

1 **LINEAR MITOCHONDRIAL GENOME IN ANTHOZOA (CNIDARIA): A CASE STUDY IN**  
2 **CERIANTHARIA**

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**32 Abstract**

33 Sequences and structural attributes of mitochondrial genomes have played a key role in the  
34 clarification of relationships among Cnidaria, a key phylum of early-diverging animals.  
35 Among the major lineages of Cnidaria, Ceriantharia ("tube anemones") remains one of the  
36 most enigmatic groups in terms of its phylogenetic position. We sequenced the  
37 mitochondrial genomes of two ceriantharians to see whether the complete organellar  
38 genome would provide more support for the phylogenetic placement of Ceriantharia. For  
39 both ceriantharian species studied, the mitochondrial gene sequences could not be  
40 assembled into a circular genome. Instead, our analyses suggest both species have  
41 fragmented mitochondrial genomes consisting of multiple linear fragments. Linear  
42 mitogenomes are characteristic of members of Medusozoa, one of the major lineages of  
43 Cnidaria, but are unreported for Anthozoa, which includes the Ceriantharia. The number of  
44 fragments and the variation in gene order between species is much greater in Ceriantharia  
45 than among Medusozoa. The novelty of the mitogenomic structure in Ceriantharia  
46 highlights the distinctiveness of this lineage but, because it appears to be both unique to and  
47 diverse within Ceriantharia, it is uninformative about the phylogenetic position of  
48 Ceriantharia relative to other anthozoan groups.

49

**50 Introduction**

51 Analyses of the mitochondrial genome have played a pivotal role in understanding  
52 relationships among Cnidaria. Foundational studies by Bridge et al. (1992, 1995) pointed to  
53 a clear division between Anthozoa and Medusozoa, with Medusozoans having the derived  
54 feature of linear mitogenomes. Subsequent studies have confirmed a circular mitochondrial  
55 genome in diverse octocorals (reviewed in Kayal et al. 2013; Figueroa and Baco 2014; Wu  
56 et al. 2016; Poliseno et al. 2017) and hexacorals (reviewed by Medina et al. 2006; Brugler  
57 and France 2007; Sinniger et al. 2007; Kayal et al. 2013; Foox et al. 2016; Shi et al. 2016;  
58 Chi and Johansen 2017; Zhang and Zhu 2017; Zhang et al. 2017) and a linear mitogenome  
59 in additional diverse medusozoans (reviewed by Smith et al. 2012; Kayal et al. 2013, 2015;  
60 Li et al. 2016; but see Takeuchi et al. 2016). Additional studies have identified other  
61 characters that support a fundamental split within Cnidaria between Medusozoa and

62 Anthozoa (e.g., Marques and Collins 2004; Daly et al. 2007; Zapata et al. 2015; Kayal et al.  
63 2017).

64 Comparative analyses of anthozoan mitogenomes have revealed structural genomic  
65 features like introns, transpositions, gene losses, homing endonucleases, and gene order  
66 rearrangements (Beagley et al. 1998; Fukami et al. 2007; Emblem et al. 2014; Foox et al.  
67 2016; Chi and Johansen 2017). The structural diversity is unexpected because anthozoan  
68 mitogenomes have some of the lowest reported rates of sequence evolution among animals  
69 (e.g., Shearer et al. 2002, Huang et al. 2008; Chen et al. 2009; Daly et al. 2010). Within  
70 Anthozoa, the sequences and structure of the mitogenome have been used to tease apart  
71 relationships that had been controversial, such as those between scleractinians and  
72 corallimorphs (Medina et al. 2006; Kitahara et al. 2014), among Actiniaria (Foux et al.  
73 2016), and the relationship of zoantharians and antipatharians to other hexacorallians  
74 (Brugler and France 2007; Sinniger et al. 2007).

75 Although mitogenomes have been more thoroughly studied in hexacorallians than in  
76 any other group of non-bilaterian metazoans (Kayal et al. 2015; Lavrov and Pett 2016), the  
77 taxonomic sampling is highly skewed towards Actiniaria and Scleractinia (Kayal et al.  
78 2013), and no complete mitogenomes have been reported for any members of order  
79 Ceriantharia. Regions of the mitogenome of ceriantharians appear to evolve under different  
80 models than those of other Anthozoa (Kayal et al. 2013, Stampar et al. 2014, Zapata et al.  
81 2015), suggesting that there are important differences between the mitochondrial genome of  
82 ceriantharians and those of other anthozoans.

83 Ceriantharia has been an especially challenging lineage to resolve in the broader  
84 cnidarian phylogeny. Historically, they were considered sibling to the Antipatharia and  
85 grouped with them as subclass Ceriantipatharia based on similarities in the larval stage (van  
86 Beneden 1897). This relationship was contested based on anatomical features by Schmidt  
87 (1974) and later based on DNA sequence data by Chen et al. (1995). At present, the most  
88 commonly cited relationship for Ceriantharia based on DNA sequences is as the sister to all  
89 other hexacorallians (e.g., Chen et al. 1995; France et al. 1996; Berntson et al. 1999; Won  
90 et al. 2001; Daly et al. 2003; Rodríguez et al. 2014; Zapata et al. 2015; Quattrini et al.  
91 2018). However, Ceriantharia has also been reconstructed as the sister to Octocorallia  
92 (Zapata et al. 2015) and as the sister to all other Anthozoa (Stampar et al. 2014).

93           The phylogenetic position of the Ceriantharia has been difficult to test because there  
94 is little sequence data, having the fewest sequences in GenBank of any hexacorallian order  
95 (411 sequences in nr database, 06/2018). In the phylogenomic analyses of Zapata et al.  
96 (2015), Ceriantharia had the lowest percent recovery of genes of any anthozoan and was  
97 equally well supported in two phylogenetic positions (sister to all other Hexacorallia or  
98 sister to Octocorallia). Some of these difficulties may stem from significant differences in  
99 evolutionary rate between Ceriantharia and other Anthozoa (Stampar et al. 2014). Taxon  
100 sampling of Ceriantharia was low in the analyses of Zapata et al. (2015) and Quattrini et al  
101 (2018) and the group is generally represented by one or two exemplars in higher-level  
102 phylogenies (e.g., France et al. 1996; Daly et al. 2003; Rodriguez et al. 2014; Zapata et al.  
103 2015; Kayal et al. 2017; Quattrini et al. 2018). This low representation is especially  
104 significant and problematic if it is the sister lineage of a much larger group, as implied by  
105 most interpretations of its phylogeny.

106           Recognizing the power of mitochondrial genomes to illuminate anthozoan  
107 relationships, we sequenced and characterized the mitogenome of the ceriantharians  
108 *Isarachnanthus nocturnus* and *Pachycerianthus magnus*. These are the first reports of  
109 mitochondrial genomes for members of this lineage. The genomes of *I. nocturnus* and *P.*  
110 *magnus* are like one another and unlike those of all other Anthozoa in being linear, but  
111 differ from one another in the arrangement of the genes and the inferred number of linear  
112 chromosomes. This surprising finding reinforces the uniqueness of Ceriantharia and  
113 underscores the difficulty in interpreting its relationship to other major groups within  
114 Cnidaria. Phylogenetic analysis of the coding regions of these mitogenomes supports  
115 interpreting Ceriantharia as the sister to Octocorallia and Hexacorallia and thus as a third  
116 major lineage within Anthozoa.

117

## 118 **Material and Methods**

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### 120 **Specimen sampling**

121 The two focal species represent the two orders of Ceriantharia (Penicillaria and Spirularia).  
122 *Isarachnanthus nocturnus* (Hartog, 1977), order Penicillaria, was collected in Sao  
123 Sebastiao Channel, Sao Paulo, Brazil (MZUSP 1478) (SISBIO 55566-1) and

124 *Pachycerianthus magnus*, order Spirularia, was collected from Taiwan, China (MZUSP  
125 1951). Specimens were preserved directly in 92% ethanol. Pieces of marginal tentacles  
126 were used for DNA extraction.

127

### 128 **Methods for obtaining and assembling genomes**

129 Libraries were prepared using an Illumina TruSeq PCR-free protocol and sequenced on the  
130 Illumina HiSeq 2500 platform yielding 250 bp paired-end reads, with an average insert size  
131 of 350 bp for *I. nocturnus* and 550 bp for *P. magnus*. The sequencing runs produced 14.2 m  
132 mate-pairs for *I. nocturnus* and 15.3 m mate-pairs for *P. magnus*. The reads were evaluated  
133 for quality and adapter-contamination using FastQC (Andrews 2016) and cleaned using  
134 Trimmomatic (Bolger et al. 2014) to remove adapters and low quality regions. 12.7 m pairs  
135 were retained for *I. nocturnus* (86.1%) and 14.9 m pairs were retained for *P. magnus*  
136 (97.7%). De novo assembly was performed using DISCOVAR *de novo* v. 52488  
137 (Weisenfeld et al. 2014) which is optimized for this type of Illumina data. The resulting  
138 assembly was converted to a BLAST database, and mitochondrial contigs identified by  
139 querying with a set of known Cnidarian mitochondrial CDS. Trimmed reads were mapped  
140 back to the identified mitochondrial contigs using the Geneious 7.1 read mapper (Kearse et  
141 al. 2012) using High Sensitivity (Medium) default settings, and the mapped reads were  
142 reassembled de novo in Geneious to validate assembly and evaluate evenness of coverage  
143 and read-agreement. We concatenated species specific mitochondrial contigs into a “pseudo  
144 contig” and mapped raw reads to determine if paired end reads would map to different  
145 mitochondrial chromosomes. Regions of sequence similarity across chromosomes were  
146 identified using LASTZ v.1.02.00 (Harris 2007) and GC content calculated for each  
147 chromosome (Richard 2018).

148

149 This pipeline was validated in previous unpublished work by the current authors on  
150 anthozoan mitochondrial genomes using identical methods of data generation, that resulted  
151 incircularized mitochondrial genomes assembled in a single DISCOVAR contig (Foux et  
152 al. 2016). However, in our study of these two ceriantharians, the assembly for both samples  
153 unexpectedly yielded numerous linear chromosomes. Since none of the contigs circularized  
154 and no paired-end reads seemed to bridge contigs, we attempted to extend contigs using

155 both IMAGE (Tsai et al. 2010) with various kmer settings and the Geneious iterative read  
156 mapper. In no case did the contigs significantly extend: reads either falsely assembled into  
157 highly discordant, non-homologous low-complexity regions or abruptly terminated. We  
158 also independently assembled the data using NOVOPlasty v 2.5.9 (Dierckxsens et al. 2016)  
159 which is explicitly designed to assemble circular, organellar genomes. In one case, this  
160 assembler extended a single contig and circularized it, however, mapping reads back to this  
161 contig revealed that the incorporated direct repeat occurs immediately after a c. 3000 bp  
162 region of minimal mapping quality, casting doubt on this assembly. We used the Phobos  
163 tandem repeat search tool (Mayer 2010) but found no definitive evidence of telomeric  
164 repeats at the ends of any linear fragment.

165

166 Contigs were annotated using DOGMA (Wyman et al. 2004), MITOS (Bernt et al. 2013)  
167 and by transferring homologous gene annotations in Geneious from a representative  
168 selection of anthozoan and medusozoan sequences from GenBank, correcting start–stop  
169 positions by hand.

170

### 171 **Phylogenetic genome and distance analysis**

172

173 Mitochondrial genomes representing all accepted cnidarian clades – except Myxozoa –  
174 were obtained from GenBank (Table 1). Sampling aimed to increase the representation of  
175 taxonomic diversity across groups when possible. Represented taxa with complete  
176 mitogenomes from each major group were chosen to maintain equal sampling across  
177 lineages where possible. The genes found in the mitochondrial genomes were partitioned  
178 for the subsequent assembly of the matrix with the equivalent order of the genes.

179 Differences in the organization of the gene sequence (Fig. 1) were not considered in the  
180 present analysis.

181

182 The combined dataset was created for all genes from the mitochondrial genomes after  
183 alignment of individual genes with MAFFT (parameter: FFT-NS-2) (Kazunori et al., 2016);  
184 this dataset was analyzed under Maximum Likelihood criteria (RAxML v8 and PhyML 3.0)  
185 (Guidon et al., 2010; Stamatakis, 2014). To evaluate nodal support and to detect if support

186 values were biased, two parametric (aLRT and aBAYES) and a non-parametric (Bootstrap)  
187 bootstrap values were computed in RAxML (500 pseudoreplicates, same parameters as the  
188 original phylogenetic analysis) and additional statistical tests were performed using PhyML  
189 with SMS to infer tree model (Lefort et al., 2017). The duplicated genes (characterized by  
190 sequence similarity) were individualized and aligned in MUSCLE and compared by p-  
191 distance model in order to calculate their respective genetic distances.

192

## 193 **Results**

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### 195 **The linear and fragmented mitochondrial genomes of Ceriantharia**

196

197 The mitochondrial genomes we present of the ceriantharians *Pachycerianthus magnus* and  
198 *Isarachnanthus nocturnus* are the first linear and fragmented mitochondrial genomes  
199 described in Anthozoa. The obtained genomes (Fig. 1) have 78,231 bp and 80,966 bp,  
200 respectively, and are organized into nine (*P. magnus*) and five (*I. nocturnus*) contigs that  
201 likely represent chromosomes. Because we did not detect a telomere sector or something  
202 similar at the end of each set of genes, we consider these probable or possible  
203 chromosomes, rather than definitive chromosomes.

204

205 A small percentage of the paired end reads mapped across the possible chromosomes.

206 There were 1,238 PE reads (1%) for *P. magnus* and 1,341 PE reads (0.5%) for *I. nocturnus*.

207 These mismatches resulted in paired ends being mapped consistently to distinct positions  
208 within the chromosomes for each species (Supplemental Figure 1). Due to the position of  
209 these mismatches, high sequence similarity across AT rich chromosomes, and potentially  
210 duplicated chromosomal regions and associated genes, this is likely an artifact of the  
211 mapping due to their relatively low occurrence.

212

213 The ceriantharian mitochondrial genomes we have sequenced are, on average, three to four  
214 times longer than those of other cnidarians, with *P. magnus* having the largest  
215 mitochondrial genome reported for an animal to date. The size (but not content) of the  
216 mitogenome of these Ceriantharia is very similar to those reported for Choanoflagellata



217 (Burger et al., 2003). The length and organization help explain several failed attempts of  
218 Long Range PCRs with diverse primers to obtain long mitochondrial sectors with previous  
219 mtDNA isolation (Abcam kit (AB65321) S. Stampar & M. Maronna, personal comm.).  
220 Previous attempts by S. S (with M. Maronna) to sequence the mitogenome sequencing by  
221 the isolation of mitochondria and subsequent sequencing on a ROCHE 454 JR resulted in  
222 similar data for *Isarachnanthus nocturnus*, but the absence or low number of reads in some  
223 sectors meant that not all chromosome sequences could be reconstructed without breaks.

224

225 Despite differences in mitogenome organization and size, the genes in the mitogenomes of  
226 *I. nocturnus* and *P. magnus* are similar in size to their homologues in other Cnidaria, except  
227 ND4L (which is as much as twice the length of that in other Anthozoa) and ND6  
228 (approximately three times the length compared to other Anthozoa). The percent of each of  
229 the ceriantharian genomes that encodes proteins or RNAs was low: 19.6% in *I. nocturnus*  
230 and 20.6% in *P. magnus*. Thus, the size of these ceriantharian mitogenomes is due to an  
231 increase in the non-coding regions.

232

233 Perhaps surprisingly given their considerable length, we found some genes common in  
234 cnidarian mitogenomes are absent in these Ceriantharia. In both *I. nocturnus* and *P.*  
235 *magnus*, we did not find the open reading frames (ORFs) polB and ORF314 or the transfer  
236 RNAs (tRNAs) methionine (trnM) and tryptophan (trnW). In *P. magnus*, ATP6, CYTb, and  
237 ND1 are duplicated, with the copies differing at 34% (ATP6), 28% (CYTb), and 19%  
238 (ND1).

239

240 Thus, although the mitogenomes of *I. nocturnus* and *P. magnus* share some characteristics  
241 (linear organization and larger size) when compared with other cnidarian lineages, the  
242 organization of the genes was quite different between the two species and it was difficult to  
243 identify any conservative gene blocks between them (Fig 2).

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## 248 **Phylogenetic genome analysis**

249

250 The best tree from our maximum likelihood analysis (PHYML - model GTR, Gamma  
251 distribution parameter 1.138, AIC=333907.267, Log-likelihood: -291618.12823,  
252 Unconstrained likelihood: -218549.49064) of the sequences in the mitochondrial genomes  
253 of Cnidaria (Fig. 3) is similar in topology to those recovered previously (e.g., Chen et al.,  
254 1995; Song & Won, 1997; Collins et al., 2006; Stampar et al., 2014; Katal et al. 2017). It  
255 includes reciprocally monophyletic Medusozoa and Anthozoa, with Ceriantharia as the  
256 sister group of Hexacorallia + Octocorallia. In this tree, the monophyly of Anthozoa and of  
257 the three subgroups within it are well supported. At the same time, the medusozoan groups  
258 have high support levels, despite only a small number of mitogenomes available from the  
259 Medusozoa (especially in Cubozoa and Staurozoa). The structure of the tree for Medusozoa  
260 had relatively short internal branches and relatively long terminal branches (Fig. 3).

261

262

## 263 **Discussion**

### 264 **Linearization, homology, and cnidarian evolution**

265

266 Mitochondrial genome architecture seems to be more variable among members of early-  
267 diverging clades of Metazoa than among members of Bilateria (Lavrov et al. 2013; Osigus  
268 et al. 2013; Lavrov and Pett 2016). Among the phyla traditionally indicated as near the base  
269 of Metazoa, only Cnidaria includes major lineages for which the mitochondrial genome is  
270 not circular (Bridge et al. 1992; Kayal et al. 2013; Lavrov and Pett 2016). In Cnidaria,  
271 linear mitochondrial genomes are compelling as a synapomorphy for Medusozoa because  
272 this feature is both fairly unusual and highly consistent (reviewed by Kayal et al. 2015).  
273 The linear mitogenomes of major medusozoan lineages are largely conserved in terms of  
274 gene order and can be related through a relatively straightforward transformation series  
275 (Kayal et al. 2015) involving fragmentation and gene re-arrangement through  
276 recombination.

277

278 A striking exception to the pattern in Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa is  
279 Myxozoa. Myxozoans are intracellular parasites with complex lifecycles. Although their  
280 phylogenetic position has been difficult to assess (reviewed by Fook et al. 2015), they are  
281 inferred to be highly modified medusozoans (Evans et al. 2008, 2010; Chang et al. 2015) or  
282 as the sister group (Endocnidozoa) to Medusozoa (Kayal et al. 2017). The linear  
283 mitochondrial genome characteristic among members of Medusozoa appears to be quite  
284 variable in Myxozoa. The mitochondrion of species in the myxozoan genus *Kudoa* are  
285 small, and the genes in them are organized into a single circular genome that is evolving  
286 more quickly than those in other Medusozoa (Takeuchi et al. 2015). The order of genes  
287 reported for the mitochondrial genome of *Kudoa* does not correspond to those published for  
288 other Medusozoans (cf. Kayal et al. 2015; Takeuchi et al. 2015). In contrast, in the  
289 myxozoan *Enteromyxum leei*, the mitogenome is organized into eight circular  
290 chromosomes (Yahalomi et al. 2017). This high within-lineage variation in genome  
291 architecture mirrors what we have discovered here in Ceriantharia.

292

293 This deviation in mitogenome structure in Myxozoa does not refute the value of linear  
294 mitochondrial genomes as synapomorphy for Medusozoa, but it does underscore that  
295 variation in mitochondrial genome structure is characteristic of Cnidaria. Likewise, the  
296 linear mitogenome of Ceriantharia we describe here should not be interpreted as proof for a  
297 particularly close relationship between Ceriantharia and Medusozoa; it is merely more  
298 evidence of plasticity in mitogenome architecture in Cnidaria. The duplication of two genes  
299 in *Pachycerianthus magnus* is a very interesting discovery, because there are some  
300 substantial distance between each copy of these genes.

301

302 The present study presents more evidence of the isolation of Ceriantharia in relation to  
303 Hexacorallia and Octocorallia but does not support a close relationship between  
304 Ceriantharia and Medusozoa. The gene order in the mitogenomes of Medusozoa are largely  
305 conserved (Kayal et al. 2013, 2015) and is wholly different in Ceriantharia. Furthermore,  
306 our phylogenetic reconstruction based on the sequences within the mitochondrial genome  
307 supports Ceriantharia as an isolated branch within Anthozoa, rather than as a close ally of  
308 Medusozoa (Fig 3). This pattern of sequence affinity despite structural difference was also

309 seen for the myxozoans *Kudoa* and *Enteromyxum* (Takeuchi et al. 2015, Yahalomi et al.  
310 2017): phylogenetic analyses of sequences place these species within or sister to the  
311 Medusozoa although the structure of their genomes is unlike those of other medusozoans.  
312

313 In contrast to the conservation of gene order generally characterizing medusozoan  
314 mitochondrial genomes, we did not identify any conservative gene blocks among  
315 ceriantharians and other anthozoans. We found no consistency between our two  
316 ceriantharian species and any other published gene order from a cnidarian mitochondrial  
317 genome. The absence of any relation to the patterns observed in Octocorallia or  
318 Hexacorallia may be an indication of the phylogenetic isolation of Ceriantharia from these  
319 two groups. These differences in gene order underscore the differences in rate of gene  
320 evolution between ceriantharians and other anthozoans reported by Stampar et al. (2014)  
321 and may bolster the contention that Ceriantharia are a third major lineage in Anthozoa.  
322

323 The composition of nucleotides in each of the ceriantharian mitogenomes did not deviate  
324 from the general pattern seen in other Cnidaria (Table 2). In some cases (e.g. ATP6,  
325 NAD4), the nucleotide composition appears to be at an intermediate stage between  
326 Medusozoa and Anthozoa. The nucleotide composition is distinct in each of these groups  
327 and the values we found for Ceriantharia lies between them. Nevertheless, conclusive  
328 interpretations will require a greater number of species of Ceriantharia and greater sampling  
329 of Medusozoa (e.g., Cubozoa, Staurozoa).

330

331 The non-coding areas in Ceriantharia are very long and account for almost 80% of the  
332 mitochondrial genome. It is in these regions that the differences between Ceriantharia and  
333 other Cnidaria are most notable. In general, noncoding regions tend to be larger in  
334 Anthozoa than in Medusozoa and represent as much as 10% of the mitochondrial genome  
335 (Octocorallia: Park et al., 2011). The marked increase in non-coding DNA in the  
336 mitochondrial genome of Ceriantharia is noteworthy even though the increases seem not to  
337 have been the result of a single event in the two ceriantharians we have studied here. The  
338 higher rate of mitochondrial gene evolution in Ceriantharia compared to other Anthozoa

339 (Stampar et al. 2014) may help to explain the generation and accumulation of the  
340 noncoding regions in Ceriantharia.

341

342

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349

### 350 **References**

351 Andrews S. FastQC: a quality control tool for high throughput sequence data. Version 1

352 [FastQC]. 2018 Jun 18. Available from:

353 <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>.

354 Beagley CT, Okimoto R, Wolstenholme D R. The mitochondrial genome of the sea  
355 anemone *Metridium senile* (Cnidaria): introns, a paucity of tRNA genes, and a near-  
356 standard genetic code. *Genetics*. 1998;148: 1091-1108.

357 Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf  
358 M, Stadler PF. MITOS: improved de novo metazoan mitochondrial genome  
359 annotation. *Mol Phylogenetics Evol*. 2013 Nov 1;69(2):313-9.

360 Berntson EA, France SC, Mullineaux LS. Phylogenetic relationships within the class  
361 Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. *Molecular*  
362 *phylogenetics and evolution*. 1999 Nov 1;13(2):417-33.

363 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence  
364 data. *Bioinformatics*. 2014 Apr 1;30(15):2114-20.

- 365 Bridge D, Cunningham CW, DeSalle R, Buss LW. Class-level relationships in the phylum  
366 Cnidaria: molecular and morphological evidence. *Mol Biol Evol.* 1995; 12(4): 679-  
367 689.
- 368 Bridge D, Cunningham CW, Schierwater B, DeSalle R, Buss LW. Class-level relationships  
369 in the phylum Cnidaria: evidence from mitochondrial genome structure. *PNAS*  
370 1992; 89: 8750-8753.
- 371 Brugler MR, France SC. The complete mitochondrial genome of the black coral  
372 *Chrysopathes formosa* (Cnidaria: Anthozoa: Antipatharia) supports classification of  
373 antipatharians within the subclass Hexacorallia. *Mol Phylogenet Evol.* 2007;42:  
374 776-788.
- 375 Burger G, Forget L, Zhu Y, Gray MW, Lang BF. Unique mitochondrial genome  
376 architecture in unicellular relatives of animals. *Proceedings of the National*  
377 *Academy of Sciences.* 2003 Feb 4;100(3):892-7.
- 378 Chang ES, Neuhof M, Rubinstein ND, Diamant A, Philippe H, Huchon D, Cartwright P.  
379 Genomic insights into the evolutionary origin of Myxozoa within Cnidaria.  
380 *Proceedings of the National Academy of Sciences.* 2015 Dec 1;112(48):14912-7.
- 381 Chen CA, Odorico DM, Lohuis ten M, et al. Systematic relationships within the Anthozoa  
382 (Cnidaria: Anthozoa) using the 5'-end of the 28S rDNA. *Mol Phylogenet Evol.*  
383 1995;4: 175–183.
- 384 Chen IP, Tang CY, Chiou CY, Hsu JH, Wei NV, Wallace CC, Muir P, Wu H, Chen CA.  
385 Comparative analyses of coding and noncoding DNA regions indicate that *Acropora*  
386 (Anthozoa: Scleractina) possesses a similar evolutionary tempo of nuclear vs.  
387 mitochondrial genomes as in plants. *Mar Biotechnol.*2009;11: 141-152.
- 388 Chi SI, Johansen SD. Zoantharian mitochondrial genomes contain unique complex group I

- 389 introns and highly conserved intergenic regions. *Gene* 2017;628: 24-31.
- 390 Chi SI, Johansen SD. Zoantharian mitochondrial genomes contain unique complex group I  
391 introns and highly conserved intergenic regions. *Gene*.2017;628: 24-31.
- 392 Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B.  
393 Medusozoan phylogeny and character evolution clarified by new large and small  
394 subunit rDNA data and an assessment of the utility of phylogenetic mixture models.  
395 *Syst biol.* 2006;55(1), 97-115.
- 396 Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC,  
397 McFadden CS, Opresko DM, Rodriguez E, et al. The phylum Cnidaria: A review of  
398 phylogenetic patterns and diversity 300 years after Linnaeus. *Zootaxa.* 2007;1668:  
399 127-182.
- 400 Daly M, Fautin DG, Cappola VA. Systematics of the hexacorallia (Cnidaria: Anthozoa).  
401 *Zoological Journal of the Linnean Society.* 2003 Nov 1;139(3):419-37.
- 402 Daly M, Gusmão LC, Reft AJ, Rodríguez. Phylogenetic Signal in Mitochondrial and  
403 Nuclear Markers in Sea Anemones (Cnidaria, Actiniaria). *Integr Comp Biol.* 2010;50:  
404 371-388.
- 405 Dierckxsens N, Mardulyn P, Smits G. NOVOPlasty: de novo assembly of organelle  
406 genomes from whole genome data. *Nucleic acids research.* 2016 Oct 24;45(4):e18.
- 407 Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high  
408 throughput. *Nucleic Acids Res.* 2004 Mar 1;32(5):1792-7.
- 409 Emblem Å, Okkenhaug S, Weiss ES, Denver DR, Karlsen BO, Moum T, Johansen SD. Sea  
410 anemones possess dynamic mitogenome structures. *Mol Phylogenet Evol.* 2014;75:  
411 184-193.

- 412 Evans NM, Holder MT, Barbeitos MS, Okamura B, Cartwright P. The phylogenetic  
413 position of Myxozoa: exploring conflicting signals in phylogenomic and ribosomal  
414 data sets. *Molecular biology and evolution*. 2010 Jun 24;27(12):2733-46.
- 415 Evans NM, Lindner A, Raikova EV, Collins AG, Cartwright P. Phylogenetic placement of  
416 the enigmatic parasite, *Polypodium hydriforme*, within the Phylum Cnidaria. *BMC*  
417 *evolutionary biology*. 2008 Dec;8(1):139.
- 418 Figueroa DF, Baco AR. Octocoral mitochondrial genomes provide insights into the  
419 phylogenetic history of gene order rearrangements, order reversals, and cnidarian  
420 phylogenetics. *Genome Biol Evol*. 2014;7(1), 391-409.
- 421 Foox J, Brugler M, Siddall ME, Rodríguez E. Multiplexed pyrosequencing of nine sea  
422 anemone (Cnidaria: Anthozoa: Hexacorallia: Actiniaria) mitochondrial genomes.  
423 *Mitochondr DNA Part A*. 2016;27: 2826-2832.
- 424 Foox J, Siddall ME. The road to Cnidaria: history of phylogeny of the Myxozoa. *J*  
425 *Parasitol*. 2015;101: 269 – 274.
- 426 France SC, Rosel PE, Agenbroad JE, Mullineaux LS, Kocher TD. DNA sequence variation  
427 of mitochondrial large-subunit rRNA provides support for a two-subclass  
428 organization of the Anthozoa (Cnidaria). *Mol Mar Biol Biotech*.1996;5: 15-28.
- 429 Fukami H, Chen CA, Chiou CY, Knowlton N. Novel group I introns encoding a putative  
430 homing endonuclease in the mitochondrial *cox1* gene of scleractinian corals. *J Mol*  
431 *Evol*. 2007;64: 591-600.
- 432 Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms  
433 and methods to estimate maximum-likelihood phylogenies: assessing the  
434 performance of PhyML 3.0. *Syst Biol*. 2010 May 1;59(3):307-21.



- 435 Harris RS. Improved pairwise alignment of genomic DNA. PhD thesis. Penn State  
436 University, Computer Science and Engineering, 2007.
- 437 Huang D, Meier R, Todd PA, Chou LM. Slow mitochondrial COI sequence evolution at the  
438 base of the metazoan tree and its implications for DNA barcoding. *J Mol*  
439 *Evol.*2008;66: 167-174.
- 440 Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment,  
441 interactive sequence choice and visualization. *Briefings in bioinformatics.* 2017 Sep  
442 6.
- 443 Kayal E, Bentlage B, Cartwright P, Yanagihara AA, Lindsay DJ, Hopcroft RR, Collins AG.  
444 Phylogenetic analysis of higher-level relationships within Hydroidolina (Cnidaria:  
445 Hydrozoa) using mitochondrial genome data and insight into their mitochondrial  
446 transcription. *PeerJ.* 2015;3: e1403.
- 447 Kayal E, Bentlage B, Pankey MS, Ohdera AH, Medina M, Plachetzki DC, et al.  
448 Phylogenomics provides a robust topology of the major cnidarian lineages and  
449 insights on the origins of key organismal traits. *BMC Evol Biol.* 2018;18(1): 68.
- 450 Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV. Cnidarian phylogenetic  
451 relationships as revealed by mitogenomics. *BMC Evol Biol.* 2013;13:1.
- 452 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper  
453 A, Markowitz S, Duran C, Thierer T. Geneious Basic: an integrated and extendable  
454 desktop software platform for the organization and analysis of sequence data.  
455 *Bioinformatics.* 2012 Apr 27;28(12):1647-9.
- 456 Kitahara MV, Lin M-F, Forêt S, Huttley G, Miller DJ, Chen CA. The “Naked Coral”  
457 hypothesis revisited – evidence for and against scleractinian monophyly. *PLoS*  
458 *ONE.* 2014;9: e94774.

- 459 Lavrov DV, Pett W, Voigt O, Wörheide G, Forget L, Lang BF, Kayal E. Mitochondrial  
460 DNA of *Clathrina clathrus* (Calcarea, Calcinea): six linear chromosomes,  
461 fragmented rRNAs, tRNA editing, and a novel genetic code. *Molecular biology and*  
462 *evolution*. 2012 Dec 6;30(4):865-80.
- 463 Lavrov DV, Pett W. Animal mitochondrial DNA as we do not know it: mt-genome  
464 organization and evolution in Nonbilaterian lineages. *Genome Biol Evol*. 2016;8:  
465 2896-2913.
- 466 Lefort V, Longueville JE, Gascuel O. SMS: smart model selection in PhyML. *Mol Biol*  
467 *Evol*. 2017 May 11;34(9):2422-4.
- 468 Li HH, Sung PJ, Ho HC. The complete mitochondrial genome of the Antarctic stalked  
469 jellyfish, *Haliclystus antarcticus* Pfeffer, 1889 (Staurozoa: Stauromedusae). *Genom*  
470 *Data*, 2016;8: 113-114.
- 471 Marques AC, Collins AG. Cladistic analysis of Medusozoa and cnidarian evolution. *Invert*  
472 *Biol*. 2004;123: 23-42.
- 473 Mayer C. Phobos 3.3.11. 2018 Jun 18. Available  
474 from:[http://www.rub.de/spezzoo/cm/cm\\_phobos.htm](http://www.rub.de/spezzoo/cm/cm_phobos.htm).
- 475 Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL. Naked corals: skeleton loss in  
476 Scleractinia. *PNAS*. 2006;103(24), 9096-9100.
- 477 Osigus HJ, Eitel M, Bernt M, Donath A, Schierwater B. Mitogenomics at the base of  
478 Metazoa. *Molecular phylogenetics and evolution*. 2013 Nov 1;69(2):339-51.
- 479 Park E, Song JI, Won YJ. The complete mitochondrial genome of *Calicogorgia granulosa*  
480 (Anthozoa: Octocorallia): potential gene novelty in unidentified ORFs formed by  
481 repeat expansion and segmental duplication. *Gene*. 2011 Oct 15;486(1):81-7.

- 482 Polisen A, Altuna A, Cerrano C, Wörheide G, Vargas S. Historical biogeography and  
483 mitogenomics of two endemic Mediterranean gorgonians (Holaxonia, Plexauridae).  
484 *Org Div Evol.* 2017;17(2): 365-373.
- 485 Quattrini AM, Faircloth BC, Dueñas LF, Bridge TC, Brugler MR, Calixto-Botía IF, DeLeo  
486 DM, Foret S, Herrera S, Lee SM, Miller DJ. Universal target-enrichment baits for  
487 anthozoan (Cnidaria) phylogenomics: New approaches to long-standing problems.  
488 *Molecular ecology resources.* 2018 Mar;18(2):281-95.
- 489 Richard, D. GC content in sliding window - GitHub repository. 2018 Jun 18. Available  
490 from: <https://github.com/DamienFr/GC-content-in-sliding-window->
- 491 Rodríguez E, Barbeitos MS, Brugler MR, Crowley LM, Grajales A, Gusmão L,  
492 Häussermann V, Reft A, Daly M. Hidden among sea anemones: the first  
493 comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria,  
494 Anthozoa, Hexacorallia) reveals a novel group of hexacorals. *PLoS One.* 2014 May  
495 7;9(5):e96998.
- 496 Schmidt H. On the evolution in the Anthozoa. In: Cameron AM, Campbell BM, Cribb AB  
497 et al. editors. *Proceedings of the 2nd international symposium on coral reefs.*  
498 Brisbane: The Great Barrier Reef Committee; 1974. pp. 533–560.
- 499 Shearer TL, Van Oppen MJH, Romano SL, Wörheide G. Slow mitochondrial DNA  
500 sequence evolution in the Anthozoa (Cnidaria). *Mol Ecol.* 2002;11(12): 2475-2487.
- 501 Shi X, Tian P, Lin R, Lan W, Niu W, Zheng X. Complete mitochondrial genome of disc  
502 coral *Turbinaria peltata* (Scleractinia, Dendrophylliidae). *Mitochond DNA.*  
503 2016;27: 962-963.

- 504 Sinniger F, Chevaldonné P, Pawlowski J. Mitochondrial genome of *Savalia savaglia*  
505 (Cnidaria, Hexacorallia) and early metazoan phylogeny. *J Mol Evol.* 2007;64: 196-  
506 203.
- 507 Smith DR, Kayal E, Yanagihara AA, Collins AG, Pirro S, Keeling PJ. First complete  
508 mitochondrial genome sequence from a box jellyfish reveals a highly fragmented  
509 linear architecture and insights into telomere evolution. *Genome Biol Evol.* 2012;4:  
510 52-58.
- 511 Song JI, Won JH. Systematic relationship of the anthozoan orders based on the partial  
512 nuclear 18S rDNA sequences. *Korean Journal of Biological Sciences.* 1997 Jan  
513 1;1(1):43-52.
- 514 Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large  
515 phylogenies. *Bioinfor.* 2014 May 1;30(9):1312-3.
- 516 Stampar SN, Maronna MM, Kitahara MV, Reimer JD, Morandini AC. Fast-Evolving  
517 mitochondrial DNA in Ceriantharia: A reflection of Hexacorallia paraphyly? *PLoS*  
518 *ONE.* 2014;9: e86612.
- 519 Takeuchi F, Sekizuka T, Ogasawara Y, Yokoyama H, Kamikawa R, Inagaki Y, Nozaki T,  
520 Sugita-Konishi Y, Ohnishi T, Kuroda M. The mitochondrial genomes of a  
521 myxozoan genus *Kudoa* are extremely divergent in metazoa. *PloS ONE.* 2015;  
522 10:e0132030.
- 523 Tsai IJ, Otto TD, Berriman M. Improving draft assemblies by iterative mapping and  
524 assembly of short reads to eliminate gaps. *Genome biology.* 2010 Apr;11(4):R41.
- 525 van Beneden E. Les Anthozoaires de la "Plankton-Expedition". *Ergebn Plankton-Exp*  
526 *Humbolt-Stiftung.* 1897;2: 1-222.

- 527 Weisenfeld NI, Yin S, Sharpe T, Lau B, Hegarty R, Holmes L, Sogoloff B, Tabbaa D,  
528 Williams L, Russ C, Nusbaum C. Comprehensive variation discovery in single  
529 human genomes. *Nature genetics*. 2014 Dec;46(12):1350.
- 530 Won J, Rho B, Song J. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on  
531 morphological and molecular characters. *Coral Reefs*. 2001 Aug 1;20(1):39-50.
- 532 Wu JS, Ju YM, Hsiao ST, Hsu CH. Complete mitochondrial genome of *Junceella fragilis*  
533 (Gorgonacea, Ellisellidae). *Mitochondr DNA*. 2016;27: 1229-1230.
- 534 Wyman SK, Jansen RK, Boore JL. Automatic annotation of organellar genomes with  
535 DOGMA. *Bioinformatics*. 2004 Jun 4;20(17):3252-5.
- 536 Yahalomi D, Haddas-Sasson M, Rubinstein ND, Feldstein T, Diamant A, Huchon D. The  
537 multipartite mitochondrial genome of *Enteromyxum leei* (Myxozoa): eight fast-  
538 evolving megacircles. *Molecular biology and evolution*. 2017 Mar 16;34(7):1551-6.
- 539 Zapata F, Goetz FE, Smith SA, Howison M, Siebert S, Church SH, Sanders SM, Ames CL,  
540 McFadden CS, France, SC. et al. Phylogenomic analyses support traditional  
541 relationships within Cnidaria. *PLoS ONE*. 2015;10: e0139068.
- 542 Zhang B, Zhang YH, Wang X, Zhang HX, Lin Q. The mitochondrial genome of a sea  
543 anemone *Bolocera* sp. exhibits novel genetic structures potentially involved in  
544 adaptation to the deep-sea environment. *Ecol Evol*. 2017;7(13): 4951-4962.
- 545 Zhang L, Zhu Q. Complete mitochondrial genome of the sea anemone, *Anthopleura midori*  
546 (Actiniaria: Actiniidae). *Mitochondr DNA* 2017;28(3): 335-336.
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548 **Table 1** - Species included in present study

|                              |                  | <b>SPECIES</b>                  | <b>SOURCE/GENBANK CODE</b> |
|------------------------------|------------------|---------------------------------|----------------------------|
| <b>CERIANTHARIA</b>          | Ceriantharia     | <i>Isarachnanthus nocturnus</i> | This study                 |
|                              |                  | <i>Pachycerianthus magnus</i>   | This study                 |
| <b>HEXACORALLIA</b>          | Actiniaria       | <i>Aiptasia pulchella</i>       | NC_022265                  |
|                              |                  | <i>Alicia sansibarensis</i>     | NC_027610                  |
|                              |                  | <i>Antholoba achates</i>        | KR051002                   |
|                              | Antipatharia     | <i>Myriopathes japonica</i>     | NC_027667                  |
|                              |                  | <i>Stichopathes lutkeni</i>     | NC_018377                  |
|                              | Corallimorpharia | <i>Corynactis californica</i>   | NC_027102                  |
|                              |                  | <i>Discosoma nummiforme</i>     | NC_027100                  |
|                              | Scleractinia     | <i>Dendrophyllia arbuscula</i>  | KR824937                   |
|                              |                  | <i>Tubastrea coccinea</i>       | KX024566                   |
|                              | Zoantharia       | <i>Palythoa heliodiscus</i>     | NC_035579                  |
| <i>Zoanthus sansibaricus</i> |                  | NC_035578                       |                            |
| <b>OCTOCORALLIA</b>          | Alcyonacea       | <i>Calicogorgia granulosa</i>   | NC_023345                  |
|                              |                  | <i>Corallium konojoi</i>        | NC_015406                  |
|                              |                  | <i>Dendronephthya suenisoni</i> | NC_022809                  |
|                              |                  | <i>Euplexaura crassa</i>        | HQ694728                   |
|                              |                  | <i>Muricea purpurea</i>         | NC_029698                  |
|                              |                  | <i>Paracorallium japonicum</i>  | NC_015405                  |
|                              |                  | <i>Paracorallium</i> sp.        | AB595189                   |
|                              |                  | <i>Alatina moseri</i>           | KJ452776 - 83              |
| <b>MEDUSOZOA</b>             | Cubozoa          |                                 |                            |
|                              | Hydrozoa         | <i>Cladonema pacificum</i>      | KT809323                   |
|                              |                  | <i>Craspedacusta sowerbyi</i>   | JN593332                   |
|                              |                  | <i>Liriope tetraphylla</i>      | KT809327                   |
|                              |                  | <i>Pennaria disticha</i>        | JN700950                   |
|                              |                  | <i>Physalia physalis</i>        | KT809328                   |
|                              |                  | <i>Aurelia aurita</i>           | DQ787873                   |
|                              | Scyphozoa        | <i>Cassiopea andromeda</i>      | JN700934                   |
|                              |                  |                                 |                            |
|                              | Staurozoa        | <i>Lucernaria janetae</i>       | JN700946                   |
|                              |                  | <i>Haliclystus antarcticus</i>  | NC_030337                  |

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552 **Table 2** Gene Properties in the mtDNA of Cnidaria after Kayal et al., 2011. \* asterisk corresponds to an incomplete stop codon.

|               | Ceriantharia |       |       |     | Anthozoa |      |       |      | Hydrozoa |      |       |     | Scyphozoa |      |       |     | Staurozoa |      |       |     | Cubozoa |      |       |     |
|---------------|--------------|-------|-------|-----|----------|------|-------|------|----------|------|-------|-----|-----------|------|-------|-----|-----------|------|-------|-----|---------|------|-------|-----|
|               | Size         | %AT   | Start | End | Size     | %AT  | Start | End  | Size     | %AT  | Start | End | Size      | %AT  | Start | End | Size      | %AT  | Start | End | Size    | %AT  | Start | End |
| <i>atp6</i>   | 702-738      | 66-72 | A/T   | A   | 695±13   | 63±2 | ATG   | A*   | 704±1    | 75±4 | A     | A*  | 704±3     | 69±4 | A     | A*  | 708       | 63±1 | A     | A   | 708±7   | 63±2 | AG    | *   |
| <i>atp8</i>   | NP           | NP    | NP    | NP  | 206±22   | 66±3 | ATG   | A    | 206±3    | 83±5 | A     | A*  | 208±7     | 73±4 | AG    | AG* | 204       | 62±4 | A     | A   | 210±2   | 64±4 | AG    | AG* |
| <i>cob</i>    | 1527-1567    | 65-70 | ATG   | A/G | 1156±13  | 64±3 | ATG   | A/G* | 1148±12  | 73±3 | AG    | A   | 1146±8    | 66±2 | A     | AG  | 1068      | 60±2 | A     | ?   | 1149    | 62±2 | A     | G   |
| <i>cox1</i>   | 1587-1773    | 58-64 | AT    | A   | 2343±512 | 61±2 | ATG   | A/G* | 1569     | 67±3 | AG    | AG  | 1580±7    | 64±3 | A     | AG  | 1578      | 61±1 | A     | A   | 1569    | 58±3 | A     | A   |
| <i>cox2</i>   | 651-759      | 61-66 | AT    | A/G | 756±74   | 62±2 | ATG   | A/G* | 744±10   | 73±4 | A     | AG  | 746±8     | 67±4 | A     | AG* | 747       | 62±1 | A     | AG  | 737±2   | 61±2 | A     | AG* |
| <i>cox3</i>   | 690-813      | 59-67 | AT    | A   | 789±4    | 61±2 | ATG   | A/G  | 786      | 72±4 | A     | AG  | 786       | 64±3 | A     | AG  | 786       | 61±1 | A     | AG  | 786     | 59±3 | A     | AG  |
| <i>nad1</i>   | 945-951      | 65-69 | AT    | A   | 977±10   | 62±1 | ATG   | A/G  | 989±4    | 73±4 | A     | AG  | 972±5     | 66±4 | AG    | A*  | 987       | 59±0 | A     | A   | 987±8   | 62±3 | AG    | AG  |
| <i>nad2</i>   | 1113-1116    | 66-71 | AT    | A   | 1148±116 | 63±3 | ATG   | A    | 1328±32  | 79±5 | A     | AG* | 1323±13   | 70±5 | A     | AG  | 1346±2    | 59±4 | A     | A   | 1341    | 63±7 | A     | G*  |
| <i>nad3</i>   | 351-363      | 69-70 | AT    | A/G | 343±14   | 63±1 | ATG*  | G/A  | 355±4    | 77±4 | A     | *   | 357±6     | 69±4 | AG    | A*  | 354       | 65±4 | A     | A*  | 351     | 62±4 | AG    | *   |
| <i>nad4</i>   | 1428-1467    | 68-72 | AT    | A   | 1467±11  | 63±1 | ATG*  | G/A  | 1458±2   | 76±4 | A     | AG* | 1441±2    | 68±5 | A     | AG* | 1461      | 61±3 | A     | AG* | 1446    | 59   | A     | G   |
| <i>nad4L</i>  | 474-675      | 68-71 | ATG   | A   | 298±2    | 68±1 | ATG*  | A    | 299±2    | 79±4 | A     | *   | 303±1     | 72±5 | AG    | A*  | 299±2     | 64±1 | A     | *   | 290±2   | 67±3 | A     | G*  |
| <i>nad5</i>   | 1824-1827    | 68-72 | ATG   | A/G | 1889±2*  | 62±1 | ATG   | AG   | 1832±2   | 76±4 | A     | AG* | 1830±19   | 68±5 | AG    | A*  | 1860      | 60±2 | A     | AG  | 1824    | 62±1 | AG    | G   |
| <i>nad6</i>   | 1395         | 70    | ATA   | A   | 582±32   | 62±3 | ATG   | A    | 556±8    | 79±5 | A     | AG* | 564±12    | 70±5 | A     | AG* | 553±2     | 62±2 | A     | A   | 542±4   | 64±3 | A     | G*  |
| <i>ORF314</i> | NP           | NP    | NP    | NP  | ?        | ?    | ?     | ?    | 291      | 78   | A     | G   | 313±7     | 73±8 | A     | A   | 288       | 62   | A     | A   | 315     | 64   | A     | A   |
| <i>polB</i>   | NP           | NP    | NP    | NP  | ?        | ?    | ?     | ?    | ?        | ?    | ?     | ?   | 969       | 70±8 | A     | A   | 1119      | 58   | ATG   | ?   | 873     | 58   | G     | A   |
| <i>rnl</i>    | 2049-2145    | 64-67 | C     | C/A | 2345±154 | 61±5 | NA    | NA   | 1746±9   | 76±4 | NA    | NA  | 1818±34   | 69±5 | NA    | NA  | 1830      | 57±1 | NA    | NA  | 769     | 57   | NA    | NA  |
| <i>rns</i>    | 1126-1127    | 62-66 | A/G   | T/G | 1128±77  | 55±1 | NA    | NA   | 910±21   | 74±2 | NA    | NA  | 950±10    | 69±5 | NA    | NA  | 914±1     | 57±1 | NA    | NA  | 672     | 62   | NA    | NA  |
| <i>trnM</i>   | NP           | NP    | NP    | NP  | 71±0     | 55±2 | NA    | NA   | 71±1     | 69±2 | NA    | NA  | 71±0      | 64±5 | NA    | NA  | 69±0      | 53±2 | NA    | NA  | -       | -    | NA    | NA  |
| <i>trnW</i>   | NP           | NP    | NP    | NP  | 70±0     | 49±2 | NA    | NA   | 70±1     | 65±3 | NA    | NA  | 70±0      | 64±5 | NA    | NA  | 71±0      | 52±2 | NA    | NA  | -       | -    | NA    | NA  |

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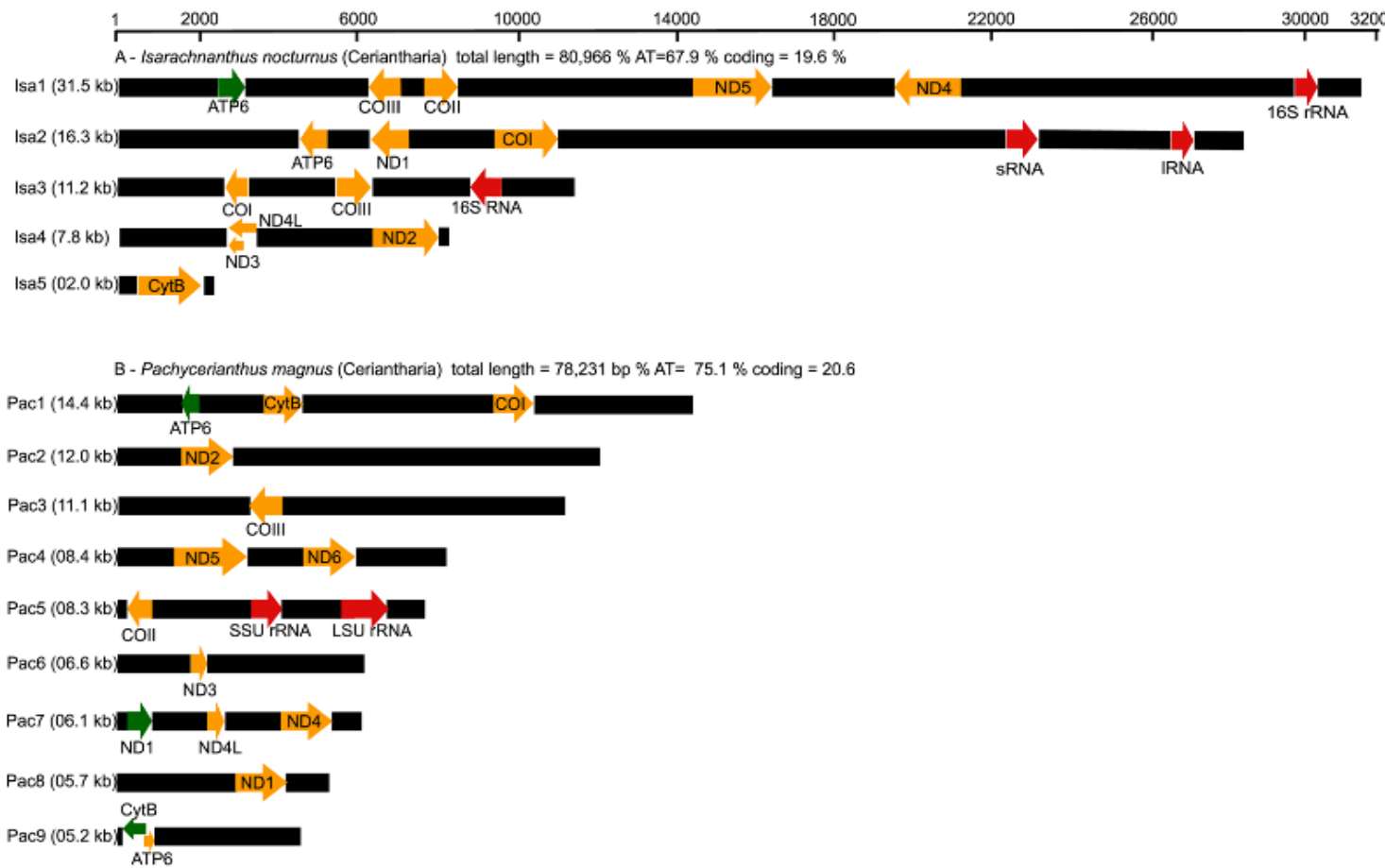
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556 **Figure 1 – Architecture of mitochondrial genome of *Isarachnanthus nocturnus* (ISA1 –**557 **ISA6) and *Pachycerianthus magnus* (Pac1-Pac9)**

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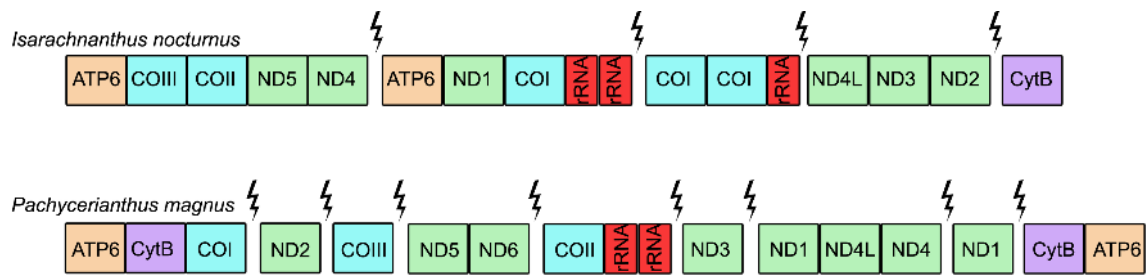
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562 **Figure 2 – Sequence of genes in mitochondrial genome of Ceriantharia**

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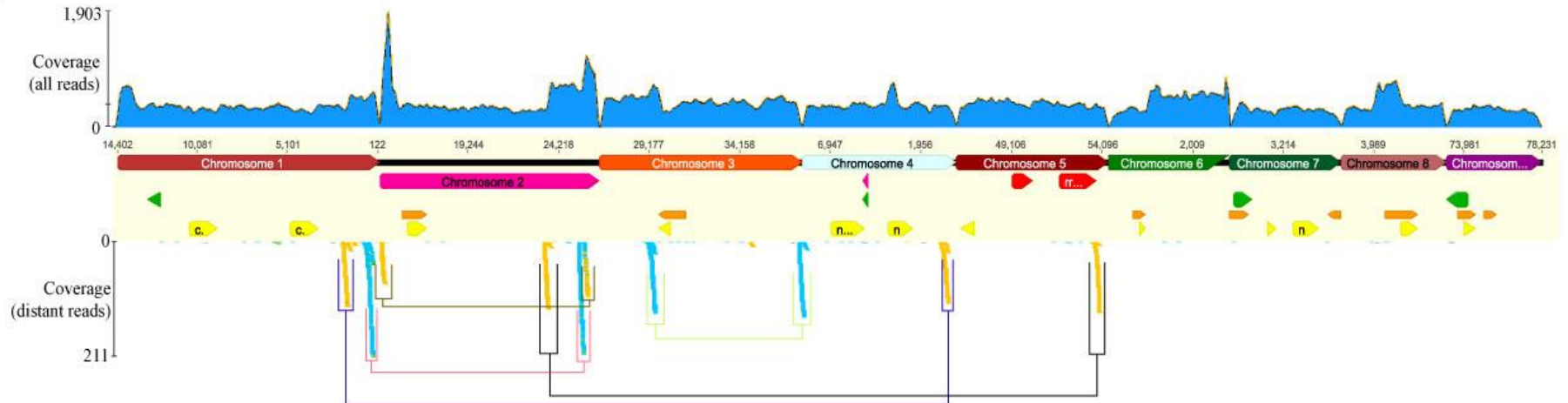
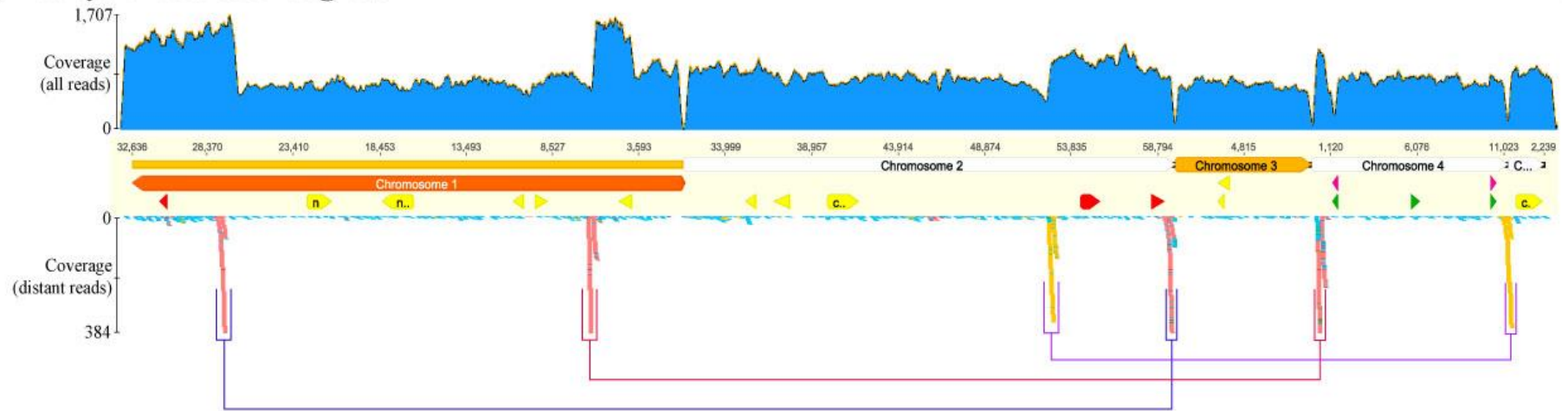
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572 **Suppl. Figure 1**A) *Isarachnanthus nocturnus*B) *Pachycerianthus magnus*

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