Korean J. Syst. Zool. Vol. 26, No. 3: 197-201, November 2010

The Complete Mitochondrial Genome of *Dendronephthya gigantea* (Anthozoa: Octocorallia: Nephtheidae)

Eunji Park, Boa Kim and Yong-Jin Won*

Division of EcoScience, Ewha Womans University, Seoul 120-750, Korea

ABSTRACT

We sequenced the whole mitochondrial genome of *Dendronephthya gigantea* (Anthozoa: Octocorallia: Nephteidae), the first mitochondrial genome sequence report in the Family Nephtheidae. The mitochondrial genome of *D. gigantea* was 18,842 bp in length, and contained 14 protein coding genes (*atp6* and 8, *cox1-3*, *cytb*, *nd1-6* and *4L*, and *msh1*), two ribosomal RNAs, and only one transfer RNA. The gene content and gene order is identical to other octocorals sequenced to date. The portion of the noncoding regions is slightly larger than the other octocorals (5.08% compared to average 3.98%). We expect that the information of gene content, gene order, codon usage, noncoding region and protein coding gene sequence could be used in the further analysis of anthozoan phylogeny.

Keywords: Octocorallia, Nephtheidae, mitochondrial genome, Korea

INTRODUCTION

The subclass Octocorallia (Cnidaria: Anthozoa) comprises approximately 3,000 extant species of soft corals, sea pens, gorgonians and blue corals (Daly et al., 2007). To elucidate the evolutionary relationships within octocorals, much research has been conducted using 18S rRNA, *cox1*, *nd2* or *msh1* genes (Berntson et al., 1999, McFadden et al., 2006). However, because of the lack of enough amount of nucleotide substitution due to slow evolution of mitochondrial and nuclear ribosomal genes, a clear phylogenetic relationship has remained unsolved (Hellberg, 2006).

Mitochondrial genomes (mitogenomes) have been used for phylogenetic studies in diverse animal groups (Brugler and France, 2007, Sinniger et al., 2007, Wang and Lavrov, 2007). Not only protein coding genes, but also gene order, gene content, and non-coding regions can be used in phylogenetic analyses (Gissi et al., 2008). Currently, only six octocoral mitogenomes have been sequenced, in contrast with 27 hexacoral mitochondrial genome sequences available on GenBank. For the phylogenetic analysis of octocorals, more mitochondrial genome information is needed. In a previous study we constructed anthozoan phylogeny and estimated divergence time between hexacorals and octocorals using 13 protein coding mitochondrial genes including *D. gigantea* (Kim et al., 2008) reported in this paper. The result showed that the first splitting event among four octocoral species had occurred around 50-79 million years ago (Ma). In contrast, 16 different hexacoral species showed relatively long history of divergence (> 240 Ma). The discrepancy mainly arose from the very limited taxon sampling of the octocorals compared to the hexacorals (Kim et al., 2008). These results provided insights into the anthozoan evolution and also highlighted the need and importance of more extensive taxon sampling, particularly in octocorals, for better understanding of phylogenetic relationships among anthozoans. Here, we determined and described the complete mitochondrial genome sequence of *D. gigantea*, the first report from the Family Nephtheidae.

*To whom correspondence should be addressed
Tel: 82-2-3277-4471, Fax: 82-2-3277-4514
E-mail: won@ewha.ac.kr

MATERIALS AND METHODS

Sample collection and DNA extraction

D. gigantea specimen was collected at a depth of 20 m on the cliff of Munseom Island, south of the Jejudo Island, Korea in 2005. Several 2 cm long branches were dissected and stored in 95% ethanol at −50°C until use. Total genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's protocol.

PCR amplification and sequencing

Thirty-one primer pairs were newly designed to amplify the complete octocoral mitochondrial genome (Table 1), selecting

Table 1. The 31 primer pairs used to amplify the whole mitochondrial genome of *Dendronephthya gigantea*

Primer	Sequence	Primer	Sequence
1F	ATGAACAAATATCTTACACG	1R	ATAARTGCTGRAATAAAAT
2F	CAATGTTATTGACAGATAGA	2R	GCTAAACCCAAGAAATG
3F	ACAGGTTATAGTTATAATGA	3R	GTCTGCTGGCACTTAGTTAG
4F	CTGGTCGAAGATGCGTAGTA	4R	TGTGCTAACACTGGGTTAGA
5F	TGGATTAAGTCAGGTGTC	5R	ATAGCTAATCATTTGAGC
6F	GTACTAGCTGATATTAATGT	6R	YACTGCATCTAAACCTATCA
7F	ACAGGAATTCTAGGAATGGG	7R	GACATTTGTCCCCAAGGTAA
8F	ATATTTTAAGAGACGTTAAT	8R	CTCTACTGGATTAGCCCCTA
9F	ATCCTTTAGTAACTCCTG	9R	TTGGCCAGAAGGCACCTA
10F	TGCTAGTTTGGTGCTACTAG	10R	TCGGCAGCTGCGACAGTTAA
11F	AGGTATTATTCTTAATAGAA	11R	TTACAACTAGGAGARTAAAC
12F	YTRCTTCAAATGGGGTTTCC	12R	AGAATTGTAACACTCGGG
13F	CTTAGTAAAATATTTCAAAG	13R	TGTATCTTGAAAYACAATAT
14F	TGGGCYGAACARAYTTCAAA	14R	TAARCTGTTATAATTAGCTA
15F	GCAGGAATGTATGTAGCTGC	15R	AGACTTTACTCAGTTCCACT
16F	CTATTTTAGGYTGGAAGAGA	16R	ACTTCCTGTTTGTCTAAGTT
17F	ACTGGTGTAGTAAGACTA	17R	TTTCCTCTTGAGACAGTA
18F	TGACCGTGATAATGTAGCAC	18R	GGCACCTTATTAATCCCWAA
19F	TGGTGACACAGCTCGGTT	19R	GCACGATAGATAATAGCGCA
20F	ATTRTTATTTAAAGTATCTG	20R	ATATTTGTTATTACTAAAGG
21F	GTTTTTAACTAARTGGTATR	21R	TCCCAACCRATAAATARTTG
22F	ATTCTACAAGTTATATGAGA	22R	GCATGAATRATTGAGCCTGC
23F	AGTTTATATCAYYTACTAAC	23R	TATCATTAATGCATAATTAA
24F	ATGCCTGGGAGTTTAATC	24R	AGAAGAAATAATAAGCAGCT
25F	TTTGAAAGTATATTAATACC	25R	GTACTAGTWGAAAAAGCAGC
26F	ATGGTRTTTACTTTAGCTAA	26R	GCTGCTAGTTGGTATTGGCA
27F	CTAAGARCCCCACCARTAAA	27R	TATCACCCTTATCATYTAGT
28F	TGAAAATATARTACTGAGCC	28R	TCWACAGCTAAYAAGGGAAC
29F	GTAAATACRTAGGGAAATAG	29R	CATTAGSTATTAAAATGGAT
30F	GAGTGATTAGCGCCACATAA	30R	GGAGCCTATATCCTTGRGAT
31F	TGGAGTTTTCATTCTTCTCT	31R	CCAATCATTACTGGCATTAC

conserved sites among four octocorallian genome sequences available on GenBank (accession IDs: DQ640649, DQ640646, AF064823, and AF063191). Each primer pair was designed to amplify an approximate 700-bp segment, and each amplicon was overlapped by about 70 bp with both 5′ and 3′ adjacent segments. PCR reactions were conducted using the following conditions: initial denaturation step at 94˚C for 1_ min, 35_cycles at 92˚C for 40 sec, 50˚C for 1 min, 72˚C for 1 min, followed by a final extension step at 72˚C for 7 min. PCR products were purified with a LaboPass PCR purification kit (Cosmo Genetech Inc. Seoul, Korea), and sequenced using an ABI3730XL instrument (50-cm capillary).

Gene annotation and sequence analysis

To construct a complete mitogenome contig, 31 sequences were aligned and assembled using AlignIR (LiCoR). Protein coding genes and rRNA genes were identified with a blast search based on sequence similarity and confirmed by ORF Finder (NCBI). Transfer RNA genes were searched with the tRNA scan-SE program (Lowe and Eddy, 1997), and codon usage was calculated using DnaSP 5.0 (Librado and Rozas, 2009).

RESULTS

Genome composition and gene order

The mitochondrial genome of *D. gigantea* consisted of 18,842 bp in length (GenBank accession ID: FJ372991, Fig. 1) and contained 14 protein coding genes (*atp6* and *8*, *cox1-3*, *cytb*, *nd1-6* and *4L*, and *msh1*), two rRNA genes (large and small subunit ribosomal RNA), and one tRNA gene (for methionine), which was identical to the typical octocoral species reported so far in terms of genome composition (McFadden et al., 2010). Additionally, the gene order was identical to other octocorals except for the deep-sea bamboo coral species of the Family Isididae (Brugler and France, 2008).

Protein coding genes and codon usage

In general, metazoan mitochondrial genomes consist of 13 protein coding genes (*atp6* and *8*, *cox1-3*, *cytb nd1-6* and *4L*),

however, the octocoral mitochondrial genome contained an additional bacterial *mutS* homolog *msh1* gene, which has not been documented in other metazoans, even in the sponge and hexacoral mitochondrial genomes (Pont-Kingdon et al., 1998). Among the 14 protein coding genes, ten genes (*cox1*, *nd1*, *cytb*, *nd6*, *nd4*, *nd4L*, *msh1*, *nd2*, *nd5*, and *nd4*) were encoded in the heavy strand and the remaining four genes (*cox3*, *apt6*, *atp8*, and *cox2*) were encoded in the light strand (Table 2). All genes are inferred to start with an ATG codon and terminated with a TGA or TAA stop codon except *cox1*, which ended with an ATTT sequence, and is considered to make a TAA stop codon by adding multiple adenines to the 3′ end of the mRNA strand (Anderson et al., 1981). According to the codon usage program, UUA for Leu (RSCU 3.30) and AGA for Arg (RSCU 2.65) were highly preferred. In contrast, UCA for Trp (RSCU 0.07) and CUC for Leu RSCU 0.15) were highly avoided (Table 3).

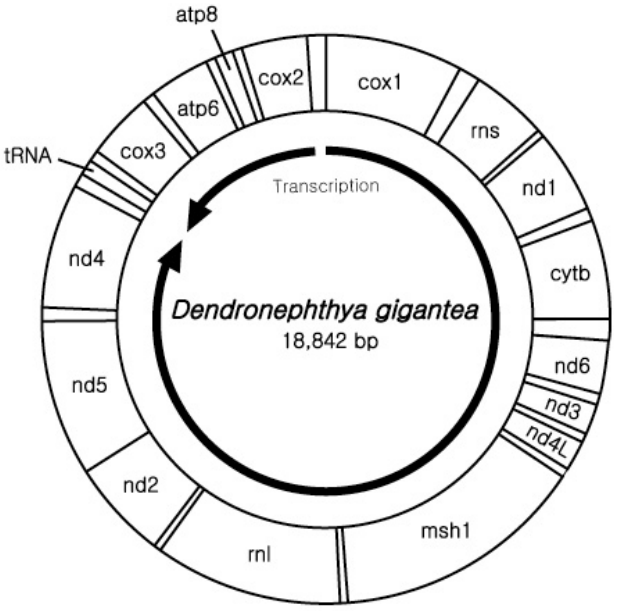

Fig. 1. Circular map of *Dendronephthya gigantea* mitochondrial genome.

Ribosomal and transfer RNA genes

The *D. gigantea* mitochondrial genome encoded 12S and 16S rRNA genes of 923 and 2,171 bp, respectively. The small subunit ribosomal RNA was located between the *cox1* and *nd1* genes, and the large subunit ribosomal RNA was found between the *msh1* and *nd2* genes. Compared to typical metazoan mitochondrial genomes, which have 22 tRNAs (Gissi et al., 2008), all six octocorals sequenced to date have only one type of $tRNA^{Met}$. The tRNA for methionine of *D. gigantea* which is 71 bp in length, was located between *nd4* and *cox3* (Fig. 2).

Non-coding region

Non-coding intergenic regions occupied 5.08% (957 bp/18,842 bp) of the total length of the *D. gigantea* mitogenome and showed a large proportion of noncoding DNA/total DNA compared to the other five octocoral mitogenomes (average 3.96%, McFadden et al., 2010). All genes were separated by non-coding regions with a range of 4 bp to

Table 2. Organization of the *Dendronephthya gigantea* mitochondrial genome

Gene	Position		Length		Codon		Strand	Intergenic nucleotides
	Start	Stop	Nucleotides	Amino acids	Start	Stop		
cox1	1	1597	1597	533	ATG	T(AA)[a]	+	151
rns	1749	2671	923				+	4
nd1	2676	3647	972	324	ATG	TAG	+	82
cytb	3730	4866	1137	379	ATG	TAG	+	199
nd6	5066	5623	558	186	ATG	TAG	+	43
nd3	5667	6020	354	118	ATG	TAG	+	19
nd4L	6040	6333	294	98	ATG	TAA	+	30
msh1	6347	9286	2940	980	ATG	TAA	+	9
rnl	9296	11466	2171				+	4
nd2	11471	12628	1158	386	ATG	TAG	+	−13[b]
nd5	12616	14433	1818	606	ATG	TAG	+	84
nd4	14518	15966	1449	483	ATG	TAA	+	72
$tRNA^{Met}$	16039	16109	71				−	39
cox3	16149	16934	786	262	ATG	TAG	−	64
atp6	16999	17706	708	236	ATG	TAA	−	24
atp8	17731	17946	216	72	ATG	TAG	−	22
cox2	17969	18730	762	254	ATG	TAG	−	111

(+) and (−) indicate heavy strand and light strand, respectively. [a]The stop codon "TAA" is formed at the end of the gene by polyadenylation to the end of *cox1* mRNA which ends with "T". [b]Negative value indicates overlapping region of two adjacent genes.

Table 3. Codon Usage

Amino acids	Codon	No.	RSCU	Amino acids	Codon	No.	RSCU	Amino acids	Codon	No.	RSCU
Phe	UUU	221	1.51	Thr	ACU	113	1.82	Cys	UGU	51	1.65
	UUC	72	0.49		ACC	33	0.53		UGC	11	0.35
Leu	UUA	405	3.30		ACA	85	1.37	Trp	UGA	3	0.07
	UUG	82	0.67		ACG	18	0.29		UGG	87	1.93
	CUU	94	0.77	Ala	GCU	161	1.87	Arg	CGU	29	1.20
	CUC	18	0.15		GCC	68	0.79		CGC	8	0.33
	CUA	109	0.89		GCA	94	1.09		CGA	18	0.74
	CUG	29	0.24		GCG	22	0.26		CGG	11	0.46
Ile	AUU	240	1.41	Tyr	UAU	192	1.63	Ser	AGU	97	1.49
	AUC	51	0.30		UAC	43	0.37		AGC	52	0.80
	AUA	218	1.28	Stop	UAA	4	0.62	Arg	AGA	64	2.65
Met	AUG	146	1.00		UAG	9	1.38		AGG	15	0.62
Val	GUU	130	1.56	His	CAU	76	1.42	Gly	GGU	93	1.10
	GUC	32	0.38		CAC	31	0.58		GGC	48	0.57
	GUA	103	1.23	Gln	CAA	108	1.66		GGA	100	1.19
	GUG	69	0.83		CAG	22	0.34		GGG	96	1.14
Ser	UCU	124	1.90	Asn	AAU	125	1.52				
	UCC	28	0.43		AAC	40	0.48				
	UCA	62	0.95	Lys	AAA	99	1.41				
	UCG	28	0.43		AAG	41	0.59				
Pro	CCU	90	1.80	Asp	GAU	90	1.42				
	CCC	48	0.94		GAC	37	0.58				
	CCA	45	0.90	Glu	GAA	88	1.09				
	CCG	18	0.36		GAG	73	0.91				

Relative Synonymous Codon Usage (RSCU) is the observed frequency divided by the expected frequency under the assumption of equal usage of synonymous codons. An RSCU of 1 indicates equal usage of codons for an amino acid.

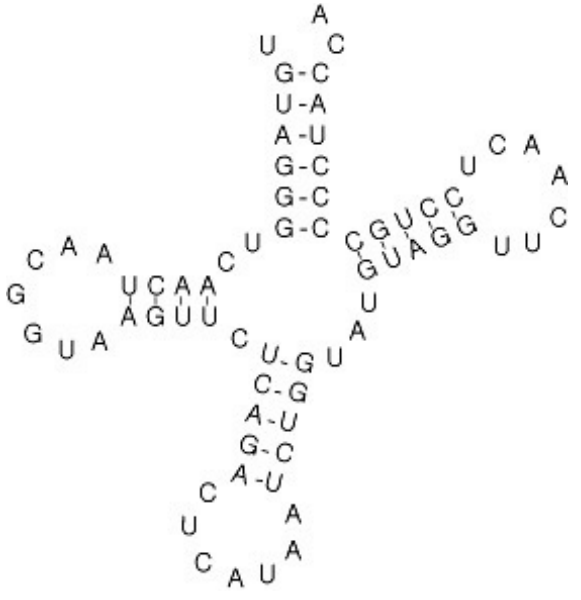

Fig. 2. The secondary structure of $tRNA^{Met}$.

199 bp except between *nd2-nd5* where the two genes a overlapped by 13 bp. The longest non-coding region was located between *cytb* and *nd6*.

DISCUSSION

The *D. gigantea* mitochondrial genome was of 18,842 bp in length, and comprised 14 protein coding genes, two rRNAs, and only one tRNA. In general, mitochondrial genes, such as *cox1* and *nd2*, are widely used for phylogenetic analysis due to high substitution rates compared to those of nuclear genes (Gissi et al., 2008). However, mitochondrial genes of corals are inappropriate to reveal evolutionary relationships because of the low genetic variation and substitution rate (Hellberg, 2006; McFadden et al., 2006). Therefore, a large number of informative DNA sequences are needed to better understand octocoral phylogeny. For this reason, whole mitochondrial genomes are a good resource for phylogenetic information, including gene content and gene order as well as DNA sequences. The study using 13 protein coding genes from 17 hexacorals and 3 octocorals suggested that one of the two clades of scleractinia is closely related to the corallimorpharia than to the other clade of scleractinia hence the corallimorpharia should be included in the scleractinia (Med-

ina et al., 2006). Our previous study including the protein coding genes from *D. gigantea* estimated the divergence time among four octocorals approximately 50-79 Ma (Kim et al., 2008). These studies contributed to better understand of the anthozoan evolution. However, the insufficient taxon sampling acts as a limit for better understanding of the evolutionary relationships and molecular evolutionary patterns of mitogenome of anthozoans. Therefore more mitochondrial genome sequencings and characterizations are needed for further study.

ACKNOWLEDGEMENTS

We thank Jun-Im Song for her species identification of *D. gigantea*. This work was supported by the Korea Research Foundation Grant (KRF-2005-070-C00124) to Y.J. Won.

REFERENCES

Anderson, S., A. Bankier, B. Barrell, M. De Bruijn, A. Coulson, J. Drouin, I. Eperon, D. Nierlich, B. Roe and F. Sanger, 1981. Sequence and organization of the human mitochondrial genome. Nature, 290(5806): 457-465.

Berntson, E., S. France and L. Mullineaux, 1999. Phylogenetic relationships within the Class Anthozoa (Phylum Cnidaria) based on nuclear 18S rDNA sequences. Mol. Phylogenet. Evol., 13(2): 417-433.

Brugler, M. and S. France, 2007. The complete mitochondrial genome of the black coral Chrysopathes formosa (Cnidaria: Anthozoa: Antipatharia) supports classification of antipatharians within the subclass Hexacorallia. Mol. Phylogenet. Evol., 42(3): 776-788.

Brugler, M. and S. France, 2008. The mitochondrial genome of a deep-sea bamboo coral (Cnidaria, Anthozoa, Octocorallia, Isididae): genome structure and putative origins of replication are not conserved among octocorals. J. Mol. Evol., 67(2): 125-136.

Daly, M., M. Brugler, P. Cartwright, A. Collins, M. Dawson, D. Fautin, S. France, C. McFadden, D. Opresko and E. Rodrigues, 2007. The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa, 1668: 127-182.

Gissi, C., F. Iannelli and G. Pesole, 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity, 101(4): 301-320.

Hellberg, M., 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol. Biol., 6(1): 24.

Kim, B., S.-R. Kong, J.-I. Song and Y.-J. Won, 2008. Molecular phylogeny and divergence time estimation of the soft coral *Dendronephthya gigantea* (Alcyonacea: Nephtheidae). Korean J. Syst. Zool., 24, 327-332.

Librado, P. and J. Rozas, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11): 1451.

Lowe, T. and S. Eddy, 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res., 25(5): 955.

McFadden, C., S. France, J. Sanchez and P. Alderslade, 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. Mol. Phylogenet. Evol., 41(3): 513-527.

McFadden, C., J. Sanchez and S. France, 2010. Molecular Phylogenetic Insights into the Evolution of Octocorallia: A Review. Integrative and Comparative Biology, doi:10.1093/icb/icq056.

Pont-Kingdon, G., N. Okada, J. Macfarlane, C. Beagley, C. Watkins-Sims, T. Cavalier-Smith, G. Clark-Walker and D. Wolstenholme, 1998. Mitochondrial DNA of the coral Sarcophyton glaucum contains a gene for a homologue of bacterial MutS: a possible case of gene transfer from the nucleus to the mitochondrion. J. Mol. Evol., 46(4): 419-431.

Sinniger, F., P. Chevaldonne and J. Pawlowski, 2007. Mitochondrial genome of Savalia savaglia (Cnidaria, Hexacorallia) and early metazoan phylogeny. J. Mol. Evol., 64(2): 196-203.

Wang, X. and D. Lavrov, 2007. Mitochondrial genome of the homoscleromorph *Oscarella* carmela (Porifera, Demospongiae) reveals unexpected complexity in the common ancestor of sponges and other animals. Mol. Biol. Evol., 24(2): 363.

Received September 20, 2010
Accepted November 12, 2010