Conservation Genet Resour
DOI 10.1007/s12686-017-0692-6

TECHNICAL NOTE

Complete mitochondrial genome sequence for the green humphead parrotfish *Bolbometopon muricatum*

Wei-Chuan Chiang[1] · Chia-Hao Chang[2] · Hung-Hung Hsu[1] · Nian-Hong Jang-Liaw[3]

Received: 28 December 2016 / Accepted: 3 February 2017

Abstract Here we describe the complete mitochondrial genome sequence of humphead parrotfish, *Bolbometopon muricatum*. The circle genome (16,788 bp) consists of 13 protein coding, 22 tRNA, 2 rRNA genes and 1 control region. Generally it has the typical vertebrate mitochondrial gene arrangement but for the relative positions of the $tRNA^{Ile}$, $tRNA^{Gln}$, and $tRNA^{Met}$, and a $tRNA^{Met}$ psuedogene was detected. This work provides fundamental molecular data which will be useful for species identification, forensic genetics and further researches on genetic diversity and conservation of this vulnerable marine fish.

Keywords Complete mitochondrial genome · *Bolbometopon muricatum* · Humphead parrotfish

The humphead parrotfish (*Bolbometopon muricatum*) is the largest species of parrotfish, growing to ~4 feet (1.3 m) in length and ~100 lbs (46 kg) in weight (Randall 2005). They live in coral reef habitats from ~3 to 100 feet (1–30 m) in the central and western Pacific and Indo-Pacific, and frequent barrier and fringing reefs during the day, but rest in caves or shallow sandy lagoon flats at night (Donaldson and Dulvy 2004). Though being a favorite of divers and conservationists, humphead parrotfish is threatened to overfishing. Shoaling and group sleeping behaviour render this fish highly vulnerable to spear fishing (Donaldson and Dulvy 2004). In Taiwan, the government promulgated the "Wildlife Conservation Act" to protect rare and endangered animals and the humphead parrotfish has been on the list since 2014, which means it is not allowable for public sale or any kinds of consuming currently. In this study, the specimen was taken from a Taitung County government seizure. It was found dead floating on sea surface of Orchid Island, eastern Taiwan on 2nd, July 2016 and was deposited in the Research Museum of Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, with the specimen number ASIZP0063367. Here we present the complete mitochondrial genome of *B. muricatum* that would provide useful information to future evolutionary research of parrotfishes, and would thus contribute towards the identification and conservation of this species.

Genomic DNA of *B. muricatum* was extracted using the Quick Gene DNA Tissue Kit S (Fujifilm, Tokyo, Japan). In the beginning the partial mitochondrial *CYTB* and *COX1* sequences were superlatively amplified with three different pairs of primers: one pair of the *CYTB* primers were designed on the basis of the *B. muricatum CYTB* sequences (EU601357), and the other two pairs of the *COX1* primers were designed by Chang et al. (2016). PCR were performed in a mixture with a final volume of 50 µl containing 20 ng template DNA, 20 µmol of each specific primer, 12.5 µl of Fast-Run™ Advanced *Taq* Master Mix (ProTech, Taiwan), and distilled water. The thermal cycling began with one cycle at 94 °C for 4 min; subsequently 35 cycles of denaturation at 94 °C for 0.5 min, 50–60 °C for 0.5 min, and 72 °C for 1 min; and finally, a single extension step at 72 °C for 10 min. PCR products were purified using a PCR DNA Fragments Extraction Kit (Geneaid, Taiwan) and were sequenced on an ABI 3730 DNA Analyzer (Applied

✉ Nian-Hong Jang-Liaw
taco.tw@gmail.com

[1] Eastern Marine Biology Research Center of Fisheries Research Institute, Taitung 96143, Taiwan

[2] Department of Animal Biology, University of Illinois, Champaign, IL 61802, USA

[3] Conservation and Research Center, Taipei Zoo, Taipei 11656, Taiwan

Published online: 18 February 2017

Biosystems, Foster, California, USA). Then, the obtained *CYTB* and *COX1* sequences were used to designed two pairs of long PCR primers (CytbF: 5′-GGCTCTCTCCTAGGACTCTGCCTAGC-3′ and COX1R: 5′-CTACGGATGCGCCTGCATGTGCAAGA-3′; COX1F-CTTGCACATGCAGGCGCATCCGTAGA-3′ and CytbR: 5′-GCGATATCAGAGGTGTAATGCATGGCTA-3′). PCR were performed in a mixture with a final volume of 50 µl containing 20 ng template DNA, 20 µmol of each specific primer, 1 µl of PrimeSTAR GXL DNA polymerase (TAKARA, Japan), 4 µl dNTP mixture (2.5mM each) and distilled water. The thermal cycling began with one cycle at 94 °C for 4 min; subsequently 30 cycles of denaturation at 98 °C for 10 s, 60 °C for 15 s, and 68 °C for 10 min; and finally, a single extension step at 68 °C for 20 min. PCR products were purified using a PCR DNA Fragments Extraction Kit (Geneaid,

Table 1 The genome organization of the humphead parrotfish (*Bolbometopon muricatum*) mtDNA

Gene	From	To	Length (bp)	Codon		Intergenic nucleotides	Strand
				Start	Stop		
tRNA^{Phe}	1	68	68			–	H
12 S rRNA	69	1013	945			0	H
tRNA^{Val}	1014	1085	72			0	H
16 S rRNA	1087	2767	1681			1	H
tRNA$^{Leu(UUR)}$	2768	2841	74			0	H
ND1	2845	3807	963	ATG	TAA	3	H
tRNA^{Ile}	3819	3888	70			11	H
tRNA^{Met}	3899	3968	70			10	H
tRNA^{Gln}	3975	4045	71			6	L
ND2	4112	5158	1047	ATG	TAG	66	H
tRNA^{Trp}	5168	5237	70			9	H
tRNA^{Ala}	5240	5308	69			2	L
tRNA^{Asn}	5312	5384	73			3	L
O_L	5385	5424	40			0	
tRNA^{Cys}	5425	5491	67			0	L
tRNA^{Tyr}	5492	5562	71			0	L
COX1	5564	7114	1551	GTG	TAA	1	H
tRNA$^{Ser(UCN)}$	7115	7185	71			0	L
tRNA^{Asp}	7189	7261	73			3	H
COX2	7269	7959	691	ATG	T	7	H
tRNA^{Lys}	7960	8033	74			0	H
ATP8	8035	8202	168	ATG	TAA	1	H
ATP6	8193	8876	684	ATG	TAA	−10	H
COX3	8876	9661	786	ATG	TAA	−1	H
tRNA^{Gly}	9661	9731	71			−1	H
ND3	9733	10,084	352	ATG	T	1	H
tRNA^{Arg}	10,085	10,153	69			0	H
ND4L	10,154	10,450	297	ATG	TAA	0	H
ND4	10,444	11,821	1378	ATG	TAA	−7	H
tRNA^{His}	11,825	11,893	69			3	H
tRNA$^{Ser(AGY)}$	11,895	11,963	69			1	H
tRNA$^{Leu(CUN)}$	11,977	12,048	72			13	H
ND5	12,049	13,887	1839	ATG	TAA	0	H
ND6	13,884	14,405	522	ATG	TAG	−4	L
tRNA^{Glu}	14,407	14,474	68			1	L
CYTB	14,480	15,620	1141	ATG	T	5	H
tRNA^{Thr}	15,621	15,692	72			0	H
tRNA^{Pro}	15,693	15,762	70			0	L
CR	15,763	16,788	1026			0	–

Taiwan) and were sequenced by primer walking on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster, California, USA). The sequenced fragments were assembled into the whole mitochondrial genome sequence deposited in GenBank with the accession number KY235362.

The organization of mitochondrial genome of *B. muricatum* is shown in Table 1, which was sequenced and determined to be 16,788 bp in size, including 13 typical vertebrate protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes, and a control region (CR). A physical map of the genome was generated using OGDRAW (http://ogdraw.mpimp-golm.mpg.de/) (Lohse et al. 2013, Fig. 1). All genes were encoded on the H-strand with the exception of one protein-coding gene (*ND6*) and eight tRNA genes [$tRNA^{Gln}$, $tRNA^{Ala}$, $tRNA^{Asn}$, $tRNA^{Cys}$, $tRNA^{Tyr}$, $tRNA^{Ser(UCN)}$, $tRNA^{Glu}$, and $tRNA^{Pro}$]. The base composition was counted using MEGA6 (Tamura et al. 2013). The overall base composition in descending order is A (29.19%), T (27.2%), C (27.95%), G (15.66%) with 43.61% GC content. The positions of RNA genes were predicted by the MITOS (Bernt et al. 2012) and the locations of protein-coding genes were identified by comparing with the homologous genes of several fishes including 2 species of Scaridae (Mabuchi et al. 2004; Jang-Liaw et al. 2013, 2016; Chang et al. 2016; Han et al. 2016). The 22 tRNA genes range from 67 to 74 bp in length and can fold into a typical cloverleaf secondary structure that was estimated by the online software tRNAscan-SE v2 (Lowe and Chan 2016). Similar with another parrotfish *Chlorurus sordidus, B. muricatum* had a gene rearrangement in an IMQtRNA gene-cluster region ($tRNA^{Ile}$-$tRNA^{Met}$-$tRNA^{Gln}$) followed by a putative tRNA pseudogene, which is different from

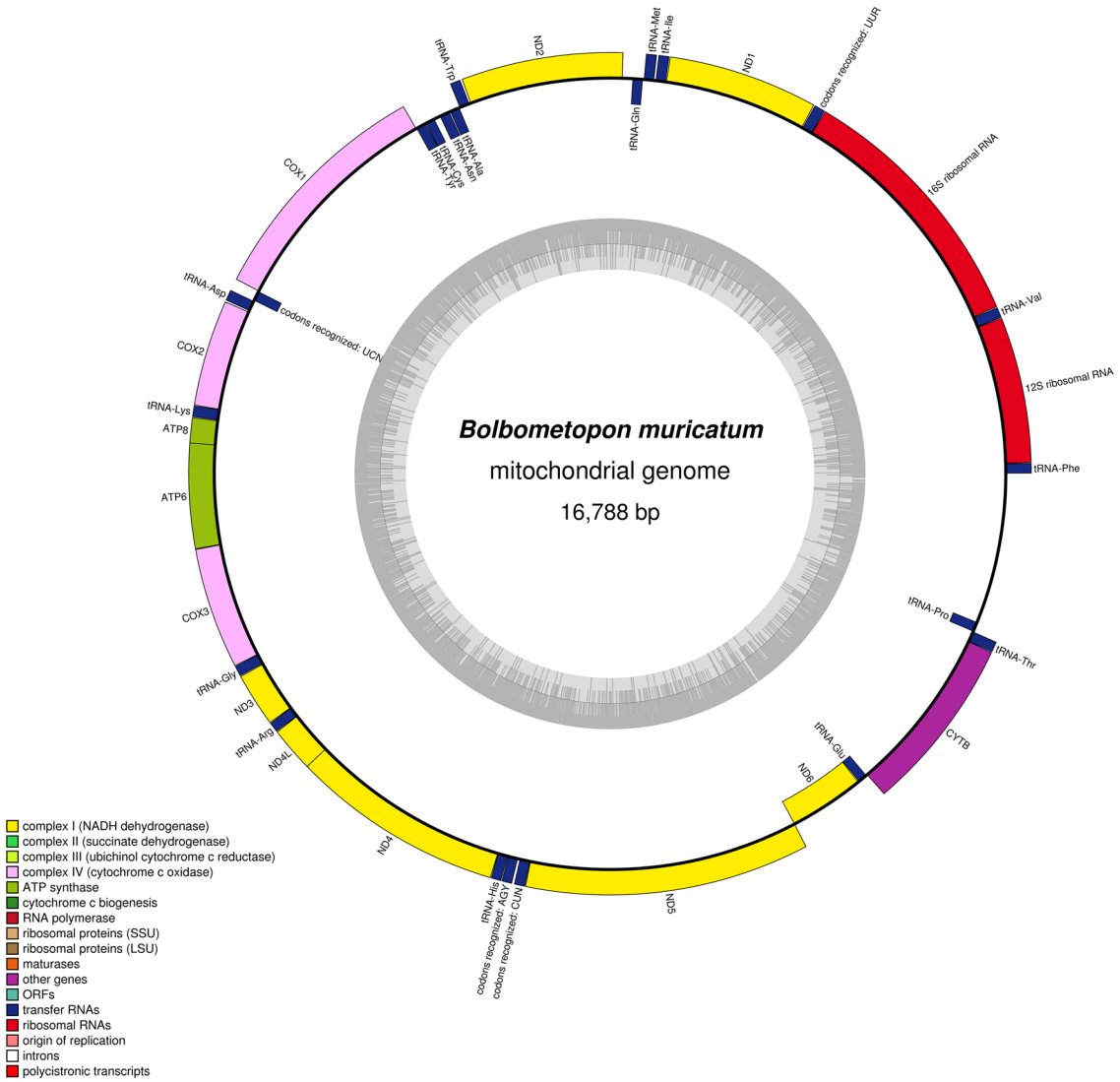

Fig. 1 Physical map of the mitochondrial genome of *Bometopon muricatum*

the typical IQMgene order of vertebrates (Mabuchi et al. 2004). The two ribosomal RNA genes, *12S rRNA* (945 bp) and *16S rRNA* (1681 bp), located between $tRNA^{Phe}$ and $tRNA^{Leu(UUR)}$ genes and were separated by the $tRNA^{Val}$ gene as seen in other vertebrates (Table 1). *ND5* and *ND6* overlap by 5 nucleotides, whereas they are encoded on the opposing strand. Except for *COX1* with a GTG start codon, the remaining 12 protein-coding genes start with an ATG codon. Ten protein-coding genes in humphead parrotfish mitochondrial genome end with complete stop codons, TAA (*ND1, COX1, ATP8, ATP6, COX3, ND4L, ND4* and *ND5*), and TAG (*ND2* and *ND6*). The remaining protein-coding genes end with the incomplete stop codons representing as 'T' (*COX2, ND3* and *CYTB*). The origin of L-strand replication (O_L) was located between the $tRNA^{Asn}$ and $tRNA^{Cys}$ genes within a cluster of five tRNA genes (WANCY region, Table 1) as in most vertebrates, which is 40 bp long. CR is 1026 bp long and no repeat set was found (in total 15,763–16,788; checked by online software "TANDEM REPEATS FINDER"; Benson 1999).

Acknowledgements We are grateful to Taitung County government for providing the mussel material. This work was supported by Animal Adoption Plan of Taipei Zoo (105_CR_01) and Ministry of Science and Technology, Taiwan (MOST 105-2917-I-564-075).

References

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27:573–580

Bernt M, Donath A, Jühling F et al (2013) MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69:313–319

Chang CH, Jang-Liaw NH, Lin YS, Carlisle A, Hsu HH, Liao YC, Shao KT (2016) The complete mitochondrial genome of the salmon shark, *Lamna ditropis* (Chondrichthyes, Lamnidae). Mitochondr DNA Part A 27:316–317

Donaldson TJ, Dulvy NK (2004) Threatened fishes of the world: *Bolbometopon muricatum* (Valenciennes 1840) (Scaridae). Environ Biol Fishes 70:373

Han Y, Chen G, Luo J, Wen X, Li W, Wang J (2016) The complete mitochondrial genome of *Cheilinus undulates* based on high-throughput sequencing technique. Mitochondrial DNA A DNA Mapp Seq Anal 27:1897–1899

Jang-Liaw NH, Tsai CL, Watanabe K (2013) Complete mitochondrial genome of the Kikuchi's minnow *Aphyocypris kikuchii* (Teleostei, Cyprinidae). Mitochondr DNA 24:11–13

Jang-Liaw NH, Wen ZH, Chen IS (2016) The complete mitochondrial genome of the Kinmen's population of endangered minnow *Metzia mesembrinum* (Cypriniformes, Cyprinidae) from Taiwan. Mitochondrial DNA A DNA Mapp Seq Anal 27:622–623

Lohse M, Drechsel O, Kahlau S, Bock R (2013) Organellar Genome DRAW-a suite of tools for generating physical maps of plastid and mitochondrial genomes and visualizing expression data sets. Nucleic Acids Res 41:W575–W581

Lowe TM, Chan PP (2016) tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44:W54–W57

Mabuchi K, Miya M, Satoh TP, Westneat MW, Nishida M (2004) Gene rearrangements and evolution of tRNA pseudogenes in the mitochondrial genome of the parrotfish (Teleostei: Perciformes: Scaridae). J Mol Evol 59:287–297

Randall JE (2005) Reef and shore fishes of the South Pacific: New Caledonia to Tahiti and the Pitcairn Islands. University of Hawaii Press, Honolulu

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729