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The Complete Mitochondrial Genome Sequence and Phylogenetic Analysis of *Saurogobio punctatus* (Cypriniformes: Cyprinidae: Gobioninae)

Juan Guo, Yu Zeng*, Jin Li, Ling Mao and Yu Shuang Zhao

China West Normal University, Nanchong 637000, People's Republic of China

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YZ conceived and designed the research. JL, LM, YZ and JG were involved in the analysis and interpretation of the data. JG wrote the drafting of the paper and revised the manuscript. YZ critically reviewed the article regarding its intellectual content. All authors agree to be accountable for all aspects of the work.

Key words
***Saurogobio punctatus*, Mitochondrial genome, Genetic structure and composition, Phylogenetic**

ABSTRACT

Saurogobio punctatus is a small-sized freshwater fish, widely distributed in eastern Asia. Presently, eight species are recognized as valid in *Surogobio*, among which the complete mitochondrial genome information had been known for seven species, except for *S. punctatus*. The lack of information was partly due to the recent discovery and establish of *S. punctatus* in the genus *Saurogobio* only in 2018. In the present study, the complete mitochondrial genome of *S. punctatus* was determined and phylogenetic of *Saurogobio* was analyzed using the complete mitochondrial genome sequence. The genome was 16,600 bp in length, with 55.98% of (A + T) content. It consisted of 13 protein-coding genes, 2 ribosomal RNAs, 22 tRNAs and a control region. The gene composition and the structural arrangement of the *S. punctatus* complete mtDNA were identical to other *Saurogobio* species. Phylogenetic analyses indicated that *S. punctatus* was closely related to *S. dabryi*, with strong statistical support, and was regarded as the sister taxon of the clade formed by *S. xiangjiangensis*, *S. gracilicaudatus* and *S. dabryi*, distinctly separated from all other *Saurogobio* species. It was inconsistent with previous results; the possible reason could be the different sequence utilized in each study. Moreover, phylogenetic results based on whole mitochondrial genomes were more reliable than partial mitochondrial.

INTRODUCTION

Saurogobio (Cypriniformes: Cyprinidae: Gobioninae) is a small-sized freshwater fish endemic to eastern Asia, including China, Russia, North Korea and northern Vietnam (Yang *et al*., 2003). Presently, eight species are recognized as valid, i.e., the *Saurogobio dabryi* (Bleeker, 1871), *S. dumerili* (Bleeker, 1871), *S. gracilicaudatus* (Yao and Yang, 1977), *S. gymnocheilus* (Lo *et al*., 1998), *S. immaculatus* (Koller, 1927), *S. xiangjiangensis* (Tang, 1980), *S. lissilabris* (B˘an˘arescu and Nalbant, 1973) and *S. punctatus* (Tang, 2018) (Nelson *et al*., 2016; Tang *et al*., 2018). The complete mitochondrial genome information on complete mitochondrial genome of *Saurogobio* fish had been published for seven species, except for *S. punctatus* (Xu *et al*., 2015; Tong and Fu, 2019; Wan *et al*., 2015). Currently, studies on *S. punctatus* are quite limited, including the first report of *S. punctatus* by Tang *et al*. (2018) and the population genetic structure and geographic differentiation of *S. punctatus* in the Yangtze River basin by Li *et al*. (2018a). Moreover, the results were all based on partial mitochondrial genes, while the whole mitochondrial genome of this species is still unavailable. Based on previous result we found differences between partial and complete mitochondrial genome of the results. Therefore, we want to use the mitochondrial genome to explore the status of *S. punctatus* in the Gobioninae.

Mitochondrial genome has the characteristics of simple genomic structure, small size, rapid evolution, maternal inheritance and low level of recombination. It was widely used in phylogenetics, population genetic variation and species identification (Brown *et al*., 1979; Moritz *et al*., 1987). The complete mitochondrial genes of seven species of *Saurogobio* had been published and could be obtained on NCBI, except *S. punctatus*. The lack of sequence data has largely hindered the research and conservation of *S. punctatus* on population genetics and species identification. Thus, the study of whole mitochondrial genome in *S. punctatus* provides important information for its phylogenetic evaluation and molecular

* Corresponding author: zengyu@cwnu.edu.cn
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evolution.

In this study, we sequenced and evaluated complete mitochondrial genomes of *S. punctatus*. We also conducted phylogenetic analyses using the obtained genomes of *S. punctatus* and 11 complete mitogenomes retrieved for the subfamily Gobioninae. The results helped to clear the phylogenetic position of *S. punctatus*, and contribute to reconstructing interspecific phylogenetic relationships within the genus *Saurogobio*.

MATERIALS AND METHODS

The specimen of *S. punctatus* was collected from Peng'an section of the Jialing River (31°2′14″N, 106°23′45″E) by cage net. *S. punctatus* was identified from fish samples according to the morphological descriptions by Tang *et al.* (2018). The specimen was preserved in 100% ethanol until DNA extraction. DNA was extracted with DNeasy Blood and Tissue kit (Qiagen, Germany) following the manufacturer's protocol. DNA of *S. punctatus* then delivered to the Tsingke Biological Technology Company (Chengdu, China) for Illumina sequencing. Methods of sequence assembly, annotation and sequence analysis could refer to Mao *et al.* (2021).

The outgroup taxa and close relatives of *Saurogobio* fishes were selected, based on the results of previous studies (Tang *et al.*, 2011; Li *et al.*, 2018b). *Pseudogobio esocinus*, *P. guilinensis*, *P. vaillanti* and *Abbottina rivularis* were used as outgroups, seven valid *Saurogobio* species were used as close relatives. Mitogenomes of 11 species belonging to the subfamily Gobioninae were retrieved from GenBank to study the phylogenetic position of *S. punctatus* and phylogenetic relationships within the Gobioninae (Table I). We used PhyloSuite (Zhang *et al.*, 2020) to conduct, manage and streamline the analyses of 12 sequences, with the help of several plug-in programs. 13 sequences were aligned in batches with MAFFT (Katoh and Standley, 2013) using 'auto' strategy and codon alignment mode. The alignments were refined using the codon-aware program MACSE v. 2.03 (Ranwez *et al.*, 2018), which preserved reading frame and allowed incorporation of sequencing errors or sequences with frameshifts. Ambiguously aligned fragments of 13 alignments were removed in batches using Gblocks (Talavera and Castresana, 2007) with the following parameter settings. Minimum number of sequences for a conserved/flank position (7/7), maximum number of contiguous non-conserved positions (8), minimum length of a block (10), allowed gap positions (with half). Model Finder (Kalyaanamoorthy *et al.*, 2017) was used to select the best-fit model using BIC criterion. Maximum likelihood phylogenies were inferred using IQ-TREE (Nguyen *et al.*, 2015) under the model automatically selected by IQ-TREE (Auto option in IQ-TREE) for 20,000 ultrafast (Minh *et al.*, 2013) bootstraps, as well as the Shimodaira Hasegawa like approximate likelihood-ratio test (Guindon *et al.*, 2010). Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist *et al.*, 2012) under GTR+I+G+F model (2 parallel runs, 5000000 generations), in which the initial 25% of sampled data were discarded as burn-in. The complete mitochondrial genome sequence then was submitted to NCBI (GenBank: ON041156). Strand skew values were calculated according to the formulae given by Perna and Kocher (1995): AT skew = (A - T)/ (A + T) and GC skew = (G -C)/ (G + C), where A, T, C, G were the four bases.

Table I. List of the 11 Gobioninae species in phylogenetic analysis, with their GenBank accession numbers.

Species	Subfamily	GenBank No	Size (bp)
Saurogobio gymnocheilus	Gobioninae	NC 050400	16,604
Saurogobio lissilabris	Gobioninae	NC 050401	16,594
Saurogobio dabryi	Gobioninae	KF612272	16,601
Saurogobio immaculatus	Gobioninae	NC 033919	16,988
Saurogobio gracilicaudatus	Gobioninae	NC 050398	16,608
Saurogobio xiangjiangensis	Gobioninae	NC 050399	16,600
Saurogobio dumerili	Gobioninae	NC 022187	16,601
Pseudogobio esocinus	Gobioninae	NC_013759	16,609
Pseudogobio guilinensis	Gobioninae	MN883565	16,609
Pseudogobio vaillanti	Gobioninae	NC 032292	16,605
Abbottina rivularis	Gobioninae	NC 023781	16,597

RESULTS

Mitogenome organization

The complete mitochondrial genome of *S. punctatus* was a circular molecule of 16,600 bp and showed a typical teleost mitochondrial order, which included 2 rRNA genes (12s rRNA and 16s rRNA), 22 transfer RNA genes (tRNA), 13 protein-coding genes (PCGs) and the control region (D-loop). The nucleotide composition was: A 30.07%, T 25.91%, G 16.68%, C 27.34%. The genome had an overall AT content of 55.98% and GC content of 44.02%, and it showed a biased A + T ratio. The composition was skewed away from A in favored of T (the AT-skew was +0.074), but was almost balanced for G and C (the GC-skew was -0.242). A negative GC and AT skew value meant more Cs or Ts in coding strand, respectively. To the opposite, a positive GC and AT skew value indicated the coding strand had more Gs or As, respectively. The mitochondrial

genome map and gene characteristics were shown in Figure 1 and Table II, respectively.

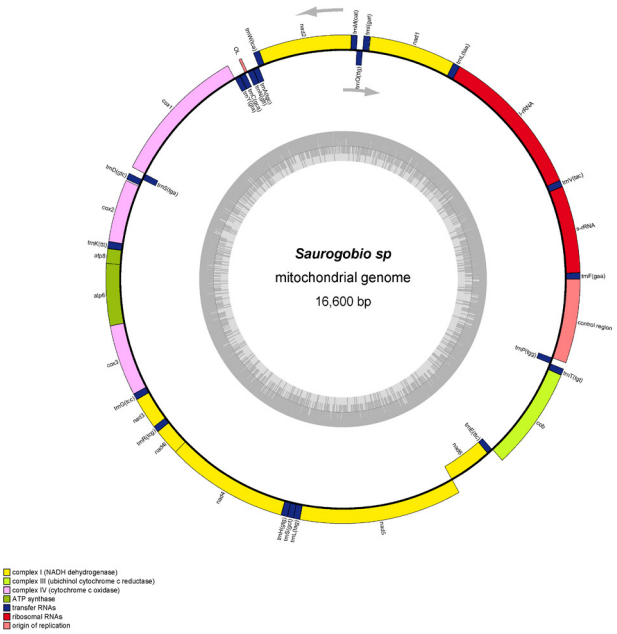

Fig. 1. The map of the mitochondrial genome of *S. punctatus*. Genes encoded on the light and heavy strand are shown inside and outside the circle respectively. The gray histogram represents the GC content of the genome

Most genes were encoded on the heavy strand (H strand), except for the ND6 and eight tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu, tRNA-Pro). Two types of start codons (ATG and GTG) and four types of stop codons (TAA, TAG, TA and T) were used in protein-coding genes. Among the 13 protein-coding genes, 12 PCGs used ATG as regular initiation codon, while only COX1 gene started with GTG. Six genes (ND2, COX1, ATP6, ATP8, ND4L, ND5) made use of the regular stop codon TAA, ND1, ND3 and ND6 used TAG, whereas the remaining 4 genes (COX2, COX3, ND4, CYTB) used incomplete codons TA or T as termination codons (Table II). Four pairs of adjacent PCGs, ATP8-ATP6, ATP6-COIII, ND4L-ND4, and ND5-ND6 had the overlapped size of 7, 1, 7, and 4 bp, respectively. And there were eleven regions of gene overlap totaling 28 bp (ranging from 1 to 7 bp) and 12 intergenic spacer regions totaling 38 bp (ranging from 1 to 13 bp). The two rRNA genes combined were 2648 bp in length. The location of 12S rRNA gene (958 bp) was between tRNA-Phe and tRNA-Val, and 16S rRNA gene (1690 bp) was located between tRNA-Val and tRNA-Leu. In addition, the 22 tRNA genes, ranging from 69 to 76 bp, were distributed across the mitogenome of *S. punctatus*. The noncoding control region (D-loop), with the length of 926 bp, was located between the tRNA-Phe and tRNA-Pro genes.

Although BI and ML analyses relied on different methods, they produced almost the same phylogenetic trees. Both topological structures indicated the existence of two major clades: One group involved *S. dumerili*, *S. lissilabris*, *S. gymnocheilus*, and *S. immaculatus*, and another group consisted of *S. dabryi*, *S. gracilicaudatus*, *S. xiangjiangensis* and *S. punctatus*. But compared with Tang's findings, the location of *S.dabryi* is different. In this study, *S. dabryi* and *S. punctatus* were closely related, while in Tang *et al*. (2018) study the two are separated by *S. gracilicaudatus* and *S. xiangjiangensis*.

DISCUSSION

The composition and arrangement of the mitochondrial genome of *S. puntatus* were similar to other Gobioninae species (Mao *et al*., 2021; Tong and Fu, 2019). The use of codons was also similar to other fishes: start with ATG and GTG, end with TAA, TAG, TA-, T-. Similar patterns in the gene arrangements and codon use had also been observed in published mitochondrial genomes of other *Saurogobio* and *Squalidus* species (Wan *et al*., 2015; Xu *et al*., 2015; Chai and Fu, 2020). Presumably, TAG and incomplete codons in ND2 would be completed to TAA by post-transcriptional polyadenylation (Ojala *et al*., 1981). At the same time, the GC content (44.02%) of *S. puntatus* mitochondrial genomes was lower than AT content (55.98%), which was frequently observed in other Cyprinid species (Yue *et al*., 2006). The higher GC content of DNA, the more stable was the doublestranded helical molecule. On the contrary, the more unstable (Bhagavan, 2002).

S. punctatus is a bony fish belongs to Cypriniformes, Cyprinidae, Gobioninae, and according to previous results *Saurogobio* is a monophyletic group. The results of Bayesian analysis and maximum likelihood tree showed a topology with strong posterior probability values, suggesting that the phylogenetic tree was well-supported. The *Saurogobio* was a monophyletic group which could be divided into two major groups (Fig. 2). *S. punctatus* was regarded as the sister taxon of the clade formed by *S. xiangjiangensis*, *S. gracilicaudatus* and *S. dabryi*, distinctly separated from all other *Saurogobio* species. The topology of the phylogenetic tree further supported the results of previous studies, which confirmed the basic relationships in the *Saurogobio* genus (Tong and Fu, 2019). The phylogenetic tree proved that *S. punctatus* and *S. dabryi* were closely related, while Tang *et al*. (2018) research was not the case. A possible reason could be

Table II. Characteristics of the complete mitochondrial genome of *S. punctatus.*

Gene	Start	Stop	Strand	Length	Codon start/ stop	Anti codon	Intergenic region
tRNA-Phe	1	69	+	69		GAA	0
12S rRNA	70	1027	+	958			0
tRNA-Val	1030	1101	+	72		TAC	2
16S rRNA	1102	2791	+	1690			0
tRNA-Leu	2792	2867	+	76		TAA	0
ND1	2868	3842	+	975	ATG/TAG		0
tRNA-Ile	3847	3918	+	72		GAT	4
tRNA-Gln	3917	3987	-	71		TTG	-2
tRNA-Met	3989	4057	+	69		CAT	1
ND2	4058	5104	+	1047	ATG/TAA		0
tRNA-Trp	5104	5174	+	71		TCA	-1
tRNA-Ala	5177	5245	-	69		TGC	2
tRNA-Asn	5321	5351	+	31		GTT	1
rep-origion	5247	5319	-	73			1
tRNA-Cys	5351	5418	-	68		GCA	-1
tRNA-Tyr	5421	5488	-	68		GTA	2
COX1	5490	7040	+	1551	GTG/TAA		1
tRNA-Ser	7041	7111	-	71		TGA	0
tRNA-Asp	7115	7186	+	72		GTC	3
COX2	7200	7890	+	691	ATG/T		13
tRNA-Lys	7891	7966	+	76		TTT	0
ATP8	7968	8132	+	165	ATG/TAA		1
ATP6	8126	8809	+	684	ATG/TAA		-7
COX3	8809	9593	+	785	ATG/TA		-1
tRNA-Gly	9593	9663	+	71		TCC	-1
ND3	9664	10014	+	351	ATG/TAG		0
tRNA-Arg	10013	10082	+	70		TCG	-2
ND4L	10083	10379	+	297	ATG/TAA		0
ND4	10373	11751	+	1379	ATG/TA		-7
tRNA-His	11755	11824	+	70		GTG	3
tRNA-Ser	11825	11893	+	69		GCT	0
tRNA-Leu	11893	11965	+	73		TAG	-1
ND5	11966	13801	+	1836	ATG/TAA		0
ND6	13798	14319	-	522	ATG/TAG		-4
tRNA-Glu	14320	14388	-	69		TTC	0
CYTB	14393	15533	+	1141	ATG/T		4
tRNA-Thr	15534	15605	+	72		TGT	0
tRNA-Pro	15605	15674	-	70		TGG	-1
D-loop	15675	16600	+	926			0

Note: + and - represent heavy strand and light strand, respectively. In the intergenic region column, negative numbers indicate an overlap between two adjacent genes.

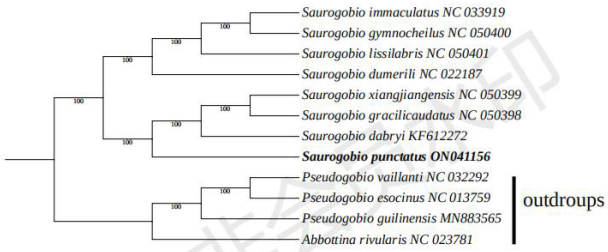

Fig. 2. Maximum likelihood tree showing the phylogenetic position of *Saurogobio punctatus* (bolded) among *Saurogobio* species. Only Bootstrap support (BP) greater than 50% are shown

the different sequence utilized in each study, that Tang *et al*. (2018) used the partial mitochondrial genome (cytb), whereas this study used the whole mitochondrial genome. Previous research had also confirmed this, Mao *et al*. (2021) research on phylogenetic analysis with the whole mitochondrial genome were inconsistent with Yang *et al*. (2006) with cytb. In addition, Arnason and Gullberg (1996) found the phylogenetic relationships based on cytb among the five clades were not resolved in the bootstrap analysis, whereas in Arnason's later study (Arnason *et al*., 2004), he used the whole mitochondrial genome to figure out their relationships. These articles demonstrated that the phylogenetic results based on ctyb and the whole mitochondrial genome were different, and the results of the latter were more reliable.

In summary, we reported the complete mitochondrial genome of *S. punctatus* for the first time. The full-length of *S. punctatus* mitochondrial genome was 16,600 bp long, and it showed a similar order and characteristic with other *Saurogobio* fishes. Our findings could provide novel molecular resources for further population genomics, evolution, and conservation studies of *S. punctatus*.

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DECLARATIONS

Disclosure statement

The creators report no irreconcilable circumstance. The writers are liable for the substance and composing of the article.

Data availability statement

The complete mitochondrial genome sequence of Glyphoglossus yunnanensis is deposited in the GenBank database under the accession number ON041156. The associated BioProject number, SRA accession number, and BioSample number are PRJNA846592, SRR19576530, and SAMN28898293, respectively.

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Ethical approval

The specimen for this paper were approved by the Animal Ethics Committee at China West Normal University. All animals handling and processing by the Law of the People's Republic of China on the Protection of Wildlife and approved by the Animal Care Committee of CIB, CAS.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Arnason, U., and Gullberg, A., 1996. Cytochrome b nucleotide sequences and the identification of five primary lineages of extant cetaceans. *Mol. Boil. Evolut*., **13**: 407-417. https://doi.org/10.1093/oxfordjournals.molbev.a025599

Arnason, U., Gullberg, A., and Janke, A., 2004. Mitogenomic analyses provide new insights into cetacean origin and evolution. *Gene*, **333**: 27-34. https://doi.org/10.1016/j.gene.2004.02.010

Bhagavan, N.V., 2002. Chapter 23- Nucleic Acid Structure and Properties of DNA *Medical biochemistry (Fourth Edition)*. Academic Press. https://doi.org/10.1016/B978-012095440-7/50025-1

Brown, W.M., Geogre, M., and Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. *Proc. natl. Acad. Sci. U. S. A*., **76**: 1967-1971. https://doi.org/10.1073/pnas.76.4.1967

Chai, J., and Fu, C.Z., 2020. Three mitochondrial genomes of freshwater fishes in the genus *Squalidus*. *Mitochondr. DNA B Resour*., **5**: 3761-3763. https://doi.org/10.1080/23802359.2020.1835583

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol*., **59**: 307-321. https://doi.org/10.1093/sysbio/syq010

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Haeseler, A.V., Jermiin, L.S., 2017. Model Finder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods*, **14**: 587-589. https://doi.org/10.1038/nmeth.4285

Katoh, K., and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evolut.*, **30**: 772-780. https://doi.org/10.1093/molbev/mst010

Li, X.B., Tang, Q.Y., Yu, D. and Liu, H.Z., 2018a. Population genetic structure and geographic differentiation of *Saurogobio punctatus* (Teleostei, Cypriniformes, Cyprinidae) in the Yangtze River Basin based on cytochrome b gene sequences. *Sichuan J. Zool.*, **37**: 251-259.

Li, Y.H., Cao, K., and Fu, C.Z., 2018b. Ten fish mitogenomes of the tribe Gobionini (Cypriniformes: Cyprinidae: Gobioninae). *Mitochondr. DNA B Resour.*, **3**: 803-804. https://doi.org/10.1080/23802359.2018.1467236

Mao, L., Zeng, Y., Li, J., Zhang, F.B., Liu, Y.Y. and Gong, J.H., 2021. The complete mitochondrial genome sequence and phylogenetic analysis of *Gnathopogon herzensteini* (Cypriniformes, Cyprinidae, Gobioninae). *Biologia*, **76**: 1087-1094. https://doi.org/10.2478/s11756-020-00667-9

Minh, B.Q., Nguyen, M.A., and Haeseler, V.A., 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol.*, **30**: 1188-1195. https://doi.org/10.1093/molbev/mst024

Moritz, C., Ted, Brown, W.M., 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. *Annu. Rev. Ecol. Syst.*, **18**: 269-292. https://doi.org/10.1146/annurev.es.18.110187.001413

Nelson, J.S., Grande, T.C., and Wilson, M.V.H., 2016. *Fishes of the world, 5th edition*. John Wiley and Sons, Hoboken, NJ. https://doi.org/10.1002/9781119174844

Nguyen, L.T., Schmidt, H.A., and Haeseler, V.A. and Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.*, **32**: 268-274. https://doi.org/10.1093/molbev/msu300

Ojala, D., Montoya, J., and Attardi, G., 1981. TRNA punctuation model of RNA processing in human mitochondria. *Nature*, **290**: 470-474. https://doi.org/10.1038/290470a0

Perna, N.T., and Kocher, T.D., 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. mol. Evol.*, **41**: 353-358. https://doi.org/10.1007/BF01215182

Ranwez, V., Douzery, E.J.P., Cambon, C., Chantret, N. and Delsuc, F., 2018. MACSE v2: Toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. *Mol. Biol. Evol.*, **35**: 2582-2584. https://doi.org/10.1093/molbev/msy159

Ronquist, F., Teslenko, M., Mark, V.P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, **61**: 539-542. https://doi.org/10.1093/sysbio/sys029

Talavera, G., and Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.*, **56**: 564-577. https://doi.org/10.1080/10635150701472164

Tang, K.L., Agnew, M.K., Chen, W.J., Hirt, M.V., Raley, M.E., Sado, T., Schneider, L.M., Yang, L., Bart, H., He, S.P., Liu, H.Z., Miya, M., Miya, M., Saitoh, K., Simons, A.M., Wood, R.M. and Mayden, R.L., 2011. Phylogeny of the gudgeons (Teleostei: Cyprinidae: Gobioninae). *Mol. Phylogenet. Evol.*, **61**: 103-124. https://doi.org/10.1016/j.ympev.2011.05.022

Tang, Q.Y., Li, X.B., Yu, D., Zhu, Y.R., Ding, B.Q., Liu, H.Z. and Danley, P.D., 2018. *Saurobobio punctatus* sp. nov, a new cyprinid gudgen (Teleostei: Cypriniformes) from the Yangtze River, based on both morphological and molecular data. *J. Fish Biol.*, **92**: 347-364. https://doi.org/10.1111/jfb.13498

Tong, J., and Fu, C.Z., 2019. Four complete mitochondrial genomes of *Saurogobio* fishes (Cypriniformes: Gobionidae). *Mitochondr. DNA B Resour.*, **4**: 2175-2176. https://doi.org/10.1080/23802359.2019.1623126

Wan, Q., Ma, Z., Pan, D.D., Wei, M. and Tao, G., 2015. The complete mitochondrial genome of *Saurogobio dumerili* (Cypriniformes: Cyprinidae). *Mitochondr. DNA*, **26**: 757-758. https://doi.org/10.3109/19401736.2013.855737

Xu, H., Xue, Y., Li, X.Y., Li, X.Y., Luo, X., Li, Y. and Li, Y., 2015. Complete mitochondrial genome of the Chinese lizard gudgeon, *Saurogobio dabryi* (Teleostei: Cypriniformes: Cyprinidae). *Mitochondr. DNA,* **26**: 840-841. https://doi.org/10.3109/19401736.2013.855919

Yang, J., He, S., Freyhof, J., Witte , K. and Liu, H.Z., 2006. The phylogenetic relationships of the Gobioninae (Teleostei: Cyprinidae) inferred from

mitochondrial cytochrome b gene sequences. *Hydrobiologia*, **553**: 255–266. https://doi.org/10.1007/s10750-005-1301-3

Yang, X.P., Zhang, M.Y., and Liu, H.Z., 2003. Studies on morphometricis of the genus *Saurogobio*. *Acta hydrobiol. Sin*., **27**: 164-169.

Yue, G.H., Liew, W.C., and Orban, L., 2006. The complete mitochondrial genome of a basal teleost, the Asian arowana (*Scleropages formosus*, Osteoglossidae). *BMC Genomics*, **7**: 242. https://doi.org/10.1186/1471-2164-7-242

Zhang, D., Fang, L.G., Jakovlić, I., Zou, H., Zhang, J., Li, W.X. and Wang, G.T., 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour*., **20**: 348–355. https://doi.org/10.1111/1755-0998.13096