

# The Mitochondrial Genome of the Sea Anemone *Stichodactyla haddoni* Reveals Catalytic Introns, Insertion-like Element, and Unexpected Phylogeny

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**Figure S1:** Amino acid alignment of orfA protein in *Stichodactyla haddoni* (Sha; 174 aa), *Anthopleura midori* (Ami; 617 aa) and *Urticina eques* (Ueq; 644 aa). See Table S2 for accession numbers. Identical residues are indicated (\*) above aligned sequences.

**Figure S2:** Mitochondrial transcripts from Illumina paired read libraries in *Stichodactyla helianthus* [Rivera-de-Torre et al. 2018] mapped to the *S. haddoni* mitogenome sequence (MW760873). (a) Histograms of estimated normalized read numbers (NGS reads/ kb). See legends to Figures 1 and 6 in the main manuscript for mitochondrial gene abbreviations. (b) Sashimi plot of ND5 splicing junction, consistent with perfect splicing of the ND5-717 intron. 21 reads mapped unambiguously to the ligated sequence region. (c) Sashimi plot of COI splicing junction, consistent with perfect splicing of the COI-884 intron. 100 reads map unambiguously to the ligated sequence region.

**Ref:** Rivera-de-Torre, E.; Martinez-del-Pozo, A.; Garb, J.E. *Stichodactyla helianthus*' de novo transcriptome assembly: discovery of a new actinoporin isoform. *Toxicon* **2018**, *150*, 105-114. doi:10.1016/j.toxicon.2018.05.014.

**Figure S3:** Phylogeny of sea anemones assessed by mitogenome protein sequences. Maximum-likelihood (ML) phylogenetic tree is shown based on alignments of 3,237 amino acid residues derived from concatenated genes. Bootstrap values (%) of 500 replications for alternative phylogenetic inference methods are shown at the internal nodes (ML/NJ/ME). Red filled circles indicate highly significant branch points (bootstrap values of 100%) across the ML, NJ and ME tree construction methods. Members of the Actiniidae family, including *Stichodactyla haddoni* and *Phymanthus crucifer* are marked by a green box. NJ, Neighbor Joining; ME, Minimal evolution.

**Table S1:** Annotation of the complete mitogenome sequence of *S. haddoni*.

**Table S2:** Key features of sea anemone mitogenomes included in this study.

**Figure S1.** Amino acid alignment of orfA protein in *S. haddoni* (Sha), *A. midori* (Ami) and *U. eques* (Ueq).

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Ami  MSQALAKNLIKRWLSVIIISYLFIVVKSGGDNFICVKVTLLSLAVLLTHYPWLSFSL
Ueq  MRKLSLRNLIRKKWLGIIISYFVFIVAISGGDNFICVKVTLLSLAILLTHYPWLSFGLL

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Ami  IISVLMNLLKGYLLTIFILFFLIMFRVALYKKVKGIRWLILIIYWSALILMWTGTIVISY
Ueq  IISVLMNLLKGYLLTIFILFFLIMFRVALYKKVKGIWWLILIIYWSAFTLMCAGTIVTFC

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Ami  WGETLFKFLSYNQIGPEPFGLSMSFAQSEASSFKLAPEVWARPSWIYSVGALSIVEGFAQ
Ueq  WGGTFIEYLPFSQVGPEPLGLHGPFQNEAPPFSLAPKVWERPSWVSSVGALSIVEGFAQ

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Ami  TPFENMTYLLEVKGLTGTGFPSSRASLAQVEIDPLISKLESTGLFPFKPSVIENFRGLKDW
Ueq  TPFEDMTYHLDVKGSTTGFPCRASLAQVEIDPLISRLERTGLFPFKPSVIDNFRDLLMDW

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Ami  HCEPLLFLSNFFEAHLSKIALVEEKLQSIGLYQKKLFGLHLYNDWLVLTTYDECTRIGIN
Ueq  HCEPLLFFSNFFDVHLPKALMEEEKCKSIGLYQDTLLGLHLYNDWLVLTTYDECTRIGIN

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Ami  SMTPMGPLDTSQLTDIKIFGSNIKLMFGERQIEIALSNLRETKALGLQELWDNSPPEPFA
Ueq  SMTPTGPLYASQLTDIKAFESNIKLMFGERQIEIALSNLRESKVLGLQELWNNSP&PNF

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Ami  FDNPNFVHGDNLNLHKGPRPNSWLIERFTPPRGSNPFVDSFSQFMGQKVNVIFPLINNGE
Ueq  YYDPVFVHSELDSFRGPRQDTWAIERYTTSPGSNPLVHSFSQFFGKQVQNILIPPMENGE

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Ami  GVLHITLADGSLSPFVEVKVGNTPSSWSPRLELMPERINFVSPRFFMFLTPPTGFFMPT
Ueq  GILDVHFTDGSFLSPFVEVKVGQTPSSWDFKVELWPDITINIVWADHFMFLKPPTGIFMPN
Sha  MFLTPPTGWFECK

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Ami  NQNILDIPLNWVGPPLFYKGVITGNPFDFTDDYDHPGALNLTKKITESNIFHDMSVYQT
Ueq  NGTVFDIPLNCVWGAPLYYKGAIAGNPLNFAADYNHPGALNLIKKVTESNIFHDMSLCQT
Sha  EGTFLDIPINFVWGPPLFYKGVIVGDPFNFVSDYKHPAALGLVKKMTETKIFQEMSLFQR

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Ami  KGQGQGSQGILKNFETFLKNFNKEELAQCQDLPPVIKLPREELFSRGIQPSAAVRLWLY
Ueq  KGQGQGDIGILRNFKTFWKNFNKKELARCNEAHPMIKIPRQELFRMGHIQPMTALKLWLY
Sha  KGEQGQDILKNYKTFKLNFIKKELSRSTVPPFIKIPWKELFLGEYIQPITALKLWLW

* * * *
Ami  -----SLRQQILKSEGSCQVSP
Ueq  RGKLLSPNTSYELARKALSLPKKLFIVQSFRRQILKSGPSSDES
Sha  RGELYSSDTSYGYACKVLGLPKNQFIIRAFRRQIVRSNQIS

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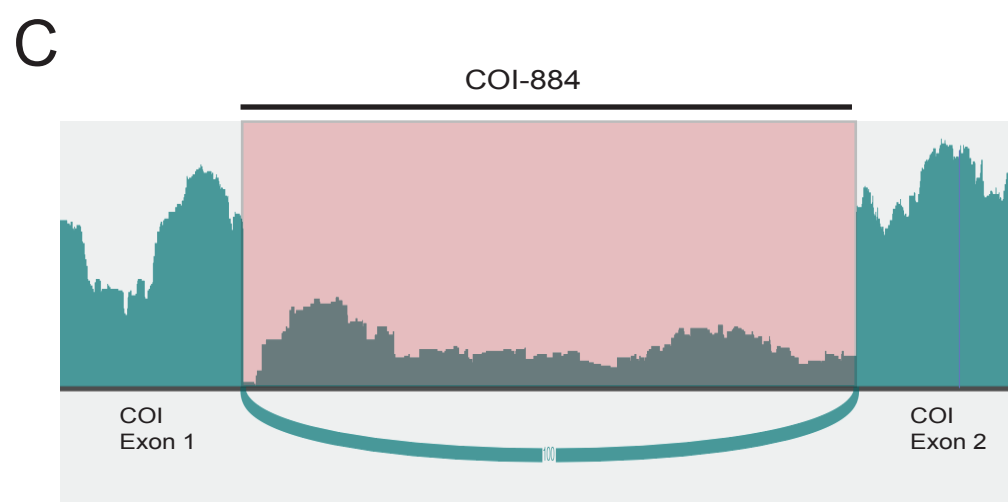
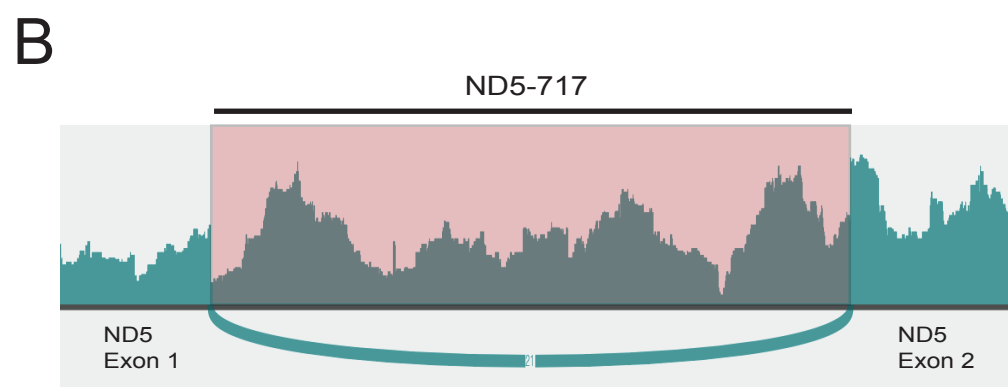
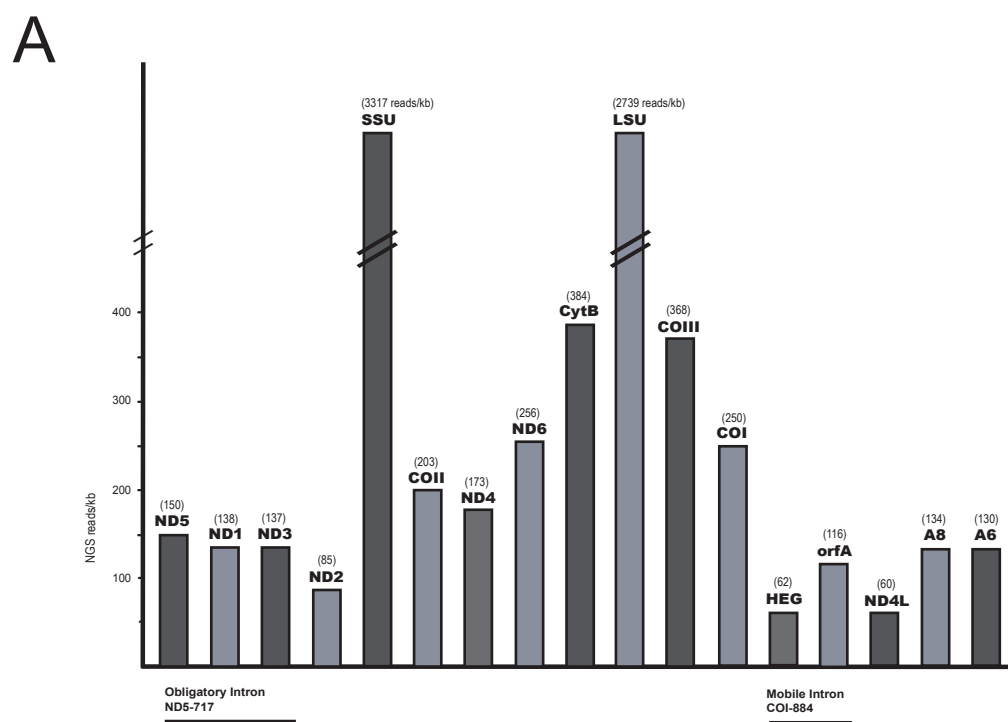


Figure S2

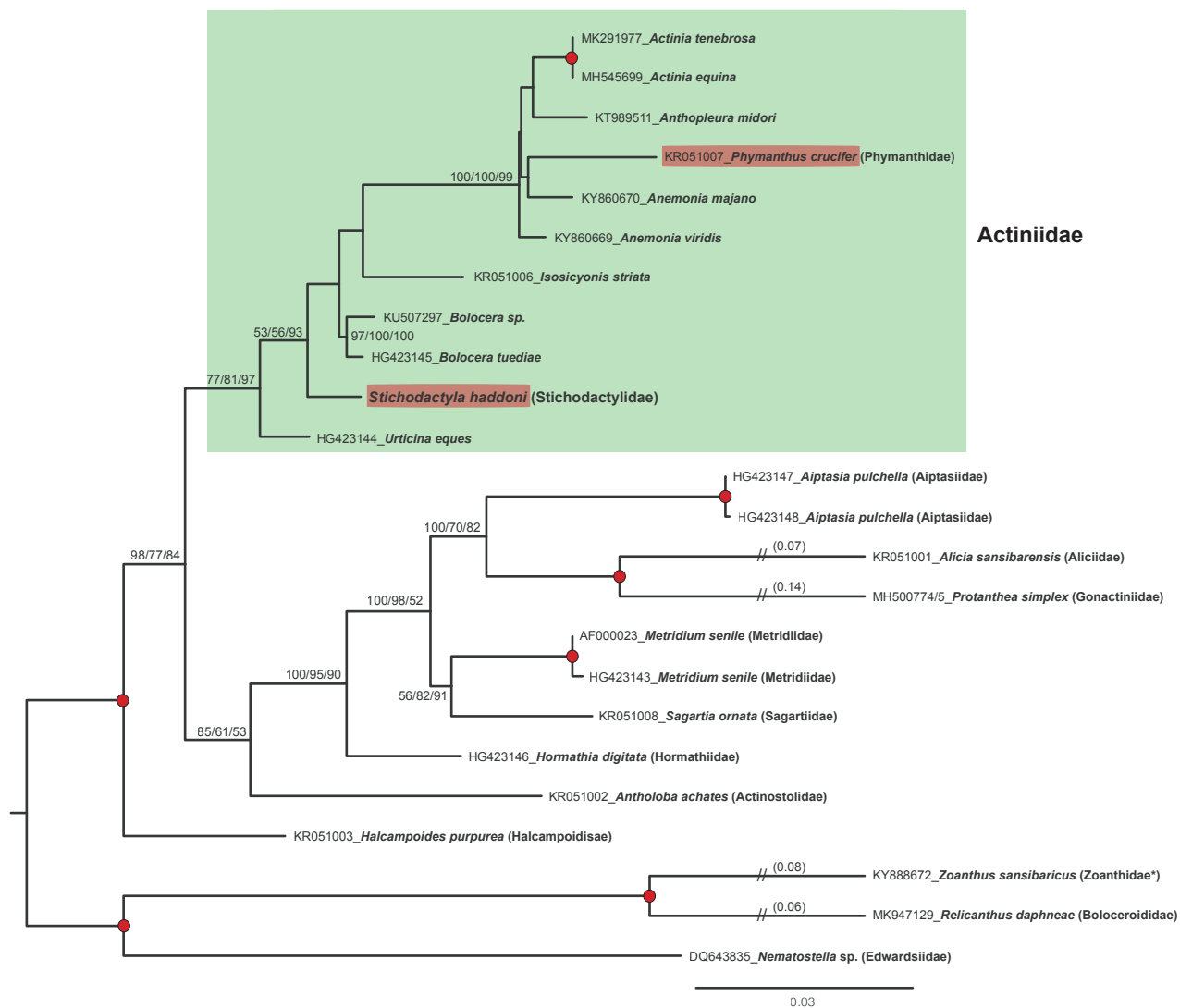


Figure S3

**Table S1: Annotation of *Stichodactyla haddoni* complete mitochondrial genome**

<b>Genes &amp; Regions</b>	<b>Pos</b>	<b>Size nt</b>	<b>Size aa</b>
ND5-exon 1	1-717	717	239
ND5-717, 5' ribozyme	718-940	223	
ND1	941-1924	984	327
IGR-1	1925-1930	6	
ND3	1931-2287	357	118
ND5-717, 3' ribozyme	2288-2397	111	
ND5-exon 2	2398-3513	1116	371
ND5		1833	610
IGR-2	3514-3809	296	
W	3810-3879	70	
IGR-3	3380-3993	614	
ND2	3994-5376	1383	461
IGR-4	5377-5425	49	
SSU	5426-6483	1058	
IGR-5	6484-6535	52	
COII	6536-7282	741	246
IGR-6	7283-7287	5	
ND4	7288-8763	1467	488
IGR-7	8764-8767	4	
ND6	8768-9376	609	202
IGR-8	9377-9403	27	
CytB	9404-10555	1152	383
IGR-9	10556-11033	418	
M	11034-11104	71	
LSU	11105-13321	2248	
IGR-10	13322-13392	71	
COIII	13393-14181	789	262
IGR-11	14182-14349	176	
COI-exon 1	14350-15242	893	
Intron COI-884	15243-16107	865	
HEG <sup>1</sup>	14350-16095	1746	581
COI-exon 2	16108-16789	682	
COI		1575	524
IGR-12	16790-17718	285	
OrfA	17196-17729	534	177
ND4L	17719-18018	300	99
IGR-13	18019-18043	24	
A8	18043-18258	216	71
IGR-14	18259-18290	33	
A6	18291-18980	690	229
IGR-15	18981-18999	19	

<sup>1</sup> Reading frame fused to COI 5' exon

**Table S2.** Key features of sea anemone mitogenomes included in this study**A: Sea anemones (Order Actiniaria)**

Species	Acc.	Mt Size	COI intron	HEG fusion	orfA
<b>Family Actiniidae</b>					
<i>Actinia equina</i>	MH545699	20,690 bp	COI+	-	+ (IGR-6)
<i>Actinia tenebrosa</i>	MK291977	20,691 bp	COI+	-	+ (IGR-6)
<i>Anemonia majano</i>	KY860670	19,545 bp	COI+	+ (pseudo)	+ (IGR-6)
<i>Anemonia viridis</i>	KY860669	20,108 bp	COI+	+ (pseudo)	+ (IGR-6)
<i>Anthopleura midori</i>	KT989511	20,039 bp	COI+	+ (pseudo)	+ (IGR-6)
<i>Bolocera tuediae</i>	HG423145	19,143 bp	COI+	+ (in frame)	-
<i>Bolocera</i> sp.	KU507297	19,463 bp	COI+	+ (in frame)	-
<i>Isosicyonis striata</i>	KR051006	19,001 bp	COI+	+ (in frame)	+ (IGR-6)
<i>Urticina eques</i>	HG423144	20,458 bp	COI+	+ (in frame)	+ (IGR-6)
<b>Family Actinostolidae</b>					
<i>Antholoba achates</i>	KR051002	17,816 bp	COI+	+ (in frame)	+ (IGR-6)
<b>Family Aiptasiidae</b>					
<i>Aiptasia pulchella</i> <sup>4</sup>	HG423147	19,791 bp	COI+	-	+ (IGR-6)
<i>Aiptasia pulchella</i> <sup>4</sup>	HG423148	19,790 bp	COI+	-	+ (IGR-6)
<b>Family Aliciidae</b>					
<i>Alicia sansibarensis</i>	KR051001	19,575 bp	COI-	-	-
<b>Family Boloceroididae</b>					
<i>Relicanthus daphneae</i>	MK947129	17,727 bp	COI+	+ (in frame)	-
<b>Family Edwardsiidae</b>					
<i>Nematostella</i> sp.	DQ643835	16,389 bp	COI-	-	-
<b>Family Gonactiniidae</b>					
<i>Protanthea simplex</i>	MH500774/75	21,326 bp	COI-	-	-
<b>Family Halcampoidisae</b>					
<i>Halcampoides purpurea</i>	KR051003	18,038 bp	COI+	-	-
<b>Family Hormathiidae</b>					
<i>Hormathia digitata</i>	HG423146	18,754 bp	COI+	-	+ (IGR-6)
<b>Family Metridiidae</b>					
<i>Metridium senile</i>	HG423143	17,444 bp	COI+	-	-
<i>Metridium senile</i>	AF000023	17,743 bp	COI+	-	-
<b>Family Phymanthidae</b>					
<i>Phymanthus crucifer</i>	KR051007	19,727 bp	COI+	-	+ (IGR-6)
<b>Family Sagartiidae</b>					
<i>Sagartia omata</i>	KR051008	17,446 bp	COI+	-	-
<b>Family Stichodactylidae</b>					
<i>Stichodactyla haddoni</i>	Unpublished <sup>5</sup>	18,999 bp	COI+	+ (in frame)	+ (IGR-12)

**B: Colonial anemones (Order Zoantharia)**

Species	Acc. no	Mt Size	COI intron	HEG fusion	orfA
<b>Family Zoanthidae</b>					
<i>Zoanthus sansibaricus</i>	KY888672	20,972 bp	COI+	-	-

Notes: 1) COI+ or -, COI intron present or absent. 4) The sea anemone *Aiptasia pulchella* may also be annotated as *Exaiptasia pallida*. 5 Information from our unpublished complete mitochondrial genome sequence of *Stichodactyla haddoni*.