Novel gene rearrangement in the mitochondrial genome of *Muraenocesox cinereus* and the phylogenetic relationship of Anguilliformes

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The structure and gene sequence of the fish mitochondrial genome are generally considered to be conservative. However, two types of gene arrangements are found in the mitochondrial genome of Anguilliformes. In this paper, we report a complete mitogenome of *Muraenocesox cinereus* (Anguilliformes: Muraenocidae) with rearrangement phenomenon. The total length of the *M. cinereus* mitogenome was 17,673 bp, and it contained 13 protein-coding genes, two ribosomal RNAs, 22 transfer RNA genes, and two identical control regions (CRs). The mitochondrial genome of *M. cinereus* was obviously rearranged compared with the mitochondria of typical vertebrates. The genes ND6 and the conjoint trnE were translocated to the location between trnT and trnP, and one of the duplicated CR was translocated to the upstream of the ND6. The tandem duplication and random loss is most suitable for explaining this mitochondrial gene rearrangement. The Anguilliformes phylogenetic tree constructed based on the whole mitochondrial genome well supports Congridae non-monophyly. These results provide a basis for the future Anguilliformes mitochondrial gene arrangement characteristics and further phylogenetic research.

Anguilliformes is a kind of ecologically diverse fish, mainly marine fish. Its body is very slender, its cross-sectional area is reduced, and it generally lacks ventral fins1,2. Traditional morphological taxonomy divides the eel-shaped order (the largest one) into three sub-orders: Anguilloidei, Congroidei and Muraenoidae3. However, previous phylogenetic studies of Anguilliformes relationship based solely on morphological data have failed to resolve the relationship between these three suborders4–5. For example, recent molecular analysis based on mitochondrial and nucleic acid data has raised questions about the taxonomic status of Anguilliformes6,7. The phylogenetic relationship of Anguilliformes is still unclear. In particular, the family-level classification needs to be revised, because many known families or genera may be multi-lineage, especially the well-known Congridae and Nettastomatidae8,9. *M. cinereus* belongs to the Muraenoidae genus in the Muraenocidae family, also known as dagger-tooth pike conger. *M. cinereus* is located as far north as Japan and South Korea, and south as far as the Arafura Sea and North Australia10. *M. cinereus* mainly inhabits soft bottoms, but was also common in estuaries. *M. cinereus* was an important economic fish and one of the most popular moray eels in China11.

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extremely low, and the replacement rate was faster than that of nuclear DNA. Therefore, mitochondrial markers have become the most commonly used inference target for molecular phylogeny of fish species. However, variations in this gene sequence have been found in various vertebrate lineages, including amphibians, reptiles, birds, marsupials, and fish. In recent years, with the increase in the number of mitochondrial genome sequencing and the improvement of technology, people have discovered more and more gene rearrangements.

However, the bony fish with the largest number of published complete mitochondrial genomes showed only a partial gene rearrangement. Gong summarized the complete sequence of 1255 fish mitochondrial genomes in the National Center for Biotechnology Information (NCBI) database, and found that 52 fish species have rearranged mitochondrial genomes, including shuffling, translocation, and inversion, involving 15 subjects and 34 families. The probability of rearrangement of genes in the fish mitochondrial genome is low.

In general, the mitochondrial genome structure of fish, especially in the order of genes, is highly conserved. However, with the gradual increase of mtDNA sequence data of fish, there have been reports of rearrangement of mtDNA, which is characterized by involvement in DNA strand breakage and repair. The random deletion of duplicates has been proposed to explain the mitochondrial gene rearrangement of two kinds of millipedes. The difference between this model and the TDRL model is that this loss is non-random, and it depends on the transcription polarity and location of the gene. Shi et al. raised a new model, the double replication random loss (DRRL) model to explain the rearrangement of the flatfish Samariscus latus genome. According to this model, the control region (CR) was usually copied and shifted. Then, the two CRs successively initiated the double replication of the mitochondrial genome, leading to gene replication between the two CRs. Finally, one of each pair of duplicated genes was randomly lost.

Previous studies have shown that rearrangement of the mitochondrial genome can provide important clues to the evolution and origin of species. In this paper, the gene structure and gene rearrangement of the mitochondrial genome of M. cinereus (Muraenidae) common in Chinese waters were reported, and the relationship between the mitochondrial genome rearrangement and phyletic evolution of Anguilla was further discussed based on previous report. Based on the similarities and differences of the gene rearrangement order in the mitochondrial genome, the possible rearrangement process was discussed in order to have a better understanding of the rearrangement events and evolutionary mechanisms of the eel mitochondrial genome.

Results and discussion

Genome structure and composition. The complete mitochondrial genome of M. cinereus is 17,673 bp (GenBank accession number MT571331), which is within the published length range of the bony fish mitochondrial genome (16,417–18,369 bp) (Table 1). The structure of the moray mitochondrial genome was different from other bony fishes, it includes 13 PCGs, 22 tRNAs, two rRNAs (12S and 16S rRNA), a light chain replication source (OL) and two control-region s (CR) (Fig. 1). And the gene rearrangement was different from some other fish mitochondria. To be precise, ND6 binding trnE was transferred between trnAT and trnAP, and a replicated CR was transferred upstream of the ND6 gene (Fig. 1, Table 2). In the vertebrate mitochondrial genome, the presence of replicated CR was considered a special feature. The base composition of M. cinereus mitochondrial genome was: A = 32.1%, T = 27.6%, G = 23.8% and C = 16.6%, respectively (Table 3). Overall, the mitotic genome was very compact. However, 89 base pairs of 12 gene spacers were found in the mitochondrial genome of moray eel, ranging in length from 1 to 35 bp. Most of the gaps were found in the area where rearrangement occurred, including 35 bp between Cytb and trnI, 4 bp between trnT and CR1, 1 bp between trnL and trnP, and 23 bp between CR2 and trnP. The AT-skew of the mitochondrial genome was positive and the GC-skew was negative, respectively 0.076 and -0.179, indicating that As and Cs are more abundant than Ts and Gs.

PCGs and codon usage. The total length of the 13 PCGs in the M. cinereus mitochondrial genes were 11,454 bp, and they encode 3,818 amino acids. Genes encoding 13 proteins include seven NADH dehydrogenases (ND1-ND6 and ND4L), three cytochrome c oxidases (COI-COII), two ATPases (ATP6 and ATP8) and one cytochrome b (cyt b). The 13 PCGs range in size from 168 bp (ATP8) to 1857 bp (ND5) (Table 3). Like the typical mitochondrial genome of vertebrates, there are twelve genes in the H-strand and only ND6 genes in the L-strand.

The initiation codon of the 13 protein-coding genes used the typical initiation codon ATG, except for GTG for the COI gene. Seven PCGs (ND1, COI, ATP8, ATP6, COII, ND4L, and ND5) were terminated with a stop codon TAA, three (ND2, ND3, and ND6) were terminated with TAG, and one (Cyt b) was terminated with AGA. In addition, ND4 and COII terminate with an incomplete nucleotide T- (Table 2). This result was very similar to that of Lu et al. Whether in vertebrate or vertebrate mitochondrial genes, the presence of incomplete stop codons is
a common phenomenon. For genes with TAA as the stop codon, one of the most common interpretations is that produced by polyadenylation after transcription. In the 13 PCGS, the values of COI, ATP6, COII, ND3, and ND4 of the AT-skew and GC-skew are negative, and the rest is both positive and GC-skew is negative (Table 3).

According to the codon degeneracy 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. This result also appears in the research results of Vandana et al. The most used amino acids are Leu (15.82%), Ile (8.07%) and Ala (7.96%), the few most used amino acids are Asp (2.12%), Arg (1.94%) and Cys (0.84%) (Fig. 2a). The relative synonymous

| Family          | Species                          | Length (bp) | Accession No | References |
|-----------------|----------------------------------|-------------|--------------|------------|
| Nemichthyidae   | Nemichthys scolopaceus           | 17,457      | NC_013620    | 64         |
|                 | Labichthys carinatus             | 16,683      | NC_013626    | 68         |
| Serrivomeridae  | Serrivomer sector                | 16,099      | NC_013436    | 13         |
|                 | Sternoptychus hypomelas          | 16,566      | NC_013628    | 13         |
| Anguillidae     | Anguilla dieffenbachi            | 16,687      | NC_06538     | 13         |
|                 | Anguilla megastoma               | 16,714      | NC_006541    | 13         |
|                 | Anguilla japonica                | 16,685      | NC_006530    | 13         |
|                 | Anguilla reithardtii             | 16,690      | NC_006546    | 13         |
|                 | Anguilla marmorata               | 16,745      | NC_06540     | 13         |
|                 | Anguilla interioris              | 16,713      | NC_006539    | 13         |
|                 | Anguilla obscura                 | 16,704      | NC_006545    | 13         |
|                 | Anguilla bicolor bicolor         | 16,700      | NC_006534    | 13         |
|                 | Anguilla bicolor pacifica        | 16,693      | NC_065035    | 13         |
| Moringuidae     | Moringua microcir                | 15,858      | NC_013602    | 13         |
|                 | Moringua edwardsi                | 16,841      | NC_013622    | 68         |
| Chlopsidae      | Kaupichthys hyporoids            | 16,662      | NC_013607    | 68         |
|                 | Robensia catherinae              | 16,627      | NC_013633    | 68         |
| Synaphobranchidae| Synaphobranchus capiti           | 16,166      | NC_005805    | 13         |
|                 | Synenchelys parasitica           | 16,689      | NC_013605    | 68         |
|                 | Ilyophis bruneus                 | 16,682      | NC_013634    | 13         |
| Heterenchelyidae| Pyhonichthys microphthalmus      | 17,042      | NC_013601    | 13         |
| Myrocongridae   | Myroconger compressus            | 16,642      | NC_013631    | 68         |
| Muraenidae      | Scuticaria tigrina               | 16,521      | KP874183     | 13         |
|                 | Gymnomuraena zebra               | 16,576      | NC_027240    | 13         |
|                 | Gymnothorax formosus             | 16,558      | KP874184     | 13         |
|                 | Gymnothorax kidalo               | 16,579      | NC_04417     | 13         |
|                 | Rhinomuraena quaesita            | 16,566      | NC_013610    | 68         |
|                 | Gymnothorax minor                | 16,574      | NC_036175    | 13         |
| Derichthyidae   | Derichthys serpentinus           | 17,077      | NC_013611    | 68         |
|                 | Coloconger cadenati              | 17,755      | NC_013606    | 68         |
|                 | Nessorhamphus ingflianus         | 17,782      | NC_013608    | 68         |
| Nettastomatidae | Facciodia oxyrhyncha             | 17,789      | NC_013621    | 68         |
|                 | Hoplannis punctata               | 17,828      | NC_013623    | 68         |
|                 | Leptocephalus sp                 | 18,037      | NC_013615    | 68         |
| Congridae       | Conger myriaster                 | 18,705      | NC_027261    | 68         |
|                 | Conger japonicus                 | 17,778      | NC_027186    | 68         |
|                 | Heteroconger hassi               | 17,768      | NC_013629    | 68         |
|                 | Paraconger notialis              | 17,729      | NC_013630    | 68         |
| Muraenesocidae  | Muraenesox bogio                 | 18,247      | NC_013614    | 68         |
|                 | Muraenesox cinereus              | 17,673      | MT571331     | This study |
| Ophichthidae    | Ophichthus rotundus              | 17,785      | KY081397     | 68         |
|                 | Ophichthus brevirostris          | 17,723      | MK189459     | 13         |
|                 | Ophisaurus macourbychus          | 17,843      | NC_005802    | 13         |
|                 | Myrichthys macrourbychus         | 17,859      | NC_013635    | 68         |
| Saccopharyngida | Saccopharynx pelecanoides        | 18,978      | NC_005299    | 68         |
| Saccopharyngida | Saccopharynx lavenbergi          | 18,495      | NC_005298    | 68         |

Table 1. List of 44 Anguilliformes species and 2 outgroups used in this paper.
codon usage (RSCU) value for *M. cinereus* for the third position is shown in Fig. 2b. The usage of both two- and four-fold degenerate codons was biased toward the use of codons abundant in A, while there was an overall bias against G.

**Transfer RNAs, ribosomal RNAs and CR.** There were also 22 tRNAs in the mitochondrial genes of *M. cinereus*, like those of other vertebrates. Of these 22 tRNAs, 14 tRNAs were encoded on the heavy chain 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 light chain (Fig. 1). Among the 22 tRNAs, except that tRNA-Ser lacking the entire dihydrouridine arm, all other tRNAs have a typical clover structure (Fig. 3). In this case, tRNA-Ser lacks the dihydrouridine arm and is often seen in the mitochondrial of other vertebrates. The length of *M. cinereus* mitochondrial tRNA is 1564 bp, and the length of 22 tRNA is between 66 and 76 bp; the base composition was A = 32.0%, T = 25.3%, C = 23.1% and G = 19.6% (Tables 1, 3). The AT and GC skew values of tRNA genes were 0.116 and −0.081, respectively, which indicated that As and Cs were more abundant than Ts and Gs. The origin of light chain replication (OL) was usually located within a WANCY cluster, approximately two-thirds of the genomic distance from CR, and can fold into a stable stem ring secondary structure. Among the 22 tRNAs encoding 20 amino acids, two amino acids have two anti-codon sites, these two amino acids were Ser and Leu, and their anticodons were TGA/GCT and TAA/TAG, respectively. The small coding subunit (12S rRNA) and large coding subunit (16S rRNA) appeared on both sides with *trnF* and *trnL(UGA)*, which were located on the H-chain and separated by the *trnV* gene. The two rRNA genes are 2,668 bp in total length, and the base composition is A = 35.1%, T = 21.7%, C = 22.6%, and G = 20.6%. The AT-skew value was positive (0.236) and the GC-skew value was 0.116.
value was negative (− 0.047), which indicates that there were more adenine and cytosine nucleotides in rRNAs (Table 3).

The lengths of the two CRs were 902 bp and 944 bp, respectively, and the total length was 1846 bp, of which the ratio of AT was 66.3% (Tables 1, 3). The AT ratio of the CR region is higher than that of other parts of mitochondrial genes, so the CR region is also called the “AT-rich region”, which was also common in the mitochondria of other fish. Both AT and GC skew values were 0.092 and − 0.066, indicating that the number of adenine and cytosine nucleotides was higher than that of thymine and guanine nucleotides. The palindrome sequence motifs “TACAT” and “ATGTA” related to the termination of heavy chain replication were found in both CRs, and had been reported in other study56 (Fig. 4).

Gene rearrangement. Compared with the gene arrangement in the vertebrate mitochondrial genome, the gene order in the moray mitochondrial genome obviously rearranged57 (Fig. 1). The position of the three genes (ND6, trnE and CR) in the moray M. cinereus mitochondria had changed. In general, ND6 and the bound trnE

| Gene          | Position From | Length (bp) | Amino acid | Start/Stop codon | Anticodon | Intergenic region (bp) | Strand |
|---------------|---------------|-------------|------------|------------------|-----------|------------------------|--------|
| tRNA-Phe (F)  | 1             | 72          |            |                  | GAA       | 0                      | H      |
| 12S RNA       | 73            | 1034        | 962        |                  |           | 0                      | H      |
| tRNA-Val (V)  | 1035          | 1104        | 70         | TAC              |           | 0                      | H      |
| 16S RNA       | 1105          | 2810        | 1706       |                  |           | 0                      | H      |
| tRNA-Leu<sup>UUA</sup> (L<sub>1</sub>) | 2811 | 2886 | 76 | TAA | 0 | H |
| ND1           | 2887          | 3855        | 960        | 323              | ATG/TAA   | 0                      | H      |
| tRNA-Ile (I)  | 3863          | 3934        | 72         | GAT              |           | 7                      | H      |
| tRNA-Gln (Q)  | 3935          | 4005        | 71         |                  |           | 0                      | L      |
| tRNA-Met (M)  | 4005          | 4074        | 70         | CAT              |           | −1                     | H      |
| ND2           | 4075          | 5118        | 1044       | 348              | ATG/TAG   | 0                      | H      |
| tRNA-Trp (W)  | 5117          | 5186        | 70         | TCA              |           | −2                     | H      |
| tRNA-Ala (A)  | 5188          | 5256        | 69         | TGC              |           | 1                      | L      |
| tRNA-Asn (N)  | 5258          | 5330        | 73         | GTT              |           | 1                      | L      |
| tRNA-Cys (C)  | 5331          | 5369        | 39         |                  |           | −7                     | H      |
| tRNA-Oxy (C)  | 5381          | 5446        | 66         | GCA              |           | 7                      | L      |
| tRNA-Tyr (T)  | 5447          | 5517        | 71         |                  |           | GCA                    | 0      |
| COI           | 5519          | 7121        | 1603       | 534              | GTG/TAA   | 1                      | H      |
| tRNA-Ser<sup>UCA</sup> (S<sub>1</sub>) | 7122 | 7192 | 71 | TGA | 0 | L |
| tRNA-Asp (D)  | 7198          | 7265        | 68         |                  |           |                        |        |
| COII          | 7269          | 7959        | 691        | 230              | ATG/T     | 3                      | H      |
| tRNA-Lys (K)  | 7960          | 8034        | 75         |                  |           | TTT                    | 0      |
| ATP8          | 8036          | 8203        | 168        | 56               | ATG/TAA   | 1                      | H      |
| ATP6          | 8194          | 8877        | 684        | 228              | ATG/TAA   | −10                    | H      |
| COIII         | 8877          | 9662        | 786        | 262              | ATG/TAA   | −1                     | H      |
| tRNA-Gly (G)  | 9662          | 9731        | 70         | TCC              |           | −1                     | H      |
| ND3           | 9732          | 10,082      | 351        | 117              | ATG/TAG   | 0                      | H      |
| tRNA-Arg (R)  | 10,081        | 10,150      | 70         | TCG              |           | −2                     | H      |
| ND4L          | 10,151        | 10,447      | 297        | 99               | ATG/TAA   | 0                      | H      |
| ND4           | 10,441        | 11,821      | 1381       | 460              | ATG/T     | −7                     | H      |
| tRNA-His (H)  | 11,822        | 11,890      | 69         |                  | GTG       | 0                      | H      |
| tRNA-Ser<sup>AGC</sup> (S<sub>2</sub>) | 11,891 | 11,961 | 71 | GCT | 0 | H |
| tRNA-Leu<sup>CUA</sup> (L<sub>2</sub>) | 11,962 | 12,033 | 72 | TAC | 0 | H |
| ND5           | 12,034        | 13,890      | 1857       | 619              | ATG/TAA   | 0                      | H      |
| Cyt b         | 13,926        | 15,065      | 1140       | 380              | ATG/AGA   | 35                     | H      |
| tRNA-Thr (T)  | 15,070        | 15,141      | 72         | TGT              |           | 4                      | H      |
| CR1           | 15,142        | 16,043      | 902        |                  |           | 0                      | H      |
| ND6           | 16,044        | 16,559      | 516        | 172              | ATG/AGG   | 0                      | L      |
| tRNA-Glu (E)  | 16,561        | 16,629      | 69         | TGC              |           | 1                      | L      |
| tRNA-Pro (P)  | 16,653        | 16,729      | 77         | TGG              |           | 23                     | L      |
| CR2           | 16,730        | 17,673      | 944        |                  |           | 0                      | H      |

Table 2. Features of the mitochondrial genome of Muraenesox cinereus.
repeated genes leading to the existence of intergenic spacers or pseudogenes\textsuperscript{23,60,61}. Therefore, in this study, the well the rearrangement of genes with redundant genes. The TDRL model was due to the incomplete deletion of \textit{M. cinereus}. This tandem duplication and random loss (TDRL) model explains of molecular rearrangement of parthenogenetic lizards\textsuperscript{62}. The evidence of the TDRL model was indicated by the presence of pseudogenes or observed in the mitochondrial genome of \textit{M. cinereus}, as described previously in the mitochondrial genome of \textit{M. cinereus}, in the Anguillaridae, we to further study the evolutionary status of \textit{M. cinereus} in the Anguillaridae, we selected 14 closely related families and two outgroups (\textit{Saccopharynx lavenbergi} and \textit{Eurypharynx pelecanoides}) to construct evolutionary trees (BI and ML) to analyze phylogenetic relationships. After removing highly differentiated regions, a phylogenetic tree was constructed with 10,987 bp sequence. The results show that the topological structure of the ML tree and the BI tree are basically the same. Therefore, we merge two trees together to form a tree. In addition, the BI tree has a higher support value than the ML tree (Fig. 6). Both trees clearly show that \textit{M. cinereus} and \textit{M. bagio} were the closest in relationship, and that these two species form the Muranoseoxide branches (BI posterior probabilities [PP] = 1; ML bootstrap [BP] = 100). The mitochondrial genome structures of \textit{M. cinereus} and \textit{M. bagio} were very similar. However, there was no other gene between \textit{iRNA-Thr} and ND6 gene in \textit{M. bagio}\textsuperscript{46} mitochondrial genome, but CR1 gene existed between \textit{tRNA-Thr} and ND6 gene in \textit{M. cinereus} mitochondrial genome. In the \textit{M. bagio} mitochondrial genome, ND6 combined with \textit{iRNA-Glu} rearranged and

| Mitogenome | A | T | C | G | A+T\% | AT-skew | GC-skew | Length (bp) |
|------------|---|---|---|---|--------|---------|---------|-------------|
| ND1 | 28.8 | 28.6 | 26.9 | 15.7 | 57.4 | 0.004 | −0.264 | 969 |
| ND2 | 34.9 | 28.7 | 24.0 | 12.4 | 63.6 | 0.096 | −0.321 | 1044 |
| COI | 26.4 | 31.1 | 24.0 | 18.5 | 57.5 | −0.082 | −0.128 | 1603 |
| COII | 30.1 | 28.5 | 24.5 | 16.9 | 58.6 | 0.027 | −0.182 | 691 |
| ATP8 | 35.1 | 30.4 | 25.6 | 8.9 | 65.5 | 0.073 | −0.483 | 168 |
| ATP6 | 30.3 | 32.6 | 24.4 | 12.7 | 62.9 | −0.037 | −0.315 | 684 |
| COI | 28.1 | 30.7 | 23.9 | 17.3 | 58.8 | −0.043 | −0.160 | 786 |
| ND3 | 28.2 | 33.3 | 24.8 | 13.7 | 61.5 | −0.083 | −0.289 | 351 |
| ND4 | 31.3 | 29.8 | 23.6 | 15.4 | 61.0 | 0.025 | −0.212 | 1381 |
| ND4L | 25.9 | 27.6 | 29.3 | 17.2 | 53.5 | −0.031 | −0.261 | 297 |
| ND5 | 32.5 | 28.4 | 25.5 | 13.6 | 60.9 | 0.067 | −0.304 | 1857 |
| Cytb | 28.7 | 27.9 | 26.4 | 17.0 | 56.6 | 0.014 | −0.216 | 1140 |
| ND6 | 44.6 | 14.1 | 28.3 | 13 | 58.7 | 0.520 | −0.083 | 351 |
| tRNA | 32.0 | 25.3 | 23.1 | 19.6 | 57.3 | 0.116 | −0.081 | 1564 |
| rRNA | 35.1 | 21.7 | 22.6 | 20.6 | 56.7 | 0.236 | −0.047 | 2668 |
| CR | 36.2 | 30.1 | 18.0 | 15.8 | 66.3 | 0.092 | −0.066 | 1846 |

Table 3. Composition and skewness of \textit{Muraenesox cinereus} mitogenome.
transferred between the tRNA-Thr and tRNA-Pro genes. However, in the mitochondria of *M. cinereus*, not only ND6 and tRNA-Glu were rearranged, but also the CR gene was rearranged. Inoue et al.'s complete mitochondrial data studies and Santini et al.'s tandem dataset (mitochondrial and nuclear genes) studies also support Congridae's non-singularity. Regarding the unity of Nettastomatidae, the ML tree showed that all Nettastomatidae species were grouped into a clade, and supported the origin of single lines. However, the BI tree divided Nettastomatidae into two clades, indicating that Nettastomatidae was non-singleton, which was consistent with the results of Inoue and Lu et al. Our results indicated that both Derichthyidae and Chlopsidae were monophyletic, but Santini et al.'s results were contrary to ours. For the main relationship between the Anguilliformes lineage and different families, our results were basically consistent with previous molecular studies.

In our research, it was found that Chlopsoidei, Muraenoidei and Anguilloidei all had typical vertebrate mitochondrial genome sequences, but Congroidei had two different patterns of gene arrangement: (1) with typical vertebrate gene order (Nemichthyidae, Serrivomeridae and Synaphobranchidae) (2) with gene rearrangement (Derichthyidae, Nettastomatidae, Congridae, Muraenesocidae and Ophichthidae). The four families (Ophichthidae, Derichthyidae, Muraenesocidae and Nettastomatidae) studied by Inoue et al. also had mitochondrial genes lacking ND6 and trnE, which were also clustered, which was also consistent with our results. In this study, we found an interesting phenomenon. The five families with gene rearrangement at the bottom formed a
branch, and those without gene rearrangement formed a separate branch at the top of the tree (The cyan ellipse in Fig. 6 indicates that no gene rearrangement had occurred; the red ellipse indicated that gene rearrangement

Figure 3. The secondary structure of tRNA in the mitochondrial genome of *Muraenesox cinereus.*
had occurred). These results indicate that the origin of eels is a diverse evolution. If the new gene sequence originates from a single ancestral species in the Congroidei suborder, then this pattern of presence/absence may be a good phylogenetic marker for identifying monoline populations as Kumazawa and Nishida\textsuperscript{68} and Macey et al.\textsuperscript{18} suggested, they had a higher vertebrate relationship. Therefore, more advanced methods can be considered to classify the controversial Anguilliformes species. There are still some phylogenetic mismatches based on morphological and molecular data, so more eel mitochondrial genomes should be sequenced to support this hypothesis in future research.

**Conclusion**

With the advancement of genetics research, many people believe that the richness of molecular information is superior to morphological data, and molecular analysis has become the most commonly used methods in the study of biological system development. Therefore, in this study, we sequenced and assembled the complete...
mitochondrial gene of *M. cinereus* and described its characteristics, which contains 37 genes and two control regions. After comparing with the typical vertebrate mitochondrial genes, we found that *M. cinereus* mitochondrial gene rearrangement obviously occurred, the rearrangement part of the gene ND6 and trnE were transferred between trnT and trnP, accompanied by CR repeat. The most suitable model to explain this rearrangement phenomenon is the duplication-random loss model. The two phylogenetic trees (BI and ML) created using the mitochondrial genomes from 46 Anguilliformes were basically consistent with previous molecular studies on the interrelationships between the main Anguilliform lineages and different families, although some of these families were slightly related differently. Both phylogenetic trees strongly support the non-monophyly of Congridae, providing a basis for the more advanced classification of Anguilliformes. In addition, our research results provide a theoretical basis for in-depth understanding of the mechanism and evolution of *M. cinereus* gene rearrangement and phylogenetic studies of eel.

**Figure 6.** Phylogenetic analysis based on the nucleotide sequences of the 12 PCGs in the mitogenome. The numbers beside the nodes are posterior probabilities (BI, bottom) and bootstrap (ML, top). Red ellipse: gene rearrangement occurs; cyan ellipse: no gene rearrangement occurs.
Materials and methods

Sample collection permit and experimental approval. All procedures in this study were performed under the guidelines of the Regulations for the Administration of Laboratory Animals (Decree No. 2 of the State Science and Technology Commission of the People's Republic of China, November 14, 1988), and were approved by the Animal Ethics Committee of Zhejiang Ocean University (Zhoushan, China).

Fish sample, DNA extraction, PCR amplification and sequencing. Individual *M. cinereus* specimens were collected by a commercial trawl fishing method in Zhoushan City, Zhejiang Province, China (30° 40′ 30″ N, 121° 20′ 28″ E) and immediately preserved with 95% ethanol. Total genomic DNA was extracted using the SQ tissue DNA kit (OMEGA) according to the manufacturer’s protocol. After extraction, the DNA was stored in −4 °C refrigerator. The polymerase chain reaction (PCR) primers used in this experiment designed 10 pairs of primers for the amplification of the complete mitochondrial genome of *M. cinereus* based on the complete mitochondria published by the predecessors (Table S1). The PCR was carried out in a 25 μl reaction volume containing 2.0 mM MgCl₂, 0.4 mM of each dNTP, 0.5 μM of each primer, 1.0 U of Taq polymerase (Takara, China), 2.5 μl of 10 × Taq buffer, and approximately 50 ng of DNA template. Using the following cycling conditions: (1) initial activation step for 5 min at 95 °C; (2) 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C (as the case may be) for 30 s and extension at 72 °C for 30 s; and (3) a final extension of 5 min at 72 °C. The sequences were determined using an ABI genetic analyzer (Applied Biosystems, China).

Sequence assembly, annotation and analysis. The obtained sequence fragment was passed through CodonCode Aligner 9.0.1 (CodonCode Corporation, Dedham, MA) was assembled into a complete mitochondrial genome. The assembled mitochondrial genome is annotated by Sequin (version 15.10, http://www.ncbi.nlm.nih.gov/Sequin/). The boundaries of protein coding and ribosomal RNA genes were determined by NCBI-BLAST (http://blast.ncbi.nlm.nih.gov). The tRNA genes were verified using MITOS WebServer (http://mitos2.bioinf.uni-leipzig.de/index.py) using the default setting. Composition skew values were calculated according to the following formulas: AT skew = (A − T)/(A + T); GC skew = (G − C)/(G + C). The base composition and relative synonymous codon usage (RSCU) were obtained using MEGA X. The mitochondrial gene map of *M. cinereus* was generated online by using CGView.

Phylogenetic analyses. Download 46 complete Anguilliformes mitochondrial genomes from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) for phylogenetic studies (Table 1). Saccopharyngiformes was considered to be an intimately related species to Anguilliformes; therefore, we selected two species of *Neocyema erythrosoma* and *Saccopharynx lavenbergi* in Saccopharyngiformes as the outgroup in this study. The 12PCGs sequences used for phylogenetic analysis were extracted from DAMBE version 7.2.35. The 13 PCGs used did not include ND6 and were not used because of their heterogeneous base composition and consistent poor phylogenetic performance. Sequences were aligned with default parameters using Clustal X and manually checked using BioEdit. Use software Gbblock to eliminate ambiguous sequences. Substitution vs. the Tamura-Nei (TN93) genetic distance in pairwise comparisons was used to test for substitution saturation using DAMBE version 7.2.35. The third codon position shows significant saturation (Fig. S1), so it is only defined as purine and pyrimidine. Based on Bayesian inference (BI) and maximum likelihood (ML) two methods, using PhyML and MrBayes software for phylogenetic analyses. Based on the Akaiki information criteria (AIC), parallel uses Modeltest 3.7 of 56 models for ML analysis, and MrModeltest 2.2 of 24 models for BI analysis to determine the best evolutionary model and points out that GTR + G + I was the analysis dataset of best-fit alternative models. Perform Bootstrap analysis (1,000 repetitions) to assess the relative level of support for ML analysis. Use 'Lset’ and ‘Preset’ for Bayesian phylogenetic analysis and allow the program to converge to the best estimate of model parameters. Other parameter settings are as follows: each Markov chain starts from a random tree, runs for 2 million generations, and samples a tree every 100 generations (a total of 20,000 trees are sampled) to ensure the independence of the samples. In order to improve the mixing ability of the Markov chain, the Metropolis coupling Markov chain Monte Carlo (MCMC) method was used, and three heating chains (temperature = 0.5) and one cold chain were operated simultaneously. To guarantee the stationarity had been reached, the average standard deviation of split frequencies was set below 0.01. The phylogenetic tree was viewed in FigTree v1.4.0.

Ethical standards. Ethics Committee approval was obtained from the Institutional Ethics Committee of Zhejiang Ocean University to the commencement of the study.

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References

1. Mehta, R. S. Ecomorphology of the moray bite: Relationship between dietary extremes and morphological diversity. *Physiol. Biochem. Zool.* **82**, 90–103 (2009).
2. Mehta, R. S. & Wainwright, P. C. Raptorial jaws in the throat help moray eels swallow large prey. *Nature* **449**, 79–82 (2007).
3. Robins, C. R. The phylogenetic relationships of the anguilliform fishes. *Fishes Western N. Atl.* **1**, 9–23 (1989).
4. Greenwood, P. H. Notes on the anatomy and classification of elopomorph fishes. *Bull. Mus. Comp. Zool.* **32**, 65–102 (1977).
7. Santini, F.
6. Inoue, J. G.
8. Inoue, J. G., Miya, M., Tsukamoto, K. & Nishida, M. Complete Mitochondrial DNA Sequence of
10. Russell, B. & Houston, W. Offshore fishes of the Arafura Sea.
11. Chen, D., Ye, Y., Chen, J., Zhan, P. & Lou, Y. Molecular nutritional characteristics of vinasse pike eel (Muraenosox cinereus) during pickling. Food Chem. 224, 359–364. https://doi.org/10.1016/j.foodchem.2016.12.089 (2017).
12. Boore, J. L. Animal mitochondrial genomes. Nucleic Acids Res. 27, 1767–1780 (1999).
13. Lu, Z. Z.
15. Anderson, S. B.
19. Zhang, J. Y., Zhang, L. P., Yu, D. N., Storey, K. B. & Zheng, R. Q. Complete mitochondrial genomes of
21. Liu, J., Yu, J., Zhou, M. & Yang, J. Complete mitochondrial genome of
20. Yan, J., Li, H. & Zhou, K. Evolution of the mitochondrial genome in snakes: Gene rearrangements and phylogenetic relationships.
23. Eberhard, J. R. & Wright, T. F. Rearrangement and evolution of mitochondrial genomes in parrots.
24. Pääbo, S., Thomas, W. K., Whitfield, K. M., Kumazawa, Y. & Wilson, A. C. Rearrangements of mitochondrial transfer RNA genes in the vertebrate mitochondrial genome. J. Mol. Evol. 14, 545–554 (1979).
25. Gong, L., Shi, W., Yang, M., Li, D. & Kong, X. Novel gene arrangement in the mitochondrial genome of conger myriaster. Fish. Sci. 66, 1186–1188 (2002).
26. Miya, M., Kawaguchi, A. & Nishida, M. Mitogenomic exploration of higher teleostean phylogenies: A case study for moderate-scale evolutionary genomics with 38 newly determined complete mitochondrial DNA sequences. Mol. Biol. Evol. 18, 1993–2009 (2001).
28. Kong, X., et al. A novel rearrangement in the mitochondrial genome of tongue sole, Cynoglossus semilaevis: Control region translocation and a tRNA gene inversion. Genome. 52, 975–984. https://doi.org/10.1139/g09-069 (2009).
29. Gong, L., Shi, W., Li, Z. & Kong, X. Y. Rearrangement of mitochondrial genome in fishes. Zool. Res. 34, 666–673 (2013).
30. Inoue, J. G., Masaki, M., Katsumi, T. & Mutsumi, N. Evolution of the deep-sea gulper eel mitochondrial genomes: Large-scale gene rearrangements originated within the eels. Mol. Biol. Evol. 20, 1917–1924 (2003).
31. Ishikawa, K., Kimura, Y., Tokai, T., Tsukamoto, K. & Nishida, M. Gene rearrangement around the control region in the mitochondrial genome of conger myriaster. Fish. Sci. 66, 1186–1188 (2002).
32. Miya, M., Nagahashi, M., Nishiya, Y. Rearrangements of Agamidae species. Mol. Biol. Evol. 21, 136–142 (2004).
33. Mau, D. S., Gower, D. J., Rafael, Z. & Mark, W. A hotspot of gene order rearrangement by tandem duplication and random loss in the vertebrate mitochondrial genome. Mol. Biol. Evol. 23, 227–234 (2006).
34. Lavrov, D., Boone, J. L. & Brown, W. M. Complete mtDNA sequences of some millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. Mol. Biol. Evol. 19, 163–169 (2002).
35. Arndt, A. & Smith, M. J. Mitochondrial gene rearrangement in the sea cucumber genus Cucumaria. Mol. Biol. Evol. 8, 1009–1016 (1998).
36. Moritz, C., Dowling, T. E. & Brown, W. M. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18, 269–292 (1987).
37. Sammler, S., Bleidorn, C. & Tiedemann, R. Full mitochondrial genome sequences of two endemic Philippine hornbill species (Aves: Bucerotidae) provide evidence for pervasive mitochondrial DNA recombination. BMC Genom. 12, 35. https://doi.org/10.1186/1471-2164-12-35 (2011).
38. Atsushi, K.
39. Arndt, A. & Smith, M. J. Mitochondrial gene rearrangement in the sea cucumber genus Cucumaria. Mol. Biol. Evol. 8, 1009–1016 (1998).
40. Moritz, C., Dowling, T. E. & Brown, W. M. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annu. Rev. Ecol. Syst. 18, 269–292 (1987).
41. Erin, E. S. et al. Multiple independent origins of mitochondrial control region duplications in the order Psittaciformes. Mol. Phylogenet. Evol. 64, 342–356. https://doi.org/10.1016/j.ympev.2012.04.009 (2012).
42. Mauro, D. S., Gower, D. J., Rafael, Z. & Mark, W. A. A hotspot of gene order rearrangement by tandem duplication and random loss in the vertebrate mitochondrial genome. Mol. Biol. Evol. 23, 227–234 (2006).
43. Lavrov, D. V., Boone, J. L. & Brown, W. M. Complete mtDNA sequences of some millipedes suggest a new model for mitochondrial gene rearrangements: Duplication and nonrandom loss. Mol. Biol. Evol. 19, 163–169 (2002).
45. Schierup, M. H. & Hein, J. Consequences of recombination on traditional phylogenetic analysis. Genetics 156, 879–891 (2000).
46. Zhi, L. et al. Comparative mitochondrial genomics of snakes: Extraordinary substitution rate dynamics and functionality of the duplicate control region. BMC Evol. Biol. 7, 123. https://doi.org/10.1186/1471-2148-7-123 (2007).
47. Shi, W., Dong, X. L., Wang, Z. M., Miao, X. G. & Kong, X. Y. Complete mitogenome sequences of four flatfishes (Pleuronectiformes) reveal a novel gene arrangement of r-strand coding genes. BMC Evol. Biol. 13, 173 (2013).
48. Kumazawa, Y., Ota, H., Nishida, M. & Ozawa, T. The complete nucleotide sequence of a snake (Dinodon semicarinatus) mitogenome with two identical control regions. Genetics 150, 313–329 (1998).
49. Liu, Y. et al. Mitochondrial genome of the yellow catfish Pelteobagrus fulvidraco and insights into Bagridae phylogenetics. Genomics 111, 1258–1265. https://doi.org/10.1016/j.ygeno.2019.08.005 (2019).
50. Gong, L., Li, Z. M., Guo, B. Y., Ye, Y. & Liu, L. Q. Characterization of the complete mitochondrial genome of the tidewater goby, Eucyclogobius newberryi (Gobiiformes; Gobiidae; Gobiionellinae) and its phylogenetic implications. Conserv. Genet. Resour. 10, 93–97 (2017).
51. Lin, J. P. et al. The first complete mitochondrial genome of the sand dollar Sphaerechinus caurinus (Echinoidea: Clypeasteroida). Sci. Rep. 6, 1686–1693. https://doi.org/10.1038/srep16864 (2016).
52. Prabhuth, V. R. Characterization of the complete mitochondrial genome of Barilius malabaris and its phylogenetic implications. Genomics 112, 2154–2163. https://doi.org/10.1016/j.genom.2019.12.009 (2020).
53. Xu, T. J., Cheng, Y. Z., Sun, Y. N., Shi, G. & Wang, R. X. The complete mitochondrial genome of bighead croaker, Colichthys saurus (Perciformes, Sciaenidae): Structure of control region and phylogenetic considerations. Mol. Biol. Rep. 38, 4673–4685. https://doi.org/10.1007/s11033-010-0602-4 (2011).
54. Ojala, D., Montoya, J. & Attardi, G. tRNA punctuation model of RNA processing in human mitochondria. Nature 290, 470–474. https://doi.org/10.1038/290470a0 (1981).
55. Vandana, R. P. et al. Characterization of the complete mitochondrial genome of Barilius malabaris and its phylogenetic implications. Genomics 112, 2154–2163 (2019).
56. Wang, X., Wang, J., He, S. & Mayden, R. L. The complete mitochondrial genome of the Chinese hook snout carp Opsariichthys goodei (Teleostei: Cypriniformes) and insights into the evolution of the Cyprinidae. Gene 655, 75–83 (2019).
57. Shi, W., Guo, L., Wang, Z. M., Miao, X. G. & Kong, X. Y. Tandem duplication and random loss for mitogenome rearrangement in symphurus (Teleostei: Pleuronectiformes). BMC Genom. 16, 355 (2015).
58. Gong, L. et al. Large-scale mitochondrial gene rearrangements in the hermit crab Pagurus nigrofascia and phylogenetic analysis of the Anomura. Gene 695, 75–83 (2019).
59. Wang, Z. W. et al. Complete mitochondrial genome of Parasesarma affine (Brachyura: Sesarmidae): Gene rearrangements in Sesarmidae and phylogenetic analysis of the Brachyura. Int. J. Biol. Macromol. 118, 31–40 (2018).
60. Inoue, G. J., Miyai, M., Tsukamoto, K. & Nishida, M. Evolution of the deep-sea gulfperch eel mitochondrial genomes: Large-scale gene rearrangements originated within the eels. Mol. Biol. Evol. 20, 1917–1924. https://doi.org/10.1093/molbev/msv206 (2003).
61. Inoue, G. J. et al. Deep-ocean origin of the freshwater eels. Biol. Lett. 6, 363–366. https://doi.org/10.1098/rsbl.2010.0899 (2010).
62. Chen, J. N., López, J. A., Lavoué, S., Miyai, M. & Chen, W. J. Phylogeny of the Eleopomorphia (Teleostei): Evidence from six nuclear and mitochondrial markers. Mol. Phylogenet. Evol. 70, 152–161 (2014).
63. Reece, J. S., Bowen, B. W., Smith, D. G. & Larson, A. Molecular phylogenetics of moray eels (Muraenidae) demonstrates multiple origins of a shell-crushing jaw (Gymnomuraena, Echidna) and multiple colonizations of the Atlantic Ocean. Mol. Phylogenet. Evol. 57, 829–835 (2010).
64. Johnson, G. D., Ida, H., Sakaue, J., Sado, T. & Asahida, T. A “living fossil” eel (Anguilliformes: Muraenidae). BMC Genom. 14, 319–333 (2013).
65. Eucyclogobius newberryi and its phylogenetic implications. Zoological Studies 50, 313–329 (1998).
66. Lee, J. S., Wishart, D. S. Circular genome visualization and exploration using CGView. Nucleic Acids Sympos. Ser. 27, 537–549 (1999).
67. Paul, S. & Wishart, D. S. Circular genome visualization and exploration using CGView. Bioinformatics 21, 537–549 (2005).
68. Nelson, J. S. Fishes of the World 4th edn. (Wiley, New York, 2006).
69. Xia, X. DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Mol. Biol. Evol. 35, 1550–1552. https://doi.org/10.1093/molbev/msz009 (2018).
70. Shi, W. et al. Next generation sequencing yields the complete mitochondrial genome of the longfang moray, Enchelynassa canina (Anguilliformes: Muraenidae). Mitochondrial DNA Part A 27, 2431–2432 (2015).
71. Loh, K. H. et al. Next generation sequencing yields the complete mitochondrial genome of the Zebra moray, Gymnomuraena zebra (Anguilliformes: Muraenidae). Mitochondrial DNA Part A 27, 1–2 (2015).
72. Perna, N. T. & Kocher, T. D. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J. Mol. Evol. 41, 353–358 (1995).
73. Sudhir, K. et al. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549 (2018).
74. Paul, S. & Wishart, D. S. Circular genome visualization and exploration using CGView. Bioinformatics 21, 537–549 (2005).
75. Jordan, J. M. & Brown, R. J. Phylogenetic analysis of reptiles as phylogenetic markers. Mol. Biol. Evol. 12, 759–772. https://doi.org/10.1093/jme/12.5.759 (1995).
76. Loh, K. H. et al. Next generation sequencing yields the complete mitochondrial genome of the longfang moray, Enchelynassa canina (Anguilliformes: Muraenidae). Mitochondrial DNA Part A 27, 2431–2432 (2015).
77. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948. https://doi.org/10.1093/bioinformatics/btm404 (2007).
78. Hall, T. BioEdit: A user-friendly biological sequence alignment program for Windows 95/98/NT. Nucleic Acids Sympos. Ser. 41, 95–98 (1999).
79. Gerard, T. & Jose, C. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577 (2007).
80. Gascuel, O. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321 (2010).
81. Huelsenbeck, J. P. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542 (2012).
82. Posada, D. & Crandall, K. A. MODELTEST: Testing the model of DNA substitution. Bioinformatics 14, 817–818 (1998).
83. Nylander, J. A. A., Fredrik, R., Huelsenbeck, J. P. & Nieves-Aldrey, J. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67 (2004).
84. Sitnikova, T., Rzhetsky, A. & Nei, M. Interior-branch and bootstrap tests of phylogenetic trees. Mol. Biol. Evol. 12, 319–333 (1995).
85. Antezana, M. When being “most likely” is not enough: Examining the performance of three uses of the parametric bootstrap in phylogenetics. J. Mol. Evol. 56, 198–222 (2003).
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K.Z., K.H.Z., Y.E.L. and B.J.L. conceived and designed research. K.Z., K.H.Z., Y.E.L., H.Z., L.G., L.H.J., L.Q.L., Z.M.L. and B.J.L. conducted experiments, analysed data and wrote the manuscript. Authors critically reviewed and approved the manuscript.

Competing interests
The authors declare no competing interests.

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