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# Comparative mitochondrial genome analysis of Cynoglossidae (Teleost: Pleuronectiformes) and phylogenetic implications

Bilin Hu[1, 2], Tingqi Jiang[1, 2], Liming Wei[1, 2], Nannan Zhang[1, 2], Kaixin Wang[1, 2], Liqin Liu[1, 2], Bingjian Liu[1, 2], Jing Liu[1, 2], Zhenming Lü[1, 2], Li Gong[1, 2*]

[1] National Engineering Laboratory of Marine Germplasm Resources Exploration and Utilization, Zhejiang Ocean University, Zhoushan 316022, China

[2] Marine Science and Technology College, Zhejiang Ocean University, Zhoushan 316022, China

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**Abstract**

Generally, a teleostean group (e.g., family or genus) owns one type or a set of similar mitochondrial gene arrangement. It is interesting, however, that four different types of gene arrangement have been found in the mitochondrial genome (mitogenome) of Cynoglossidae species. So far, the possible mechanisms of mitogenomic gene rearrangement and its potential implications have aroused widespread attention and caused lots of controversy. Here, a total of 21 Cynoglossidae mitogenomes and a newly sequenced mitogenome of *Cynoglossus puncticpes* (Pleuronectiformes: Cynoglossidae) were compared. The length ranges from 16 417 bp to 18 369 bp, which is mainly caused by the length heteroplasmy of control region (CR). Further analysis reveals that the difference of tandem repeats acts as a determining factor resulting in the length heterogeneity. Like most gene rearrangements of Cynoglossinae mitogenomes, *tRNA-Gln* gene encoded by the L-strand has translocated to the H-strand (*Q* inversion), accompanied by the translocation of CR in *C. puncticpes* mitogenome. The typical *IQM* order (*tRNA-Ile-Gln-Met*) changed to *QIM* order. Tandem duplication/random loss and mitochondrial recombination were accepted as the most possible models to account for the rearrangements in *C. puncticpes* mitogenome. Phylogenetic trees showed a strong correlation between the gap spacer in the rearranged *QIM* area and phylogeny, which provides a fresh idea for phylogenetic studies in future.

**Key words:** mitogenome, gene rearrangement, tandem duplication/random loss, mitochondrial inversion, control region, phylogenetic study

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## 1 Introduction

The vertebrate mitochondrial genome (mitogenome) is typically a double-stranded circular DNA molecule, with a size of 14–22 kb. It contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (12S and 16S), an L-strand replication origin ($O_L$) and a putative control region (CR) (Boore, 1999). Generally, gene numbers and their arrangements in the mitogenome are considered highly conserved. But with the growing number of mitogenomes in recent decades, increasing gene rearrangements have been observed in various vertebrate mitogenomes, such as amphibians, reptiles and fish (Zhang et al., 2018; Yan et al., 2008; Lü et al., 2019). Gong et al. estimated the rearrangement rate was about 4% in fish mitogenomes, mainly existing in Anguilliformes, Myctophiformes, Lophiiformes and Pleuronectiformes mitogenomes (Gong et al., 2013a).

There are three main rearrangement types in vertebrate mitogenomes, including shuffling, translocation and inversion. A single rearrangement type or multiple rearrangement events may occur in mitogenomes. So far, four main mechanisms have been proposed to account for mitogenomic rearrangements, including tandem duplication/random loss (TDRL) model (Moritz and Brown, 1987), intra-mitochondrial recombination model (Poulton et al., 1993), tRNA mis-priming model (Cantatore et al., 1987) and tandem duplication/non-random loss (TDNL) model (Lavrov et al., 2002). TDRL and intra-mitochondrial recombination are two most accepted models. The former has been widely used to explain the translocation of genes encoded on the same strand (Lü et al., 2019; Zhang et al., 2022a), and the latter is commonly used to account for inversion event occurring between different strands (from light strand to heavy strand, and vice versa) (Kong et al., 2009; Gong et al., 2019a).

Tongue soles (Pleuronectiformes: Cynoglossidae), the most recently diverged taxa of flatfish, derived large numbers of morphologically similar species. According to morphological taxonomy, Cynoglossidae is divided into two subfamilies, Symphurinae and Cynoglossinae. Due to their special morphological characteristics, the classification of tongue soles (especially the Cynoglossinae) has long been a controversial issue (Li and Wang, 1995; Menon, 1977; Chapleau, 1988). For example, early tax-

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*Corresponding author, E-mail: gongli1027@163.com

onomists divided the speciose Cynoglossinae into two genera (*Paraplagusia* and *Cynoglossus*) mainly based on the characters of fringes on the lips and the number of lateral lines on the ocular side. While some researchers suggested divide the original genus *Cynoglossus* into seven new genera mainly based on the number of lateral line and left nostril. Although Li and Wang (1995) supported the similar taxonomic characters, they reduced these seven genera to the level of subgenera. Menon (1977) divided *Cynoglossus* into six groups mainly based on the number of scales between the lateral lines, type of scales, location of mouth and number of caudal fins. However, there was no detailed description about the classification of *Cynoglossus* in Nelson et al. (2016) taxonomy system. Recently, plenty of molecular evidence supported the non-monophyly of *Cynoglossus* (Mu et al., 2015; Gong et al., 2020; Wang et al., 2020a), raising doubts about the rationality of this genus. Unfortunately, there is still a lack of comprehensive and systematic study of these controversial taxa.

Generally, a teleostean group (e.g., family or genus) possesses only one type or a set of similar mitochondrial gene arrangement (Miya et al., 2003; Luo et al., 2019). Taking Pleuronectiformes for example, which possess the most diversified gene rearrangement patterns in teleost mitogenomes, Bothidae, Samaridae, Citharidae and Poecilopsettidae exclusively own its unique rearrangement representing the corresponding families (Luo et al., 2019; Shi et al., 2013, 2014a). Interestingly, there have been four different types of rearrangement found in Cynoglossidae mitogenomes, including one typical arrangement type (no rearrangement) and three rearrangement types (Shi et al., 2015a; Gong et al., 2020; Kong et al., 2009). *Symphurus plagiusa* (Cynoglossidae: Symphurinae) shares the typical arrangement type with most vertebrate mitogenomes, while its close relative, *S. orientalis* captures a diverse rearrangement type (Shi et al., 2015a). Cynoglossinae mitogenomes shared other two rearrangement types, both of which were featured by the translocation of CR and inversion of *tRNA-Q* gene. The main difference between these two types of mitogenomes lies in the *IQM* cluster (*tRNA-Ile-Gln-Met*) order. Most of the Cynoglossinae mitogenomes are presented by *QIM* order, while 3 Cynoglossinae mitogenomes (*Cynoglossus melampetalus*, *C. interruptus* and *C. robustus*) are featured by *IMQ* order (Kong et al., 2009; Gong et al., 2020).

Accordingly, the complete mitogenome sequence of *C. puncticpes* (Cynoglossidae: Cynoglossinae) was newly determined and annotated in this study. Combined with other 21 published Cynoglossidae mitogenomes, the mitogenome composition, control region structure, rearrangement types, possible rearrangement processes and interpretations were comparatively analyzed. Also, the relationship between mitogenomic rearrangement and phylogeny of Cynoglossidae were explored. These results will contribute to better understanding the characteristics of flatfish mitogenomes, rearrangement mechanisms and its potential application in phylogenetic studies.

## 2 Materials and methods

### 2.1 *Sample collection and genome survey sequencing*

Specimens were collected from Zhuhai, Guangdong, China, (22°00′15″N, 113°18′17″E). Muscle tissue was stored in 95% ethanol at –80℃. Total genomic DNA was extracted using a standard phenol-chloroform method for muscle tissue. DNA was treated with RNase A to produce pure, RNA-free DNA. Two paired-end DNA libraries were constructed with insert size of 350 bp, and then sequenced using the Illumina HiSeq4000 platform following the manufacturer's protocol. The library construction and sequencing were performed at Onemore Technology Company (Wuhan, China).

### 2.2 *K-mer analysis and mitochondrial genome assembly*

After removing low quality reads, all clean data were used to perform K-mer analysis using software GCE v1.0.0 (Liu et al., 2013). Based on the results of the K-mer analysis, information on peak depth and the number of predicted best K-mer were obtained and used to estimate the size of the genome. Its relationship was expressed by using the following algorithm: Genome size = kmer_num/peak_depth, where K-mer_num indicates the total number of predicted best K-mer, and peak_depth indicates the expected value of the K-mer depth. Also, the heterozygosity ratio and repeat sequence ratio were estimated based on the K-mer analysis. Subsequently, the filtered clean data were assembled and mapped to complete mitogenome sequence using NOVOPlasty v4.3.1 (Dierckxsens et al., 2017).

### 2.3 *Mitogenome annotation and sequence analysis*

The newly complete mitogenome of *C. puncticeps* was annotated using the software of Sequin (version 15.10, http://www.ncbi.nlm.nih.gov/Sequin/). The PCGs were determined by their open reading frame following the vertebrate mtDNA translation table. The predicted boundaries of ribosomal RNA genes were determined using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov). Transfer RNA genes were determined the location and the secondary structure predicted by the MITOS Web Server and tRNA scan-SE 1.21. CR was determined by the locations of adjacent genes. Tandem repeat units of the CR were identified with Tandem Repeats Finder 4.09. The base composition, sequence similarity and genetic distance were analyzed with MEGA X. The strand asymmetry was calculated using the following formulas: AT-skew = (A – T)/(A + T); GC-skew = (G – C)/(G + C). Genome sequence similarity of the *C. puncticeps* mitogenome with other Cynoglossidae mitogenomes was performed using the CG View Comparison Tool (CCT). CCT uses BLAST to compare a query genome with all other genomes and then presents the results as a circular map.

### 2.4 *Phylogenetic analysis*

A total of 22 Cynoglossidae mitogenomes were used to reconstruct the phylogenetic relationships, including 21 mitogenomes downloaded from the GenBank database and one newly determined sequence in this study (*C. puncticeps*). Soleidae species have been thought to be most closely related to Cynoglossidae; therefore, two Soleidae species, *Zebrias zebrinus* and *Z. crossolepis*, were selected as the outgroup. Fasta files with the nucleotide sequences for all 13 PCGs were extracted from the GenBank files using PhyloSuite (Zhang et al., 2020). Sequences were aligned using Clustal X 2.0 (Larkin et al., 2007) with the default parameters and manually checked with BioEdit (Hall, 1999). The ambiguous sequences were eliminated using Gblocks (Talavera and Castresana, 2007). Subsequently, the sequences were concatenated into a single alignment and converted into input files (Phylip and Nexus format) for phylogenetic analyses. Both maximum likelihood (ML) and Bayesian inference (BI) were employed for phylogenetic analyses. ML analysis was conducted using IQ-TREE (Nguyen et al., 2015), under an ML + rapid bootstrap (BS) algorithm with 1 000 replicates. The BI analysis was carried out in MrBayes 3.2.6 (Ronquist et al., 2012) with default parameters. Two independent runs of four Markov chain Monte Carlo (MCMC) chains (one cold chain and three hot chains) were simultaneously run for $3 \times 10^6$ generations, with sampling conduc-

ted every 1 000 generations and the first 25% of the generations were discarded as burn-in. To guarantee the stationarity had been reached, the average standard deviation of split frequencies was set below 0.01. (Katoh et al., 2002; Lanfear et al., 2017)

## 3 Results and discussion

### 3.1 *Genome structure, composition and skewness*

The complete mitogenome of *C. puncticeps* (GenBank accession number OP_573760) is a closed-circular molecule of 17 158 bp in length. The gene content is typical with other teleostean mitogenomes, including 13 PCGs, 22 tRNA genes, 2 rRNA genes and a putative CR. Except *ND6* and seven tRNAs (*tRNA-Pro, Ala, Asn, Cys, Tyr, Ser*$_1$, *Glu*), which are distributed on the light (L-) strand, the remaining genes are distributed on the heavy (H-) strand (Fig. 1, Table 1).

In this study, the sequence features of the newly determined *C. puncticeps* mitogenome and other 21 Cynoglossidae mitogenomes were compared. The mitogenomes have obvious variation in size, ranging from 16 417 bp (*C. abbreviates*) to 18 369 bp (*C. trigrammus*). All mitogenomes are rich in As and Ts, with the A+T content ranging from 53.8% (*Symphurus plagiusa*) to 62.0% (*C. puncriceps*). AT-skew and GC-skew of all mitogenomes are shown in Table 1, which are consistent with most metazoan mitogenomes (positive AT-skew and negative GC-skew) (Gong et al., 2019b; Zhang et al., 2021; Miya et al., 2003). Using *C. puncticeps* mitogenome as the reference sequence, all Cynoglossidae mitogenomes were compared using CCT. Obviously, the inner

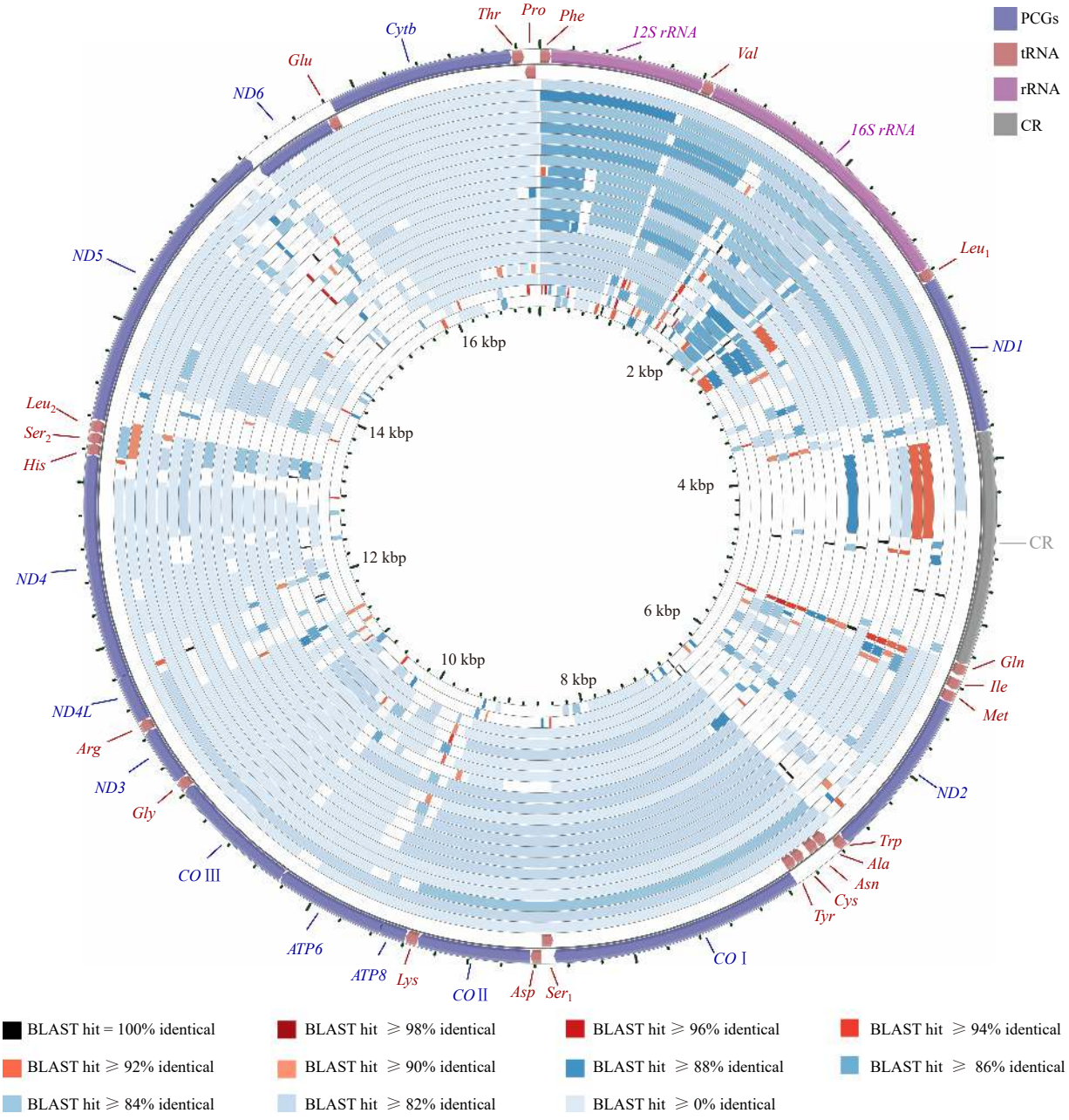


**Fig. 1.** The comparison map of all Cynoglossidae mitogenomes. The species order from the outermost to the innermost ring is presented as that in Table 1, taking *Cynoglossus puncticeps* mitogenome as the reference. Colors of the block denote the degree of sequence similarity.

**Table 1.** List of 22 Cynoglossidae species and two outgroups used in this paper

| Name | Abbreviation | AT/% | AT-skew | GC-skew | Length/bp | Accession No. | Reference |
|---|---|---|---|---|---|---|---|
| *Cynoglossus puncticeps* | *C. pun* | 62.0 | 0.012 | −0.237 | 17 158 | OP_573760 | this study |
| *Paraplagusia bilineata* | *P. bil* | 60.2 | 0.025 | −0.225 | 16 985 | NC_023227 | unpublished |
| *Cynoglossus monpus* | *C. mon* | 61.2 | 0.007 | −0.239 | 16 425 | MT798589 | Wang et al., 2020a |
| *Cynoglossus joyneri* | *C. joy* | 60.4 | 0.022 | −0.239 | 16 428 | NC_030256 | Bo et al., 2017 |
| *Cynoglossus melampetalus* | *C. mel* | 61.0 | 0.029 | −0.278 | 16 641 | MN082377 | Gong et al., 2020 |
| *Paraplagusia blochii* | *P. blo* | 60.1 | 0.012 | −0.216 | 16 611 | NC_023228 | Li et al., 2016 |
| *Cynoglossus robustus* | *C. rob* | 59.9 | 0.012 | −0.244 | 16 720 | LC482305 | Song et al., 2019b |
| *Cynoglossus interruptus* | *C. int* | 60.6 | 0.007 | −0.239 | 17 262 | LC482306 | Song et al., 2019a |
| *Paraplagusia japonica* | *P. jap* | 59.6 | 0.019 | −0.216 | 16 694 | NC_021376 | Si et al., 2017 |
| *Cynoglossus abbreviates* | *C. abb* | 60.4 | 0.025 | −0.259 | 16 417 | JQ349004 | Shi et al., 2016a |
| *Cynoglossus roulei* | *C. rou* | 60.8 | 0.028 | −0.273 | 16 598 | MN966658 | Wang et al., 2020b |
| *Cynoglossus nanhaiensis* | *C. nan* | 60.0 | 0.012 | −0.243 | 17 130 | MT117229 | Tian et al., 2020 |
| *Cynoglossus gracilis* | *C. gra* | 61.6 | 0.019 | −0.248 | 16 565 | KT809367 | Wei et al., 2016 |
| *Cynoglossus semilaevis* | *C. sem* | 60.6 | 0.029 | −0.265 | 16 731 | EU366230 | Kong et al., 2009 |
| *Cynoglossus trigrammus* | *C. tri* | 60.6 | 0.057 | −0.304 | 18 369 | KP057581 | Mu et al., 2015 |
| *Cynoglossus sinicus* | *C. sin* | 60.8 | 0.035 | −0.252 | 16 478 | JQ348998 | Shi et al., 2015b |
| *Cynoglossus senegalensis* | *C. sen* | 59.9 | 0.044 | −0.240 | 16 519 | NC_045034 | Gietbong et al., 2018 |
| *Cynoglossus bilineatus* | *C. bil* | 59.8 | 0.068 | −0.240 | 16 454 | NC_023226 | Shi et al., 2016b |
| *Cynoglossus itinus* | *C. iti* | 58.8 | −0.007 | −0.201 | 16 915 | NC_023446 | unpublished |
| *Cynoglossus zanzibarensis* | *C. zan* | 57.5 | −0.003 | −0.193 | 16 569 | NC_030364 | Shi et al., 2014b |
| *Symphurus orientails* | *S. ori* | 58.5 | −0.042 | −0.177 | 17 498 | KP992899 | Shi et al., 2015a |
| *Symphurus plagiusa* | *S. pla* | 53.8 | 0.010 | −0.240 | 17 034 | JQ639061 | Shi et al., 2015a |
| *Zebrias crossolepis* | *Z. cro* | 54.6 | 0.037 | −0.320 | 16 734 | KJ433564 | Gong et al., 2016 |
| *Zebrias zebrinus* | *Z. zeb* | 54.8 | 0.049 | −0.326 | 16 758 | JQ700100 | Wang et al., 2013 |

most two circles (*S. orientails* and *S. plagiusa* mitogenomes) show great difference between other 20 circles in most regions due to their divergent gene arrangements (Fig. 1). Overall, all Cynoglossinae mitogenomes are observed to be conserved, except the similarity of the entire control region, 3′ end of *ND2* and *ND5*, and 5′ end of *ND6* (Fig. 1).

### 3.2 *PCGs and codon usage*

The start/stop codons of 22 Cynoglossidae mitogenomes were compared. The results reveal that there are five start codons (ATG, ATT, ATA, ATC and GTG), three complete stop codons (TAA, TAG and AGA) and two incomplete termination codons (TA and T). ATG and GTG are two most frequently used start codons almost occurring in all PCGs, and an unusual start codon (ATC) is found exclusively in *C. melampetalus* mitogenome (*ND3*) (Table 2).

Due to base-composition asymmetry of the mitogenomes, value of GC-skew can act as a signal of the origin and end point of the leading strand, and the transformation from leading strand to lagging strand during DNA replication. Hence, the coding strand of certain gene can be confirmed by the value of GC-skew. Generally, positive GC-skew value indicates the gene is encoded by the H-strand; whereas, negative GC-skew value indicates the L-strand. In this study, the value of GC-skew of 13 PCGs of 22 Cynoglossidae mitogenomes was calculated. The result shows that except for *ND6* gene has a positive GC-skew value, the remaining 12 PCGs have a negative GC-skew value, suggesting the *ND6* gene is encoded by L-strand, and the remaining 12 PCGs are encoded by H-strand (Fig. 2).

The mitochondrial *COI* gene has long been acted as a DNA barcoding for species identification. To investigate possible synonyms in these Cynoglossidae species, genetic distance and sequence similarity of *COI* gene among 22 Cynoglossidae mitogenomes were calculated. The maximum genetic distance value is 0.732 (between *S. orientalis* and *C. zanzibarensis*), and the corresponding sequence similarity has a lowest value (71.5%). The minimum genetic distance value is 0.002 (between *C. interruptus* and *C. robustus*), and their sequence similarity reaches up to 99.8%, suggesting that they might be the same species. The overall sequence similarity of the complete mitogenomes (96.5%) and the phylogenetic relationship of these two species also support this point (Table 3).

### 3.3 *Control region*

CR is the most variable region in mitogenomes because of its fast evolution rate compared with other genes. It is typically located between *tRNA-Pro* and *tRNA-Phe* genes, with an extremely high AT content and abundant tandem repeat units, performing an important function in replication and transcription. However, the CR of all 20 Cynoglossinae mitogenomes have translocated downstream to the 3′ end of *ND1* gene (Fig. 3). In order to better understand the variations of CR, 22 Cynoglossidae CRs were compared. The result reveals a high AT content, ranging from 64.1% (*C. zanzibarensis*)–70.6% (*S. orientalis*). The length also has a significant variation ranging from 655 bp (*C. bilineatus*) to 1 433 bp (*C. puncticeps*), which is the main contributor for length heteroplasmy of the complete mitogenomes of Cynoglossidae species. Further analysis shows that the difference of tandem repeats acts as a determining factor resulting in the length heterogeneity. Except *C. senegalensis* and *C. sinicus*, the remaining 20 CRs possess diverse tandem repeats differing in motifs and number of copies, which ranges from 8 bp to 73 bp and 1.9 to 40.2 copies, respectively. Although CR in most vertebrate mitogenomes (e.g., amphibians, reptiles, birds, fish and mammals) captures several conserved sequence blocks (White and Martin, 2009; Zhao et al., 2006; Brown et al., 1986; Gong et al., 2015), no obvious conserved sequences were found in Cynoglossidae mitogenomes due to its high variation (Fig. S1).

**Table 2.** The start codon and stop codon of 13 PCGs in Cynoglossidae mitogenomes

| Species | Start codon / Stop codon | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *ND1* | *ND2* | *CO Ⅰ* | *CO Ⅱ* | *ATP8* | *ATP6* | *COⅢ* | *ND3* | *ND4L* | *ND4* | *ND5* | *ND6* | *Cyt b* |
| *Cynoglossus puncticeps* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TAA |
| *Praplagusia bilineata* | ATG/TAA | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATA/TAA | ATG/TA | ATA/T | ATG/TAA | ATG/TAG | ATG/TAA | ATG/TAA | ATG/TAA |
| *Cynoglossus monopus* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/TAA | ATG/TAA | ATG/TAG | ATG/TAG |
| *Cynoglossus joyneri* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/T |
| *Cynoglossus melampetalus* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATC/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |
| *Praplagusia blochii* | ATG/TAA | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATA/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus robustus* | ATG/TAA | ATG/TAG | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TAA |
| *Cynoglossus interruptus* | ATG/TAA | ATG/TAG | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TAA |
| *Praplagusia japonica* | ATG/TAA | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATA/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TAA |
| *Cynoglossus abbreviatus* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/TAG | ATG/TAA | ATG/TAG | ATG/TAG |
| *Cynoglossus roulei* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus nanhaiensis* | ATG/TAA | ATG/TAG | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/TAA | ATG/TAA | ATG/TAA | ATG/TAA | ATG/TAA | ATG/TAA |
| *Cynoglossus gracilis* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus semilaevis* | ATG/TAA | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATA/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus trigrammus* | ATG/TAG | ATG/TAA | GTG/TAA | ATG/T | GTG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/TAA | ATG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus sinicus* | ATG/TAG | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | GTG/TAA | ATG/TAG | ATG/TAA |
| *Cynoglossus senegalensis* | ATG/TAA | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/T | GTG/T | ATG/TAG | ATG/TAA |
| *Cynoglossus bilineatus* | ATG/TAG | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATA/T | ATG/TAA | ATG/TAG | ATG/TAG | ATG/TAA | ATG/TAA |
| *Cynoglossus itinus* | GTG/TAA | ATG/TAG | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATT/T | ATG/TAA | ATG/TAG | ATG/AGA | ATG/TAG | ATG/TAA |
| *Cynoglossus zanzibarensis* | ATG/TAG | ATG/TAA | GTG/TAA | ATG/T | ATG/TAA | GTG/TAA | ATG/TA | ATG/T | GTG/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TAA |
| *Symphurus orientails* | ATG/TAA | ATG/AGA | ATT/TAA | ATG/T | ATG/TAA | ATG/TAA | ATG/TA | ATG/T | ATG/TAA | ATT/TAG | ATG/TAA | ATG/TAG | ATG/T |
| *Symphurus plagiusa* | ATG/TAA | ATG/AGA | GTG/TAA | ATG/AGA | ATG/TAA | ATA/TAA | ATG/TAA | ATG/T | ATG/TAA | ATG/T | ATG/TAA | ATG/TAG | ATG/TAA |

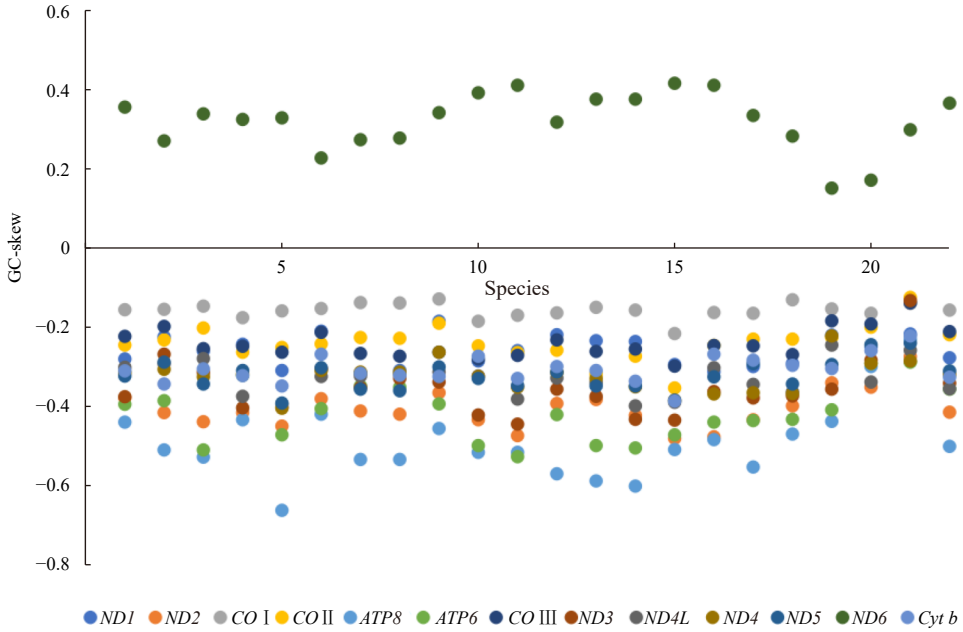


**Fig. 2.** The GC-skew value of 13 PCGs of Cynoglossidae mitogenomes. The species order on the horizontal axis is presented as that in Table 1.

### 3.4 *Gene rearrangement*

Gene order in 22 Cynoglossidae mitogenomes were compared. Except that in *S. plagiusa* (Symphurinae) mitogenome, the remaining 21 mitogenomes underwent varying degrees of gene rearrangement events compared with that in ancestral vertebrate mitogenomes. Three types of mitogenomic rearrangement were found, represented by (Ⅰ) *S. orientalis* (Symphurinae) mitogenome (Fig. 4b); (Ⅱ) *C. puncticeps* and other 16 Cynoglossinae mitogenomes (Fig. 4c); and (Ⅲ) *C. melampetalus*, *C. robustus* and *C. interruptus* (Cynoglossinae) mitogenomes (Fig. 4d).

Gene arrangements in *S. orientalis* mitogenome is featured by the translocation of *tRNA-Val* and *tRNA-Met* genes downstream to the 3' end of the *16S rRNA* gene. It is a unique rearrangement phenomenon in Pleuronectiformes mitogenomes and was first reported by Shi et al. (2015a). The authors explained that the double $O_L$-like structures-initiated DNA synthesis twice during mitochondrial replication, causing tandem duplication of the genes located between the CR and the WANCY region. Generally, mitogenome only maintains one set of functional genes, the duplicated genes would randomly lose their functions and became pseudogenes, shorter non-coding sequences or even be eliminated from the genome (Fig. 4b). Six large intergenic spacers (over 20 bp) in the rearranged regions suggested a duplication and deletion process during mitochondrial replication.

All Cynoglossinae mitogenomes underwent remarkable gene rearrangements. The *tRNA-Gln* gene encoded by the L-strand has translocated to the H-strand (*Q* inversion) and the control region translocated downstream to the place between *ND1*. Interestingly, two types of gene rearrangements coexist in Cynoglossinae mitogenomes. The difference between these two types of mitogenomes lies in the rearrangement of *IQM* cluster (*tRNA-Ile-Gln-Met*). Most of the Cynoglossinae mitogenomes (17/20, including *C. puncticeps* in this study) are presented by *QIM* order (Fig. 4c), while only 3 Cynoglossinae mitogenomes are featured by *IMQ* order (Fig. 4d). Kong et al. (2009) reported the first gene rearrangement phenomenon in Cynoglossidae mitogenomes, followed by increasingly uniform patterns. Intra-mitochondrial recombination and tandem duplication/random loss model were widely accepted to explain the rearrangement events (Mu et al., 2015; Wang et al., 2020a; Gong et al., 2013b). In *C. puncticeps* and other 16 Cynoglossinae mitogenomes, the typical *IQM* cluster (ancestral teleosts gene order with no rearrangement) underwent an inverted duplication, forming an *M'- Q'- I'- I- Q- M* dimeric block (the symbol' indicates inverted gene). In the next step, the redundant genes lost their functions through random deletion, namely *M'- Q'- I'- I- Q- M* (the symbol indicates the deleted gene). Thus, a new *Q'IM* gene order was generated (Fig. 4c). Gong et al. (2020) found the first unusual rearrangement in *C. melampetalus* mitogenome, which was a unique phenomenon in Cynoglossidae mitogenomes. They adopted the same models to account for the distinctive rearrangement. It was speculated that the *Q'IM* cluster in most Cynoglossinae mitogenomes formed a *Q'- I- M- Q'- I- M* dimeric block after duplication, then followed by random deletion of the supernumerary genes, *Q'- I- M- Q'- I- M*. Thus, a new *IMQ'* gene order in Cynoglossidae mitogenome emerged (Fig. 4d). Identical gene rearrangements were found in *C. robustus* and *C. interruptus* mitogenomes.

### 3.5 *Phylogenetic analysis of Cynoglossidae and non-monophyly of Cynoglossu*

In this study, the phylogenetic relationship of Cynoglossidae species was reconstructed based on the nucleotide sequences of 13 PCGs using ML and BI methods. ML tree and BI tree produced an identical topological structure. Here, only one topology (ML) with both support values is displayed (Fig. 5). It is comprised of two clades. *Symphurus orientalis* and *S. plagiusa* form the Symphurinae clade, locating in the base of the tree. Three *Paraplagusia* species (*P. bilineata*, *P. japonica* and *P. blochii*) and 17 *Cynoglossus* species constitute the Cynoglossinae clade. It is worth noting that three *Paraplagusia* species cluster into a group, while the other 17 *Cynoglossus* species form a paraphyletic relationship. Among them, *C. monopus* and *C. puncticeps* form a sister clade with three *Paraplagusia* species, suggesting these two *Cynoglossus* species have a closer relationship with *Paraplagusia*

**Table 3.** The genetic distance (bottom left) and sequence similarity (upper right) based on *COI* gene in 22 Cynoglossidae mitogenomes

| | *C. abb* | *C. bil* | *C. gra* | *C. int* | *C. iti* | *C. joy* | *C. mel* | *C. mon* | *C. nan* | *C. pun* | *C. rob* | *C. rou* | *C. sem* | *C. sen* | *C. sin* | *C. tri* | *C. zan* | *P. bil* | *P. blo* | *P. jap* | *S. ori* | *S. pla* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. abb* | | 80.20 | 90.00 | 83.00 | 79.40 | 84.00 | 82.70 | 81.40 | 81.40 | 81.90 | 83.10 | 88.80 | 88.40 | 80.90 | 80.60 | 80.70 | 80.30 | 79.90 | 80.90 | 80.60 | 71.90 | 73.60 |
| *C. bil* | 0.199 | | 81.30 | 80.30 | 81.10 | 82.10 | 81.30 | 80.70 | 80.40 | 82.70 | 80.30 | 81.50 | 82.10 | 84.50 | 83.00 | 79.90 | 82.30 | 78.70 | 80.50 | 80.20 | 72.80 | 73.50 |
| *C. gra* | 0.100 | 0.188 | | 82.90 | 81.80 | 83.70 | 84.10 | 82.00 | 82.10 | 82.80 | 83.00 | 89.60 | 89.10 | 81.20 | 80.30 | 80.20 | 79.90 | 79.80 | 81.60 | 82.00 | 71.50 | 73.90 |
| *C. int* | 0.171 | 0.198 | 0.172 | | 81.40 | 83.40 | 86.40 | 82.20 | 81.50 | 82.70 | 99.80 | 82.00 | 82.20 | 80.50 | 80.10 | 80.50 | 81.60 | 80.70 | 81.60 | 82.10 | 70.80 | 73.40 |
| *C. iti* | 0.207 | 0.191 | 0.183 | 0.187 | | 83.60 | 81.10 | 81.10 | 84.50 | 82.00 | 81.40 | 80.80 | 80.40 | 81.60 | 80.50 | 79.70 | 80.50 | 79.30 | 80.50 | 81.10 | 71.20 | 74.30 |
| *C. joy* | 0.161 | 0.180 | 0.164 | 0.167 | 0.165 | | 85.00 | 84.30 | 82.90 | 85.30 | 83.50 | 84.00 | 83.60 | 83.20 | 82.10 | 82.20 | 81.70 | 81.60 | 82.90 | 83.00 | 72.20 | 75.50 |
| *C. mel* | 0.174 | 0.188 | 0.160 | 0.137 | 0.191 | 0.151 | | 82.40 | 81.70 | 83.60 | 86.40 | 83.60 | 82.90 | 81.60 | 81.30 | 81.50 | 80.50 | 80.70 | 81.40 | 81.40 | 72.20 | 73.40 |
| *C. mon* | 0.187 | 0.194 | 0.182 | 0.179 | 0.190 | 0.158 | 0.177 | | 80.80 | 84.80 | 82.10 | 82.40 | 81.80 | 80.70 | 81.10 | 80.50 | 80.20 | 81.10 | 82.20 | 82.20 | 71.50 | 72.80 |
| *C. nan* | 0.187 | 0.197 | 0.180 | 0.186 | 0.156 | 0.172 | 0.184 | 0.193 | | 81.40 | 81.50 | 81.60 | 81.20 | 80.50 | 82.00 | 81.40 | 80.90 | 79.10 | 82.10 | 81.90 | 72.10 | 74.90 |
| *C. pun* | 0.182 | 0.174 | 0.173 | 0.174 | 0.182 | 0.148 | 0.165 | 0.153 | 0.187 | | 82.70 | 82.20 | 82.10 | 80.90 | 80.20 | 81.00 | 80.70 | 81.40 | 82.90 | 82.90 | 72.40 | 73.60 |
| *C. rob* | 0.170 | 0.198 | 0.171 | 0.002 | 0.187 | 0.166 | 0.136 | 0.180 | 0.186 | 0.174 | | 81.90 | 82.10 | 80.50 | 80.10 | 80.30 | 81.60 | 80.70 | 81.70 | 82.10 | 70.80 | 73.40 |
| *C. rou* | 0.110 | 0.183 | 0.101 | 0.178 | 0.190 | 0.158 | 0.162 | 0.174 | 0.182 | 0.176 | 0.179 | | 96.20 | 80.90 | 81.80 | 80.70 | 79.60 | 79.60 | 80.90 | 81.80 | 71.30 | 73.50 |
| *C. sem* | 0.113 | 0.177 | 0.106 | 0.176 | 0.194 | 0.161 | 0.169 | 0.180 | 0.185 | 0.176 | 0.176 | 0.038 | | 80.20 | 80.90 | 79.90 | 80.10 | 79.30 | 81.60 | 82.00 | 70.90 | 73.50 |
| *C. sen* | 0.192 | 0.156 | 0.189 | 0.196 | 0.185 | 0.169 | 0.185 | 0.194 | 0.196 | 0.193 | 0.196 | 0.190 | 0.196 | | 85.20 | 79.20 | 81.40 | 77.80 | 79.10 | 79.50 | 72.80 | 72.60 |
| *C. sin* | 0.195 | 0.171 | 0.198 | 0.200 | 0.196 | 0.180 | 0.188 | 0.190 | 0.182 | 0.200 | 0.200 | 0.180 | 0.189 | 0.149 | | 79.40 | 81.80 | 78.80 | 80.50 | 79.00 | 72.30 | 72.90 |
| *C. tri* | 0.192 | 0.200 | 0.198 | 0.194 | 0.202 | 0.177 | 0.184 | 0.194 | 0.185 | 0.189 | 0.196 | 0.189 | 0.197 | 0.207 | 0.205 | | 79.30 | 79.00 | 79.70 | 79.40 | 71.70 | 74.00 |
| *C. zan* | 0.200 | 0.178 | 0.202 | 0.185 | 0.196 | 0.184 | 0.196 | 0.199 | 0.192 | 0.194 | 0.185 | 0.202 | 0.197 | 0.187 | 0.183 | 0.206 | | 79.70 | 80.70 | 79.50 | 71.50 | 74.10 |
| *P. bil* | 0.202 | 0.214 | 0.203 | 0.194 | 0.208 | 0.185 | 0.194 | 0.190 | 0.210 | 0.187 | 0.194 | 0.202 | 0.206 | 0.224 | 0.213 | 0.209 | 0.204 | | 85.20 | 86.30 | 72.30 | 73.50 |
| *P. blo* | 0.192 | 0.196 | 0.185 | 0.185 | 0.196 | 0.172 | 0.187 | 0.179 | 0.180 | 0.172 | 0.184 | 0.189 | 0.182 | 0.211 | 0.196 | 0.203 | 0.194 | 0.149 | | 87.50 | 72.40 | 74.20 |
| *P. jap* | 0.195 | 0.200 | 0.181 | 0.180 | 0.191 | 0.171 | 0.187 | 0.179 | 0.182 | 0.172 | 0.180 | 0.180 | 0.178 | 0.206 | 0.211 | 0.207 | 0.206 | 0.138 | 0.126 | | 72.10 | 73.60 |
| *S. ori* | 0.727 | 0.720 | 0.725 | 0.720 | 0.725 | 0.722 | 0.715 | 0.731 | 0.730 | 0.727 | 0.721 | 0.719 | 0.718 | 0.727 | 0.722 | 0.719 | 0.732 | 0.731 | 0.721 | 0.714 | | 76.50 |
| *S. pla* | 0.268 | 0.266 | 0.267 | 0.272 | 0.260 | 0.250 | 0.275 | 0.280 | 0.256 | 0.267 | 0.272 | 0.266 | 0.268 | 0.276 | 0.274 | 0.261 | 0.262 | 0.271 | 0.262 | 0.268 | 0.726 | |

Note: Abbreviations of species names are presented as that in Table 1.

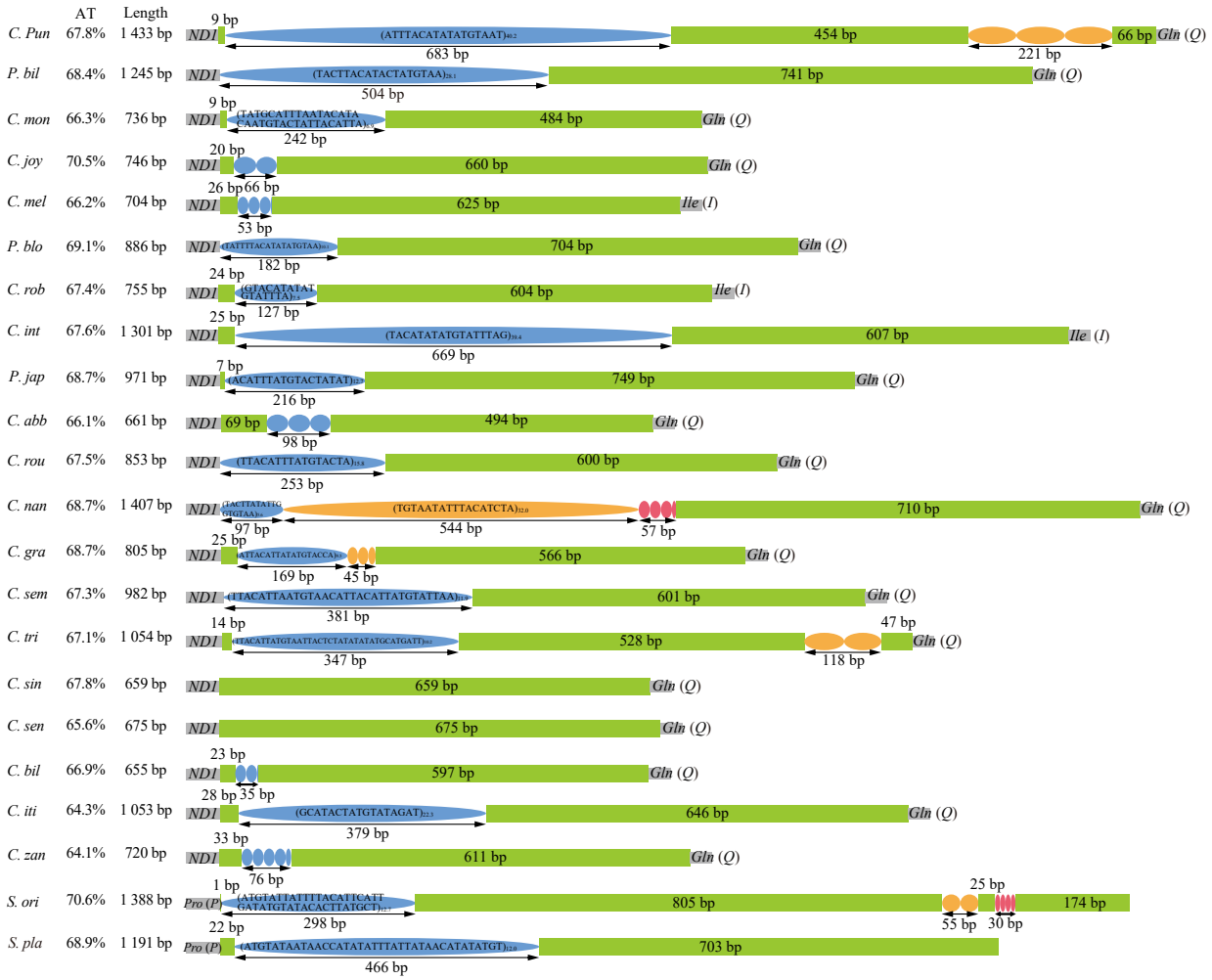


**Fig. 3.** Structure of control region in 22 Cynoglossidae mitogenomes. The colored ovals indicate the tandem repeats; the remaining regions are shown with green boxes. Tandem repeat with copy number exceeding 5 is displayed in the format of $(motif)_n$.

than with other *Cynoglossus* species.

Many studies have contributed to the classification and phylogeny of tongue soles (Cynoglossidae) based on morphological taxonomy (Chapleau, 1988; Menon, 1977). Chapleau (1988) provided the first exhaustive comparative osteological study of Cynoglossidae, and proposed two subfamilies (Symphurinae and Cynoglossinae) of Cynoglossidae. Since then, almost all of the studies followed this classification system. However, increasing number of molecular evidence revealed that Symphurinae were quite different from their closely related species, Cynoglossinae. For example, the mitogenomic rearrangements in these two taxa are strikingly different (Fig. 4). Generally, a teleostean group (belonging to the same order or family) possesses only one type or a set of similar mitochondrial gene arrangement (Zhang et al., 2022b; Luo et al., 2019; Montaña-Lozano et al., 2022). It is a rare phenomenon that two sets of various mitochondrial gene arrangement coexist in a family, especially in teleostean mitogenomes (Lü et al., 2019; Shi et al., 2015a). Besides, the genetic distance and the branch length in the phylogenetic tree also suggest that these two taxa are raised to the rank of family. Of course, an examination of the morphology and molecular data of more species belonging to Symphurinae are needed to further test this proposal.

The speciose Cynoglossinae are divided into two genera (*Paraplagusia* and *Cynoglossus*) mainly based on the characters of fringes on the lips (the former possesses fringes on the lips on eyed side; the latter lacks fringes on the lips). Numerous studies showed *Parapaglusia* was monophyletic, while no evidence was found to support a similar status for *Cynoglossus*. In fact, growing number of molecular researches including our results corroborate the non-monophyly of *Cynoglossus* (Mu et al., 2015; Wang et al., 2020a; Gong et al., 2020). With the increasing number of tongue soles, the classification of *Cynoglossus* has been largely disputed (Li and Wang, 1995; Nelson et al., 2016; Chapleau, 1988; Menon, 1977). Previous researchers suggested divided the original genus *Cynoglossus* into seven new genera manily based on the number of lateral line and left nostril, including *Cynoglossus* Hamilton 1822, *Arelia* Kaup 1858, *Trulla* Kaup 1858, *Icania* Kaup 1858, *Areliscus* Jordan et Snyder 1900, *Dollfusichthys* Chabanaud 1931 and *Cynoglossoides* Smith 1953. While Li and Wang (1995) degraded these genera to the rank of subgenus. However, Menon (1977) did not regard lateral line and left nostril as the effective taxonomic characters. Instead, number of scales between the lateral lines, type of scales, location of mouth and number of caudal fins were synthesized to divide *Cynoglossus* into six groups, including canariensis, kopsii, arel, cynoglossus, carpenter and heterolepis. While there was no detailed description about the classification of *Cynoglossus* in Nelson et al.'s taxonomy system (Nelson et al., 2016). According to WoRMS database (https://www.marinespecies.org/aphia.php?p=taxdetails&id=126112), the

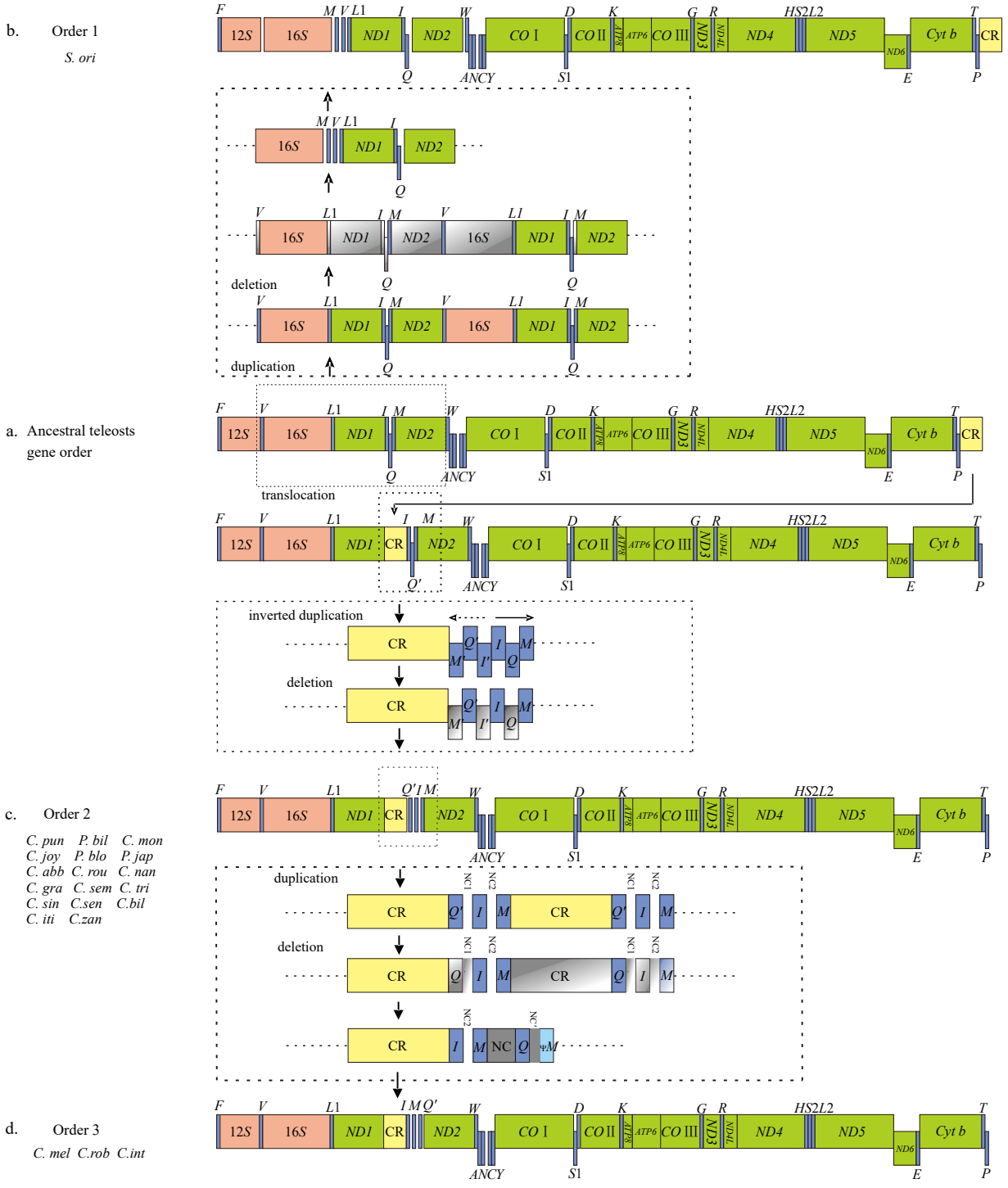


**Fig. 4.** Inferred intermediate steps between the gene order of ancestral teleosts and 22 Cynoglossidae mitogenomes. a. The ancestral gene order of teleosts; b. gene order of *Synglossus orientalis* (Symphurinae) mitogenome; c. gene order of 17 Cynoglossinae mitogenomes; d. gene order of 3 Cynoglossinae mitogenomes.

genus *Cynoglossus* possesses about 112 species. More purposeful research is needed on each of these subgenera or groups with more integrated approaches to clarify the speciose taxa.

### 3.6 *Implications of QIM spacers*

The typical *IQM* order in ancestral vertebrate mitogenomes changed to *QIM* or *IMQ* order in all Cynoglossinae mitogenomes. Previous studies suggested that gene rearrangements and length of gap spacer in the rearranged area could reflect the evolutional trace of mitogenomes, and further revealed the phylogenetic relationships (Gong et al., 2020; Zhang et al., 2022b; McKnight and Shaffer, 1997). Here, the length of gap spacer in the rearranged area were compared. In *C. puncticeps* and other 16 Cynoglossinae mitogenomes, the intergenic space between *Q* and *I* (G1) ranged from 3 bp (*C. joyneri*) to 160 bp (*C. zanzibarensis*); 5 bp (*P. bilineata* and *P. blochii*) to 35 bp (*C. gracilis*) between *I* and *M* (G2). That between *M* and *ND2* (G3) was consistent in all 17 Cynoglossinae species (1 bp). In *C. melampetalus*, *C. interrup-*

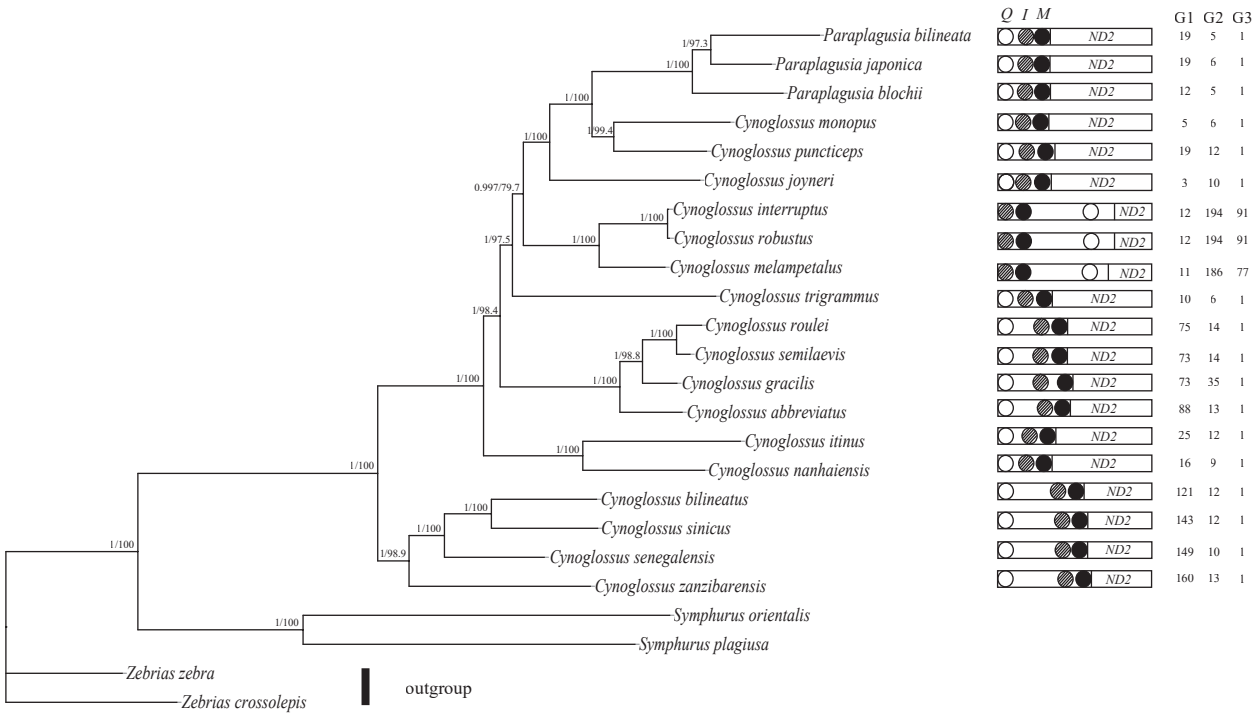


**Fig. 5.** The relationship of Cynoglossidae species and intergenic space between *tRNA* and *QIM*. G1, G2 and G3 indicate the intergenic space between *Q* and *I*, *I* and *M*, *M* and *ND2*, respectively.

*tus* and *C. robustus* mitogenomes featured with *IMQ* gene order, large gaps existed between *M* and *Q* (186–194 bp), *Q* and *ND2* (77–91 bp), and that between *I* and *M* was comparatively small (11–12 bp). Although no obvious correlation was found between G2, G3 and the phylogenetic relationship, the intergenic space between *Q* and *I* (G1) had a decreasing trend with the evolution process (excluding the gap spacers in *IMQ* cluster in *C. melampetalus*, *C. interruptus* and *C. robustus* mitogenomes). In the base of phylogenetic tree (*C. zanzibarensis*, *C. senegalensis*, *C. sinicus*, and *C. bilineatus*), the intergenic space of G1 showed the largest lengths (121–160 bp). In the middle branches of the tree, the intergenic space reduced to a moderate length range (73–88 bp). Reversely, in the top of the tree, the intergenic space decreased to the smallest gaps (3–19 bp). This trend was in accordance with our previous result (Gong et al., 2020), suggesting intergenic spacer in the rearranged area could reveal the phylogenetic relationships in some certain. However, the position of the two newly sequenced *C. nanhaiensis* and *C. itinus* challenged this trend in this study. Accordingly, more taxon samplings and more new molecular markers are needed to uncover the authentic phylogenetic relationships and the potential application prospect of gene rearrangements.

## References


Bo Zhang, Lei Jia, Liu Kefeng, et al. 2017. The complete mitochondrial genome of *Cynoglossus joyneri* and its novel rearrangement. Mitochondrial DNA Part B, 2(2): 581–582, doi: 10.1080/23802359.2017.1365642

Boore J L. 1999. Animal mitochondrial genomes. Nucleic Acids Research, 27(8): 1767–1780, doi: 10.1093/nar/27.8.1767

Brown G G, Gadaleta G, Pepe G, et al. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. Journal of Molecular Biology, 192(3): 503–511, doi: 10.1016/0022-2836(86)90272-X

Cantatore P, Gadaleta M N, Roberti M, et al. 1987. Duplication and remoulding of tRNA genes during the evolutionary rearrangement of mitochondrial genomes. Nature, 329(6142): 853–855, doi: 10.1038/329853a0

Chapleau F. 1988. Comparative osteology and intergeneric relationships of the tongue soles (Pisces; Pleuronectiformes; Cynoglossidae). Canadian Journal of Zoology, 66(5): 1214–1232, doi: 10.1139/z88-177

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: *de novo* assembly of organelle genomes from whole genome data. Nucleic Acids Research, 45(4): e18

Gietbong F Z, Kim N K, Andriyono S, et al. 2018. Characterization of the mitogenome of *Cynoglossus senegalensis* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part B, 3(2): 1191–1192, doi: 10.1080/23802359.2018.1524728

Gong Li, Jiang Hui, Zhu Kehua, et al. 2019a. Large-scale mitochondrial gene rearrangements in the hermit crab *Pagurus nigrofascia* and phylogenetic analysis of the Anomura. Gene, 695: 75–83, doi: 10.1016/j.gene.2019.01.035

Gong Li, Liu Bingjian, Liu Liqing, et al. 2019b. The complete mitochondrial genome of *Terapon jarbua* (Centrarchiformes: Terapontidae) and comparative analysis of the control region among eight Centrarchiformes species. Russian Journal of Marine Biology, 45(2): 137–144, doi: 10.1134/S1063074019020068

Gong Li, Lu Xinting, Luo Hairong, et al. 2020. Novel gene rearrangement pattern in *Cynoglossus melampetalus* mitochondrial genome: new gene order in genus *Cynoglossus* (Pleuronectiformes: Cynoglossidae). International Journal of Biological Macromolecules, 149: 1232–1240, doi: 10.1016/j.ijbiomac.2020.02.017

Gong Li, Shi Wei, Si Lizhen, et al. 2013a. Rearrangement of mitochondrial genome in fishes. Zoological Research, 34(6): 666–673

Gong Li, Shi Wei, Si Lizhen, et al. 2015. The complete mitochondrial genome of peacock sole *Pardachirus pavoninus* (Pleuronectiformes: Soleidae) and comparative analysis of the control region among 13 soles. Molecular Biology, 49(3): 408–417, doi: 10.1134/S0026893315030061

Gong Li, Shi Wei, Wang Zhongming, et al. 2013b. Control region translocation and a tRNA gene inversion in the mitogenome of *Paraplagusia japonica* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA, 24(6): 671–673, doi: 10.3109/19401736.2013.773984

Gong Li, Si Lizhen, Shi Wei, et al. 2016. The complete mitochondrial

genome of *Zebrias crossolepis* (Pleuronectiformes: Soleidae). Mitochondrial DNA Part A, 27(2): 1235–1236, doi: 10.3109/19401736.2014.945534

Hall T A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98

Katoh K, Misawa K, Kuma K I, et al. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research, 30(14): 3059–3066, doi: 10.1093/nar/gkf436

Kong Xiaoyu, Dong Xiaoli, Zhang Yanchun, et al. 2009. A novel rearrangement in the mitochondrial genome of tongue sole, *Cynoglossus semilaevis*: control region translocation and a tRNA gene inversion. Genome, 52(12): 975–984, doi: 10.1139/G09-069

Lanfear R, Frandsen P B, Wright A M, et al. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution, 34(3): 772–773

Larkin M A, Blackshields G, Brown N P, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23(21): 2947–2948, doi: 10.1093/bioinformatics/btm404

Lavrov D V, Boore J L, Brown W M. 2002. Complete mtDNA sequences of two millipedes suggest a new model for mitochondrial gene rearrangements: duplication and nonrandom loss. Molecular Biology and Evolution, 19(2): 163–169, doi: 10.1093/oxfordjournals.molbev.a004068

Li Donghe, Shi Wei, Gong Li, et al. 2016. The complete mitochondrial genome of *Paraplagusia blochii* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part A, 27(1): 92–93, doi: 10.3109/19401736.2013.873918

Li Sizhong, Wang Huimin. 1995. Fauna Sinica (Osteichthyes): Pleuronectiformes (in Chinese). Beijing: Science Press

Liu Binghang, Shi Yujian, Yuan Jianying, et al. 2013. Estimation of genomic characteristics by analyzing k-mer frequency in de novo genome projects, arXiv (2013) arXiv:1308.2012v1. Available online https://arxiv.org/abs/1308.2012v1

Lü Zhenming, Zhu Kehua, Jiang Hui, et al. 2019. Complete mitochondrial genome of *Ophichthus brevicaudatus* reveals novel gene order and phylogenetic relationships of Anguilliformes. International Journal of Biological Macromolecules, 135: 609–618, doi: 10.1016/j.ijbiomac.2019.05.139

Luo Hairong, Kong Xiaoyu, Chen Shixi, et al. 2019. Mechanisms of gene rearrangement in 13 bothids based on comparison with a newly completed mitogenome of the threespot flounder, *Grammatobothus polyophthalmus* (Pleuronectiformes: Bothidae). BMC Genomics, 20(1): 792, doi: 10.1186/s12864-019-6128-9

McKnight M L, Shaffer H B. 1997. Large, rapidly evolving intergenic spacers in the mitochondrial DNA of the salamander family Ambystomatidae (Amphibia: Caudata). Molecular Biology and Evolution, 14(11): 1167–1176, doi: 10.1093/oxfordjournals.molbev.a025726

Menon A G K. 1977. A Systematic Monograph of the Tongue Soles of the Genus *Cynoglossus* Hamilton-Buchanan (Pisces, Cynoglossidae). Washington: Smithsonian Institution Press

Miya M, Takeshima H, Endo H, et al. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. Molecular Phylogenetics and Evolution, 26(1): 121–138, doi: 10.1016/S1055-7903(02)00332-9

Montaña-Lozano P, Moreno-Carmona M, Ochoa-Capera M, et al. 2022. Comparative genomic analysis of vertebrate mitochondrial reveals a differential of rearrangements rate between taxonomic class. Scientific Reports, 12(1): 5479, doi: 10.1038/s41598-022-09512-2

Moritz C, Brown W M. 1987. Tandem duplications in animal mitochondrial DNAs: variation in incidence and gene content among lizards. Proceedings of the National Academy of Sciences of the United States of America, 84(20): 7183–7187

Mu Xidong, Wang Xuejie, Liu Yi, et al. 2015. An unusual mitochondrial genome structure of the tonguefish, *Cynoglossus trigrammus*: control region translocation and a long additional non-coding region inversion. Gene, 573(2): 216–224, doi: 10.1016/j.gene.2015.07.041

Nelson J S, Grande T C, Wilson M V H. 2016. Fishes of the World. 5th ed. Hoboken: John Wiley & Sons

Nguyen L T, Schmidt H A, von Haeseler A, et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution, 32(1): 268–274, doi: 10.1093/molbev/msu300

Poulton J, Deadman M E, Bindoff L, et al. 1993. Families of mtDNA re-arrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. Human Molecular Genetics, 2(1): 23–30, doi: 10.1093/hmg/2.1.23

Ronquist F, Teslenko M, van der Mark P, et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539–542, doi: 10.1093/sysbio/sys029

Shi Wei, Dong Xiaoli, Wang Zhongming, et al. 2013. Complete mitogenome sequences of four flatfishes (Pleuronectiformes) reveal a novel gene arrangement of L-strand coding genes. BMC Evolutionary Biology, 13: 173, doi: 10.1186/1471-2148-13-173

Shi Wei, Gong Li, Kong Xiaoyu. 2016a. The complete mitochondrial genome sequence of *Cynoglossus abbreviatus* (Pleuronectiformes: Cynoglossidae) with control region translocation and *tRNA-Gln* gene inversion. Mitochondrial DNA Part A, 27(3): 2157–2158

Shi Wei, Gong Li, Wang Shuying, et al. 2015a. Tandem duplication and random loss for mitogenome rearrangement in *Symphurus* (Teleost: Pleuronectiformes). BMC Genomics, 16(1): 355, doi: 10.1186/s12864-015-1581-6

Shi Wei, Jiang Jinxia, Miao Xianguang, et al. 2015b. The complete mitochondrial genome sequence of *Cynoglossus sinicus* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA, 26(6): 865–866, doi: 10.3109/19401736.2013.861433

Shi Wei, Li Donghe, Kong Xiaoyu. 2016b. Gene rearrangements in the mitochondrial genome of *Cynoglossus bilineatus* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part A, 27(3): 2161–2162

Shi Wei, Miao Xianguang, Kong Xiaoyu. 2014a. A novel model of double replications and random loss accounts for rearrangements in the Mitogenome of *Samariscus latus* (Teleostei: Pleuronectiformes). BMC Genomics, 15(1): 352, doi: 10.1186/1471-2164-15-352

Shi Wei, Yang Min, Si Lizhen, et al. 2014b. The complete mitochondrial genome of *Cynoglossus zanzibarensis* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part A, 27(3): 2172–2173

Si Lizhen, Gong Li, Shi Wei, et al. 2017. The complete mitochondrial genome of *Pseudaesopia japonica* (Pleuronectiformes: Soleidae). Mitochondrial DNA Part A, 28(1): 129–130, doi: 10.3109/19401736.2015.1111355

Song H Y, Kim J K, Jo S, et al. 2019a. The complete mitochondrial genome of *Cynoglossus interruptus* and its novel rearrangement (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part B, 4(2): 2924–2925, doi: 10.1080/23802359.2019.1660262

Song H Y, Kim J K, Jo S, et al. 2019b. Gene rearrangements in the mitochondrial genome of robust tonguefish, *Cynoglossus robustus* (Pleuronectiformes: Cynoglossidae) and a comparative analysis with other *Cynoglossus* fishes. Mitochondrial DNA B Resour, 5(1): 353–354

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology, 56(4): 564–577, doi: 10.1080/10635150701472164

Tian Silin, Wang Chen, Yan Yunrong, et al. 2020. Characterization of the complete mitochondrial genome of *Cynoglossus nanhaiensis* (Pleuronectiformes: Cynoglossidae). Mitochondrial DNA Part B, 5(3): 2613–2614, doi: 10.1080/23802359.2020.1781582

Wang Chen, Chen Hao, Tian Shilin, et al. 2020a. Novel gene rearrangement and the complete mitochondrial genome of *Cynoglossus monopus*: insights into the envolution of the family cynoglossidae (Pleuronectiformes). International Journal of Molecular Sciences, 21(8): 6895

Wang Chen, Liu Mengzhen, Tian Silin, et al. 2020b. The complete mitochondrial genome of *Cynoglossus roulei* (Pleuronectiformes: Cynoglossidae): novel rearrangement and phylogenetic position analysis. Mitochondrial DNA Part B, 5(2): 1439–1440, doi: 10.1080/23802359.2020.1734501

Wang Zhongming, Shi Wei, Jiang Jinxia, et al. 2013. The complete mitochondrial genome of a striped sole *Zebrias zebrinus* (Pleuronectiformes: Soleidae). Mitochondrial DNA, 24(6): 633–635, doi: 10.3109/19401736.2013.772160

Wei Min, Liu Yang, Guo Hua, et al. 2016. Characterization of the complete mitochondrial genome of *Cynoglossus gracilis* and a comparative analysis with other Cynoglossinae fishes. Gene, 591(2): 369–375, doi: 10.1016/j.gene.2016.06.023

White M M, Martin H R. 2009. Structure and conservation of tandem repeats in the mitochondrial DNA control region of the Least Brook lamprey (*Lampetra aepyptera*). Journal of Molecular Evolution, 68(6): 715–723, doi: 10.1007/s00239-009-9246-1

Yan Jie, Li Hongdan, Zhou Kaiya. 2008. Evolution of the mitochondrial genome in snakes: gene rearrangements and phylogenetic relationships. BMC Genomics, 9: 569, doi: 10.1186/1471-2164-9-569

Zhang Dong, Gao Fangluan, Jakovlić I, et al. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources, 20(1): 348–355, doi: 10.1111/1755-0998.13096

Zhang Ying, Gong Li, Lu Xinting, et al. 2022a. Comparative mitochondrial genome analysis of Varunidae and its phylogenetic implications. Acta Oceanologica Sinica, 41(6): 119–131, doi: 10.1007/s13131-021-1927-7

Zhang Ying, Meng Lei, Wei Liming, et al. 2021. Different gene rearrangements of the genus *Dardanus* (Anomura: Diogenidae) and insights into the phylogeny of Paguroidea. Scientific Reports, 11(1): 21833, doi: 10.1038/s41598-021-01338-8

Zhang Ying, Meng Lei, Wei Liming, et al. 2022b. Comparative mitochondrial genome analysis of Sesarmidae and its phylogenetic implications. Acta Oceanologica Sinica, 41(8): 62–73, doi: 10.1007/s13131-021-1911-2

Zhang Jiayong, Zhang Leping, Yu Danna, et al. 2018. Complete mitochondrial genomes of *Nanorana taihangnica* and *N. yunnanensis* (Anura: Dicroglossidae) with novel gene arrangements and phylogenetic relationship of Dicroglossidae. BMC Evolutionary Biology, 18(1): 26, doi: 10.1186/s12862-018-1140-2

Zhao Jinliang, Wang Weiwei, Li Sifa, et al. 2006. Structure of the mitochondrial DNA control region of the sinipercine fishes and their phylogenetic relationship. Acta Genetica Sinica, 33(9): 793–799, doi: 10.1016/S0379-4172(06)60112-1

## Supplementary information:

**Fig. S1**. Aligned sequences of the CRs in 22 Cynoglossidae mitogenomes.

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