Novel Gene Rearrangement in the Mitochondrial Genome of Three *Garra* and Insights Into the Phylogenetic Relationships of Labeoninae

Chi Zhang¹, Kun Zhang², Ying Peng², Jianshe Zhou¹, Yifan Liu² and Bingjian Liu²*

¹Institute of Fisheries Science, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, China, ²National Engineering Laboratory of Marine Germplasm Resources Exploration and Utilization, Zhejiang Ocean University, Zhoushan, China

Complete mitochondrial genomes (mitogenomes) can provide valuable information for phylogenetic relationships, gene rearrangement, and molecular evolution. Here, we report the mitochondrial whole genomes of three *Garra* species and explore the mechanisms of rearrangements that occur in their mitochondrial genomes. The lengths of the mitogenomes’ sequences of *Garra dengba*, *Garra tibetana*, and *Garra yajiangensis* were 16,876, 16,861, and 16,835, respectively. They contained 13 protein-coding genes, two ribosomal RNAs, 22 transfer RNA genes, and two identical control regions (CRs). The mitochondrial genomes of three *Garra* species were rearranged compared to other fish mitochondrial genomes. The tRNA-Thr, tRNA-Pro and CR (T-P-CR) genes undergo replication followed by random loss of the tRNA-Thr and tRNA-Pro genes to form tRNA-Thr, CR1, tRNA-Pro and CR2 (T-CR-P-CR). Tandem duplication and random loss best explain this mitochondrial gene rearrangement. These results provide a foundation for future characterization of the mitochondrial gene arrangement of Labeoninae and further phylogenetic studies.

Keywords: Garra, gene rearrangement, complete mtDNA sequence, Labeoninae, phylogeny

INTRODUCTION

The subfamily Labeoninae is wildly distributed in Asia and Africa as one of the most diverse freshwater fishes in the family Cyprinidae (Cypriniformes). About 26 genera are distributed in China, 12 of which are endemic, with a great diversity of genera and species (Pei-Qi and Een-Duan, 1984). Once, Howes et al. (1991) and Winfield et al. (2012) proposed that the tribe Labeonini is essentially equivalent to the subfamily Labeoninae in Cyprininae (Howes et al., 1991; Winfield and Nelson, 2012). Because of the great diversity of Labeoninae orofacial morphology, it is often used as a key feature for identifying the included genera and for recovering the phylogenetic history hypothesis suite. However, since the morphological diversity and the difficulty of distinguishing the ambiguous morphological characteristics, the taxonomy of the Labeoninae has been constantly subject to revision, and the phylogenetic relationships within them remain amid change. Although some researchers erected a few new genera (e.g., *Hongshuia*, *Qianlabeo*, and *Stenorhinchus*) (Zhang and Chen, 2004; Zhang and Fang, 2005; Li et al., 2006; Zhang et al., 2008), and rearranged the generic assignment of some species based on the oromandibular morphology (Yuan et al., 2008). There was no input from molecule-based phylogenetic evidence. Furthermore, Yang and Mayden (2010) constructed the most comprehensive molecular phylogeny just including one species of each of 11
genera, supporting the monophyly of Labeoninae and proposing a model for the relationships of the major sublineages within Labeoninae (Yang and Mayden, 2010). However, limited by the number of mitogenomes, the monophyly of many genera and the validity of the nomenclature are unresolved. Therefore, re-establishing the Labeoninae phylogenetic relationship using suitable species is necessary.

Garra is a genus of fish in the carp family of the subfamily Labeoninae, distributed in southern China, across Southeast Asia, India, and the Middle East to northern and central Africa (STIASSNY and GETAHUN, 2007). Garra is usually found in swift-flowing streams, where they hold on to rocks with their highly modified mouths like suckers, including bottom-dwelling fishes. They feed on plant debris in open-water habitats and on periphyton in bottom-surface habitats (Kullander and Fang, 2004; Nebeshwar et al., 2009). Currently, 20 different Garra species have been identified from the Brahmaputra River basin, three of which in China, namely, G. dengba, G. Tibetan, and G. yajiangensis caught our attention due to the rearrangement in their mitochondrial genome (Deng et al., 2018; Gong, 2018; Gong et al., 2018; Zhang et al., 2020). They were all collected from high altitudes. Among them, G. dengba is derived from the Chayu-Qu, a stream that flows into the Brahmaputra River in eastern Tibet, China. It shares with the five Garra fishes (G. arupi, G. elongata, G. gravelyi, G. kalpangi, and G. rotundinasus) an incipient proboscis on the nose, but differs from them in its less divergent dorsal and anal fins and more perforated lateral line scales (Deng et al., 2018). Meanwhile, due to the similarity of morphological characters, G. tibetana was for a long time mistakenly identified as G. kempfi (Galtier et al., 1987). G. yajiangensis, a member of the long-nosed species group, is distinguished from other members of the group mainly by the presence of a prominent, quadrangular, slightly bilobed proboscis. Apart from G. tibetana has been reported as a complete mitochondrial sequence, current researches on these three Garra fishes are mainly stagnant in morphology while their corresponding molecular evolutions are still limited (Zhang et al., 2020). In addition, Zhang et al. (2020) identified two control regions in the mitochondrial genome of G. tibetana (Zhang et al., 2020). Also, Li et al. (2016) reported that the tRNA-Pro gene of G. kempfi is positioned between two control regions, and a 246 bp repeat unit is identified in the second control region, which is different from the typical mitochondrial genome organization in vertebrate (Li et al., 2016). However, He et al. (2016) reported that the complete mitochondrial genome of G. imberba is a classic structure (He et al., 2016). A different mitochondrial genome composition is present in Garra fish, and no one has yet identified the reason for this phenomenon. Therefore, this study reports novel gene rearrangement in the mitochondrial genome of G. dengba, G. tibetana, and G. yajiangensis, which could provide important genomic information for studying the evolutionary relationships and population genetics of the genus Garra.

The mitochondrial genome (Mitogenome) houses its DNA and encodes many key proteins for the assembling and activation of the mitochondrial respiratory complex (Yan et al., 2019). Mitogenomes as a molecule marker were used for species classification, population genetics, molecular systematic geography, molecular ecology, and other areas (Groves and Shields, 1996; Nielsen et al., 2010; Cheng et al., 2012; Zhang et al., 2018). Vertebrate mitogenomes are generally 16–20 kb in length (Boore, 1999), compared to plant mitochondrial genomes, which are shorter. The structure of the vertebrate mitogenome typically consists of 13 protein-coding genes (PCGs), 22 tRNA genes (tRNAs), and two rRNA genes (rRNAs) (Boore, 1999). In the vertebrate mitogenome, the order of these genes is conserved and generally unaltered (Bibb et al., 1981; Roe et al., 1985). The mitochondrial genome is inherited maternally in most animals and therefore has a very low rate of recombination (Brown et al., 1979). However, variants of the gene sequence have now been identified in a variety of vertebrates, including amphibians, reptiles, birds, marsupials, and fish (Pääbo et al., 1991; Macey et al., 1997; Eberhard and Wright, 2015; Liu et al., 2019a; Lü et al., 2019). In recent years, with the advancement of sequencing techniques, more and more mitochondrial rearrangements are being identified.

In general, the structure of the fish mitochondrial genome is extremely conserved (Gong et al., 2013). With the gradual increase in mitochondrial DNA sequence data for fish, reports of mitogenome rearrangements continue to appear (Miya et al., 2001; Inoue et al., 2003; Zhang et al., 2021). To date, a few models have been employed for mitochondrial gene rearrangement. These models include the tandem duplication/random loss (TDRL) (Moritz and Brown, 1987), recombination model (Lunt and Hyman, 1997), tRNA mismatch model (Cantatore et al., 1987), tandem duplication/nonrandom loss model (RDNL) (Lavrov et al., 2000) and double replication/random loss model (DRRL) (Shi et al., 2014). The TDRL model occurs by tandem duplication of certain genes that undergo rearrangement, followed by random deletion of repetitive sequences. The model has been widely applied to explain the translocation of genes encoded on the same strand (Shi et al., 2015; Wang et al., 2018). The RDNL model differs from TDRL in non-random loss and is dependent on the transcriptional polarity and location of the gene.

In this study, three mitogenomes of Garra species (G. dengba, G. tibetana, and G. yajiangensis) were sequenced and annotated. We have also characterized their mitochondrial genome structure and phylogenetic analysis within their subfamily Labeoninae. In addition to this, we have explored the mechanisms by which their mitochondrial genes undergo rearrangement. Based on the above analysis, we hope that these results will provide a better understanding of the mechanisms by which rearrangements occur in the mitochondrial genomes of Garra species, as well as their evolution.

MATERIALS AND METHODS

Sample Collection, DNA Extraction, and Sequencing

The sample of G. dengba was collected in Chayu City, Tibet, China (28°66' N, 97°46' E). Samples of G. tibetana and G. yajiangensis were collected in Motuo City, Tibet, China (29°32' N, 95°33' E). Total genomic DNA was extracted...
from muscle tissue using the Qiagen QIAamp tissue kit according to the manufacturer’s protocol. The DNA sediment was solubilized in double-distilled water, stored at 4°C, and then quantified in concentration. The G. tibetana mitochondrial genome was amplified with ten pairs of universal primer by general PCR. The general PCR cycle requirement for DNA amplification is 5 min at 94°C, [30 s at 94°C, 30 s at 55–56°C, 1 min at 72°C] X 35 cycles, and 10 min at 72°C. Sequences were sequenced using an ABI Genetic Analyzer (Applied Biosystems, China). Complete mitogenome sequencing of G. dengha and G. yajiangensis was performed on an Illumina HiSeq X Ten platform.

**Assembly, Annotation, and Analysis**

The acquired sequence PCR fragments were processed through CodonCode Aligner 9.0.1 (CodonCode Corporation, Dedham, MA) and the complete mitochondrial genome was assembled. Clean data without sequencing adapters were assembled de novo using NOVOPlasty software (Dierckxsens et al., 2016). The new mitogenome was annotated with the vertebrate mitochondrial code using the MITOS (Donath et al., 2019). The structures of the 22 tRNAs were determined using tRNA-scan 2.0 (Chan et al., 2021) and then the tRNA structures were mapped by online web software Forna (Kerpedjiev et al., 2015). The circular map of the mitochondrial genome was produced by using the OGDRAW v1.3.1 (Greiner et al., 2019). Base composition and relative synonymous codon usage (RSCU) for 13 PCGs of Garra were computed and sorted using MEGA X (Kumar et al., 2018). Composition skew values were calculated by the following formulas: AT skew = (A−T)/(A + T); GC skew = (G−C)/(G + C) (Peres and Kocher, 1995). The putative origin of L-strand replication (OL) was identified by the Mfold Web Server (Zuker, 2003).
| Gene   | A (%) | T (%) | C (%) | G (%) | A + T (%) | G + C (%) | AT-skew | GC-skew | Length (bp) |
|--------|-------|-------|-------|-------|-----------|-----------|---------|---------|-------------|
| Mitogenome | 29.36 | 28.07 | 26.86 | 15.72 | 57.42 | 42.58 | 0.0224 | -0.2616 | 11,412       |
| ND1    | 29.85 | 25.74 | 29.64 | 14.77 | 55.59 | 44.41 | 0.0738 | -0.3349 | 975          |
| ND2    | 32.22 | 23.14 | 31.26 | 13.36 | 55.35 | 44.65 | 0.1641 | -0.4004 | 1,046        |
| COI    | 26.96 | 30.56 | 24.63 | 17.86 | 57.51 | 42.49 | -0.0628 | -0.1583 | 1,551        |
| COII   | 31.4  | 26.92 | 26.05 | 15.63 | 58.32 | 41.68 | 0.0769 | -0.25   | 691          |
| ATP8   | 34.55 | 27.88 | 26.06 | 11.52 | 62.42 | 37.58 | 0.1068 | -0.3871 | 165          |
| ATP6   | 29.09 | 30.41 | 26.17 | 14.33 | 59.5  | 40.5  | -0.0221 | -0.2924 | 684          |
| COIII  | 27.61 | 26.34 | 28.75 | 17.3  | 53.94 | 46.06 | 0.0236 | -0.2486 | 786          |
| ND3    | 27.92 | 28.49 | 27.92 | 15.67 | 56.41 | 43.59 | -0.0101 | -0.281  | 351          |
| ND4L   | 24.92 | 25.93 | 31.31 | 17.85 | 50.84 | 49.16 | -0.0199 | -0.274  | 297          |
| ND5    | 31.43 | 27.23 | 27.52 | 13.83 | 58.65 | 41.35 | 0.0716 | -0.331  | 1,381        |
| ND6    | 32.8  | 26.93 | 27.32 | 12.95 | 59.74 | 40.26 | 0.0983 | -0.3569 | 1,823        |
| Cytb   | 34.98 | 29.1  | 26.64 | 14.37 | 58.98 | 41.02 | 0.0134 | -0.2991 | 1,141        |
| rRNAs  | 34.98 | 29.1  | 26.64 | 14.37 | 58.98 | 41.02 | 0.0134 | -0.2991 | 1,141        |
| tRNAs  | 34.98 | 29.1  | 26.64 | 14.37 | 58.98 | 41.02 | 0.0134 | -0.2991 | 1,141        |
| CR1    | 35.07 | 31.02 | 20.36 | 13.54 | 66.10 | 33.9  | 0.0613 | -0.2013 | 938          |
| CR2    | 45.04 | 28.72 | 21.63 | 4.61  | 73.76 | 26.24 | 0.2212 | -0.6486 | 282          |

**FIGURE 1** | The RSCU of the mitogenome in (A) *Garra dengba*, (B) *Garra tibetana* and (C) *Garra yajiangensis*. The RSCU of the three *Garra* species mitogenomes show that the usage of NNA and NNC (N for A, T, C, G) was more frequent than NNT and NNG in the *G. dengba*, *G. tibetana*, and *G. yajiangensis*. 
Phylogenetic Analyses
Rapid identification of 12 PCGs in the mitochondrial genome was performed using DAMBE v7.2.3 software (Xia, 2018). 13 PCGs were used excluding ND6 owing to its heterogeneous base composition and poor consistent phylogenetic performance (Miya et al., 2003). Sequences were compared using the default parameters of Clustal X 2.0 (Larkin et al., 2007). The ambiguous sequences were eliminated by Gblock (Talavera and Castresana, 2007). Subsequently, the results of the multiple sequence comparisons were then used to construct phylogenetic trees based on maximum likelihood (ML) and Bayesian inference (BI) analyses. The ML tree was built in PhyML 3.0 (Guindon et al., 2010), using the best model (TVM + F + R8) selected in ModelFinder with 1000 nonparametric bootstrap replications (Kalyaanamoorthy et al., 2017). Bayesian inference (BI) methods were used with the program MrBayes 3.2

FIGURE 2 | Mitochondrial genome maps of the three Garra in this study. (A) Garra dengba mitochondrial genome visualization ring diagram. (B) Garra tibetana mitochondrial genome visualization ring diagram. (C) Garra yajiangensis mitochondrial genome visualization ring diagram. Genes encoded on the heavy or light strands are shown at the outside or inside of the circular gene map, respectively. The mitochondrial genome visualization ring diagram of the three species show the same structure and all have two control regions.
of the mitochondrial genomes of these three species (Ronquist et al., 2012). Based on the Akaike information criteria (AIC), MrModeltest 2.2 (Nylander et al., 2004) was performed to determine the best evolutionary model among 24 models for BI analysis and pointed out that GTR + G + I was the analytical data set for the best-fit substitution model. BI analysis was performed using Markov chain Monte Carlo (MCMC), sampled every 1,000 generations each with three heated chains and one cold chain run for 6,000,000 generations, and the first 25% of the burns were discarded. Visualization of the tree was realized using online web tool iTOL v5 (https://itol.embl.de) (Letunic and Bork, 2021).

RESULTS AND DISCUSSION

Genome Organization and Composition

The complete mitochondrial genomes of three Garra (G. dengba, accession no. OL826794; G. tibetana accession no. NC_045032 and G. yajiangensis, accession no. OL826795) in GenBank were 16,861, 16,861 and 16,835 bp in length, respectively (Table 1). The structure of the mitochondrial genomes of these three fish species is extremely similar. The mitochondrial genomes of all three species are very similar in structure, containing 13 protein-coding genes (PCGs), two rRNAs, 22 tRNAs, a light-stranded replication initiation region (OL), and two control regions (CR). Compared to the structure of the mitochondrial genomes of most teleost fishes, the mitochondrial genomes of the three fishes in this study have an extra CR. The main reason for this is the rearrangement of mitochondrial genes in these three fish species (Figure 5). The presence of replicating CR was considered a special feature in the vertebrate mitochondrial genome (Jiang et al., 2007; Shi et al., 2013). The nucleotide composition of G. dengba, G. tibetana, and G. yajiangensis mitogenomes had a higher A + T bias of 57.42, 58.44, and 58.44%, respectively, and both showed positive AT-skew and negative GC-skew (Table 2).

Protein-Coding Genes and Codon Usage

Like the typical mitochondrial genome of vertebrates (Zhang et al., 2021b; Liu et al., 2019b), the 13 PCGs consist of one cytochrome b (Cyt b), two ATPases (ATP6 and ATP8), and three cytochrome c oxidases (COI-COIII), and seven NADH dehydrogenases (ND1-ND6 and ND4L). Twelve PCGs (ND1, ND2, COI, COII, ATP8, ATP6, COIII, ND3, ND4L, ND4, ND5, and Cytb) were coded on the heavy strand (H-strand) and the remaining ND6 gene was coded on the light strand (L-strand) (Table 1). All PCGs use the start codon ATG except for COI, which uses GTG. Most of the 13 PCGs ended with TAA or TAG, whereas four other PCGs (COI, ND4, ND6, and Cytb) use a single T- or TA-as the stop codon (Table 1). The incomplete stop codon has also been found in all other Garra species (Su et al., 2015; Xiong et al., 2016). The presence of incomplete stop codons is a common phenomenon in invertebrate and vertebrate mitochondrial genes (Liu et al., 2020; Zhang et al., 2021c). All 12 PCGs genes (ND1-ND3, COI-COIII, ATP8, ATP6, ND4L, ND4, ND5) in the three Garra mitochonmdria had negative GC-skew values and they were all encoded on the H-strand, in addition, the ND6 gene encoded on the L-strand had a positive GC-skew value (Table 2, Supplementary Table S1, S2).

Depending on the codon degeneration pattern, the amino acids serine and leucine are encoded by six synonymous codons, and the remaining amino acids are encoded by four or two codons. A count of the translated amino acid content of 13 PCGs was found. The top utilized amino acids are Leu, Ile, and Ala, and the few top utilized amino acids are Asp, Arg, and Cys (Figure S1). This phenomenon is also commonly seen in the mitochondrial genomes of other fish (Su et al., 2015; Zhang et al., 2021b). We measured the RSCU of the three Garra species mitogenomes (Figure 1) and the results showed that the usage of NNA and NNC (N for A, T, C, G) was more frequent than NNT and NNG in the G. dengba, G. tibetana, and G. yajiangensis.
Transfer and Ribosomal RNAs

Like the typical set of tRNA genes in fish mitogenomes, all three Garra species mitogenomes included 22 tRNAs, a 12S rRNA, and 16S rRNA. Of these 22 tRNAs, 14 tRNAs were encoded on the H-strand and the remaining eight (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu, and tRNA-Pro) were encoded on the L-strand (Figure 2). All of them had two kinds of tRNA-Leu and tRNA-Ser (Table 1). Secondary cloverleaf structure of tRNA genes identified in the mitogenome of G. dengba, G. tibetana, and G. yajiangensis were shown in Supplementary Figures S2–4. Out of 22 tRNAs, all have a typical cloverleaf structure except for tRNA-Ser (GCT), which lacks the entire dihydrouridine arm. The 12S rRNA gene was positioned between tRNA-Phe and tRNA-Val, and the 16S rRNA gene was located between tRNA-Val and tRNA-Leu (UUR). The lengths of 12S rRNA were 952, 951, and 950 in the mitochondrial genomes of G. dengba, G. tibetana, and G. yajiangensis, respectively. The size of the 16S rRNA in G. dengba and G. tibetana were 1,688 bp, both a little longer than in G. yajiangensis (1,687 bp). Positive AT-skew values for both tRNA and rRNA in the mitochondrial genomes of G. dengba, G. tibetana, and G. yajiangensis indicate that the As content is higher than the Ts content (Table 2, Supplementary Tables S1, S2).

L-Strand Origin of Replication and Control Region

The origin of light chain replication (OL) was usually found within a WANCY group and can fold into a stem-loop ring secondary structure (Zhang et al., 2021a). The OL of G. dengba and G. tibetana were located between tRNA-Asn and tRNA-Cys with the same length of 34 bp, and 1 bp longer than G. yajiangensis (33 bp) (Table 1). The two-structure prediction of the OL region revealed that the stem lengths in the OL regions of G. dengba, G. tibetana, and G. yajiangensis were 10, 5, and 9 bp, respectively, and the loops were 11, 15, and 10 nt, respectively (Figure 3). In these OL structures, the utilization of stem codons showed a clear asymmetry, with more pyrimidines at the 5′ end of the sequence. In contrast to other fish studies (Catanese et al., 2010), the conserved sequence 5′-GCCGG-3′ was not present in the OL region in this study, but rather a segment of the 5′-GGCGG-3′ sequence was present.
The CR is the greatest non-coding region in the fish mitogenome which is analogous to the A + T enriched region of the insect mitogenome (Liu et al., 2013). The CR1 and CR2 of mitogenome which is analogous to the A + T enriched region hairpin structure (the terminator region because of their ability to form a stable chain replication termination were found in both CRs. The ratio of AT was 73.76, 71.43, and 73.41%, respectively (Table 1), and the ratio of AT was 66.10, 66.37, and 65.75%, respectively (Table 2, Supplementary Tables S1, S2). The lengths of CR1 in the three Garra mitogenomes were 938, 901, and 914 bp (Table 1), and the ratio of AT was 73.76, 71.43, and 73.41%, respectively (Table 2, Supplementary Tables S1, S2). The palindromic sequence motifs “TACAT” and “ATGTA” related to heavy chain replication termination were found in both CRs. The “TACAT” and “ATGTA” motifs are generally referred to as the terminator region because of their ability to form a stable hairpin structure (Figure 4, in the red box). Six Garra CRs were compared to probe the conservation of sequences. The results revealed 12 conserved blocks in Figure 4 (In the yellow box). As far as we know, this is the first report of a study reporting conserved blocks of the CR in Garra mitogenomes.

**Gene Rearrangement**

By comparing the structure of the mitochondrial genomes of other vertebrates (Kim et al., 2004), we found that the mitochondrial genomes of the three Garra species had undergone partial recombination of genes. The two genes that changed position in the mitochondrial genome of these three Garra species were tRNA-Pro and CR. In general, the tRNA-Pro was positioned between the tRNA-Thr and CR, and only one CR region was positioned at the end of the mitogenome. However, the position of tRNA-Pro in the mitochondrial genome of these three Garra species changed, and a CR was copied (Figure 5). In this study, the gene sequences were identical to those of the vertebrate mitochondrial genome, except for the tRNA-Pro translocation and CR repeats.

To better characterize the mechanics of gene rearrangement that occur in the Garra mitochondrial genome, we have explored this in-depth. Several models have been proposed for the mechanism of rearrangement of the mitochondrial genome (Poulton et al., 1993; Arndt and Smith, 1998; Lavrov et al., 2002; Shi et al., 2014). The recombination model is only appropriate for the exchange and inversion of small fragments (Dowton and Campbell, 2001; Kong et al., 2009); thus, this recombination model was not suited to explaining the mitochondrial gene rearrangement of Garra. Regarding the two models, TDNL and DRRL, they are often used to explain mitochondrial gene rearrangements, but with genes aggregated in the same pole (encoded by the L- or H-strand) and with no change in relative order. Therefore, neither model can be used to explain the rearrangement mechanism that occurred in this study. The TDRL model was due to incomplete deletion of duplicated genes resulting in intergenic spacer regions or pseudogenes (Shi et al., 2015; Gong et al., 2019). This TDRL phenotype explains well the gene rearrangements with redundant genes. Evidence for the TDRL model was the presence of pseudogenes or duplicate genes and the location of gene spacer regions. Therefore, in the present study, the TDRL model was recommended to study rearrangement events, as gene rearrangements with duplicate CRs were observed in the mitochondrial genome of Garra.
### TABLE 3 | List of 61 Labeoninae species and two outgroups used in this paper.

| Organism          | Family      | Subfamily | Genus     | Length | Accession NO. |
|-------------------|-------------|-----------|-----------|--------|---------------|
| Bangana decora    | Cyprinidae  | Labeoninae| Bangana   | 16607  | NC_026221     |
| Bengana rendahlí  | Cyprinidae  | Labeoninae| Bengana   | 16586  | NC_028169     |
| Cincluso cyprinos | Cyprinidae  | Labeoninae| Cinclus   | 16588  | NC_033964     |
| Cincluso myrials | Cyprinidae  | Labeoninae| Cinclus   | 16600  | NC_017611     |
| Crossocichla atrilmes | Cyprinidae | Labeoninae| Crossochelus | 16596 | NC_029947 |
| Crossocichla langei | Cyprinidae | Labeoninae| Crossochelus | 16569 | NC_029433 |
| Crossocichla reticulatus | Cyprinidae | Labeoninae| Crossochelus | 16588 | NC_031624 |
| Crossocichla siamensis | Cyprinidae | Labeoninae| Crossochelus | 16611 | NC_031827 |
| Descogobio laticeps | Cyprinidae | Labeoninae| Descogobio | 16596 | NC_045918 |
| Descogobio longibarbatius | Cyprinidae | Labeoninae| Descogobio | 16594 | NC_036301 |
| Descogobio macrophyssalidos | Cyprinidae | Labeoninae| Descogobio | 16593 | NC_035299 |
| Descogobio tetrabarbatius | Cyprinidae | Labeoninae| Descogobio | 16596 | NC_032457 |
| Descogobio yunnanensis | Cyprinidae | Labeoninae| Descogobio | 16602 | NC_025319 |
| Garra congoensis  | Cyprinidae  | Labeoninae| Garra     | 16781  | NC_021535     |
| Garra dengba      | Cyprinidae  | Labeoninae| Garra     | 16796  | OL826794      |
| Garra flavatra    | Cyprinidae  | Labeoninae| Garra     | 16743  | NC_022953     |
| Garra imberba     | Cyprinidae  | Labeoninae| Garra     | 16600  | NC_025662     |
| Garra kempí       | Cyprinidae  | Labeoninae| Garra     | 17104  | NC_028426     |
| Garra motocensis  | Cyprinidae  | Labeoninae| Garra     | 16806  | OK375462      |
| Garra orientalis  | Cyprinidae  | Labeoninae| Garra     | 17288  | NC_021935     |
| Garra pingi pingi | Cyprinidae  | Labeoninae| Garra     | 16599  | MF958973      |
| Garra poecilura   | Cyprinidae  | Labeoninae| Garra     | 16978  | NC_031628     |
| Garra qiaojensis  | Cyprinidae  | Labeoninae| Garra     | 17096  | NC_028403     |
| Garra rufa        | Cyprinidae  | Labeoninae| Garra     | 16783  | NC_022941     |
| Garra salveenaica | Cyprinidae  | Labeoninae| Garra     | 16960  | NC_033389     |
| Garra sploita     | Cyprinidae  | Labeoninae| Garra     | 16822  | NC_022944     |
| Garra tibetana    | Cyprinidae  | Labeoninae| Garra     | 16861  | NC_045032     |
| Garra yaiangensis | Cyprinidae  | Labeoninae| Garra     | 16835  | OL826795      |
| Henicorhynchus lineatus | Cyprinidae | Labeoninae| Henicorhynchus | 16825 | NC_022950 |
| Henicorhynchus siamensis | Cyprinidae | Labeoninae| Henicorhynchus | 17010 | NC_031623 |
| Labeo alvirens    | Cyprinidae  | Labeoninae| Labeo     | 16603  | NC_029444     |
| Labeo angra       | Cyprinidae  | Labeoninae| Labeo     | 16606  | NC_022945     |
| Labeo batesi      | Cyprinidae  | Labeoninae| Labeo     | 16603  | NC_038656     |
| Labeo calbasu     | Cyprinidae  | Labeoninae| Labeo     | 16607  | AP012143      |
| Labeo cafa        | Cyprinidae  | Labeoninae| Labeo     | 16590  | NC_016892     |
| Labeo chrysophedion | Cyprinidae | Labeoninae| Labeo     | 16602  | NC_022942     |
| Labeo cyclophus   | Cyprinidae  | Labeoninae| Labeo     | 16610  | NC_022949     |
| Labeo cylindrical | Cyprinidae  | Labeoninae| Labeo     | 16602  | NC_031536     |
| Labeo dussumieri  | Cyprinidae  | Labeoninae| Labeo     | 16613  | NC_031622     |
| Labeo filiatus    | Cyprinidae  | Labeoninae| Labeo     | 16614  | NC_026217     |
| Labeo goniuz      | Cyprinidae  | Labeoninae| Labeo     | 16614  | NC_028786     |
| Labeo linea       | Cyprinidae  | Labeoninae| Labeo     | 16606  | NC_022966     |
| Labeo nasus       | Cyprinidae  | Labeoninae| Labeo     | 16601  | NC_029449     |
| Labeo pierrei     | Cyprinidae  | Labeoninae| Labeo     | 16786  | NC_022943     |
| Labeo rohita      | Cyprinidae  | Labeoninae| Labeo     | 16626  | NC_017606     |
| Labeo senegalensis | Cyprinidae | Labeoninae| Labeo     | 16604  | NC_028657     |
| Labiobarbus leptochelus | Cyprinidae | Labeoninae| Labiobarbus | 16593 | NC_022954 |
| Labiobarbus lineatus | Cyprinidae | Labeoninae| Labiobarbus | 16598 | NC_022955 |
| Labiobarbus ocellatus | Cyprinidae | Labeoninae| Labiobarbus | 16596 | NC_022947 |
| Labiobarbus spilopleura | Cyprinidae | Labeoninae| Labiobarbus | 16593 | NC_031533 |
| Osteochilus pentinalaeus | Cyprinidae | Labeoninae| Osteochilus | 16563 | NC_031625 |
| Osteochilus salisburyi | Cyprinidae | Labeoninae| Osteochilus | 16599 | NC_021385 |
| Osteochilus schlegelli | Cyprinidae | Labeoninae| Osteochilus | 16575 | NC_022851 |
| Paraspinelaboe assimilis | Cyprinidae | Labeoninae| Paraspinelaboe | 16602 | NC_025947 |
| Paraspinelaboe longicorpus | Cyprinidae | Labeoninae| Paraspinelaboe | 16596 | NC_035692 |
| Ptychidio iordani | Cyprinidae  | Labeoninae| Ptychidio | 16602  | NC_024294     |
| Ptychidio macrops | Cyprinidae  | Labeoninae| Ptychidio | 16604  | MF457481      |
| Sahyadria chalakudakensis | Cyprinidae | Labeoninae| Sahyadria | 16899  | NC_018566     |
| Sahyadria denisoni | Cyprinidae  | Labeoninae| Sahyadria | 16899  | NC_021973     |
| Semilaboe notabilis | Cyprinidae | Labeoninae| Semilaboe | 16599  | NC_045916     |
| Semilaboe obscurus | Cyprinidae  | Labeoninae| Semilaboe | 16598  | NC_037408     |
| Thynnichthys polylepis | Cyprinidae | Labeoninae| Thynnichthys | 16599 | NC_022962 |
| Thynnichthys thynnoides | Cyprinidae | Labeoninae| Thynnichthys | 16599 | NC_021609 |
Based on the putative model described above, we inferred the regions of mitochondrial gene rearrangement in three *Garra* species. First, normal mitochondrial gene blocks (T-P-CR) form gene blocks (T-P-CR-T-P-CR) after complete duplication (Figure 5A). Subsequently, after successive replications, some genes (T and P) undergo random loss events, leaving only an incompletely replicated CR (Figure 5B). Thus, after this replication and random loss, both CRs are preserved (Figure 5C).

**Phylogenetic Analysis**

To further study the evolutionary status of *G. dengba*, *G. tibetana*, and *G. yajiangensis* in the Labeoninae, we selected 58 closely related subfamilies and two outgroups (*Sahyadria chalakkudiensis* and *Sahyadria denisonii*) to construct evolutionary trees (BI and ML) to analyze phylogenetic relationships (Table 3). The results showed that the ML tree and the BI tree have the same topological structure. Thus, only one topology (BI) is displayed. In addition, the support values of
BI tree are higher than ML tree (Figure 6). The phylogenetic trees showed that the subfamily Labeoninae is divided into three major clades and 13 genera (Cirrhinus, Labeo, Henicorhynchus, Crossocheilus, Thynnichthys, Osteochilus, Labiobarbus, Garra, Bangana, Discogobio Parasiniplateo, Semilabeo, Psychiodio). First, the clade1, genera Cirrhinus, and Labeo show a sister relationship, both of them were once classified in the genus Labeonini (Yang et al., 2012). Then, the clade2, including Henicorhynchus, Crossocheilus, Thynnichthys, Osteochilus, and Labiobarbus, which belonged to “Osteochilini” (Saenjundaeng et al., 2020). The clade3 aggregates all mitochondrial genes with rearrangements in the genus Garra. Except for Bangana and Garra2, all other species of the fourth clade used to belong to the genus Semilabeonini (Stout et al., 2016). The genus Bangana belonged to the cyprinid tribe Labeonini too (Zhang and Chen, 2006; Kottelat, 2017).

In addition, four clades in this phylogenetic tree show high support values except for the value between clade3 and clade4 (posterior probabilities = 0.62; bootstrap = 3.8; Figure 6), indicating that these two clades in Labeoninae are not highly differentiated. All mitochondrial sequences of genus Garra in clade3 have two control regions (Figure 5C) (Li et al., 2016; Su et al., 2015; Xiong et al., 2016; Li et al., 2016), while G. pindi pindi and G. imberba have only one control region and clusters with other Labeoninae species in clade4 without rearrangements, which is consistent with previous studies (Figure 5A) (Zou et al., 2018). Furthermore, each of the 13 different Garra fishes in clade3 clustered into six pairs of sister branches, with only G. spiota forming a monophyletic branch. The three species from Tibet described in this study (species marked by dots in the tree), G. tibetana and G. dengba, form sister branches, showing the close relationship between them. G. yajiangensis and G. kempi cluster together, while G. qiaojensisi showed a close relationship with G. kempi in Zou et al. (2018)’s study, which may be limited by the number of mitochondria in Garra (Zou et al., 2018). It is certain that Garra1 and Garra2 are distantly related due to the rearrangement of mitochondria in Garra1 species, whereas Garra2 species are not rearranged. Thus, we also found that the species of Garra2 are all from Sichuan (He et al., 2016; Zou et al., 2018), and we speculated that none mitochondria of Garra in this region have two control regions or other rearrangements. However, due to the lack of Garra samples from Sichuan, further sample collection is needed to confirm this conjecture.

CONCLUSION

With the development of genetic investigations, the reliance on molecular info is gradually surpassing morphological data, and molecular assays have become the most used method in the study of biological system development. Therefore, in this study, we sequenced and assembled the complete mitochondrial gene of G. dengba, G. tibetana, and G. yajiangensis and determined its characteristics, which contains 37 genes and two control regions. Upon comparison with typical vertebrate mitochondrial genes, we discovered a clear rearrangement of mitochondrial genes in G. dengba, G. tibetana, and G. yajiangensis, the position of tRNA-Pro in the mitochondrial genome of these three Garra species changed, accompanied by CR repeat. The TDRL model is most suitable to explain the gene rearrangements with redundant genes in this study. The two phylogenetic trees (BI and ML) were generated based on the mitochondrial genomes of 61 Labeoninae. Both phylogenetic trees strongly favor the non-monophyly of Garra, which were divided into two different clades (rearrangement and no rearrangement), providing a more advanced classification of Labeoninae. Further, our findings provide a theoretical ground for a deeper understanding of the mechanism and evolution of genus Garra gene rearrangement and phylogenetic studies of Labeoninae.

DATA AVAILABILITY STATEMENT

The raw data is publicly accessible at GenBank under Bioproject accession number PRJNA837076 and the annotation sequences were submitted to NCBI (G. dengba, accession no. OL826794; G. tibetana accession no. NC_045032 and G. yajiangensis, accession no. OL826795).

ETHICS STATEMENT

The animal study was reviewed and approved by the Zhejiang Ocean University.

AUTHOR CONTRIBUTIONS

CZ, KZ, and BL conceived and designed research. CZ, KZ, YP, JZ, YL, and BL conducted experiments, analyzed data, and wrote the manuscript. The authors critically reviewed and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.922634/full#supplementary-material
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