue samples were taken in the field, preserved in 95% ethanol and stored at -20°C,
154 or flash frozen in liquid nitrogen and stored at -80°C. We also added historical, cataloged
155 octocorals (n=33) collected from 1886 to 2006 from locations worldwide. Most museum
156 specimens were preserved and/or stored dry or in 70% EtOH. See Supplemental Table 1 for
157 further details.
158

159 *Molecular Lab Work*

160 DNA was extracted in various ways (Table S1). Contemporary samples were extracted
161 with either a modified CTAB protocol, a salting-out protocol, a GeneJet Genomic DNA

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162 Purification kit, or a Qiagen DNEasy extraction kit. Historical samples were all extracted with a
163 Qiagen DNEasy kit. For some antipatharians and octocorals, DNA was cleaned with a Qiagen
164 Power Clean Pro kit to remove PCR inhibitors. Samples were quantified with a fluorometer,
165 either with a Quant-iT or Qubit.

166 For most samples (204 out of 242), library preparation was carried out in the Laboratories
167 of Analytical Biology at the Smithsonian Institution. The quantity of genomic DNA input into a
168 library preparation ranged from <0.65 ng to 93 ng total DNA; the average was 55±15 (SD) ng
169 DNA. Library preparation was carried out using the NEBNext Ultra II FS DNA Library Prep Kit
170 for inputs ≤ 100 ng with the following modifications: the reaction volume was reduced by half,
171 the fragmentation/end prep incubation was conducted for 10 minutes (contemporary samples) or
172 2.5 minutes (historical samples), 5 µl of iTru Y-yoke adaptor (Glenn et al., 2019) was used
173 instead of NEBNext Adaptor, adaptor ligation time was 30 minutes, bead cleanups were
174 performed with KAPA Pure Beads, iTru i5 and i7 indices (Glenn et al., 2019) were used, and 10
175 cycles of PCR enrichment were conducted. A negative control was included on each plate during
176 library preparation to test for any potential contamination. All DNA libraries were quantified and
177 assessed with a Qubit fluorometer High Sensitivity Assay and a Tapestation, and final pools
178 were created for sequencing on an Illumina NovaSeq (150 bp paired-end (PE) reads, Table S1).
179 Pool 1 contained 33 historical samples sequenced on one lane of a NovaSeq S4 with 347 other
180 invertebrate samples for a target read number of 5M PE reads per sample. Pool 2 contained 133
181 samples sequenced all together on one lane of a NovaSeq X for a target read number of 20M PE
182 reads per sample. Pool 3 contained 38 samples sequenced with 57 additional samples on one lane
183 of a NovaSeq X Plus for a target read number of 10M PE reads. To assess whether we could
184 combine data from other DNA libraries, we included 38 DNA libraries (i.e., pool 4) that were

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185 prepared with an Illumina Nextera XT2 kit for NextSeq 500 sequencing at Biopolymers Facility
186 at Harvard Medical School.
187
188 *UCE and Exon Analyses*
189 Demultiplexed reads were trimmed using Trimmomatic v 0.32 or v 0.39 (Bolger et al.,
190 2014). Trimmed reads were assembled using Spades v. 3.1 or 3.13.0 (Bankevich et al., 2012).
191 Spades assemblies were then passed to phyluce v 1.7 (Faircloth 2016) to bioinformatically
192 extract UCEs and exons using previously published bait sets for octocorals (octo-v2, Erickson et
193 al., 2020) and hexacorals (hexa-v2, Cowman et al., 2020). The phyluce pipeline was used
194 separately on octocorals and hexacorals as described in the online tutorials
195 (https://phyluce.readthedocs.io/en/latest/tutorials/tutorial-1.html) with some modifications
196 following Quattrini et al. (2018, 2020). Before aligning with MAFFT v7.130b (Katoh and
197 Stanley 2013), we combined the data from 208 octocoral samples and the zoanthid
198 *Umimayanthus* with previously published target-capture data obtained from 187 octocorals and
199 11 outgroups (Quattrini et al., 2018, 2020, Untiedt et al., 2020, Erickson et al., 2021, McFadden
200 et al., 2022). We combined the data from 30 black coral samples and the stony coral *Javania*
201 with previously published (Quattrini et al., 2018, 2020; Horowitz et al., 2022, 2023a) target-
202 capture data from 106 black corals and four outgroups. After alignment, phyluce was used to
203 create a 60% taxon-occupancy matrix for all loci, which were then concatenated separately for
204 black coral (n=141) and octocoral (n=407) datasets. Phylogenomic analyses were conducted
205 using maximum likelihood in IQTree v 2.1 (Nguyen et al., 2015) on the concatenated datasets
206 with ultrafast bootstrapping (-bb 1000, Hoang et al., 2018) and the Sh-like approximate
207 likelihood ratio test (-alrt 1000, SH-aLRT Anisimova et al., 2011). A partitioned model was used

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208 (-p). The best model of nucleotide substitution for each partition was found with ModelFinder (-
209 m TESTMERGE, Kalyaanamoorthy et al., 2017) (Table 1). One octocoral sample, *Tripalea*
210 *clavaria*, a dried museum specimen, was recovered as sister to all other octocorals. This sample
211 was likely a contaminated sequence, which was pruned from the alignment. The alignment
212 (n=406 species) was then re-run in IQTree using the abovementioned parameters.
213
214 *Mitogenome Analyses*
215 For most samples (n=204), trimmed reads were also passed to Mitofinder v. 1.4 (Allio et
216 al., 2020) for mitogenome assembly and annotation using a reference database of either
217 octocorals or hexacorals downloaded from GenBank. We used trimmed reads in the analyses
218 with the –new-genes parameter (to account for *mtMutS* and HEG) and the translation table 4 (-o).
219 For the 38 samples from pool 4, mitogenomes were previously reported in Easton and Hicks
220 (2019, 2020); thus, those results are not included in the present study.
221
222 *Nuclear rRNA Analyses*
223 We also mapped, assembled, and extracted nuclear *rRNA* genes from the genome-
224 skimming data. To obtain a reference sequence for mapping and assembly of octocoral samples,
225 an annotated nuclear *rRNA* operon sequence, including the nuclear *rRNA* genes as well as *ITS1*
226 and *ITS2*, was extracted from the NCBI-annotated *Xenia* sp. genome (RefSeq assembly
227 GCF_021976095.1, scaffold NW_025813507.1) at NCBI
228 (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/). As a reference for black corals, we
229 used a 4,721 bp sequence of *Cladopathes* cf. *plumosa* (GenBank: MT318868.1) from Barrett *et*
230 *al.* (2020) that spans *18S*, *ITS1*, *5.8S*, *ITS2*, and the majority of *28S*.

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231 Trimmed read pairs were merged using BBMerge v 38.84 (Bushnell et al., 2017) with
232 the normal merge rate and the default settings and then imported into Geneious Prime v.
233 2023.1.2 (https://www.geneious.com). Merged read pairs were mapped and assembled to the
234 reference sequences using the "Map to Reference(s)" function in Geneious with the sensitivity
235 set to "Medium-Low Sensitivity/ Fast" and with five mapping iterations. Consensus sequences
236 were generated from the resulting assemblies with the following settings. At each position, the
237 threshold was set to 90% identity across all mapped reads for base-calling, a "?" was called if the
238 coverage was less than 10 mapped reads, and the quality was assigned as the highest quality
239 from any single base. Each consensus sequence was trimmed to its reference.

240 From the consensus sequences, we extracted and analyzed the *rRNA* genes *18S, 5.8S*, and
241 *28S*. The consensus sequences were aligned using MAFFT v. 1.5.0 (algorithm E-INS-I, scoring
242 matrix 100PAM/K=2) as implemented in Geneious Prime 2023.2.1 (https://www.geneious.com).
243 Two alignments were analyzed, one including *ITS1* and *ITS2* in addition to the rRNA genes and
244 another with *ITS1* and *ITS2* removed (e.g., *18S, 5.8S*, and *28S* only). The alignments were
245 trimmed at the 5' end to the beginning of *18S* using octocorals as a reference. While we were
246 able to assemble the entirety of *28S* for octocorals, we were only able to assemble about one-half
247 of the *28S* gene in black corals, due to incompleteness of the black coral reference sequence
248 used. Partitions were created for both alignments (with and without the *ITS*). Phylogenetic
249 inference was then conducted with IQTree using the best model of evolution determined by
250 Modelfinder (-m TEST, Kalyaanamoorthy et al., 2017) and 1000 ultrafast bootstrap replicates (-
251 bb 1000).

252 In addition to analyzing these concatenated *rRNA* gene alignments, we also extracted a
253 ~400 bp DNA barcode from the consensus sequences that is targeted by anthozoan-specific

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254 meta-barcoding primers (McCartin et al., in prep). This DNA barcode was compared to
255 sequences generated via conventional PCR/Sanger sequencing for seven black coral and twenty-
256 eight octocoral samples (McCartin et al., 2023). These barcoding sequences were aligned with
257 MAFFT v. 7.49 (LINS-I method) and phylogenetic inference was conducted in the same manner
258 using IQTree as for the concatenated alignment of *rRNA* gene sequences. Best models of
259 sequence evolution for the partitioned datasets were chosen by ModelTest as implemented by
260 IQTree (-m TEST)
261
262 *Statistical Tests*
263 For historical museum specimens sequenced in pool 1, we conducted analyses to
264 determine if collection year, library concentration, or DNA concentration impacted the number
265 of reads or loci obtained. We first determined a significant correlation (r=0.58, p=0.001) between
266 DNA and library concentration and thus removed DNA concentration from further analyses (Fig.
267 2A). Then, we assessed both additive and multiplicative linear regression models on log-
268 transformed data to determine whether library concentration and collection year affected the
269 dependent variables of number of reads and loci. The multiplicative models had a higher
270 adjusted R-squared value (0.32, 0.69) than the additive models (0.24, 0.65) for tests on loci and
271 read recovery, respectively; thus, we report the results of the multiplicative model below. We
272 also tested whether the number of loci recovered was influenced by the number of reads obtained
273 per sample.
274 We also determined whether the number of reads obtained across pools 1-3 significantly
275 affected the completion of mitogenome circularization when using MitoFinder. We used a one-
276 way analysis of variance on log-transformed data for both hexacorals and octocorals.
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278 **Results**

279 *Assembly Statistics*

280 Of 242 samples, two failed sequencing with only 4,926 and 89,916 PE reads obtained;
281 thus, these samples were removed from subsequent analyses. The remaining 240 samples had
282 between 854,547 and 55,565,170 PE reads, with an average of 17,382,298 ± 8,065,341 PE reads.
283 Pool 1 had an average of 8,343,203 ± 2,922,102; Pool 2 had an average of 23,156,985 ±
284 3,323,082; Pool 3 had an average of 12,822,312 ± 5,512,007; and Pool 4 had an average of
285 9,884,551 ± 857,465 PE reads. Trimmed reads were assembled into a mean of 741,347 ±
286 484,057 SD contigs per sample (range: 13,422 to 221,4702) (Table S1).

287

288 *UCE and Exon Results*

289 UCEs and exons were successfully recovered from the genome skimming data of
290 octocorals and hexacorals. For octocorals, 7 to 2,443 loci (mean 1,837 ± 662 SD) out of 3,023
291 targeted loci were recovered from each individual. The mean locus size was 1266 ± 1048 bp with
292 a trend of increasing numbers of loci obtained with increasing numbers of PE reads until ~10M
293 PE reads, where the recovery rate reached a plateau (Fig. 1). Out of 206 octocorals, <200 loci
294 were recovered in only 3% of samples; all of these samples were from pools 1 and 4 with a range
295 of collection ages from 1960 to 2017 and a 10-fold range of obtained reads (973,960 to
296 9,534,512 PE reads).

297 We were able to recover 18 to 2,361 loci (1,422 ± 720 loci) from the historical museum
298 specimens, with 1,253 loci recovered from the oldest specimen collected in 1886 and 1,336 loci
299 recovered from the holotype of *Sibogagorgia dennisgordoni* (Fig. 2). The mean locus size,
300 however, was smaller (790 ± 578 bp) compared to the contemporary samples preserved
301 specifically for genomics (1,355 ± 1,093 bp). In general, the number of loci recovered from the

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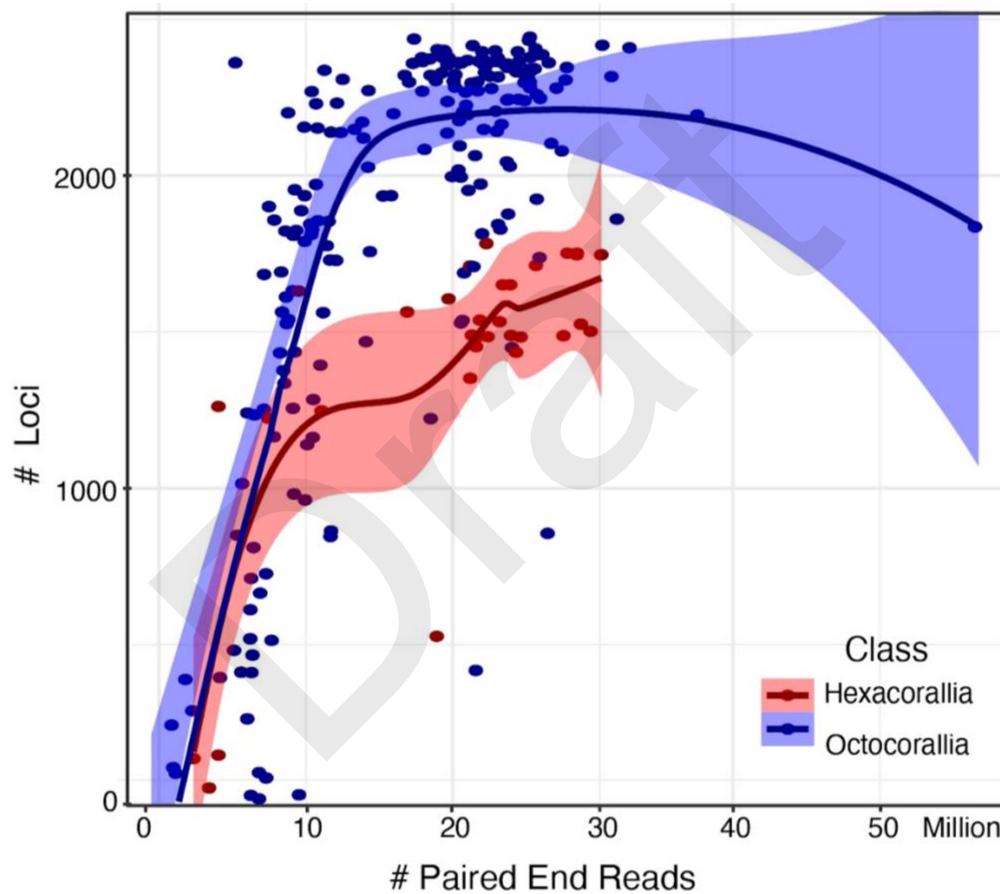
302 assemblies increased significantly (t=3.663, p=0.0009) with the number of reads obtained per
303 specimen (Fig. 2B). The number of reads increased significantly with an increase in library
304 concentration (t=2.31, p=0.028), with an interaction effect of year of collection (t=−2.25,
305 p=0.032; Fig. 2C, E). Likewise, the number of loci increased significantly with an increase in
306 library concentration (t=2.16, p=0.039), with an interaction effect of year of collection (t=-2.14,
307 p=0.041; Fig. 2D, F)



308

309 Figure 1. The number of loci recovered by the total number of paired-end reads obtained per
310 sample in both Octocorallia and Hexacorallia. A polynomial regression model with local fitting
311 was applied.

312

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313 The phylogenetic tree that included all octocoral samples from genome skimming and
314 prior target-capture work (alignment: 1,262 loci, 243,326 bp) was well supported (Fig. 3, Fig.
315 S1), and the genome-skimmed samples were recovered in the phylogeny within their respective
316 families except one dried museum specimen, *Tripalea clavaria*, which was recovered as sister to
317 all other octocorals and was thus pruned from the phylogeny. We recovered the two reciprocally-
318 monophyletic orders, Scleralcyonacea and Malacalcyonacea, and added at least 55 species to the
319 genomic-scale phylogeny of octocorals. Of 405 nodes, 96% had Sh-aLRT values over 80%, and
320 89% had bootstrap support values over 95%; most of the low values were near the tips. The
321 zoantharian used as an outgroup in the octocoral phylogeny was correctly recovered in its
322 respective order.
323

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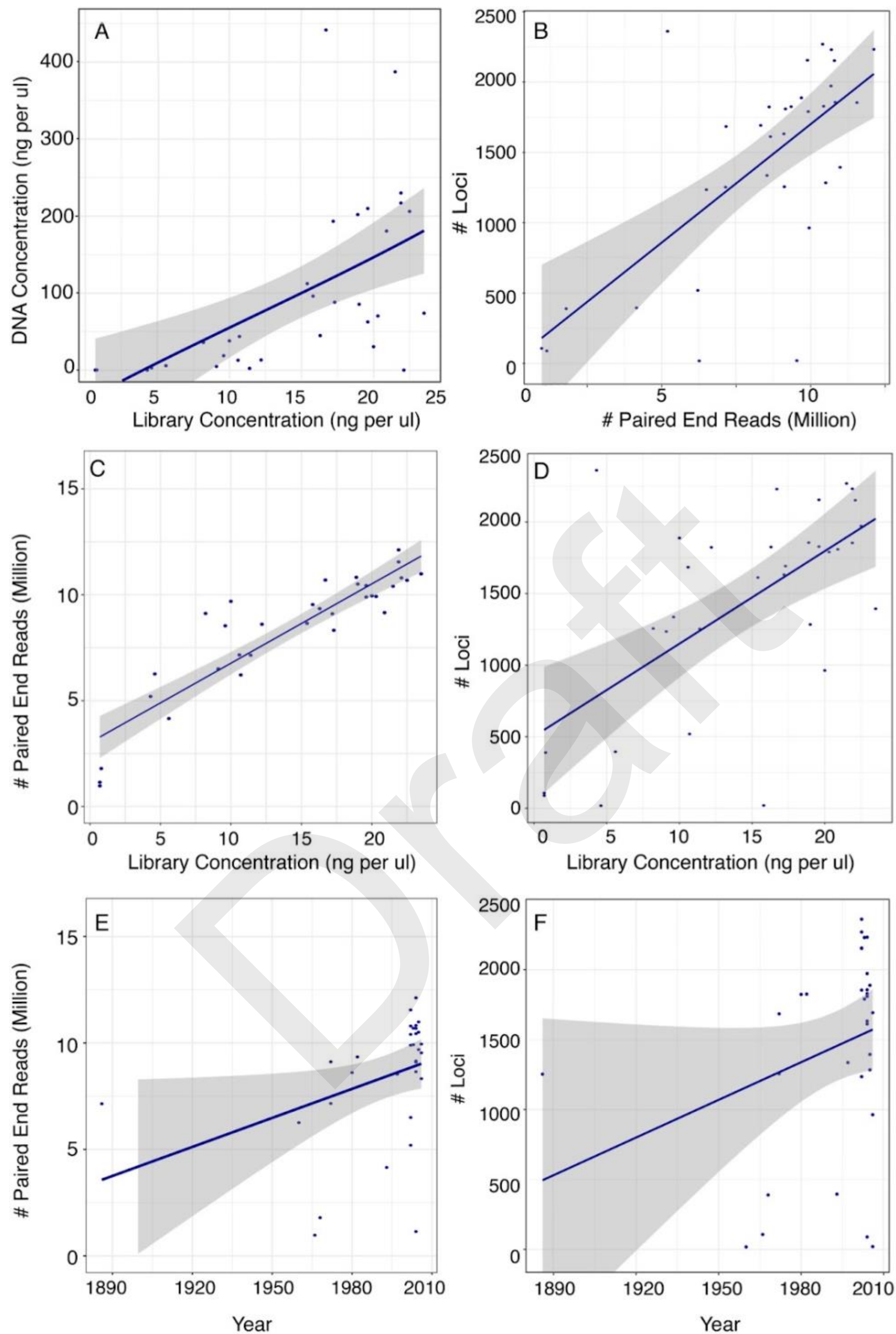

324

325 Figure 2. Data for historical museum samples sequenced in pool 1. (A) Library concentration

326 versus DNA concentration. (B) Number of loci by the number of paired-end reads. (C-F)

327 Number of reads and loci obtained by library concentration and collection year.

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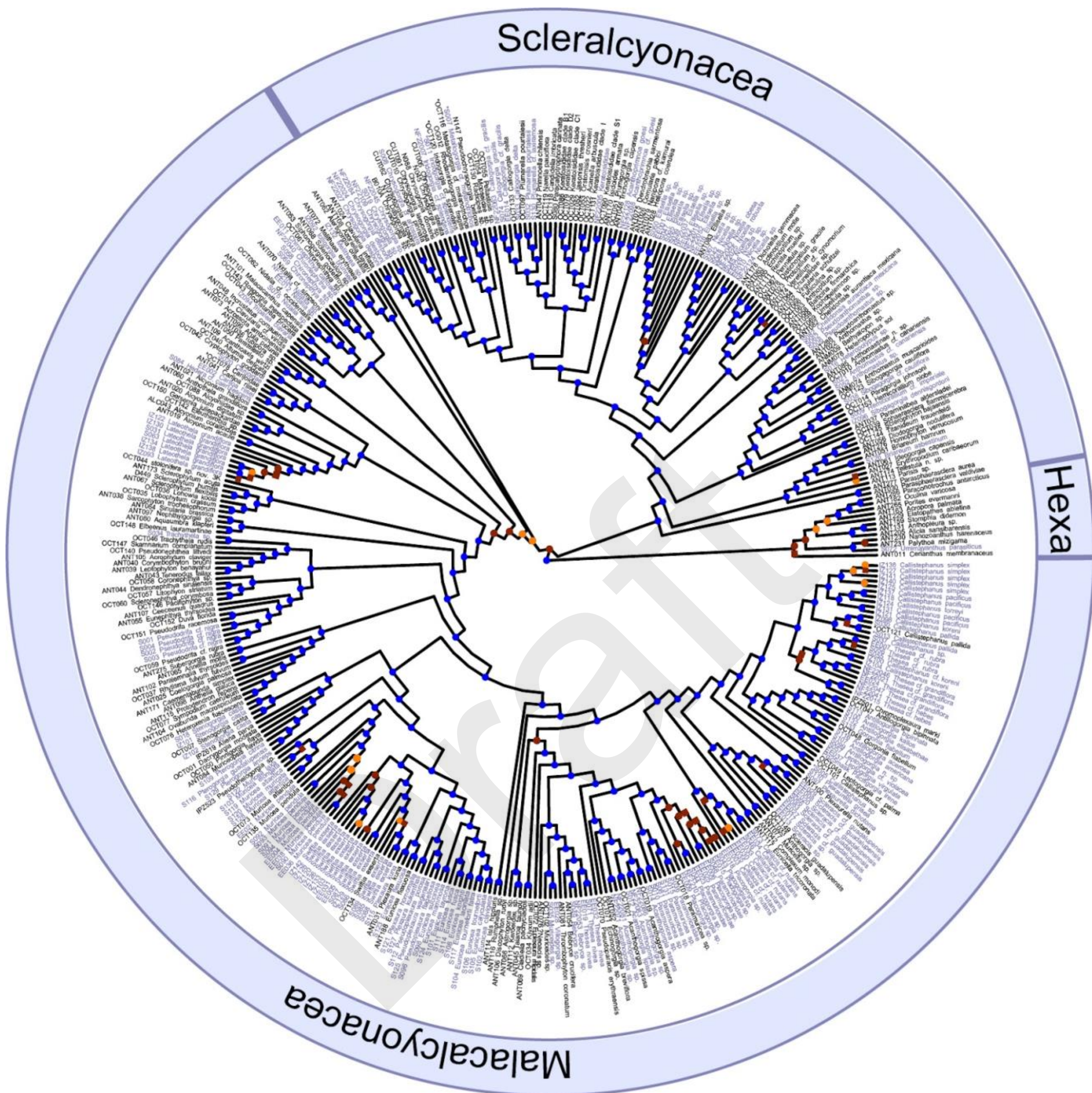

328

329 Figure 3. Maximum likelihood phylogeny of octocorals (purple=genome skim, black=target
330 capture). Outgroups include hexacorals (Hexa). Node support values, represented by circles,
331 include ultrafast bootstraps > 95% (blue), 80-95% (orange), and <80% (red). Where squares are
332 indicated, SH-aLRT values were also < 80%. *=samples genome skimmed and target-enriched

333

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334 For hexacorals, 42 to 1,783 loci (mean 1,379 ± 476 SD) out of 2,476 targeted loci were
335 recovered from each individual. The mean locus size was 2,385 ± 1961 bp with a trend of
336 increasing numbers of loci obtained with increasing numbers of PE reads until ~20M PE reads,
337 where the recovery rate slowed (Fig. 1). Out of 33 hexacorals, <200 loci were recovered in only
338 9% of samples; all of these samples were black corals collected in 2022 (sequenced in pool 2)
339 with a range of obtained reads (2,353,550 to 4,045,520 PE reads).

340 The phylogenetic tree that included all antipatharian samples (alignment: 467 loci,
341 110,353 bp) from genome skimming and prior target-capture work was well supported, and the
342 genome-skimmed samples were recovered in the phylogeny within their respective families (Fig.
343 4, Fig. S2). The newly incorporated genome-skim data (representing all seven antipatharian
344 families) reinforces the monophyletic relationships of Myriopathidae and the monogeneric
345 family, Leiopathidae. All other families are polyphyletic; notably, the new genome skim data
346 reveals that Aphanipathidae is polyphyletic, where *Distichopathes hickersonae* and *Elatopathes*
347 *abietina* are divergent from the rest of Aphanipathidae. This new dataset added at least 10
348 species to the black coral genomic-scale phylogeny. The scleractinian used as an outgroup in the
349 hexacoral phylogeny was also recovered in its correct order. Out of 140 nodes, 70% had Sh-
350 aLRT values over 80%, and 78% had bs values over 95%. In all cases, the lower node support
351 values were near the tips.

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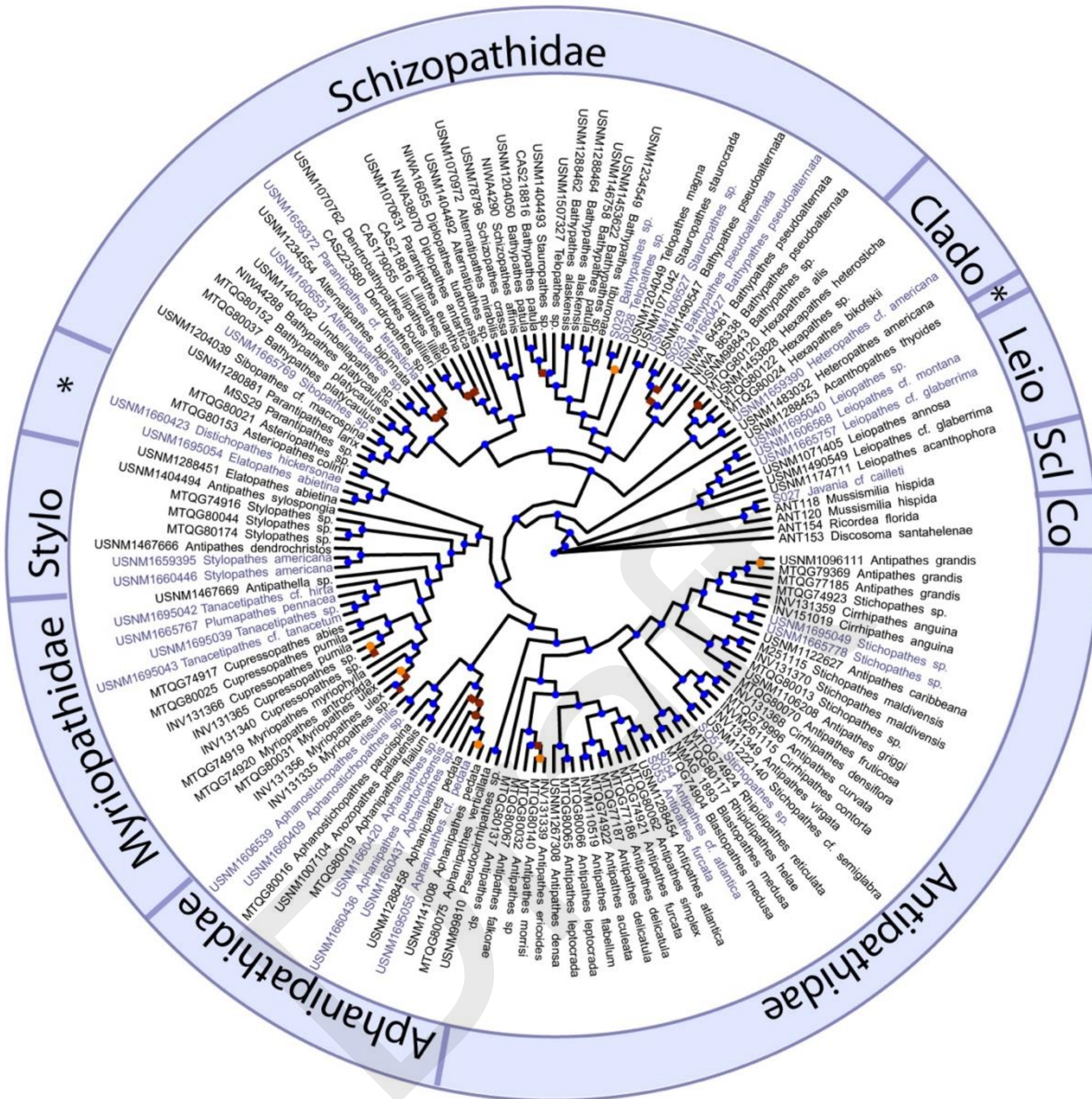

354

355 Figure 4. Maximum likelihood phylogeny of black corals (purple=genome skim, black=target
356 capture). Outgroups include Scleractinia (Scl) and Corallimorpharia (Co). Node support values,
357 represented by circles, include ultrafast bootstraps > 95% (blue), 80-95% (orange), and <80%
358 (red). Where squares are indicated, SH-aLRT values were also < 80%. Leio=Leiopathidae,
359 Clado=Cladopathidae, Stylo= Stylopathidae, and *= Species currently included within the
360 polyphyletic family Aphanipathidae.

361

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362 *Mitogenome Results*

363 All mitochondrial protein-coding genes (PCGs) and *rRNA* genes were successfully
364 retrieved from 95% of the samples targeted for mitogenome recovery. Of the 170 octocorals, we
365 recovered 14 PCGs and both rSUs in 168 individuals. Only 10 PCGs and mitochondrial rSUs
366 were recovered in two octocorals; both were museum samples collected in 1993 and 2005. The
367 *mtMutS* sequences obtained were successfully integrated with data produced from PCR/Sanger
368 sequencing, resulting in an alignment of 1074 bp (Fig. S3). Placements of taxa in the *mt MutS*
369 phylogeny were as expected, and in many cases, the sequence data were 100% identical to the
370 same species that were Sanger-sequenced. Most (70%) of octocoral mitogenomes were
371 circularized with mitofinder. The majority of these were from Pool 2, which, on average, had the
372 highest number of PE reads obtained across all pools (Fig. 5). Significantly more mitogenomes
373 were circularized with a higher number of reads obtained for octocorals (ANOVA, F=96,
374 p=0.001). For the 32 hexacorals, only one individual failed mitogenome assembly, with only
375 three PCGs obtained, yet this individual had over 3,406,440 PE reads. Only 40% of all hexacoral
376 mitogenomes were circularized with Mitofinder, with the majority of these from Pool 2. For
377 hexacorals, no significant differences were found between mitogenome circularization and
378 number of reads obtained (ANOVA, F=0.25, p>0.05).

379

380 *Nuclear rRNA Results*

381 Nuclear *rRNA* genes were successfully obtained from all but one sample. Reads mapped
382 to at least 95% of the reference sequence used, and the mean coverage was 4,317x. The length of
383 the assembled consensus sequences ranged from 4,142 to 6,136 bp, and differences in length
384 were mainly because the black coral reference did not include all of the *28S*. Across the 478 bp
385 alignment barcoding region of *28S*, sequences generated from genome skimming were 100% (p-

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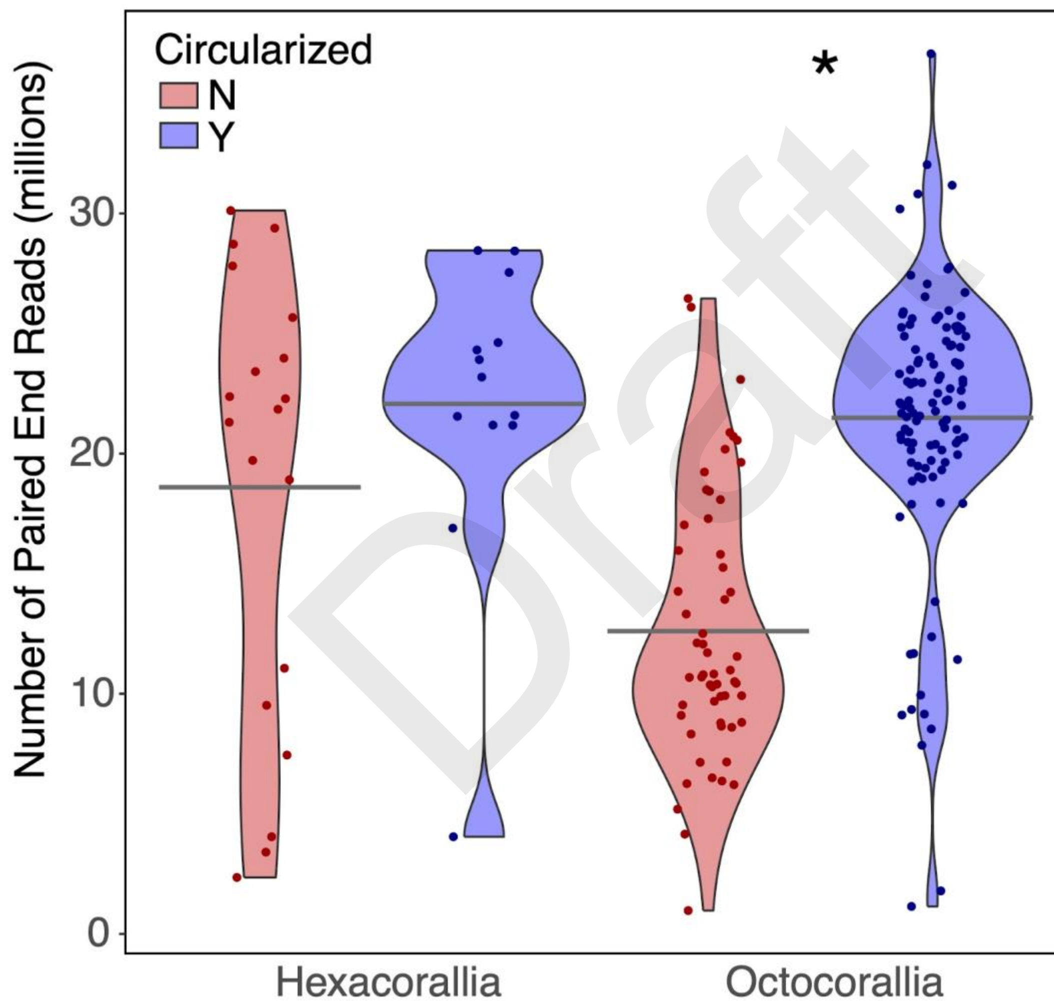
386 distance) similar to their respective Sanger sequence except in the case of one specimen of
387 *Sibogagorgia* cf. *cauliflora* (Fig. S4). This specimen had numerous ambiguous bases across the
388 478 bp alignment and was 15% divergent from the Sanger sequence. The phylogenetic tree
389 produced from the *rRNA* genes (6,031 bp alignment) included taxa in positions as expected,
390 except for *Sibogagorgia cf. caulifora* and *Plumarella pourtalesii (*Fig. S5).
391



392
393 Figure 5. Circularization of mitogenomes by the number of paired-end reads from each sample
394 for Octocorallia (n=171, 114 circular, 57 non-circular) and Hexacorallia (n=32, 13 circular, 19
395 non-circular). (*p=0.001). Gray bars indicate the group mean.
396

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397 **Discussion**

398 *The Utility of Genome Skimming*

399 Genome skimming is an effective approach for obtaining a range of loci useful for
400 systematics and reference DNA barcode libraries of anthozoans. We bioinformatically obtained
401 >1400 UCE/exon loci on average from both hexacorals and octocorals, indicating the utility of
402 genome skimming for obtaining loci that are otherwise captured through a target-capture
403 enrichment process. To highlight the similarity of results obtained from genome skimming and
404 target capture, nine octocorals that were genome skimmed in this study were also target-captured
405 in prior work (McFadden et al., 2022). In all cases, the numbers of UCE loci obtained from the
406 skimmed samples were similar to or slightly higher (~200 loci) than the target-captured samples,
407 and five pairs of skimmed/target-captured samples included in the phylogeny were recovered as
408 sister taxa. In addition to obtaining UCE/exon loci, we obtained nuclear ribosomal RNA gene
409 sequences with high mapping coverage to reference sequences and most mitochondrial
410 genes. Further, with minimal bioinformatic effort (i.e., just using one assembly program), we
411 were able to obtain complete, circularized mitogenomes for 60% of all the samples. Our results
412 indicate that this approach can also be used on historical-museum samples, where most target
413 regions (i.e., mitochondrial genes, nuclear *rRNA* genes, UCES/exons) were successfully
414 obtained.

415 Historical specimens, many of which had highly-degraded and low-quantity DNA (Table
416 S1), performed very well with genome skimming. None of these historical specimens were
417 preserved specifically for genetic applications. Yet, we recovered most, if not all, mt genes,
418 nuclear *rRNA* genes, and more than 1000 UCE/exon loci from 75% of the samples. In addition,
419 this approach is useful for obtaining numerous loci from type specimens (i.e., *Sibogagorgia*

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420 *dennisgordoni)* and specimens collected over 100 years ago. Our results, however, suggest that
421 DNA concentration is directly correlated with library concentration, and higher library
422 concentrations yield more reads and, thus, more UCE/exon loci. In contrast to expectations, the
423 collection year had minimal impacts on UCE/exon loci obtained from the skimming data.
424 Museum specimens used in this study were preserved in various ways, including drying, 70%
425 EtOH, and 95% EtOH. Some specimens were likely fixed in formalin, but this information is
426 often not retained in museum records. Thus, preservation type could confound a direct
427 relationship of collection year with the number of loci obtained. Therefore, we recommend that
428 researchers try genome skimming on various museum samples, regardless of collection age or
429 preservation type. We also urge the use of type material in genome skimming studies to help
430 resolve taxonomic issues in both classes of hexacorals and octocorals. Because the first step of
431 preparing NEB genomic libraries is shearing DNA, one can skip or reduce the shearing time and
432 use just the degraded DNA that is recovered from museum specimens in the DNA library
433 preparation workflow. Our results here suggest that genome skimming is a simple genomic
434 approach that can help unlock our historical museum collections, thus ultimately helping to
435 resolve phylogenetic relationships across Metazoa.

436 There have been increasing efforts to use environmental (e)DNA sampling to
437 characterize biodiversity and monitor health across ecosystems. It is clear, however, that the
438 classification of eDNA sequences at a meaningful taxonomic resolution relies on the
439 completeness of reference databases of DNA barcodes to which eDNA can be compared (Gold et
440 al., 2021). But DNA barcodes remain missing for many metazoan taxa (e.g., Ransome et al.,
441 2017; Pappalardo et al., 2021), and there are no standard barcodes that can be used to resolve
442 species or even genera across diverse taxa, although both mitochondrial genes and nuclear *rRNA*

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443 genes are often used. Therefore, it is a critical time to increase barcode data across both taxa and
444 gene regions. Our results suggest that genome skimming is one way to improve reference
445 sequence databases simply and rapidly for applications like eDNA metabarcoding. We provide
446 evidence that *28S rRNA* sequences recovered from the genome skimming data were largely
447 congruent to sequences generated from conventional PCR amplification and Sanger sequencing.
448 Another obvious advantage of genome skimming over Sanger sequencing is the ability to
449 generate sequencing data for multiple barcoding genes simultaneously, including nuclear *rRNA*
450 and mitochondrial genes.

451 For the amount of data obtained, genome skimming is a relatively cost-effective method
452 compared to other genomic and genetic approaches. Library preparation, sequencing (10-20M
453 PE reads), and quantification cost ~$60-75 USD for this study. This same amount would
454 facilitate sequencing ~6-7 loci (approximate costs, $6-8 for sequencing, $5 for PCR reaction)
455 through traditional PCR and Sanger. Although the average costs of genome skimming are
456 relatively low compared to Sanger sequencing, the high costs and/or access to genomic
457 sequencing facilities, high-performance computing, and bioinformatics training might still be
458 prohibitive for some researchers, particularly those in low-income countries (see, e.g., Rana et
459 al., 2020; Yek et al., 2022; Whiteford et al., 2023). By collaborating across international borders,
460 we can easily pool samples from several research groups for sequencing at a genomic sequencing
461 facility, at least in situations where DNA exchange restrictions are not an issue.

462

463 *New insights into octocoral phylogeny*

464 At the genus level, the phylogeny of octocorals constructed here using a combination of
465 data obtained from target-enrichment of conserved elements and genome skimming was largely
466 congruent with that published previously using data from target-enrichment only (McFadden et

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467 al., 2022). Relationships among families were also mostly in agreement with that previous
468 analysis, with the most notable exception being the recovery of the family Cladiellidae, sister to
469 the gorgonian families Euplexauridae and Paramuriceidae, as was also found by Quattrini et al.
470 (2023). The subordinal-level clades defined by McFadden et al. (2022) were not, however, as
471 well supported by the analysis presented here (Fig. 3). These differences may be attributable to
472 differences between analyses in taxon sampling or the numbers and identities of loci included
473 (i.e., including saturated loci) and exemplify the challenges inherent in resolving the deepest
474 nodes in a group of organisms that evolved in the pre-Cambrian (McFadden et al., 2021).

475 Genomic data were obtained for the first time from representatives of 11 genera
476 (*Paracalyptrophora* Kinoshita, 1908; *Nicella* Gray, 1870; *Iciligorgia* Duchassaing, 1870;
477 *Lateothela* Moore et al., 2017; *Hedera* Conti-Jerpe & Freshwater, 2017; *Chromoplexaura*
478 Williams, 2013; *Pseudoplexaura* Wright & Studer, 1889; *Placogorgia* Wright & Studer, 1889;
479 *Villogorgia* Duchassaing & Michelotti, 1860; *Aliena* Breedy et al., 2023; and *Thesea*
480 Duchassaing & Michelotti, 1860). Phylogenetic placement of each of these genera was congruent
481 with expectations based on previous phylogenetic analyses of mitochondrial and nuclear *rRNA*
482 gene trees (Cairns & Wirshing 2018; McFadden et al., 2022; Breedy et al., 2023). The
483 phylogenomic analysis recovered *Thesea* as polyphyletic, with some species grouping in the
484 family Paramuriceidae and others in the Gorgoniidae, which is also congruent with previous
485 phylogenetic analyses (Carpinelli et al., 2022). The paraphyletic relationships of *Gorgonia* to
486 *Antillogorgia* and of *Plexaura* and *Pseudoplexaura* to *Eunicea* have also been recovered in
487 previous studies (Grajales et al., 2007; Torres-Suarez 2014), as has the polyphyly exhibited by
488 *Leptogorgia* (Poliseno et al., 2017).

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489 Molecular data were obtained for the first time for four genera, allowing their familial
490 relationships to be assessed. *Acanthoprimnoa* Cairns & Bayer, 2004, a genus whose membership
491 in Primnoidae has never been questioned (Cairns & Bayer, 2004; Cairns & Wirshing, 2018), was
492 instead found to be sister to Ifalukellidae. *Tripalea* Bayer, 1955, placed in Spongiodermidae
493 based on morphology (Cairns & Wirshing 2015), appears instead to belong to Incrustatidae in
494 the order Malacalcyonacea. Finally, *Caliacis* Deichmann, 1936 and *Pseudothelogorgia* van
495 Ofwegen, 1991, genera whose familial affinities were left *incertae sedis* by McFadden et al.
496 (2022), each occupy unique positions within the clade of malacalcyonacean gorgonians (clade 8
497 of McFadden et al., 2022), suggesting they each deserve family status. Before proposing those
498 new families, however, it will be necessary to confirm the species-level identification of the
499 material we sequenced by comparison to original type material.

500

501 *New insights into antipatharian phylogeny*

502 The black coral phylogeny is mostly congruent with previous reconstructions (Horowitz
503 et al., 2022, 2023b); however, this study includes three genera (*Distichopathes* Opresko, 2004,
504 *Plumapathes* Opresko, 2001, and *Tanacetipathes* Opresko, 2001) that have been sequenced for
505 the first time with high-throughput genomic techniques, providing new insights into
506 phylogenomic relationships within the order. *Distichopathes* Opresko, 2004 was recovered sister
507 to *Elatopathes* Opresko, 2004. Along with *Asteriopathes* Opresko, 2004, these three genera are
508 currently placed in Aphanipathidae Opresko, 2004, but they form a monophyletic clade divergent
509 from the rest of Aphanipathidae. Instead, the three genera show affinity to Stylopathidae
510 Opresko, 2006, a finding consistent with Opresko et al. (2020) based on three mitochondrial and
511 three nuclear gene regions. The recovered polyphyletic relationship of *Plumapathes* Opresko,
512 2001 and *Tanacetipathes* Opresko, 2001 (both of which reside in Myriopathidae Opresko, 2001)

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513 is notable as they possess distinctly different branching characteristics (planar in *Plumapathes* vs
514 bottlebrush in *Tanacetipathes*). However, Horowitz et al. (2023b) emphasized that smaller-scale
515 features, such as polyps and spines, are often more informative than branching
516 characteristics. Most species within the Myriopathidae have very similar spine and polyp
517 characteristics. Thus, these genera within Myriopathidae require further examination for a
518 possible taxonomic revision.

519 Six out of the seven families in the order are polyphyletic based on this and previous
520 phylogenetic reconstructions (Brugler et al. 2013, Horowitz et al. 2022, 2023a). Notably, the
521 family Aphanipathidae contains genera spread across the tree (identified by '*' in Fig. 4),
522 highlighting the need for taxonomic revisions. However, a formal taxonomic review cannot be
523 conducted because the type for Aphanipathidae by subsequent designation, *Aphanipathes*
524 *sarothamnoides* Brook, 1889 has yet to be sequenced. Therefore it is not yet certain which clade
525 represents the Aphanipathidae. This study demonstrates that genome skimming and target
526 enrichment are suitable methods to yield high phylogenetic resolution of antipatharians. All that
527 is needed now are sequence data from holotype or topotype material representing each nominal
528 and currently accepted genus to fill gaps and better support taxonomic revisions.

529
530
531 **Figure captions**
532
533 Figure 1. Number of loci recovered by total number of paired-end reads obtained per sample in
534 both Octocorallia and Hexacorallia.

535
536 Figure 2. Data for historical museum samples sequenced in pool 1. (A) Library concentration
537 versus DNA concentration. (B) Number of loci by number of paired-end reads. (C-F) Number of
538 reads and loci obtained by library concentration and collection year.

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539
540 Figure 3. Maximum likelihood phylogeny of octocorals (purple=genome skim, black=target
541 capture). Outgroups include hexacorals (hexa). Node support values, represented by circles,
542 include ultrafast bootstraps > 95% (blue), 80-95% (orange), and <80% (red). Where squares are
543 indicated, SH-aLRT values were also < 80%. *=samples genome skimmed and target-enriched
544
545 Figure 4. Maximum likelihood phylogeny of black corals (purple=genome skim, black=target
546 capture). Outgroups include Scleractinia (Scl) and Corallimorpharia (Co). Node support values,
547 represented by circles, include ultrafast bootstraps > 95% (blue), 80-95% (orange), and <80%
548 (red). Where squares are indicated, SH-aLRT values were also < 80%. Leio=Leiopathidae,
549 Clado=Cladopathidae, Stylo= Stylopathidae, and *= Species currently included within the
550 polyphyletic family Aphanipathidae.
551
552
553 Figure 5. Circularization frequency of mitogenomes by number of paired-end reads recovered in
554 each sample for Octocorallia and Hexacorallia. (*p=0.001)
555
556 **Supplemental Information**
557
558 **Supplemental Table 1.** Summary statistics for all samples genome skimmed in the present
559 study.
560
561 **Supplemental Figure 1**. Maximum-likelihood phylogenetic tree of Octocorallia with branch
562 lengths shown. Tree rooted to Hexacorallia. Tip labels in purple=genome skim, black=target
563 capture. Ultrafast bootstraps and Sh-alRT values both >95% unless indicated.
564
565 **Supplemental Figure 2**. Maximum-likelihood phylogenetic tree of Antipatharia with branch
566 lengths shown. Tree rooted to Octocorallia. Tip labels in purple=genome skim, black=target
567 capture. Ultrafast bootstraps and Sh-alRT values both >95% unless indicated.
568
569 **Supplemental Figure 3**. Maximum-likelihood phylogenetic tree of *mtMutS* sequences (1,074
570 bp alignment) generated from genome skimming data and PCR/Sanger Sequencing (McFadden
571 et al. 2022). The Scleralcyonacea is rooted to the Malacalcyonacea. Ultrafast bootstraps are
572 included.

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573
574
575 **Supplemental Figure 4:** Maximum-likelihood phylogenetic tree of *28S rRNA* barcode
576 sequences (478 bp alignment) generated from 35 samples via conventional PCR and Sanger
577 sequencing and from genome skimming data. The tree is rooted at its midpoint. Ultrafast
578 bootstraps are included.
579
580 **Supplemental Figure 5.** Maximum-likelihood phylogenetic tree of *rRNA* gene sequences (6,031
581 bp alignment) generated from genome skimming data. The tree is rooted at its midpoint.
582 Ultrafast bootstraps are included.
583

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