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

Fenfang Chen, Hongyu Ma, Chunyan Ma, Heng Zhang, Mengdi Zhao, Yongyong Meng, Hongqing Wei, Lingbo Ma

In the present study, we isolated and characterized the complete mitochondrial genome sequence of Brama japonica by polymerase chain reaction (PCR) amplification and primer-walking sequencing. The complete DNA was 17,009 bp in length and contained a typical set of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a long putative control region. The gene organization and nucleotide composition of complete mitogenome were identical to those of other Bramidae fishes. In contrast, the 12S rRNA gene contained a big poly C structure which was larger than those from other Bramidae species. Of 37 genes, twenty-eight were encoded by heavy strand, while nine were encoded by light strand. Among the 13 protein-coding genes, twelve employed ATG as start codon, while only COI utilized GTG as start codon. In the control region, 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) were identified, while the typical central conserved CSB-F could not be detected in B. japonica. The putative OL region can fold into a conserved secondary structure and the conserved motif (5’-GCCGG-3’) was found at the base of the stem in tRNACys. The overall nucleotide composition of this genome was 26.43% for A, 16.71% for G, 31.35% for C, and 25.50% for T, with a high A+T content of 51.93%. From the NJ phylogenetic tree, we can find that B. japonica was together with other five Bramidae species formed a monophyletic group among 24 species. This work provided a set of useful data for studying on population genetic diversity and molecular evolution in Bramidae and related fish species.

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

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

Fenfang Chen [1, 2], Hongyu Ma [1], Chunyan Ma [1], Heng Zhang [1], Mengdi Zhao [1, 2], Yongyong Meng [1, 2], Hongqing Wei [1, 2], Lingbo Ma [1]

[1] East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China.

[2] College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai, 201306, China.

Corresponding authors:

Hongyu Ma, and Lingbo Ma

Jungong Road 300, Shanghai, 200090 China

Email addresses: Hongyu Ma, mahongyuhome@163.com; Lingbo Ma, malingbo@vip.sina.com

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

ABSTRACT In the present study, we isolated and characterized the complete mitochondrial genome sequence of *Brama japonica* by polymerase chain reaction (PCR) amplification and primer-walking sequencing. The complete DNA was 17,009 bp in length and contained a typical set of 13 protein-coding genes, 22 transfer RNA genes, 2 ribosomal RNA genes and a long putative control region. The gene organization and nucleotide composition of complete mitogenome were identical to those of other Bramidae fishes. In contrast, the 12S rRNA gene contained a big poly C structure which was larger than those from other Bramidae species. Of 37 genes, twenty-eight were encoded by heavy strand, while nine were encoded by light strand. Among the 13 protein-coding genes, twelve employed ATG as start codon, while only COI utilized GTG as start codon. In the control region, 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) were identified, while the typical central conserved CSB-F could not be detected in *B. japonica*. The putative OL region can fold into a conserved secondary structure and the conserved motif (5'-GCCGG-3') was found at the base of the stem in $tRNA^{Cys}$. The overall nucleotide composition of this genome was 26.43% for A, 16.71% for G, 31.35% for C, and 25.50% for T, with a high A+T content of 51.93%. From the NJ phylogenetic tree, we can find that *B. japonica* was together with other five Bramidae species formed a monophyletic group among 24 species. This work provided a set of useful data for studying on population genetic diversity and molecular evolution in Bramidae and related fish species.

Keywords *Brama japonica*; mitochondrial genome; gene arrangement; light-strand replication origin; phylogenetic relationship

INTRODUCTION

The complete mitochondrial genome DNA of fishes is a compact double-stranded and closed circular molecule that ranges in size approximate from 14 to 18 kbp and replicates and transcribes autonomously (*Wolstenholme, 1992; Boore, 1999*). Although gene rearrangements have been described in some organisms *(Miya & Nishida, 1999; Shao et al., 2001; Yuan & Zhaoxia, 2009*), the gene content and organization of mitochondrial genome in fishes are quite conserved. With few exceptions, all animal mitochondrial genomes generally encode 37 genes including 13 protein coding genes, 22 transfer RNA genes and two ribosomal RNA genes, as well as a A+T rich non-coding region (*Haring et al., 1999; Moritz et al., 2003*). Complete mitochondrial genome can not only provide more information than individual gene, but also show genome-level features 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 zebra fish (*Broughton et al., 2001*), hagfish (*Rasmussen et al., 1998; Delarbre et al., 2002*), sea lamprey (*Lee & Kocher, 1995*), basal ray-finned fish (*Noack et al., 1996*), cutlass fish (*Yuan & Zhaoxia, 2009*), lungfish (*Zardoya & Meyer, 1996a*), rainbow trout (*Zardoya et al., 1995*), deep sea fish (*Miya & Nishida, 1999a*), carp (*Chang et al., 1994*) and fresh water loach (*Tzeng et al., 1992*). 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

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

(*Moritz et al., 2003*). So far, mitochondrial DNA has been extensively applied in studies on population genetics (*Avise et al., 1984; Lee et al., 2010; Ma et al., 2011*), species identification (*Rubinoff et al., 2006; Gvoždík et al., 2010*) and phylogenetic relationship (*Rasmussen & Arnason, 1999; Miya & Nishida, 2000*).

The Pacific pomfret, *Brama japonica* Hilgendorf (Perciformes: Bramidae) is widely distributed in the North Pacific Ocean (*Brodeur, 1988; Bigelow et al., 2011; Neave & Hanavan, 2011*). *B. japonica* migrates seasonally between feeding grounds and spawning grounds. From 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 (*Brodeur, 1988; Watanabe et al., 2006; Neave & Hanavan, 2011*). It is known as an epipelagic fish and undergoing 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 is thought to play an important ecological role in oceanic food webs, due to it mainly feeds on small sized squids, shrimp and fishes and itself is important prey item of larger fishes such as the swordfish and the blue shark (*Pearcy et al., 1996; 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 (*Manzer, 1972; Yabu & Ishii, 1982; Seki & Mundy, 1991; William G. Pearcy, 1993*). By now, little information is available for understanding the genetic characteristics of *B. japonica*. Although the complete mitochondrial genome can bring more critical information for the study on genome evolution and species phylogeny, it remains unavailable in *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, we described the complete mitogenome sequence of the *B. japonica* coupled with identified the tRNA secondary structures, genome organization, nucleotide composition, gene arrangement and codon usage. Moreover, we uncovered the molecular phylogenetic relationship of *B. japonica* with other 23 species within Perciformes. This study will provide insight into population genetic structure, stock identification, evolution and phylogeny and conservation genetics of *B. japonica* and related species.

MATERIALS AND METHODS

Sampling and genomic DNA extraction

Our project was approved by East China Sea Fisheries Research Institute without approval number. The specimens of *B. japonica* were collected from South China Sea (11°23'N, 114°33'E). Muscle tissues were sampled and stored in 95% ethanol at room temperature. Genomic DNA was extracted using Animal Genomic DNA Extraction Kit (TIANGEN) according to manufacturer's protocol and visualized on 1% agarose gel.

Primers design, PCR amplification and DNA sequencing

We designed a total of 17 pairs of primers 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 were used to amplify sequences of COI and 16S rRNA genes. The complete mitochondrial genome of *B. japonica* was obtained by assembling of all sequences produced by the 19 pairs of primers (Table1).

Polymerase chain reaction (PCR) was performed on a Peltier Thermal Cycler in 25 µl total volume, which included 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 were extended for 7 min at 72°C. The PCR products were separated on 1.5% agarose gels. After recovered and purified, the PCR products were directly sequenced in both directions using ABI Prism 3730 automated DNA sequencer (PE Corporation). Editing and assembly of the sequenced DNA fragments were carried out by DNAstar software.

Gene identification and analysis

A gene organization map of *B. japonica* mitochondrial genome was constructed using the CG View server (*Grant & Paul, 2008*) (http://www.stothard.afns.ualberta.ca/cgview_server/). Thirteen Protein-coding genes, two ribosomal RNA genes and non-coding regions were

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

determined by sequence comparisons with the known mitochondrial genomes of the closely related species, including *Taractes rubescens* (*Liu et al., 2015*), *Taractichthys steindachneri* (*Li et al., 2015*) and *Taractes asper*. Protein coding genes were translated into amino acid sequences using the software MEGA 4.0 (*Tamura et al., 2007*) to confirm whether the amplified 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 were also determined using MEGA 4.0. Most tRNA genes were 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 & Eddy, 1997*) with default search mode. L-strand origin (O_L) and the control region (CR) were identified by sequence homology analysis. The secondary structure of the O_L was identified using Mfold web server (*Zuker, 2003*) and RNA structure 4.5 (*Mathews et al., 1999*). The complete mitochondrial genome DNA sequence was deposited into the GenBank database using the software Sequin (http://www.ncbi.nlm.nih.gov/Sequin/).

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 except ND6 of complete mitochondrial genome was used for phylogenetic analysis. Gene ND6 was not used for phylogenetic analysis due to its high heterogeneity and poor phylogenetic performance (*Miya & Nishida, 2000*). The complete mitochondrial genomes of other 23 fish species were 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* was used as an outgroup for the analyses. The phylogenetic tree was reconstructed using neighbor-joining (NJ) algorithms in MEGA 4.0 software with 1000 bootstrap replicates (*Zardoya & Meyer, 1996*).

RESULTS AND DISCUSSION

Mitochondrial DNA genome structure

The complete mitochondrial genome of *B. japonica* was 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 were shown in Fig. 1. There was a large non-coding region spanning 1408bp between genes $tRNA^{Phe}$ and $tRNA^{Pro}$ with a high A+T content that was identified as a putative control region (Table 2). The complete genome sequence was deposited in GenBank database under the accession number KT908039. The genome organization and gene order of mitochondrial of *B. japonica* were 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*), *Eleginops Maclovinus* (*Papetti et al., 2007*) and *Gonostoma gracile* (*Miya & Nishida, 1999b*). Twenty-eight genes were encoded by heavy strand (H-strand), while the other genes were encoded by light strand (L-strand). Six overlaps were detected in this genome, of which five were on H-strand, one was on L-strand. Meanwhile, ten intergenic spacers were found, with five on H-strand, five on L-strand, respectively. The total length of overlaps and intergenic spacers were 24 bp and 62 bp, ranging from 1 to 10 bp and from 1 to 35 bp per location, respectively. The longest overlap (10 bp) occurred between genes ATP8 and ATP6, while the biggest intergenic spacer (35 bp) located between genes $tRNA^{Asn}$ and $tRNA^{Cys}$.

The overall A+T content of this mitochondrial genome was 51.93% (Table 3) that was similar to those determined in other fishes, such as *Crossostoma lacustre*, *Channel Catfish*, *Trachurus trachurus* and *Opsariichthys bidens* (*Tzeng et al., 1992; Waldbieser et al., 2003; Takashima et al., 2006; Wang et al., 2007*). The A+T content of control region and protein-coding genes were 61.22% and 47.13% respectively. The overall base composition of the complete mitochondrial genome was 26.43% for A, 31.35% for C, 16.71% for G and 25.50% for T; while it was 24.79% for A, 33.70% for C, 15.58% for G and 25.93% for T in 13 protein-coding genes.

Protein-coding gene features

The boundaries between protein-coding genes of the mitochondrial genome were determined by aligning their sequences and identifying translation initiation and termination codons with comparison to those of Bramidae fishes. *B. japonica* mitochondrial genome encoded 13 protein-coding genes with 11,407 bp in length, which accounted for 67.06% of the complete mitogenome. The lengths of protein-coding genes ranged in size from 168 (ATP8) to 1839 bp (ND5) and coded a total of 3806 amino acids. Among 13 protein-coding genes, four overlaps occurred on the same strand, whereas one presented on the opposite strands.

Base composition of this mitochondrial protein coding genes was shown in Table 3. Of the

13 protein-coding genes, twelve employed ATG as start codons, while COI utilized GTG as start codon (Table 2). COI gene was reported to use ATG as start codon in other animals, such as *Larimichthys crocea*, *Collichthys niveatus*, *Collichthys lucidus* and *Charybdis feriata* (*Cui et al., 2009; Cheng et al., 2012; Jiao et al., 2012; Ma et al., 2015*). With regards to stop codon, four genes (COI, ATP8, ND1, ND4L) used TAA, two genes (ND5, ND6) used TAG, and the remaining seven genes (ND2, ATP6, COIII, COII, ND3, ND4, Cytb) ended 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*).

Transfer and ribosomal RNA gene features

The complete mitochondrial genome contained 22 tRNA genes, which can fold into canonical clover-leaf secondary structures except $tRNA^{Ser}$ (AGC) whose paired "DHU" arm was missing (Fig. 2). This incomplete $tRNA^{Ser}$ (68bp) structure has also been found from mitogenomes of other animals such as *Scylla paramamosain* (*Ma et al., 2013*), *Pseudolabrus sieboldi* (*Oh et al., 2008*) and *Acraea issoria* (*Hu et al., 2010*). Fourteen tRNA genes were encoded by H-strand, while the remaining eight were encoded by L-strand. These 22 tRNA genes were totally 1555 bp in length and interspersed between the rRNA and the protein-coding genes with the ranges from 68 bp ($tRNA^{His}$) to 75 bp ($tRNA^{Val}$). Both $tRNA^{Leu}$ and $tRNA^{Ser}$ had two forms UUA/CUA and UCA/AGC respectively. A total of 15 unmatched base pairs were 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 was 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 tRNAs in the ribosome by adjusting its structural conformation (*Ohtsuki et al., 2002*).

The 16S and 12S ribosomal RNA genes were 1340bp and 869 bp in length. They located on the H-strand between genes tRNA$^{Leu\ (UUR)}$ and tRNA^{Phe}, separated by gene tRNA^{Val}. The 12S gene contained a remarkable Poly C (13 cytosine) structure, which was larger than the same structure from other Bramidae species. The A+T content was 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 was 30.94% for A, 27.51% for C, 20.87% for G and 20.68% for T (Table 3).

Non-coding region

A total of 12 non-coding regions were identified in the mitochondrial genome of *B. japonica*, including two larger non-coding regions light strand origin (O_L) and the control region (CR). The other nine non-coding regions were all small varying from 1 to 8 bp in length. As in most vertebrates, the putative origin of L-strand replication was located in a cluster of five tRNA genes (WANCY region) between tRNA^{Asn} and tRNA^{Cys}. This region was 35 bp long and had 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. 3). As described, the L-strand synthesis is likely initiated in a stretch of thymines in the O_L loop (*Wong & 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 was detected in Sciaenidae species and other reported teleost fishes (*Johansen et al., 1990; Zardoya et al., 1995*). C-rich or T-rich loop may indicate that primer synthesis is most probably initiated by a polypyrimidine tract (*Taanman, 1999*). Furthermore, the conserved motif (5'-GCCGG-3') was exactly shown at the base of the stem in $tRNA^{Cys}$, which was 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 were found in the loop sequences (Fig. 3). The conserved stem-loop structure indicated that it played a key role in the replication origin of mitochondrial DNA (*Desjardins and Morais, 1990*).

We identified the largest non-coding region (control region) from *B. japonica* mitogenome based on nucleotide sequence comparison with control regions from other Bramidae fishes. It was located between genes 12S rRNA and $tRNA^{Ile}$ with a length of 833 bp. The A +T content of the control region were 61.22% (Table 3), which was higher than the average value of the whole mitochondrial genome (51.93%). Further, the nucleotide composition of the control region was 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, possessed 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) which were determined by multiple homologous sequence alignment with other vertebrates (*Brown et al., 1986; Jondeung & Karinthanyakit, 2015*) (Fig. 4). A TAS motif (TACATATATGTA) was 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) were detected in the control region, while the typical central conserved CSB-E could not be found in the *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) were identified at the 3' end of the control region, which were thought to be associated with positioning RNA polymerase for both priming replication and transcription (*Clayton, 1991; Shadel &Clayton, 1997*).

Phylogenetic relationship analysis

To uncover the phylogenetic position of *B. japonica* among closely related fishes, a phylogenetic tree was constructed using the 12 concatenated protein-coding genes. The molecular phylogenetic tree with high bootstrap supports was shown in Fig. 5. As displayed from the tree topologies, we can find that the 24 species from 14 genera were mainly divided into four well-defined clades. Six species from five genera under family Bramidea including *B. japonica*, *P. aesticola*, *E. illustris*, *T. steindachneri*, *T. asper* and *T. rubescens* formed a monophyletic group, this result is identical to previous phylogeny studies by using the partial mitochondrial gene (Miya et al., 2013). Moreover, *B. japonica* was genetically closest to four Bramidae species (*E. illustris*, *T. steindachneri*, *T. asper* and *T. rubescens*) according to the phylogenetic trees.

CONCLUSION

This study characterized and determined the complete mitochondrial genome of *B. japonica*, which was 17,009 bp in length, including a typical set of 37 genes: 13 protein-coding genes, 2 rRNA genes and 22 tRNA genes, as well as a putative control region. In the control region, we identified 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), while the typical central conserved CSB-F could not be detected. Phylogenetic analysis supported that *B. japonica* is genetically closer to four Bramidae species (*E. illustris*, *T. steindachneri*, *T. asper* and *T. rubescens*). This study should be helpful for studies on population genetic structure, stock identification, evolution and phylogeny and conservation genetics in *B. japonica* and related species.

FUNDING

This work was supported by the National Science & Technology Support Plan (2013BAD13B04), the National Basic Research Special Foundation of China (2013FY110700), the Special Scientific Research Funds for Central Non-Profit Institutes (2015M07), and the National Infrastructure of Fishery Germplasm Resources.

REFERENCES

Anderson S, Bankier AT, Barrell BG, De Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG. 1981. Sequence and organization of the human mitochondrial genome. *Nature* **290:** 457-465

Avise JC, Neigel JE, Arnold J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution* **20:** 99-105

Bigelow KA, Jones JT, Dinardo GT. 2011. Growth of the Pacific pomfret, *Brama japonica*: a comparison between otolith and length–frequency (MULTIFAN) analysis. *Canadian Journal of Fisheries & Aquatic Sciences* **52:** 2747-2756

Boore JL. 1999. Animal mitochondrial genomes. *Nucleic Acids Research* **27:** 1767-1780

Brodeur RD. 1988. Zoogeography and trophic ecology of the dominant epipelagic fishes in the northern North Pacific. *Bulletin of the Ocean Research Institute-University of Tokyo* (Japan).

Broughton RE, Milam JE, Roe BA. 2001. The complete sequence of the zebrafish (*Danio rerio*) mitochondrial genome and evolutionary patterns in vertebrate mitochondrial DNA. *Genome Research* **11:**1958-1967

Brown GG, Gadaleta G, Pepe G, Saccone C. Sbisà E. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. *Journal of Molecular Biology* **192:** 503–511

Chang YS, Huang FL, Lo TB. 1994. The complete nucleotide sequence and gene organization of carp (*Cyprinus carpio*) mitochondrial genome. *Journal of Molecular Evolution* **38:**138-155

Cheng Y, Wang R, Sun Y, Xu T. 2012. The complete mitochondrial genome of the small yellow croaker and partitioned Bayesian analysis of Sciaenidae fish phylogeny. *Genetics & Molecular Biology* **35:** 191-199

Cheng Y, Xu T, Ge S, Wang R. 2010. Complete mitochondrial genome of the miiuy croaker

Miichthys miiuy (Perciformes, Sciaenidae) with phylogenetic consideration. *Marine Genomics* **3:** 201-209

Clayton DA. 1991. Nuclear gadgets in mitochondrial DNA replication and transcription. *Trends in Biochemical Sciences* **16:** 107–111

Cui Z, Liu Y, Li CP, You F, Chu KH. 2009. The complete mitochondrial genome of the large yellow croaker, *Larimichthys crocea* (Perciformes, Sciaenidae): Unusual features of its control region and the phylogenetic position of the Sciaenidae. *Gene* **432:** 33–43

Delarbre C, Gallut C, Barriel V, Janvier P, Gachelin G. 2002. Complete mitochondrial DNA of the hagfish, *Eptatretus burgeri*: the comparative analysis of mitochondrial DNA sequences strongly supports the cyclostome monophyly. *Molecular Phylogenetics and Evolution* **22:** 184-192

Desjardins P, Morais R. 1990. Sequence and gene organization of the chicken mitochondrial genome. a novel gene order in higher vertebrates. *Journal of Molecular Biology* **212:** 599-634

Grant JR, Paul S. 2008. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Research* **36:** W181-W184

Gvoždík V, Moravec J, Klütsch C, Kotlík P. 2010. Phylogeography of the Middle Eastern tree frogs (Hyla, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics & Evolution* **55:**1146–1166

Haring E, Riesing MJ, Pinsker W, Gamauf A. 1999. Evolution of a pseudo-control region in the mitochondrial genome of *Palearctic buzzards* (genus *Buteo*). *Journal of Zoological*

Systematics and Evolutionary Research **37:** 185-194

Hikaru W, Tsunemi K, Shigeyuki K. 2003. Feeding habits of Pacific pomfret *Brama japonica* in the transition zone of the central North Pacific. *Fisheries Science* **69:** 269-276

Hixson JE, Wong TW, Clayton DA. 1986. Both the conserved stem-loop and divergent 5'-flanking sequences are required for initiation at the human mitochondrial origin of light-strand DNA-replication. *Journal of Biological Chemistry* **261:** 2384-90

Hu J, Zhang D, Hao J, Huang D, Cameron S, Zhu C. 2010. The complete mitochondrial genome of the yellow coaster, *Acraea issoria* (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini): sequence, gene organization and a unique tRNA translocation event. *Molecular Biology Reports* **37:** 3431-3438

Hurst CD, Bartlett SE, Davidson WS, Bruce IJ. 1999. The complete mitochondrial DNA sequence of the Atlantic salmon, *Salmo salar*. *Gene* **239:** 237–242

Cheng J, Ma G, Miao Z, Shui B, Gao T. 2012. Complete mitochondrial genome sequence of the spinyhead croaker *Collichthys lucidus* (Perciformes, Sciaenidae) with phylogenetic considerations. *Molecular Biology Reports* **39:** 4249-4259

Johansen S, Guddal PH, Johansen T. 1990. Organization of the mitochondrial genome of Atlantic cod, *Gadus morhua*. *Nucleic Acids Research* **18:** 411–419

Jondeung A, Karinthanyakit W. 2015. Mitochondrial DNA control region of three mackerels, genus *Rastrelliger*: structure, molecular diversity and phylogenetic relationship. *Mitochondrial DNA* **29:** 1-6

Kim Y, Kweon H, Kim I, Lee Y, Kim J, Lee J. 2004. The complete mitochondrial genome of the floating goby, *Gymnogobius petschiliensis* (Perciformes, Gobiidae). *Molecules and Cells* **17:** 446-453

Lebrasseur RJ. 2011. Stomach contents of blue shark (*Prionace glauca* L.) taken in the Gulf of Alaska. *Journal of the Fisheries Board of Canada* **21:** 861-862

Lee KE, Seddon JM, Corley SW, Ellis WAH, Johnston SD, De Villiers DL, Preece HJ, Carrick FN. 2010. Genetic variation and structuring in the threatened koala populations of Southeast Queensland. *Conservation Genetics* **11:** 2091-2103

Lee WJ, Kocher TD. 1995. Complete sequence of a sea lamprey (*Petromyzon marinus*) mitochondrial genome: early establishment vertebrate genome organization. *Genetics* **139:** 873-887

Li W, Tian S, Zhu J, Wu F, Wang K, Dai X. 2015. Characterization of the complete mitochondrial genome of Sickle pomfret *Taractichthys steindachneri* (Perciformes: Bramidae). *Mitochondrial DNA* **10:** 1-2

Liu X, Tian S, Li W, Wu F, Dai X. 2015. Complete mitochondrial genome of the *Taractes rubescens* (Perciformes: Bramidae). *Mitochondrial DNA* **10:** 1-2

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res **25:** 955-964

Ma H, Ma C, Li C, Lu J, Zou X, Gong Y, Wang W, Chen W, Ma L, Xia X. 2015. First mitochondrial genome for the red crab (*Charybdis feriata*) with implication of phylogenomics and population genetics. *Scientific Reports* **5:** 11524

Ma H, Ma C, Li X, Xu Z, Feng N, Ma L. 2013. The complete mitochondrial genome sequence and gene organization of the mud crab (*Scylla paramamosain*) with phylogenetic consideration. *Gene* **519:** 120–127

Ma H, Ma C, Ma L. 2011. Population genetic diversity of mud crab (*Scylla paramamosain*) in Hainan Island of China based on mitochondrial DNA. *Biochemical Systematics and Ecology* **39:** 434-440

Manzer JI. 1972. Length–weight relationship for pomfret. *Journal of the Fisheries Research Board of Canada* **29:** 1079-1081

Mathews D, Sabina J, M, Turner D. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology* **288:** 911–940

Miya M, ., Kawaguchi A, Nishida M. 2001. Mitogenomic exploration of higher teleostean phylogenies: a case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. *Molecular Biology and Evolution* **18:** 1993-2009

Miya M, Friedman M, Satoh TP, Takeshima H, Sado T, Iwasaki W, Yamanoue Y, Nakatani M, Mabuchi K, Inoue JG, Poulsen JY, Fukunaga T, Sato Y, Nishida M. 2013. Evolutionary origin of the scombridae (tunas and mackerels): members of a paleogene adaptive radiation with 14 other pelagic fish families. *Plos One* **8:** 130-130

Miya M, Nishida M. 1999. Organization of the mitochondrial genome of a deep-sea fish, gonostoma gracile (Teleostei: Stomiiformes): first example of transfer RNA gene rearrangements in bony fishes. *Marine Biotechnology* **1:** 416-426

Miya M, Nishida M. 2000. Use of mitogenomic information in teleostean molecular phylogenetics: a tree-based exploration under the maximum-parsimony optimality criterion. *Molecular Phylogenetics and Evolution* **17:** 437–455

Moritz C, And TED, Brown WM. 2003. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* **18:** 269-292

Neave F, Hanavan MG. 2011. Seasonal distribution of some epipelagic fishes in the Gulf of Alaska region. Journal of the Fisheries Research Board of Canada **17:** 221-233

Nelson JS. 1994. Fishes of the World, 3rd edn. John Wiley and Sons: New York.

Noack K, Zardoya R, Meyer A. 1996. The complete mitochondrial DNA sequence of the bichir (*Polypterus ornatipinnis*), a basal ray-finned fish: ancient establishment of the consensus vertebrate gene order. *Genetics* **144:** 1165-1180

Oh DJ, Kim SJ, Jung YH. 2008. Comparison of the mitochondrial genomes of East Asian Pseudolabrus fishes. *Mitochondrial DNA* **19:** 453-60

Ohtsuki T, Kawai G, Watanabe K. 2002. The minimal tRNA: unique structure of *Ascaris suum* mitochondrial tRNA$^{(Ser)(UCU)}$ having a short T arm and lacking the entire D arm. *Febs Letters* **514**: 37-43

Ojala D, Montoya J, Attardi G. 1981. TRNA punctuation model of RNA processing in human mitochondrial. *Nature* **290:** 470-474

Papetti C, Liò P, Rüber L, Patarnello T, Zardoya R. 2007. Antarctic fish mitochondrial genomes lack ND6 gene. *Journal of Molecular Evolution* **65:** 519-528

Pearcy W, Anma G, Meguro T. 1996. Species associations of epipelagic nekton of the North Pacific Ocean. *Fisheries Oceanography* **5:** 1–20

Peng Z, Chen Y Q, Hui Z, Wang XL, Qu LH. 2003. The complete mitochondrial genome of a relic salamander, *Ranodon sibiricus* (Amphibia: Caudata) and implications for amphibian phylogeny. *Molecular Phylogenetics and Evolution* **28:** 620-626

Peng Z, Wang J, He S. 2006. The complete mitochondrial genome of the helmet catfish *Cranoglanis bouderius* (Silurifonnes : Cranoglanididae) and the phylogeny of otophysan fishes. *Gene* **376:** 290-297

Rasmussen AS, Arnason U. 1999. Phylogenetic studies of complete mitochondrial DNA molecules place cartilaginous fishes within the tree of bony fishes. *Journal of Molecular Evolution* **48:** 118-123

Rasmussen AS, Janke A, Arnason U. 1998. The mitochondrial DNA molecule of the hagfish (*Myxine glutinosa*) and vertebrate phylogeny. *Journal of Molecular Evolution* **46:** 382-388

Rubinoff D, Cameron S, Will K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *Journal of Heredity* **97:** 581-594

Sbisà E, Tanzariello F, Reyes A, Pesole G, Saccone C. 1997. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. *Gene* **205:** 125-140

Seki MP, Mundy BC. 1991. Some notes on the early life stages of the Pacific pomfret, *Brama japonica*, and other Bramidae from the central north Pacific Ocean. *Japanese Journal of Ichthyology* **38:** 63-68

Shadel GS, Clayton DA. 1997. Mitochondrial DNA maintenance in vertebrates. *Annual Review of Biochemistry* **66:** 409-35

Shao R, Campbell NJ, Barker SC. 2001. Numerous gene rearrangements in the mitochondrial genome of the wallaby louse, *Heterodoxus macropus* (Phthiraptera). *Molecular Biology and Evolution* **18:** 858-865

Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochimica Et Biophysica Acta* **1410:** 103–123

Takashima Y, Morita T, Yamashita M. 2006. Complete mitochondrial DNA sequence of Atlantic horse mackerel *Trachurus trachurus* and molecular identification of two commercially important species *T. trachurus* and *T. japonicus* using PCR-RFLP. *Fisheries Science* **72:** 1054-1065

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology & Evolution* **24:** 1596-1599

Tzeng CS, Hui CF, Shen SC, Huang PC. 1992. The complete nucleotide sequence of the *Crossostoma lacustre* mitochondrial genome: Conservation and variation among vertebrates. *Nucleic Acids Research* **20:** 4853-4858

Waldbieser GC, Bilodeau AL, Nonneman DJ. 2003. Complete sequence and characterization of the channel catfish mitochondrial genome. *DNA Sequence* **14:** 265-277

Wang X, Wang J, He S, Mayden RL. 2007. The complete mitochondrial genome of the Chinese hook snout carp *Opsariichthys bidens* (Actinopterygii: Cypriniformes) and an alternative pattern of mitogenomic evolution in vertebrate. *Gene* **399:** 11–19

Watanabe H. 2004. Feeding habits of neon flying squid *Ommastrephes bartramii* in the transitional region of the central North Pacific. *Marine Ecology Progress* **266:** 173-184

Watanabe H, Kubodera T, Kawahara S. 2006. Summer feeding habits of the Pacific pomfret *Brama japonica* in the transitional and subarctic waters of the central North Pacific. *Journal of Fish Biology* **68:** 1436-1450

William G, Pearcy JPF, Mary M. 1993. Biology of the Pacific Pomfret (*Brama japonica*) in the North Pacific Ocean. *Canadian Journal of Fisheries & Aquatic Sciences* **50:** 2608-2625

Wolstenholme DR. 1992. Animal mitochondrial DNA: structure and evolution. *International Review of Cytology* **141:** 173–216

Wong TW, Clayton DA. 1985. In vitro replication of human mitochondrial DNA: accurate initiation at the origin of light-strand synthesis. *Cell* **42:** 951–958

Yabu H, Ishii K, 1982. Nuclear divisions in Pacific pomfret *Brama japonica* HILGENDORF. *Nippon Suisan Gakkaishi* **48:** 505-507

Yuan L, Zhaoxia C. 2009. Complete mitochondrial genome of the Asian paddle crab *Charybdis japonica* (Crustacea: Decapoda: Portunidae): gene rearrangement of the marine brachyurans and phylogenetic considerations of the decapods. *Molecular Biology Reports* **37:** 2559-2569

Zardoya R, Meyer A. 1996. The complete nucleotide sequence of the mitchondrial genome of the lungfish (*Protopterus dolloi*) supports its phylogenetic position as a close relative of land vertibrates. *Genetics* **142:** 1249-1263

Zardoya R, Garrido-Pertierra A, Bautista JM. 1995. The complete nucleotide sequence of the mitochondrial DNA genome of the rainbow trout, *Oncorhynchus mykiss*. *Journal of*

Molecular Evolution **41:** 942-951

Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research* **31:** 3406-3415

Table Caption

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

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

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).

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

Primer name	Forward (5’-3’)	Reverse (5’-3’)	T_m (°C)
CFF/CFR	5'CRACYAAYCAYAAAGAYATYGGCA 3'	5'ACTTCWGGGTGRCCRAAGAATCA3'	52
16F/16BR	5'GCCTGT TTATCA AAA ACAT 3'	5'CCGGTCTGAACTCAGATCACGT 3'	50
1BJ	5' TAGTTCCCACTGACTCCTTGC 3'	5' GCTGAGTAAGCGGTGGATTGT 3'	52
2BJ	5' CCTCTATCGGCTCACTAATCTC 3'	5' TAAGTCATCGGGTTGTAGGG 3'	52
3BJ	5' GGGGAACCTTGAAACTGACC 3'	5' AGTGGAATCAGATGGCAAGG 3'	50
4BJ	5' ACACGCATACCACATAGTTGA	5' GTGGGAGTCATTAGGCAGTT 3'	
5BJ	3'	5' GATTATGGCAATGAGGAAAA 3'	
6BJ	5' AAAATCCCTAATCGCCTACT	5' GCCGAGGATTGAGACGATAA 3'	49
7BJ	3'	5' AAGAGGGAGGCAGTAGTCAG 3'	47
8BJ	5' ATCACCACCTGAAACTGAATAA	5' TCGTGCCATTCATACAGGTC 3'	48
9BJ	3'	5' CCGCCTGTAAAGATAATGGTGT 3'	51
10BJ	5' CTGATAAGACTTGCGGGATA 3'	5' TTCCGCAGTTGGGTTTGGTT 3'	50
11BJ	5' GTTCCATTGAAATCGGCTCT 3'	5' GGAAGTGGCAGAGTGGATGA 3'	52
12BJ	5' CAACGGACCGAGTTACCCTA 3'	5' GTTGCTATTAGTGGCAGGAC 3'	52
13BJ	5' CGTCCGCCCTTCAACTTCCT 3'	5' TCCCGTATCCCAACTCCTAT 3'	53
14BJ	5' TGCTCACAGACCGAAACCTA 3'	5' ACTTCGGCTCATTACTTGGA 3'	52
15BJ	5' CAACTTCCCTCACCACTAACC 3'	5' TGACGGTAGCACCTCAGAAT 3'	48
16BJ	5' TCAATGAAAACAACCCGACA 3'	5' CGCTTTACGCCGATGTCTGTC 3'	50
17BJ	5' AGGGAGAAGTAGAGGAGGGA 3'	5'CTATAACTAGGTTCGGTAGGTCTG	50
	5' AAAACCCAAGCACTAATCACG 3'	3'	53
	5' CCCCGTTTCCCAACTCTTATTT		54
	3'		
	5’ AACAAGGAGCAGGCATCAGG 3’		

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	9655-10003	349	ATG	T-	116		0	H
tRNA^{Arg}	10004-10072	69				TCG	0	H
ND4L	10073-10369	297	ATG	TAA	99		-7	H
ND4	10363-11743	1381	ATG	T--	460		0	H
tRNA^{His}	11744-11813	70				GTG	0	H
tRNA^{Ser}(AGC)	11814-11881	68				GCT	4	H
tRNA^{Leu} (CUA)	11886-11958	73				TAG	0	H
ND5	11959-13797	1839	ATG	TAG	613		-4	H
ND6	14315-13794	522	ATG	TAG	174		0	L
tRNA^{Glu}	14384-14316	69				TTC	4	L

Cyt*b*	14389-15529	1141	ATG	T--	380		0	H
$tRNA^{Thr}$	15530-15601	72				TGT	-1	H
$tRNA^{Pro}$	15670-15601	70				TGG	0	L
D-loop	15602-17009	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.

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

PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

Figure Legend

Fig. 1. Gene map of *Brama japonica* mitochondrial genome. 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.

Fig. 2. Putative secondary structures of 22 tRNAs encoded by the mitochondrial.

Fig. 3. 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’ GCCGG 3’ in the tRNA^{Cys}.

Fig. 4. Partial sequence of mitochondrial control region of *Brama japonica* . The sequence is presented as the L-strand sequence from the 5’ to 3’ end. In the control region, the termination associated sequence (TAS), central conserved sequence blocks (CSB-E, CSB-D), and conserved sequence blocks (CSB-1, CSB-2 and CSB-3) are boxed and marked.

Fig. 5. The phylogenetic relationship of *Brama japonica* within marine pelagic based on 12 protein-coding genes.

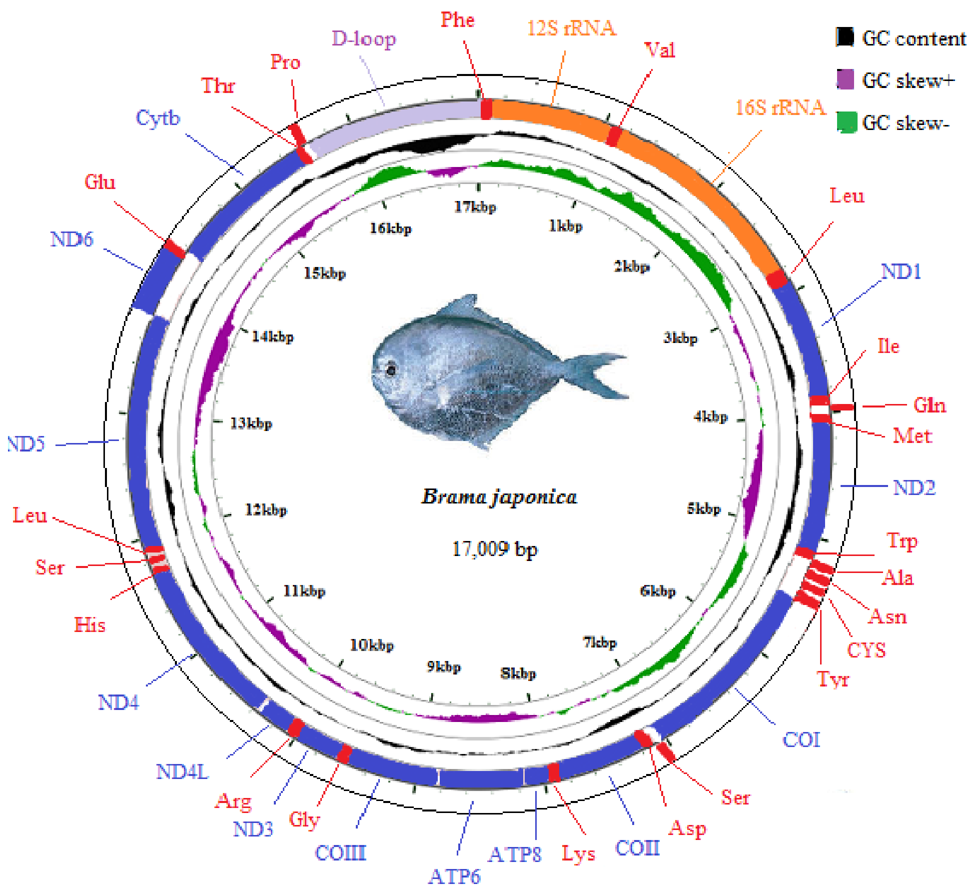
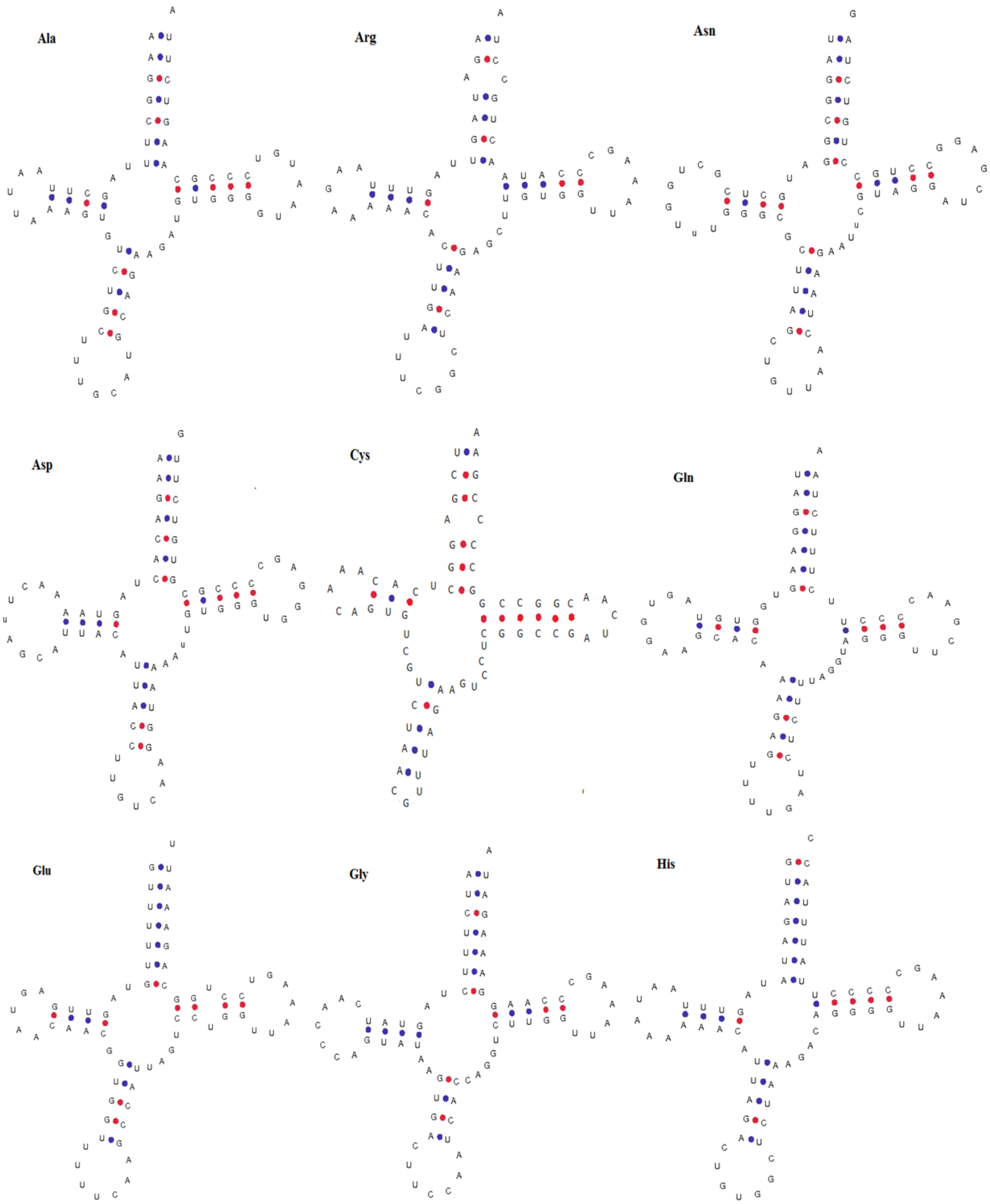
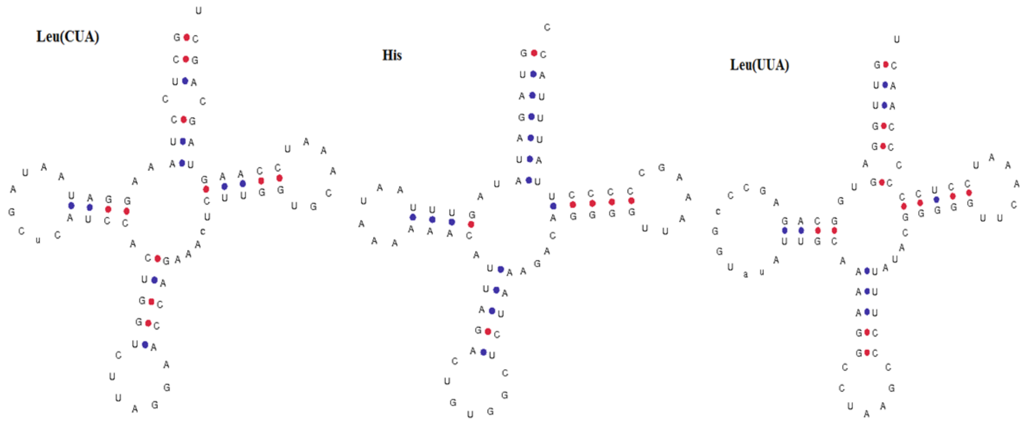
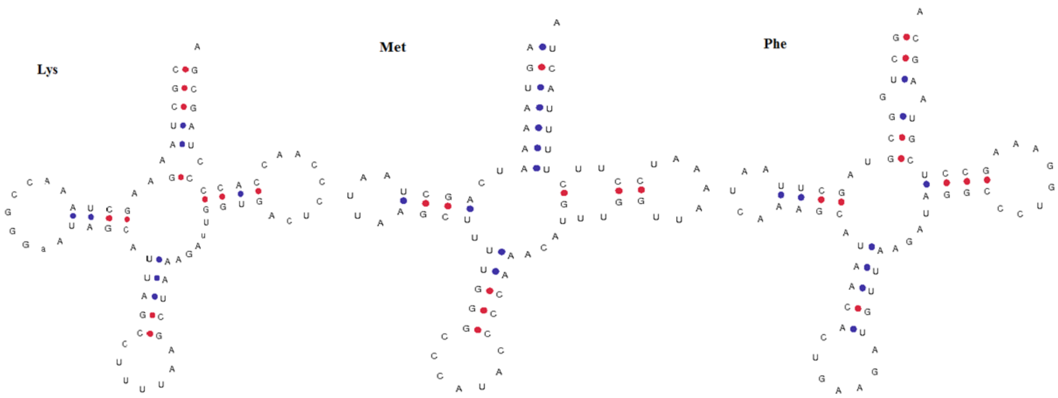
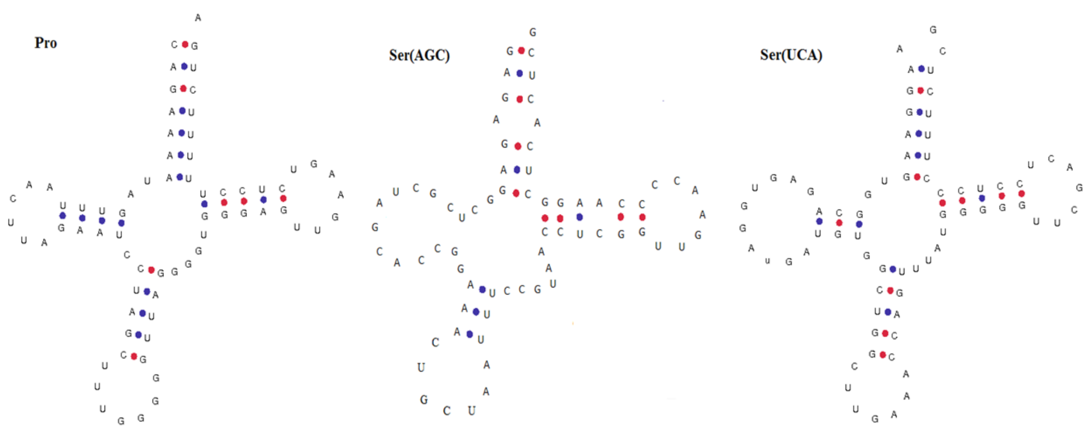


Fig. 1. Circular gene map of *Brama japonica* mitochondrial genome. Genes encoded on the heavy or light strands are shown inside or outside the circular gene map, respectively. The inner ring indicates the GC skew, the middle ring indicates the GC content. The figure was initially generated with GC viewer 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.


Ala
Arg
Asn
Asp
Cys
Gln
Glu
Gly
His







PeerJ PrePrints | https://doi.org/10.7287/peerj.preprints.1667v1 | CC-BY 4.0 Open Access | rec: 21 Jan 2016, publ: 21 Jan 2016

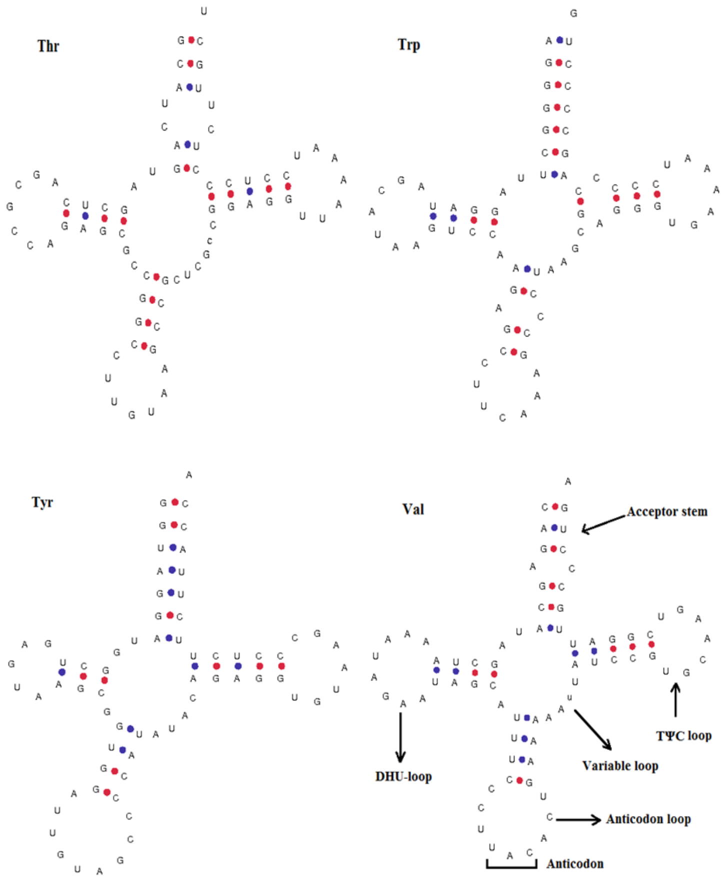


Fig. 2. Putative secondary structures of 22 tRNAs encoded by the mitochondrial.

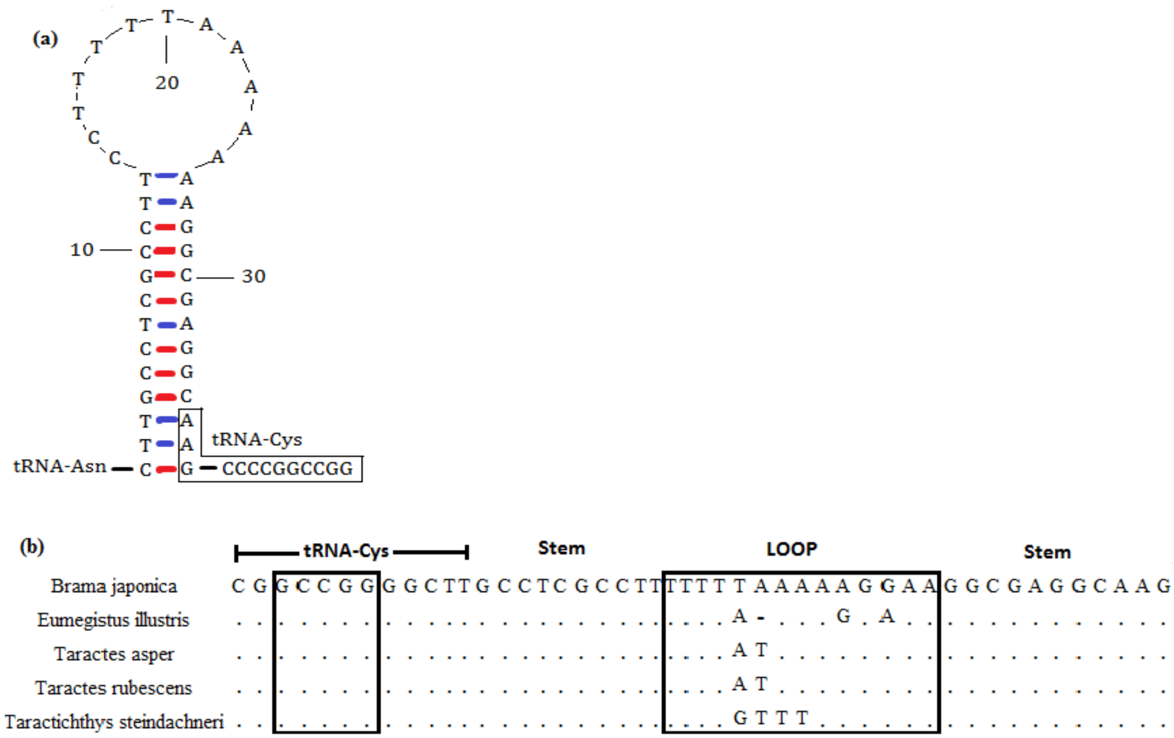


Fig. 3. A conserved secondary structure (a) as a putative replication origin (OL) in a non-coding region located between tRNA^{Asn} and tRNA^{Cys} of *Brama japonica*, and a comparison of nucleotide sequences of related species (b). The box represents the sequence of the loop of the OL secondary structure and the conserved motif 5’ GCCGG 3’ in the tRNA^{Cys}.

CAGAAAAAGGAGACTTCAACTCCCACCCCTAACCCCCAAAGCTAGGATTCTAAGTTAAACTATTTTCT
GCCGCCCCACCCCGGCCCCACCCCGGCCCCACCCCATACATGTCCGTTATGAACATGTATGTATATAGTA
TAS
CTTATAACTATTTTAATCTATTTACTATGCTTTTATCCTTTATATGTATTATCAACATTACTGATTTTAACCA
AATTATGAATGAAAGTCATACCTCCATAATCAGCATTCTTATTTAATAAGCATTTCTCATACACTCGAAG
TGGAGTATTCGTCATAGACATCCCCCCTCGCTTGCTTCTCCTTTAAGAAAGGAAAACGTTTACCATATCA
TCCTCGGATCCTTGAGCCATTGGCGCACAAGATATATACCTGGTCATCAACTCATCTAACCATACGTTTA
CSB-E
ATGAAGGGTCAGGGGCAAGAATTGTGGGGGTAGTGCCTCGTGAACTATTACTGGCCTCTGGCTTCTCT
CSB-D
TTCAGGTCCATATGGAGAGCCTCGTGCTTGTTACCGGCATTCAACGAGGGCCTCTTGGTTGATGGCTGT
CAACACTCCTCACAAGCACTACGGCAAGGAATGATATCCACAGGGGTCAGGTTTTTTCTCTCACTTTT
CAGTCAACATGCCCATGGCTGTTCAATTACAGGAATAAGGTAGAACTCTTCCTTGGGTTAGAAGACCA
GAAATTAATGTTGGAATGACATTCCATTAAGAATTGCATACAGATATATCATGAGCATATATGATATTTTT
CSB-1
TGCCCCCCTTTTCTTAACAAAATTCAATCGGGTTTTGTTCGTAAAAACCCCCTTACCCCCTTAACTCG
CSB-3 CSB-2
AGACATATTTAATATTCCTGAAAACCCCCGAAACAGGAAAGTCTCGACTTAATTTTATCTCCACTCCAT

Fig. 4. Partial sequence of mitochondrial control region of *Brama japonica* . The sequence is presented as the L-strand sequence from the 5’ to 3’ end. In the control region, the termination associated sequence (TAS), central conserved sequence blocks (CSB-E, CSB-D), and conserved sequence blocks (CSB-1, CSB-2 and CSB-3) are boxed and marked.

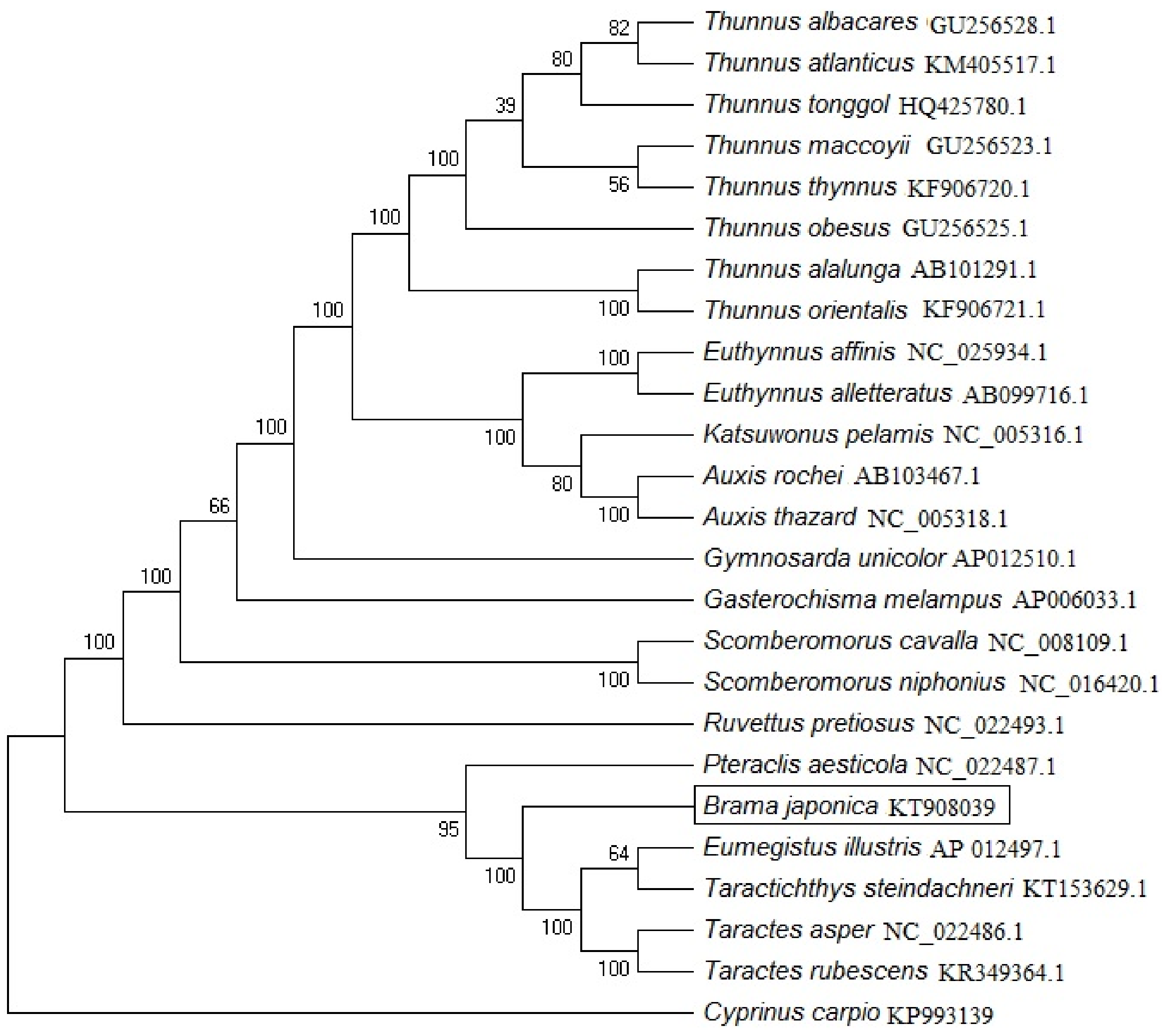


Fig. 5. The phylogenetic relationship of *brama japonica* within Perciform based on 12 protein-coding genes.