

Characteristics and phylogenetic analysis of the complete mitochondrial genome of *Glyptothorax pallozonus* (Siluriformes, Sisoridae)

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ABSTRACT

Glyptothorax pallozonus Lin, 1934 is a small benthic fish belonging to the Sisoridae family that is distributed in the Dongjiang and Rongjiang Rivers of China. In the present study, we sequenced and characterized the complete mitochondrial genome of *G. pallozonus* for the first time. The complete mitogenome of *G. pallozonus* is 16,542 bp in length and includes 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNA (rRNAs), and a control region (CR). The mitogenome architecture was identical to that of other teleosts. Maximum likelihood (ML) phylogenetic analysis strongly supported the monophyly of *Glyptothorax*, which contains two clades. These results advance our understanding of the molecular phylogeny of the genus *Glyptothorax*.

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Introduction

Glyptothorax is the most species-rich and widely distributed genus in the Sisoridae family and currently comprises 101 recognized species (Bánki et al. 2023). *Glyptothorax pallozonus* is a small benthic fish (Figure 1) that is endemic to the eastern region of Guangdong Province, China, and is characteristically found in the Dongjiang and Rongjiang Rivers (Zheng 1991). The species, like other sisorids, live in fast-moving streams, where they have adapted to using an adhesive apparatus on their underside to attach themselves to rocks and prevent themselves from being washed away (Ng and Rachmatika 2005). Over the past three decades, *G. pallozonus* has experienced a decline in population size due to environmental degradation and habitat changes. To date, this species is only occasionally caught in the wild; therefore, studies of this species are limited. Previous studies have mainly focused on the morphological, distributional, and systematic characteristics of *G. pallozonus* (Zheng 1991; Chu and Mo 1999; Jiang et al. 2011), and genetic information on this species is scarce. In the present study, the complete mitochondrial genome and the phylogenetic relationships of *G. pallozonus* were determined for the first time.





Materials and methods


A live specimen of *G. pallozonus* was collected from Fengshun, Guangdong Province, China (23°44'57" N,

116°9'26"E). The specimen was morphologically identified as described by Zheng (1991) and Chu and Mo (1999). In particular, it could be distinguished from its congeners by the presence of a bright white triangular patch on the base of the dorsal fin (Figure 1). The specimen was deposited at the ichthyological museum of Freshwater Fisheries Research Institute of Jiangsu Province, China (Dr Liqiang Zhong, e-mail: lqzhongffri@hotmail.com) under the voucher number JSFFRI-18003.



Figure 1. Specimen of *Glyptothorax pallozonus* was collected from the Fengshun county, Guangdong Province, China. The photo was taken by shujie liu on Mar 9, 2018.

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Table 1. Species and GenBank accession number of mitogenomes used in this study.

No.	Species	Accession ID	References
1	<i>Creteuchiloglanis gongshanensis</i>	KP872697	(Ma et al. 2015)
2	<i>Creteuchiloalanis kamengensis</i>	MN396886	(Ma et al. 2020)
3	<i>Creteuchiloalanis macropterus</i>	KP872682	(Ma et al. 2015)
4	<i>Exostoma gaoligongense</i>	MW256713	(Gong et al. 2021)
5	<i>Exostoma labiatum</i>	JQ026255	(Ma et al. 2015)
6	<i>Exostoma tenuicaudatum</i>	ON641841	(Gong et al. 2022)
7	<i>Exostoma tibetanum</i>	ON641840	(Gong et al. 2022)
8	<i>Glyptothorax annandalei</i>	MN396887	(Lin et al. 2021)
9	<i>Glyptothorax cavia</i>	KY230517	(Li 2017)
10	<i>Glyptothorax fokiensis</i>	JQ917224	(Zhou et al. 2012)
11	<i>Glyptothorax granosus</i>	KP872684	(Li et al. 2023)
12	<i>Glyptothorax lanceatus</i>	KP872685	(Li et al. 2023)
13	<i>Glyptothorax laosensis</i>	KP872686	(Huang et al. 2017)
14	<i>Glyptothorax longinema</i>	KP872687	(Li et al. 2023)
15	<i>Glyptothorax macromaculatus</i>	MH213458	(Lv et al. 2018)
16	<i>Glyptothorax pallozonus</i>	OP723308	This study
17	<i>Glyptothorax sinensis</i>	KJ739617	(Yan et al. 2016)
18	<i>Glyptothorax trilineatus</i>	JQ026262	(Ma et al. 2015)
19	<i>Glyptothorax zainaensis</i>	KU212205	(Li et al. 2016)
20	<i>Oreoglanis immaculatus</i>	KP872690	(Ma et al. 2015)
21	<i>Oreoglanis jingdongensis</i>	KP872691	(Ma et al. 2015)
22	<i>Oreoglanis macropterus</i>	JQ026261	(Ma et al. 2015)
23	<i>Odontobutis haifengensis</i>	MF383619	(Zhong et al. 2018)

Methods

Genomic DNA was extracted using the Ezup Column Animal Genomic DNA Kit (Sangon, China) following the manufacturer's protocol. The mitogenome was amplified using 30 sets of fish universal primers (Miya and Nishida 1999), and gaps were filled with self-designed primers based on the mitogenomes of other sisorids (Supplementary material, Table S1). The PCR products were sequenced by Sanger sequencing using the same primers.

Raw sequences were manually checked and assembled. The assembled sequences were annotated and visualized using MitoAnnotator (Zhu et al. 2023). Based on the concatenated sequences of 13 protein-coding genes (PCGs) from 23 fish (Table 1), a maximum likelihood (ML) phylogenetic tree was constructed with 1000 bootstrap assemblies and the GTR + G + I model using MEGA 11 (Tamura et al. 2021).

Results

The complete mitochondrial genome of *G. pallozonus* (GenBank accession number: OP723308) is 16,542 bp long and includes 13

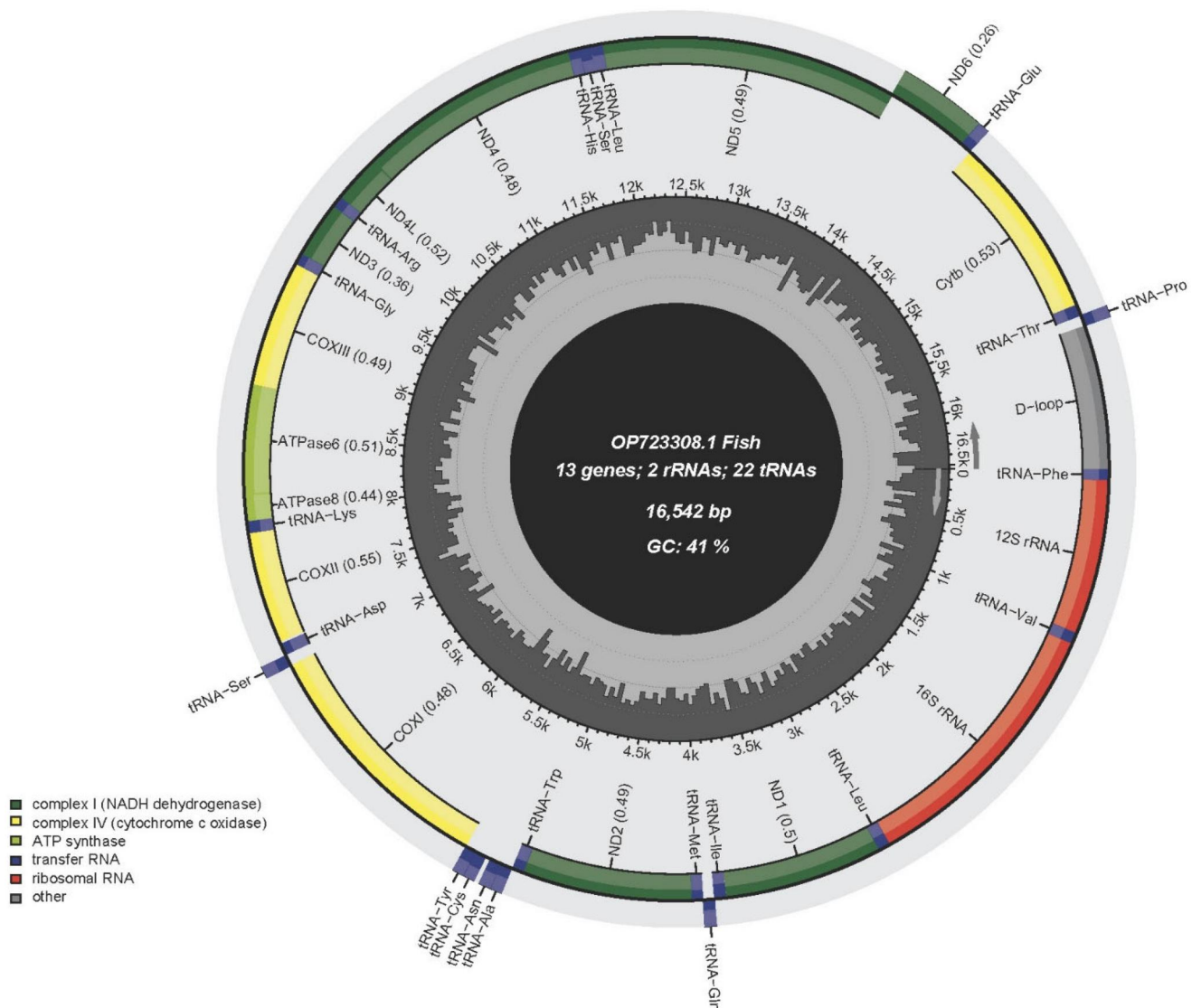


Figure 2. Gene map of the mitochondrial genome of *Glyptothorax pallozonus* (GenBank accession number: OP723308), with 13 protein coding genes, 22 tRNAs, 2 rRNAs, and a control region. Genes encoded on light strand and heavy strand were shown on the inner and outer sides of the ring, respectively.

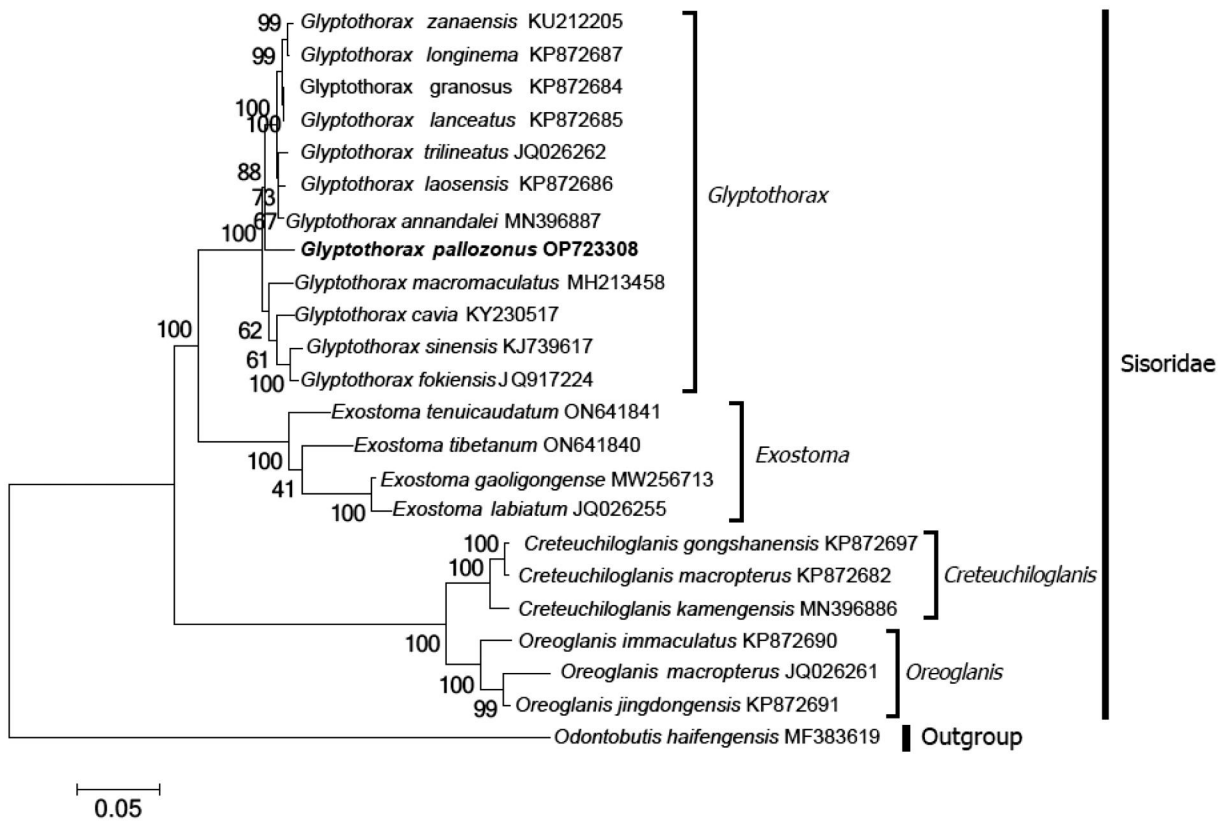


Figure 3. Maximum-likelihood (ML) phylogenetic tree was reconstructed based on the concatenated 13 protein-coding genes of *Glyptothorax pallozonus* and other 22 fishes. Accession numbers were indicated after the species name. Numbers at the nodes indicated bootstrap support values from 1000 replicates.

PCGs, two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and one control region (CR) (Figure 2). The mitogenome architecture and gene-coding strands are similar to those of the teleost consensus (Ma et al. 2015). We observed a small AT-rich characteristic (59.2%), with a nucleotide composition of 27.1% T, 25.9% C, 32.1% A, and 14.9% G. The *ND6* gene and eight tRNAs (*tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, *tRNA^{Gln}*, *tRNA^{Glu}*, *tRNA^{Pro}*, *tRNA^{Ser}*, and *tRNA^{Trp}*) were encoded on the light strand, whereas the other components were located on the heavy strand. Twelve PCGs started with ATG, while the *COI* gene began with GTG. Six genes ended with the complete stop codon TAA (*ND1*, *COI*, *ATPase8*, *ND4L*, *ND5*, and *ND6*), whereas the remaining genes had incomplete stop codons TA (*ATPase 6*) or T (*ND2*, *COII*, *COIII*, *ND3*, *ND4*, and *Cytb*). The origin of light-strand replication was 32 bp in length and located in the WANCY cluster between *tRNA^{Asn}* and *tRNA^{Cys}*. The CR was 890 bp in length and contained a termination-associated sequence (TAS), three central conserved sequence blocks (CSB-D, CSB-E, and CSB-F), and three conserved sequence blocks (CSB-1, CSB-2, and CSB-3).

The ML phylogenetic tree indicated the monophyly of the genus *Glyptothorax* with high support scores (Figure 3). Two clades were identified within the genus *Glyptothorax*; *G. pallozonus* is grouped with *G. zanaensis*, *G. longinema*, *G. granosus*, *G. lanceatus*, *G. trilineatus*, *G. laosensis*, and *G. annandalei*, and eventually forms a clade. The remaining four species, *G. macromaculatus*, *G. cavia*, *G. sinensis*, and *G. fokiensis*, form a separate clade.

Discussion and conclusion

The structure of the complete mitochondrial genome of *G. pallozonus* is similar to that of other teleosts (Miya et al. 2003).

In particular, the mitogenome structures of the genus *Glyptothorax* are highly conserved, suggesting that the mitochondrial genome is a perfect tool for population genetics and phylogenetic studies of these fishes. The ML phylogenetic tree strongly supported the monophyly of *Glyptothorax*, which was consistent with the results of a previous study based on the single gene sequences of *RAG2*, *COI*, and *Cyt b* (Jiang et al. 2011). Previous phylogenetic studies have included only four to seven species in the genus *Glyptothorax* and thus, have generated only a limited phylogeny of these fishes. Based on phylogenetic analysis, these fishes can be divided into two main clades: *G. zanaensis* and *G. trilineatus*, and *G. macromaculatus*, *G. sinensis*, and *G. fokiensis* (Huang et al. 2017; Li 2017; Lv et al. 2018). The new mitogenome discovered in this study supports the placement of *G. pallozonus* in the *Glyptothorax* genus. To the best of our knowledge, this is the first report on the evolutionary position of this species. At the same time, in the present study, the phylogenies of all 12 available fishes of *Glyptothorax* with complete mitogenomes were reconstructed, which would be useful in understanding the relationships within the *Glyptothorax* genus. However, the complete mitochondrial genome of most fishes in *Glyptothorax* (approximately 89 species) is still unavailable, and to elucidate the phylogenetic relationships of the *Glyptothorax* genus, extensive sampling and additional molecular information is necessary.

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Author contributions

QFG and ZLQ conceived this study; QFG and YS conducted the experiments, LAP and WQ analyzed the data; QFG wrote the drafting of the paper; QFG and ZLQ revised it critically. All authors contributed to the article and approved the submitted version.

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Data availability statement

The mitochondrial genome sequence is available on GenBank of NCBI at www.ncbi.nlm.nih.gov with the accession number of OP723308.

Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical approval

The species used in this study is not protected under CITES or wildlife laws in China, and its status is data deficient by the IUCN. Therefore, no specific permission or license is required for research sampling according to Regulations of the People's Republic of China. The live samples were obtained in accordance with the guidelines of the animal care and Ethical Committee of Freshwater Fisheries Research Institute of Jiangsu Province and Animal Experiments of Jiangsu Agri-animal Husbandry Vocational College.

References

- Bánki O, Roskov Y, Döring M, Ower G, Vandepitte L, Hobern D, Remsen D, Schalk P, DeWalt RE, Keping M, et al. 2023. Catalogue of Life Checklist (Version 2023-02-07). Catalogue of Life doi: [10.48580/dfq](https://doi.org/10.48580/dfq).
- Chu XL, Mo TP. 1999. Sisoridae. In: Chu XL, Zheng BS, Dai DY, editors. Fauna sinica osteichthyes siluriformes. Beijing (China): Science Press; p. 114–181.
- Gong Z, Jiang W, Feng H, Liu Y, Zhu T. 2022. Comparative mitogenomics of Two sympatric catfishes of *Exostoma* (Siluriformes: Sisoridae) from the lower Yarlung tsangpo River and its application for phylogenetic consideration. *Genes (Basel)*. 13(9):1615. doi: [10.3390/genes13091615](https://doi.org/10.3390/genes13091615).
- Gong Z, Lin F, Luo Z, Chen X. 2021. The complete mitochondrial genome of *Exostoma gaoligongense* (Siluriformes: Sisoridae) and its phylogenetic analysis within glyptosternine catfishes. *Mitochondrial DNA B Resour.* 6(4):1424–1425. doi: [10.1080/23802359.2021.1912670](https://doi.org/10.1080/23802359.2021.1912670).
- Huang FJ, Liu MD, Yu LX, Liu SP. 2017. The complete mitochondrial genome of *Glyptothorax laosensis* (Siluriformes, Sisoridae). *Mitochondrial DNA A DNA Mapp Seq Anal.* 28(1):60–61. doi: [10.3109/19401736.2015.1110798](https://doi.org/10.3109/19401736.2015.1110798).
- Jiang WS, Ng HH, Yang JX, Chen XY. 2011. Monophyly and phylogenetic relationships of the catfish genus *Glyptothorax* (Teleostei: Sisoridae) inferred from nuclear and mitochondrial gene sequences. *Mol Phylogenet Evol.* 61(2):278–289. doi: [10.1016/j.ympev.2011.06.018](https://doi.org/10.1016/j.ympev.2011.06.018).
- Li B. 2017. The complete mitochondrial genome of *Glyptothorax cavia* (Siluriformes, Sisoridae, Glyptothorax): genome characterization and phylogenetic analysis. *Mitochondrial DNA B Resour.* 2(1):259–260. doi: [10.1080/23802359.2017.1307701](https://doi.org/10.1080/23802359.2017.1307701).
- Li B, Tian ZF, Qin Y, Hao M, Zhang JB. 2016. The complete mitochondrial genome of *Glyptothorax zainaensis* (Siluriformes, Sisoridae, Glyptothorax): genome characterization and phylogenetic analysis. *Mitochondrial DNA B Resour.* 1(1):56–57. doi: [10.1080/23802359.2015.1137821](https://doi.org/10.1080/23802359.2015.1137821).
- Li JS, Peng Y, Zhang SF, Liu YF, Zhang K, Chen J, Zhang H, Zhang C, Liu BJ. 2023. The complete mitochondrial genome of *Parachiloglanis hodgarti* and its phylogenetic position within Sisoridae. *J Ocean Limnol.* 41(1):267–279. doi: [10.1007/s00343-021-1319-z](https://doi.org/10.1007/s00343-021-1319-z).
- Lin XW, Li L, Jin HY, Jin X, Ma B. 2021. Complete mitochondrial genome sequence of *Glyptothorax annandalei* in the Yarlung Zangbo River, Tibet. *Mitochondrial DNA B Resour.* 6(4):1397–1398. doi: [10.1080/23802359.2021.1911701](https://doi.org/10.1080/23802359.2021.1911701).
- Lv Y, Li Y, Ruan Z, Bian C, You X, Yang J, Jiang W, Shi Q. 2018. The complete mitochondrial genome of *Glyptothorax macromaculatus* provides a well-resolved molecular phylogeny of the Chinese sisorid catfishes. *Genes (Basel)*. 9(6):282. doi: [10.3390/genes9060282](https://doi.org/10.3390/genes9060282).
- Ma QZ, Li L, Lin XW, Jin HY, Jin X, Ma B. 2020. Complete mitochondrial genome sequence of *Pareuchiloglanis kamengensis* in the Yarlung Zangbo River, Tibet. *Mitochondrial DNA B Resour.* 5(1):368–369. doi: [10.1080/23802359.2019.1703576](https://doi.org/10.1080/23802359.2019.1703576).
- Ma XH, Kang JL, Chen WT, Zhou CJ, He SP. 2015. Biogeographic history and high-elevation adaptations inferred from the mitochondrial genome of Glyptosternoid fishes (Sisoridae, Siluriformes) from the southeastern Tibetan Plateau. *BMC Evol Biol.* 15(1):233. doi: [10.1186/s12862-015-0516-9](https://doi.org/10.1186/s12862-015-0516-9).
- Miya M, Nishida M. 1999. Organization of the mitochondrial genome of a deep-sea fish, *Gonostoma gracile* (Teleostei: Stomiiformes): first example of transfer RNA gene rearrangements in bony fishes. *Mar Biotechnol.* 1(5):416–426. doi: [10.1007/PL00011798](https://doi.org/10.1007/PL00011798).
- Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, et al. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Mol Phylogenet Evol.* 26(1):121–138. doi: [10.1016/S1055-7903\(02\)00332-9](https://doi.org/10.1016/S1055-7903(02)00332-9).
- Ng HH, Rachmatika I. 2005. *Glyptothorax exodon*, a new species of rheophilic catfish from Borneo (Teleostei: Sisoridae). *Raffles Bull Zool.* 53(2): 251–255.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol.* 38(7):3022–3027. doi: [10.1093/molbev/msab120](https://doi.org/10.1093/molbev/msab120).
- Yan SH, Xiong MH, Que YF, Tang HY, Zhu B, Shao K. 2016. The complete mitochondrial genome of *Glyptothorax sinense* (Siluriformes, Sisoridae). *Mitochondrial DNA A DNA Mapp Seq Anal.* 27(2):886–887. doi: [10.3109/19401736.2014.919489](https://doi.org/10.3109/19401736.2014.919489).
- Zheng CY. 1991. Sisoridae. In: Pan JH, Zhong L, Zheng CY, Wu HL, Liu JH, editors. The freshwater fishes of Guangdong Province. Guangzhou (China): Guangdong Science and Technology Press; p. 319–322.
- Zhong LQ, Wang MH, Li DM, Tang SK, Zhang TQ, Bian WJ, Chen XH. 2018. Complete mitochondrial genome of *Odontobutis haifengensis* (Perciformes, Odontobutiae): A unique rearrangement of tRNAs and additional noncoding regions identified in the genus *Odontobutis*. *Genomics.* 110(6):382–388. doi: [10.1016/j.ygeno.2017.12.008](https://doi.org/10.1016/j.ygeno.2017.12.008).
- Zhou CJ, Wang DQ, Yu ML, He SP. 2012. The complete mitochondrial genome of *Glyptothorax fukiensis fukiensis* (Teleostei, Siluriformes: Sisoridae). *Mitochondrial DNA A.* 23(6):414–416. doi: [10.3109/19401736.2012.710211](https://doi.org/10.3109/19401736.2012.710211).
- Zhu T, Sato Y, Sado T, Miya M, Iwasaki W. 2023. MitoFish, MitoAnnotator, and MiFish pipeline: updates in 10 years. *Mol Biol Evol.* 40(3): msda035. doi: [10.1093/molbev/msad035](https://doi.org/10.1093/molbev/msad035).