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Sequencing and characterization of mitochondrial DNA genome for *Brama japonica* (Perciformes: Bramidae) with phylogenetic consideration

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ABSTRACT

In this study, the complete mitochondrial genome sequence of *Brama japonica* is isolated and characterized by PCR and primer-walking sequencing techniques. The complete DNA is 17,009 bp in length and contained 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a long putative control region. The gene organization and nucleotide composition are identical to those of other Bramidae fishes. In contrast, the 12S rRNA gene contains a big poly C structure which is larger than those from other Bramidae species. Of 37 genes, twenty-eight are encoded by heavy strand, while nine are encoded by light strand. Among the 13 protein-coding genes, twelve employ ATG as start codon, while only one (COI) utilizes GTG as start codon. The terminal associated sequence (TAS), the central and conserved sequence block (CSB-E and CSB-D) and a variable domain (CSB-1, CSB-2 and CSB-3) are identified in the control region, while the typical central conserved CSB-F is not detected. From the phylogenetic tree, we find that *B. japonica*, together with other five Bramidae species form a monophyletic group among 24 species. This work provides a set of useful data for studies on population genetic diversity and molecular evolution in Bramidae fish species.

1. Introduction

The complete mitochondrial genome DNA of fish is a compact double-stranded and closed circular molecule that ranges approximately from 14 to 18 kbp and replicates and transcribes autonomously (Boore, 1999). Although gene rearrangements have been described in some organisms (Shao et al., 2001; Liu and Cui, 2009), the gene content and organization of mitochondrial genome in fish is quite conserved. With few exceptions, animal mitochondrial genomes generally encode 37 genes including 13 protein coding genes, 22 transfer RNA genes and two ribosomal RNA genes (Moritz and Brown, 2003). The mitochondrial genome does not only provide more information than single gene, but also show genome-level features

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including gene content, gene arrangement, base composition, modes of replication and transcription that make it become a very powerful tool for inferring genome evolution and phylogenetic relationships (Anderson et al., 1981).

Up to now, researches on mitochondrial DNA have become increasingly prevalent, and the complete mitochondrial genome sequences have been reported for numerous vertebrates, such as sea lamprey (Lee and Kocher, 1995), rainbow trout (Zardoya et al., 1995), basal ray-finned fish (Noack et al., 1996), lungfish (Zardoya and Meyer, 1996), zebra fish (Broughton et al., 2001), hagfish (Delarbre et al., 2002), cutlass fish (Liu and Cui, 2009), and tille trevally (Ma et al., 2015a). Compared to nuclear DNA, mitochondrial genome is typically of limited recombination and includes genes with comparatively fast evolution rates, as well as maternal inheritance mode (Moritz and Brown, 2003). So far, mitochondrial DNA has been extensively applied in population genetics (Lee et al., 2010; Ma et al., 2011), species identification (Rubinoff et al., 2006; Gvoždík et al., 2010) and phylogenetic relationship (Miya and Nishida, 2000).

The Pacific pomfret, *Brama japonica* Hilgendorf (Perciformes: Bramidae) is widely distributed in the North Pacific Ocean (Bigelow et al., 2011; Neave and Hanavan, 2011). It migrates seasonally between feeding and spawning grounds. Since late spring and through the summer periods, this species carries out a northward feeding migration along the subarctic frontal zone. From autumn, it migrates rapidly to subtropical frontal zone for spawning and stays there during whole winter and early spring (Watanabe et al., 2006; Neave and Hanavan, 2011). *B. japonica* is known as an epipelagic fish, and it undergoes extensive daily vertical migrations, occurring in epipelagic surface waters of around 0–100 m at night and descending to mesopelagic depths of around 400 m in daytime. Pacific pomfret plays an important ecological role in oceanic food webs, because it mainly feeds on small sized squids, shrimp and fishes and itself is important prey item of larger fishes such as the sword fish and the blue shark (Hikaru et al., 2003; Watanabe, 2004; Lebrasseur, 2011). Although it is an abundant fish species, researches are still limited on biomass, early life history and morphological classification (Seki and Mundy, 1991; Pearcy et al., 1993). By now, little information is available for understanding the genetic characteristics of *B. japonica*. The lack of complete mitochondrial genome has limited the development of population genetic diversity and molecular evolution for this species.

The family Bramidae is a group of marine fishes, which is widespread and occurring in all tropical and temperate seas. Due to their characteristic body shapes, relatively large heads and high meristic counts of vertebrae and fin rays easy identifiably, scientists can separate 22 recent species into seven genera within two subfamilies (Nelson, 1994). The classification of these seven genera remains in doubt and question, together with that of the origin of the group, deserve further study.

In this study, the complete mitogenome sequence of *B. japonica* is described as well as the molecular phylogenetic relationship of *B. japonica* with other 23 species within Perciformes. This study provides insights into the identification, evolution, phylogeny and conservation genetics of *B. japonica* and related species.

2. Materials and methods

2.1. Sampling and genomic DNA extraction

Our project is approved by East China Sea Fisheries Research Institute. The specimens of *B. japonica* are collected from South China Sea (11°23′N, 114°33′E). Muscle tissues are sampled and stored in 95% ethanol at room temperature. Genomic DNA is extracted using Animal Genomic DNA Extraction Kit (TIANGEN) according to manufacturer's protocol and visualized on 1% agarose gel.

2.2. Primers design, PCR amplification and DNA sequencing

A total of 17 pairs of primers are designed according to the multiple alignments of complete mitochondrial genome sequences of four closely related fish species including *Taractes asper*, *Taractes rubescens*, *Taractichthys steindachneri* and *Eumegistus illustris*. Besides, two pairs of universal PCR primers are used to amplify COI and 16S rRNA genes. The overlaps of two near amplicons are between 80 and 539 bp. The complete mitochondrial genome of *B. japonica* is obtained by assembling of all sequences produced by the 19 pairs of primers (Table 1).

Polymerase chain reaction (PCR) is performed on a Peltier Thermal Cycler in 25 μl total volume, which includes 0.75 μl each primer (10 μM), 2.0 μl dNTP (2.5 μM), 2.5 μl 10 × PCR buffer (Mg^{2+} plus), 2.5 U *Taq* polymerase, 17.5 μl sterilized distilled water and approximately 1 μl template DNA under the following conditions: one cycle of denaturation at 94 °C for 5 min; 35 cycles of 30 s at 94 °C, 45 s at a primer-specific annealing temperature, and 1.5 min at 72 °C. Finally, products are extended for 7 min at 72 °C. PCR products are separated on 1.5% agarose gels. After recovered and purified, PCR products are directly sequenced in both directions using ABI Prism 3730 automated DNA sequencer (PE Corporation). DNA sequences are edited and assembled by DNAstar software.

2.3. Gene identification and analysis

The mitochondrial genome map is constructed using the CG View server (Grant and Paul, 2008) (http://www.stothard.afns.ualberta.ca/cgview_server/). Thirteen Protein-coding genes, two ribosomal RNA genes and non-coding regions are determined by sequence comparisons with the known mitochondrial genomes of the closely related species, including *Taractes rubescens* (Liu et al., 2016), *Taractichthys steindachneri* (Li et al., 2016) and *Taractes asper*. Protein coding genes are translated into amino acid sequences using the software MEGA 4.0 (Tamura et al., 2007) to confirm whether the amplified

Table 1
Primers used for amplifying the complete mitochondrial genome of *Brama japonica*.

Primer name	Primer sequence	T_m (°C)	Amplification length
CFF	5′CRACYAAYCAYAAAGAYATYGGCA3′	52	680
CFR	5′ACTTCWGGGTGRCCRAAGAATCA3′		
16BF	5′GCCTGTTTATCAAAAACAT3′	50	613
16BR	5′CCGGTCTGAACTCAGATCACGT3′		
1BJ-F	5′ TAGTTCCCACTGACTCCTTGC 3′	52	1355
1BJ-R	5′ GCTGAGTAAGCGGTGGATTGT 3′		
2BJ-F	5′ CCTCTATCGGCTCACTAATCTC3′	52	1388
2BJ-R	5′ TAAGTCATCGGGTTGTAGGG 3′		
3BJ-F	5′ GGGGAACCTTGAAACTGACC3′	50	811
3BJ-R	5′ AGTGGAATCAGATGGCAAGG 3′		
4BJ-F	5′ ACACGCATACCACATAGTTGA 3′	49	1270
4BJ-R	5′ GTGGGAGTCATTAGGCAGTT 3′		
5BJ-F	5′ AAAATCCCTAATCGCCTACT 3′	47	1149
5BJ-R	5′ GATTATGGCAATGAGGAAAA 3′		
6BJ-F	5′ ATCACCACCTGAAACTGAATAA 3′	48	1307
6BJ-R	5′ GCCGAGGATTGAGACGATAA 3′		
7BJ-F	5′ CTGATAAGACTTGCGGGATA 3′	51	641
7BJ-R	5′ AAGAGGGAGGCAGTAGTCAG 3′		
8BJ-F	5′ GTTCCATTGAAATCGGCTCT 3′	50	1288
8BJ-R	5′ TCGTGCCATTCATACAGGTC 3′		
9BJ-F	5′ CAACGGACCGAGTTACCCTA 3′	55	896
9BJ-R	5′ CCGCCTGTAAAGATAATGGTGT 3′		
10BJ-F	5′ CGTCCGCCCTTCAACTTCCT 3′	52	1518
10BJ-R	5′ TTCCGCAGTTGGGTTTGGTT 3′		
11BJ-F	5′ TGCTCACAGACCGAAACCTA 3′	53	995
11BJ-R	5′ GGAAGTGGCAGAGTGGATGA 3′		
12BJ-F	5′ CAACTTCCCTCACCACTAACC 3′	52	1540
12BJ-R	5′ GTTGCTATTAGTGGCAGGAC 3′		
13BJ-F	5′ TCAATGAAAACAACCCGACA 3′	48	950
13BJ-R	5′ TGCCCTCCCTCCTCTACTTC3′		
14BJ-F	5′ AGGGAGAAGTAGAGGAGGGA 3′	50	1598
14BJ-R	5′ ACTTCGGCTCATTACTTGGA 3′		
15BJ-F	5′ AAAACCCAAGCACTAATCACG 3′	50	941
15BJ-R	5′ TGACGGTAGCACCTCAGAAT 3′		
16BJ-F	5′ CCCCGTTTCCCAACTCTTATTT 3′	53	2032
16BJ-R	5′ CGCTTTACGCCGATGTCTGTC 3′		
17BJ-F	5′CGTTCCCTTACGGTACTTTC3′	54	1071
17BJ-R	5′CTATAACTAGGTTCGGTAGGTCTG 3′		

domains are functional with no frame-shifting or no premature stop codons. The codon usage of protein-coding genes and the nucleotide composition of the mitochondrial genome are also determined using MEGA 4.0. Most tRNA genes are identified by their proposed clover-leaf secondary structure and anti-codons using the web-based tRNA-scan SE 1.21 program (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997) with default search mode. L-strand origin (O_L) is identified by sequence homology analysis. The secondary structure of the O_L is identified using Mfold web server (Zuker, 2003) and RNA structure 4.5 (Mathews et al., 1999). The complete mitochondrial genome DNA sequence is deposited into the GenBank database using the software Sequin (http://www.ncbi.nlm.nih.gov/Sequin/).

2.4. Phylogenetic relationship analysis

In order to evaluate the phylogenetic position of *B. japonica* among Perciformes, a total of 10,885 bp sequence data representing 12 concatenated protein-coding genes are used for phylogenetic analysis. Gene ND6 is not used for phylogenetic analysis due to its high heterogeneity and poor phylogenetic performance (Miya and Nishida, 2000). The complete mitochondrial genomes of other 23 fish species are downloaded from GenBank database, including *Auxis rochei*, *Auxis thazard*, *Eumegistus illustris*, *Euthynnus affinis*, *Euthynnus alletteratus*, *Gasterochisma melampus*, *Gymnosarda unicolor*, *Katsuwonus pelamis*, *Pteraclis aesticola*, *Ruvettus pretiosus*, *Scomberomorus cavalla*, *Scomberomorus niphonius*, *Taractes asper*, *Taractes rubescens*, *Taractichthys steindachneri*, *Thunnus alalunga*, *Thunnus albacores*, *Thunnus atlanticus*, *Thunnus maccoyii*, *Thunnus obesus*, *Thunnus orientalis*, *Thunnus thynnus*, and *Thunnus tonggol*. *Cyprinus carpio* is used as outgroup for analysis.

Twelve protein-coding genes are aligned by clustal W in MEGA 5.1 with default settings and saved to a single multiple sequence alignment. Then, the RAXML web-servers (http://embnet.vital-it.ch/raxml-bb/index.php) are employed to format and analyze these sequences alignments (Stamatakis et al., 2008). The model CAT is selected for estimating the evolutionary rate of the 12 protein-coding genes. Maximum likehood (ML) analysis is conducted after bootstraps. Finally, the phylogenetic tree is viewed and edited by the software FigTree v1.4.2.

3. Results and discussion

3.1. Mitochondrial DNA genome structure

The complete mitochondrial genome of *B. japonica* is 17,009 bp in size, including 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a putative control region. The gene order and GC content of mitochondrial genome are shown in Fig. 1. There is a large non-coding region spanning 1408 bp between genes $tRNA^{Phe}$ and $tRNA^{Pro}$ with a high A + T content that is identified as a putative control region (Table 2). The complete genome sequence is deposited in GenBank database under the accession number KT908039. The genome organization and gene order are similar to those of other fishes (Hurst et al., 1999; Miya et al., 2001; Cheng et al., 2010), but different from those of *Scylla paramamosain* (Ma et al., 2013) and *Eleginops maclovinus* (Papetti et al., 2007). Twenty-eight genes are encoded by heavy strand (H-strand), while the other ones are encoded by light strand (L-strand). Six overlaps are detected, of which five are on H-strand, and one is on L-strand. Meanwhile, ten intergenic spacers are found, with five on H-strand, five on L-strand, respectively. The total length of overlaps and intergenic spacers are 24 bp and 62 bp, ranging from 1 to 10 bp and from 1 to 35 bp, respectively. The longest overlap (10 bp) occurs between genes ATP8 and ATP6, while the biggest intergenic spacer (35 bp) locates between genes $tRNA^{Asn}$ and $tRNA^{Cys}$.

The overall A + T content of the mitochondrial genome is 51.93% (Table 3) that is similar to those determined in other fishes, such as *Channel catfish*, *Trachuru strachurus* and *Opsariichthys bidens* (Waldbieser et al., 2003; Takashima et al., 2006; Wang et al., 2007). The A + T content of control region and protein-coding genes are 61.22% and 47.13%. The overall base composition of the complete mitochondrial genome is 26.43% for A, 31.35% for C, 16.71% for G and 25.50% for T; while it is 24.79% for A, 33.70% for C, 15.58% for G and 25.93% for T in 13 protein-coding genes.

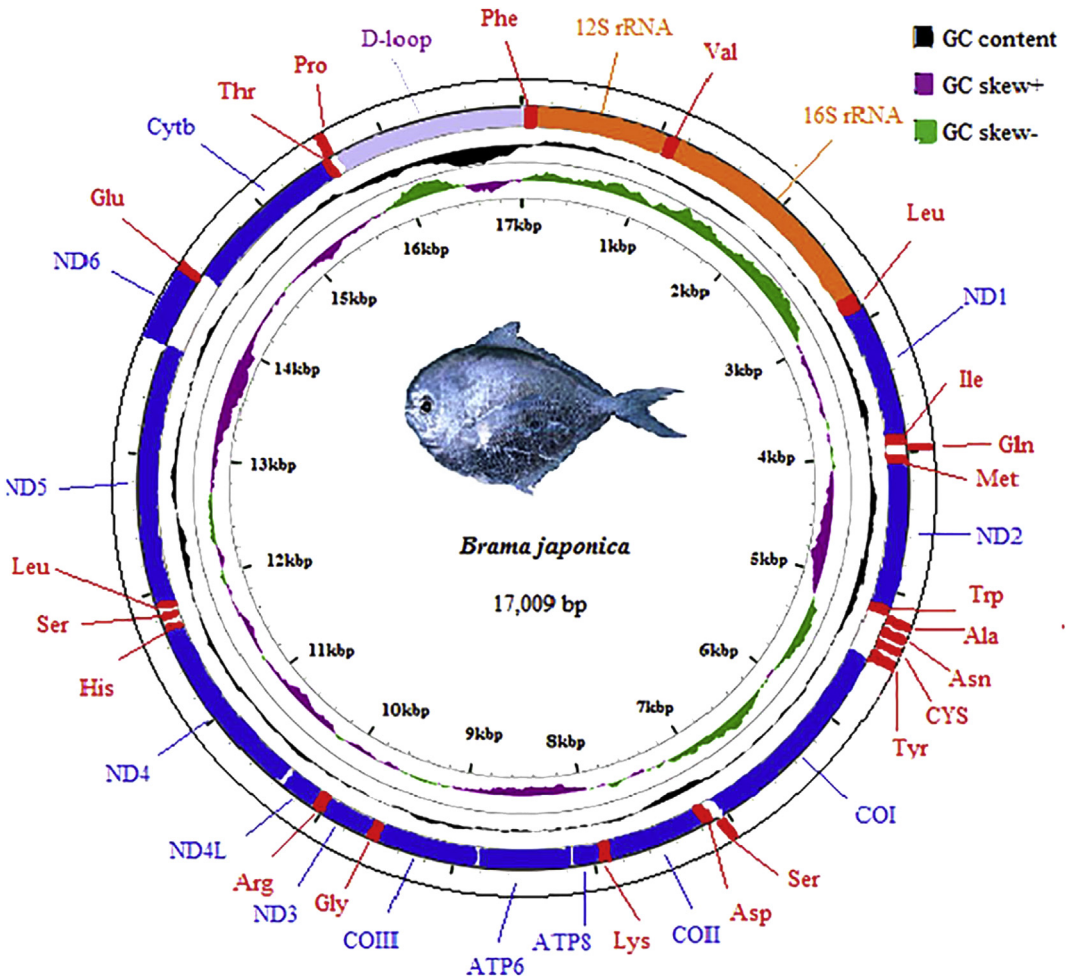

Fig. 1. Circular gene map of *Brama japonica* mitochondrion. Genes encoded on the heavy or light strands are shown inside or outside the circular gene map, respectively. The inner ring indicates the GC skew, the middle ring indicates the GC content. The figure was initially generated with GC viewer and modified manually. The abbreviations for the genes are as follows: COI, COII and COIII refer to the cytochrome oxidase subunits, Cytb refers to cytochrome *b*, and ND1-6 refers to NADH dehydrogenase components.

Table 2
Characteristics of the complete mitochondrial genome of *Brama japonica*.

Gene	Position (5′–3′)	Size (bp)	Codon			Anti-codon	Intergenic nucleotide[b] (bp)	Strand[c]
			Start	Stop[a]	Amino acid			
$tRNA^{Phe}$	1–68	68				GAA	0	H
12S rRNA	69–1026	958					0	H
$tRNA^{Val}$	1027–1098	72				TAC	0	H
16S rRNA	1099–2790	1692					0	H
$tRNA^{Leu\ (UUA)}$	2791–2865	75				TAA	0	H
ND1	2866–3840	975	ATG	TAA	325		4	H
$tRNA^{Ile}$	3845–3916	72				GAT	−1	H
$tRNA^{Gln}$	3986–3916	71				TTG	−1	L
$tRNA^{Met}$	3986–4054	69				CAT	0	H
ND2	4055–5100	1046	ATG	TA-	348		0	H
$tRNA^{Trp}$	5100–5170	71				TCA	1	H
$tRNA^{Ala}$	5240–5172	69				TGC	1	L
$tRNA^{Asn}$	5314–5242	73				GTT	35	L
$tRNA^{Cys}$	5350–5416	67				GCA	0	L
$tRNA^{Tyr}$	5483–5417	67				GTA	1	L
COI	5485–7035	1551	GTG	TAA	517		0	H
$tRNA^{Ser\ (UCA)}$	7107–7036	72				TGA	3	L
$tRNA^{Asp}$	7111–7183	73				GTC	8	H
COII	7192–7882	691	ATG	T-	230		0	H
$tRNA^{Lys}$	7883–7956	74				TTT	1	H
ATP8	7958–8125	168	ATG	TAA	56		−10	H
ATP6	8116–8798	683	ATG	TA-	227		0	H
COIII	8799–9583	785	ATG	TA-	261		0	H
$tRNA^{Gly}$	9584–9654	71				TCC	0	H
ND3	9655–10,003	349	ATG	T-	116		0	H
$tRNA^{Arg}$	10,004–10,072	69				TCG	0	H
ND4L	10,073–10369	297	ATG	TAA	99		−7	H
ND4	10,363–11,743	1381	ATG	T–	460		0	H
$tRNA^{His}$	11,744–11,813	70				GTG	0	H
$tRNA^{Ser\ (AGC)}$	11,814–11,881	68				GCT	4	H
$tRNA^{Leu\ (CUA)}$	11,886–11,958	73				TAG	0	H
ND5	11,959–13,797	1839	ATG	TAG	613		−4	H
ND6	14,315–13,794	522	ATG	TAG	174		0	L
$tRNA^{Glu}$	14,384–14,316	69				TTC	4	L
Cyt*b*	14,389–15,529	1141	ATG	T–	380		0	H
$tRNA^{Thr}$	15,530–15601	72				TGT	−1	H
$tRNA^{Pro}$	15,670–15601	70				TGG	0	L
D-loop	15,602–17,009	1408						–

[a] TA- and T– represent incomplete stop codons.
[b] Numbers correspond to the nucleotides separating adjacent genes. Negative numbers indicate overlapping nucleotides.
[c] H and L indicate that the gene is encoded by the H or L strand.

3.2. Protein-coding gene features

B. japonica mitochondrial genome encodes 13 protein-coding genes with 11,407 bp in length that accounts for 67.06% of the complete mitogenome. The lengths of protein-coding genes ranges in size from 168 (ATP8) to 1839 bp (ND5) and encodes a total of 3806 amino acids. Among 13 protein-coding genes, four overlaps occur on the same strand, whereas one presents on the opposite strands.

Base composition of protein coding genes is shown in Table 3. Of the 13 protein-coding genes, twelve employ ATG as start codons, while COI utilizes GTG as start codon (Table 2). COI gene usually uses ATG as start codon in other animals, such as *Larimichthys crocea*, *Collichthys lucidus* and *Charybdis feriata* (Cui et al., 2009; Cheng et al., 2012; Ma et al., 2015b). With regards to stop codon, four genes (COI, ATP8, ND1, and ND4L) use TAA, two genes (ND5 and ND6) use TAG, and the remaining seven genes (ND2, ATP6, COIII, COII, ND3, ND4, and Cytb) end with incomplete codons. Termination codons seem to have a tendency to be variable in fish mitogenomes (Kim et al., 2004; Peng et al., 2006). This feature is common among vertebrate mitochondrial protein-coding genes, and these incomplete stop codons are presumably due to post-transcriptional modifications during the mRNA maturation process such as polyadenylation (Ojala et al., 1981).

3.3. Transfer and ribosomal RNA gene features

The complete mitochondrial genome contains 22 tRNA genes, which can fold into canonical clover-leaf secondary structures except $tRNA^{Ser}$ (AGC) whose paired "DHU" arm is missing (File S1). This incomplete $tRNA^{Ser}$ (68 bp) structure has also been found from mitogenomes of other animals such as *Scylla paramamosain* (Ma et al., 2013), *Pseudolabrus sieboldi* (Oh

Table 3
The base composition in different regions of the mitochondrial genome of *Brama japonica* (the genes which are encoded by the L-strand are converted to complementary strand sequences).

Region	Base composition (%)				A + T content (%)
	A	C	G	T	
Protein-coding gene					
ND1	22.87	34.97	16.41	25.74	48.61
ND2	23.61	39.48	13.38	23.52	47.13
COI	23.79	28.37	18.89	28.95	52.74
COII	27.21	29.52	15.92	27.35	54.56
ATP8	30.95	35.12	11.90	22.02	52.97
ATP6	24.45	33.38	13.03	29.14	53.59
COIII	23.57	33.25	17.58	25.61	49.18
ND3	19.20	35.53	15.47	29.80	49.00
ND4L	21.21	34.68	15.82	28.28	49.49
ND4	23.90	35.41	15.35	25.34	49.24
ND5	26.92	33.33	14.36	25.39	52.31
ND6	35.25	36.40	15.33	13.03	48.28
Cytb	23.40	33.48	15.16	27.96	51.36
Overall of Protein-coding gene	24.79	33.70	15.58	25.93	50.72
tRNA gene					
$tRNA^{Phe}$	33.82	20.59	25.00	20.59	54.41
$tRNA^{Val}$	34.72	22.22	18.06	25.00	59.72
$tRNA^{Leu\ (UUA)}$	24.00	28.00	25.33	22.67	46.67
$tRNA^{Ile}$	22.22	29.17	27.78	20.83	43.05
$tRNA^{Gln}$	25.35	15.49	26.76	32.39	57.74
$tRNA^{Met}$	31.88	21.74	13.04	33.33	65.21
$tRNA^{Trp}$	29.58	28.17	26.76	15.49	45.07
$tRNA^{Ala}$	26.09	15.94	26.09	31.88	57.97
$tRNA^{Asn}$	17.81	20.55	32.88	28.77	46.58
$tRNA^{Cys}$	17.91	25.37	32.84	23.8823.8	41.79
$tRNA^{Tyr}$	25.37	29.85	20.90	8	49.25
$tRNA^{Ser\ (UCA)}$	19.44	19.44	33.33	27.78	47.22
$tRNA^{Asp}$	28.77	19.18	24.66	27.40	56.17
$tRNA^{Lys}$	32.43	27.03	20.27	20.27	52.70
$tRNA^{Gly}$	33.80	22.54	16.90	26.76	60.56
$tRNA^{Arg}$	33.33	17.39	20.29	28.99	62.32
$tRNA^{His}$	35.71	17.14	18.57	28.57	64.28
$tRNA^{Ser\ (AGC)}$	25.00	30.88	25.00	19.12	44.12
$tRNA^{Leu\ (CUA)}$	31.51	24.66	20.55	23.29	54.80
$tRNA^{Glu}$	23.29	14.49	24.64	36.23	59.52
$tRNA^{Thr}$	18.06	34.72	26.39	20.83	49.25
$tRNA^{Pro}$	24.29	12.86	28.57	34.29	58.58
Overall of tRNA gene	29.77	26.30	20.64	23.28	53.05
rRNA gene					
16S rRNA	31.97	26.48	20.45	21.10	53.07
12S RNA	29.12	29.33	21.61	19.94	49.06
Overall of rRNA gene	30.94	27.51	20.87	20.68	51.62
Control region	28.20	25.00	13.78	33.03	61.22
Overall of the genome	26.43	31.35	16.71	25.50	51.93

et al., 2008) and *Acraea issoria* (Hu et al., 2010). Fourteen tRNA genes are encoded by H-strand, while the remaining eight are encoded by L-strand. These 22 tRNA genes are totally 1555 bp in length and intersperse between the rRNA and the protein-coding genes with the ranges from 68 ($tRNA^{His}$) to 75 bp ($tRNA^{Val}$). Both $tRNA^{Leu}$ and $tRNA^{Ser}$ have two forms UUA/CUA and UCA/AGC, respectively. A total of 15 unmatched base pairs are found in stem regions, including A-C in $tRNA^{Arg}$, $tRNA^{Cys}$, $tRNA^{Leu(UUA)}$, $tRNA^{Lys}$, $tRNA^{Ser(AGC)}$, $tRNA^{Ser(UCA)}$, $tRNA^{Trp}$, and $tRNA^{Val}$; C-C in $tRNA^{Leu(CUA)}$ and $tRNA^{Thr}$; U-U in $tRNA^{Met}$ and $tRNA^{Thr}$; U-C in $tRNA^{Ile}$; G-A in $tRNA^{Phe}$; A-A in $tRNA^{Ser(AGC)}$. The overall A + T content of 22 tRNAs is 53.05%, with the biggest rate (65.21%) for $tRNA^{Met}$ and the lowest rate (41.79%) for $tRNA^{Cys}$. Aberrant tRNA can work in a similar way as usual tRNA in the ribosome by adjusting its structural conformation (Ohtsuki et al., 2002).

The 16S and 12S ribosomal RNA genes are 1340 bp and 869 bp in length. They locate on H-strand between genes $tRNA^{Leu(UUR)}$ and $tRNA^{Phe}$, separated by gene $tRNA^{Val}$. The 12S gene contains a remarkable Poly C (13 cytosine) structure, which is larger than the same structure from other Bramidae species. The A + T content is 53.07% for 16S rRNA gene (A = 31.97%; G = 20.45%; T = 21.10%; C = 26.48%), and 49.06% for 12S rRNA gene (A = 29.12%; G = 21.61%; T = 19.94%; C = 29.33%), respectively. The base composition of the two rRNA genes is 30.94% for A, 27.51% for C, 20.87% for G and 20.68% for T (Table 3).

3.4. Non-coding region

A total of 12 non-coding regions are identified in the mitochondrial genome of *B. japonica*, including two large non-coding regions: light strand origin (O_L) and the control region. The other nine non-coding regions are all small, varying from 1 to 8 bp in length. As in most vertebrates, the putative origin of L-strand replication is located in a cluster of five tRNA genes (WANCY region) between $tRNA^{Asn}$ and $tRNA^{Cys}$. This region is 35 bp long and has the potential to fold into a stem-loop secondary structure, with a stem of 13 paired nucleotides and a loop of 12 bp nucleotides (Fig. 2). As described, the L-strand synthesis is likely initiated in a stretch of thymines in the O_L loop (Wong and Clayton, 1985). This condition is typical in tetrapods, whereas in fish the O_L loop contains a polypyrimidine tract (Hurst et al., 1999; Peng et al., 2003). On the other hand, C-rich loop is detected in Sciaenidae species and other reported teleost fishes (Johansen et al., 1990; Zardoya et al., 1995). C-rich or T-rich

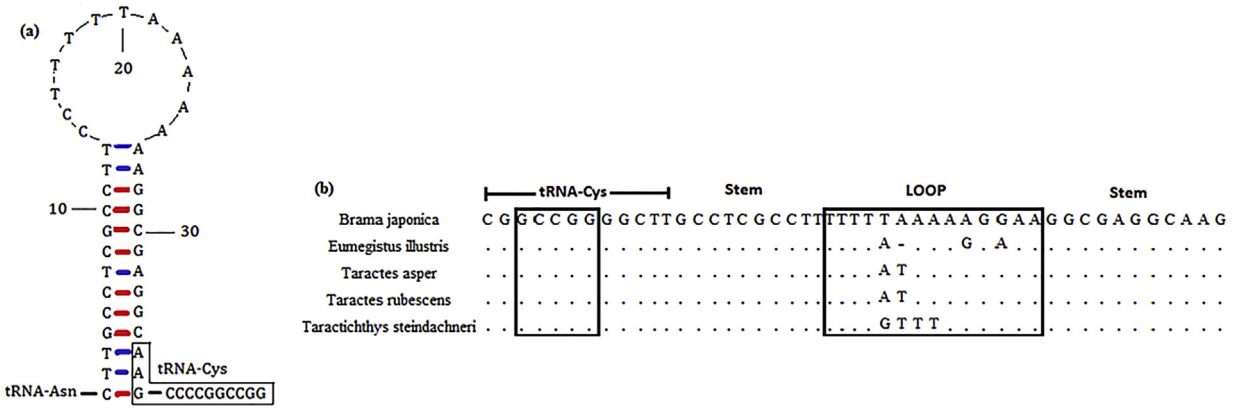

Fig. 2. A conserved secondary structure (a) as a putative replication origin (OL) in a non-coding region located between $tRNA^{Asn}$ and $tRNA^{Cys}$ of *Brama japonica*, and a comparison of nucleotide sequences of related species (b). The box represents the sequence of the loop of the OL secondary structure and the conserved motif 5′GCCGG3′ in the $tRNA^{Cys}$.

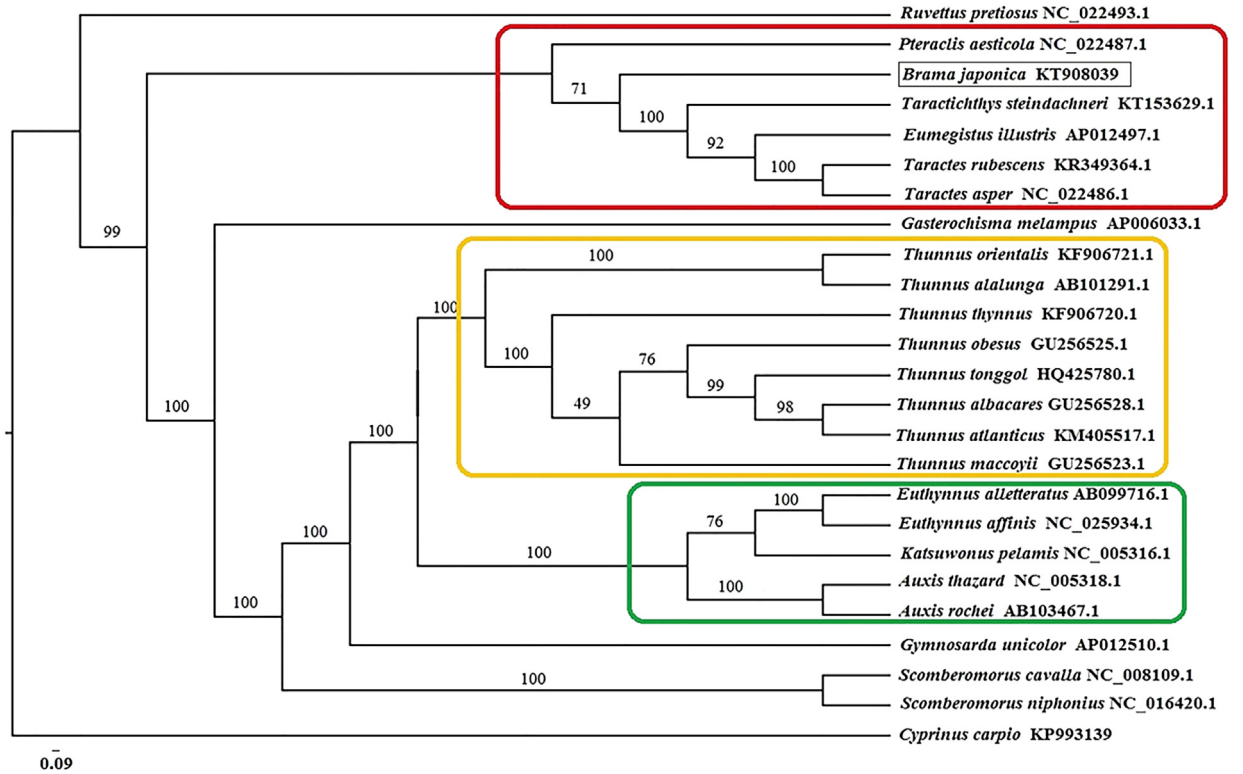

Fig. 3. Phylogenetic tree of *Brama japonica* within Perciform based on 12 protein-coding genes using maximum likelihood (ML) analysis.

loop may indicate that primer synthesis is most probably initiated by a polypyrimidine tract (Taanman, 1999). Furthermore, the conserved motif (5′-GCCGG-3′) is exactly shown at the base of the stem in $tRNA^{Cys}$, which is associated with the transition from RNA to DNA synthesis (Hixson et al., 1986). The O_L sequence in Bramidae mitogenomes has accordant stem region and complementary structure. However, slight variations are found in the loop sequences (Fig. 2). The conserved stem-loop structure indicates that it plays a key role in the replication origin of mitochondrial DNA (Desjardins and Morais, 1990).

The largest non-coding region is identified from *B. japonica* mitogenome based on nucleotide sequence comparison with control regions from other Bramidae fishes. It is located between genes 12S rRNA and $tRNA^{Ile}$ with a length of 833 bp. The A + T content of the control region is 61.22% (Table 3), which is higher than the average value of the whole mitochondrial genome (51.93%). Further, the nucleotide composition of the control region is 28.20% for A, 25.00% for C, 13.78% for G, and 33.03% for T, respectively.

The control region, characterized by discrete and conserved sequence blocks, possesses the typical tripartition with a terminal associated sequence (TAS), a central and conserved sequence block (CSB) domains containing the conserved sequence blocks CSB-F, CSB-E and CSB-D (Sbisà et al., 1997), and a variable domain consists of three conserved sequence blocks (CSB-1, CSB-2, CSB-3) (Brown et al., 1986; Jondeung and Karinthanyakit, 2016) (File S2). A TAS motif (TACATATATGTA) is found at the 5′ end of the control region. The TAS may work as a recognizable site for terminating the synthesis of the heavy strand (Cheng et al., 2010). Meanwhile, two central conserved sequence blocks (CSB-F and CSB-D) are detected in the control region, while the typical central conserved CSB-E is not found in *B. japonica*. They might add to the knowledge for examining the structure-function relationships of the control region (Cui et al., 2009). In addition, three conserved sequence blocks (CSB-1, CSB-2 and CSB-3) are identified at the 3′ end of the control region, which are thought to be associated with positioning RNA polymerase for both priming replication and transcription (Clayton, 1991; Shadel and Clayton, 1997).

3.5. Phylogenetic relationship analysis

To uncover the phylogenetic position of *B. japonica* among closely related fishes, a phylogenetic tree is constructed using the 12 concatenated protein-coding genes. The molecular phylogenetic tree using maximum-likehood (ML) analysis is shown in Fig. 3. As displayed from the tree topologies, we find that the 24 species from 14 genera are mainly divided into three well-defined clades which are boxed with color line. Six species from five genera under family Bramidea including *B. japonica*, *P. aesticola*, *E. illustris*, *T. steindachneri*, *T. asper* and *T. rubescens* form a monophyletic group. This result is identical to previous phylogeny studies by using the partial mitochondrial gene (Miya et al., 2013). Moreover, *B. japonica* is genetically closest to four Bramidae species (*E. illustris*, *T. steindachneri*, *T. asper* and *T. rubescens*) according to the phylogenetic tree.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2016.06.012.

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