The First High-Quality Genome Assembly and Data Analysis of the Malaysian mahseer (*Tor tambroides*)

Melinda Mei Lin Lau^a, Leonard Whye Kit Lim^a, Hung Hui Chung^{a*} and Han Ming Gan^{b,c}

^aFaculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.

^bGeneSEQ Sdn Bhd, Bandar Bukit Beruntung, 48300 Rawang, Selangor, Malaysia

^cCentre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia

*Corresponding author: Hung Hui Chung (hhchung@unimas.my)

Abstract

The Malaysian mahseer (*Tor tambroides*), one of the most valuable freshwater fish in the world, is mainly targeted for human consumption. The mitogenomic data of this species is available to date, but the genomic information is still lacking. For the first time, we sequenced the whole genome of an adult fish on both Illumina and Nanopore platforms. The hybrid genome assembly had resulted in a sum of 1.5 Gb genomic sequence from the 44,726 contigs found with 44 kb N50 length and BUSCO genome completeness of 84.3%. Four types of SSRs had been detected and identified within the genome with a greater AT abundance than that of GC. Predicted protein sequences had been functionally annotated to public databases, namely GO, KEGG and COG. A maximum likelihood phylogenomic tree containing 53 Actinopterygii species and two outgroups was constructed, providing first insights into the genome-based evolutionary relationship of *T. tambroides* with other ray-finned fish. These data are crucial in facilitating the study of population genomics, species identification, morphological variations, and evolutionary biology, which are helpful in the conservation of this species.

Keywords: Genome; Gene annotation; *Tor tambroides*; Phylogenetic; Functional annotation

Introduction

The Malaysian mahseer, *Tor tambroides* (Bleeker, 1854), one of the members of the family Cyprinidae, is a widespread species found in aquaculture and fisheries mainly targeted for human consumption (Kottelat *et al.*, 2018). It is commonly named Kelah or Empurau in Malaysia and Jurung, Indonesia (Jaafar *et al.*, 2021). As a true mahseer (*Tor* spp.), it can be found in rapidly-flowing waters with rocky bottoms (Shreshtha, 1997). Together with *Tor tambra* and *Tor dourenensis*, it is one of the three *Tor* spp. found in freshwaters of Malaysia, and among the 16 *Tor* spp. found worldwide (Ng, 2004).

Like other *Tor* spp., *T. tambroides* is endangered by environmental degradation within their habitat, causing an elevating shrink in their population size in recent years (Ingram et al., 2005). Anthropogenic modification of rivers, including agricultural activities, logging, and deforestations, had interrupted and reduced the water flow within its habitat. Consequently, such actions not only shrink the population size of *T. tambroides* and other *Tor* spp., but it is also associated with impacts on the aquatic environment. Despite environmental issues, *T. tambroides* are also at threat from overfishing with the usage of hooks, nets, and dynamites. However, there is currently no information on the rate of these losses (Kottelat et al., 2018).

It resides a wide range of freshwaters, including Brunei Darussalam, Yunnan China, Indonesia (Kalimantan, Jawa, and Sumatera), Lao, Malaysia (Peninsular, Sabah, and Sarawak) and Thailand (Kottelat 2018). Despite its comprehensive habitat coverage, its distant population can probably be different, leaving its taxonomy still vague. Its body is covered with scales depending on locality, such as silver, bronze, and reddish.

Although the presence of an upper median projection can be observed in *T. tambroides*, its other features, including equal caudal fin lobe, long lower median lobe, sub-terminal mouth position and pointed rostrum hood, were shared closely with other *Tor* spp., making it difficult to be distinguished (Jaafar et al., 2021). Therefore, disentangling *Tor* spp. via lips polymorphisms and median lobes resulting from direct environmental influences is discouraged due to lack of direct evidence (Roberts & Khaironizam, 2008). Unpublished research had emphasised the reduction in lips and lobe size and the loss of red colour when the fishes from Sungai Tembat, Terengganu, were kept in captivity for more than two years (Walton et al., 2019). However, such polymorphism is yet to be observed in nature and thus remains deficient.

The accumulation of significant genetic and/or morphological differences among *Tor* spp distributed across Indonesia and Malaysia urged the revision of *Tor* taxonomy. Studies had been done aiming to resolve the ambiguous taxonomical status of *Tor* spp. by looking into its phylogenetic relationship through mitochondrial DNA, Cytochrome c Oxidase Subunit I (*COXI*) gene, Cytochrome b, ATPase 6 /8 gene, 16S rRNA gene, microsatellite, and SNP markers (Walton et al., 2017; Jaafar et al., 2021; Lim et al., 2021a). However, to date, the whole genome and transcriptome sequences of *Tor* spp. are still unavailable, except for a few studies that had reviewed its conservation status, conducted a gut metagenomic analysis, as well as sequenced the transcriptome of *T. tambra* (Lau et al., 2021a; Lau et al., 2021b; Lau et al., 2021c). Genomic and transcriptomic sequencing of *Tor* species is necessary to provide a more powerful tool that continues to resolve and address questions of species identification, evolutionary biology, morphological variations, sequences related to sex differentiation, growth, reproduction, and immune, which is helpful for further conservation of *Tor* species (Jaafar et al., 2021).

The rapid growth and expansion of the aquaculture industry had improved fish production as compared to traditional fishing. *T. tambroides* is one of the notable species found in the aquaculture industry due to its high nutritional value and unique flesh taste. However, it has been found that the genetic variability decreases in captivity-held fishes, causing them to be more vulnerable to infections. Thus, it is necessary to comprehend the fish immune system and its underlying mechanisms in response to its exposure to ecotoxicological chemicals (Lim et al., 2018; Lim et al., 2021b). However, in general, the studies of the fish immune

response are limited due to its complexity and lack of suitable reagents for classical immunological assays (Salinas & Magadán, 2017). Similarly, as a slow grower fish, it is necessary to venture into the growth-related aspect of the *T. tambroides* genome as well, which is in line with the goal of fish farming of this species which faces knowledge-scarcity on growth improvement to date. Omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, can develop high throughput outcomes and facilitate more novel findings.

In the recent years, with the booming of long-read sequencing technologies (Heather & Chain, 2016), the implementation of combining both Illumina reads and Nanopore/PacBio reads can be found in several studies (Austin et al., 2017; Tan et al., 2018; Lim et al., 2021c; Lim et al., 2022). Integration of short but accurate Illumina reads with long but less accurate Nanopore/PacBio reads could enhance the completeness of the assembled genome than assemblies based on Illumina reads only (Austin et al., 2017; Tan et al., 2018; Lim et al., 2021c; Lim et al., 2022). Thus, in this study, we sequenced and reported on the first genomic data of *T. tambroides*. We also functionally annotated the genome of *T. tambroides* to KEGG, GO, and COG databases and further identified its immune-related genes. Furthermore, we inferred the phylogenetic relationship of *T. tambroides* with other Actinopterygii fishes based on the BUSCO supermatrix data. It is hoped that these data generated from this study can be channeled for the improvement studies that are driven along with the conservation endeavors of this fish species.

Experimental Design, Materials and Methods

Sampling and DNA extraction

An adult *T. tambroides* (voucher ID: ASD03018) was sampled from a local aquaculture farm, with its locality reported in previous studies (Lau et al., 2021c; Lim et al., 2021). The fish had been deposited as voucher specimens in the fish museum located at the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak. The fish was euthanised, and 50 mg of its muscle tissue was used for genomic DNA extraction using the DTAB-CTAB DNA extraction kit (GeneReach Biotechnology Corp) according to the manufacturer's instructions.

Species Verification

Cytochrome c oxidase subunit I (COI) gene was amplified, and the PCR product was purified and sent for Sanger sequencing. The sequences were analysed using NCBI BLASTn and found to possess 100% similarity with the mitogenome sequences of *T. tambroides* reported by Lim et al. (2021) with the GenBank accession number MW471071.1.

Library Construction and Whole Genome Sequencing

Approximately one ug of gDNA was sheared to 350 bp using a Bioruptor and directly used for PCR-free library preparation using the NEB Ultra Illumina library preparation kit (NEB, Ipswich, MA). The library was quantified with a Qubit (Invitrogen) and sequenced on a NovaSEQ6000 (Illumina, San Diego, CA) with 2 x 150 bp run configuration. Similarly, for Nanopore sequencing, one ug of unsheared gDNA was used as the input for LSK109 library

preparation (Oxford Nanopore, UK) according to the manufacturer's instructions. The library was sequenced on two MinION flowcells. Nanopore reads were base-called from their fast5 files using Guppy version 4.4.1 (high accuracy mode).

Sequence Data Processing and Assembly

Illumina reads were trimmed with fastp (Chen et al., 2018), while the Nanopore reads were trimmed with porechop (Wick et al., 2017). The trimmed Nanopore reads and paired-end Illumina reads were used to perform a hybrid *de novo* assembly using Wengan v0.2 (Di Genova et al., 2021). Jellyfish v2.3.0 was used to obtain a frequency distribution of k-mer counting with the clean reads, producing kmer frequency distributions of 31-mers (Marçais & Kingsford, 2011). These histograms were subsequently processed using GenomeScope, which estimates genomic size, repeat content and heterozygosity via kmer-based statistical approach (Vurture et al., 2017). QUAST v5.0.2 was used to evaluate various metrics of the *T. tambroides* genome (Mikheenko et al., 2018) using the parameter: read length 150 bp and max K-mer coverage 1,000. BUSCO v5.2.2 was used to evaluate the completeness of the assembled *T. tambroides* genome based on the single-copy orthologs represented in the actinopterygii_odb10 database (Manni et al., 2021).

Detection of Repetitive Sequences and Prediction of Protein-Coding Genes

Repetitive sequences in the assembled genomes were identified and masked using RepeatModeler2 and RepeatMasker, respectively. Previously generated transcriptomic reads (Lau et al., 2021b) were aligned to the repeat-masked genome assembly using HiSAT2 (Kim et al., 2015). The transcriptome alignment BAM file and the repeat-masked genome assembly were used as the input for protein-coding gene prediction in BRAKER2 (Bruna et al., 2021).

SSR Analysis

Simple sequence repeats (SSRs) analysis was identified using Kmer-SSR (<https://github.com/ridgelab/Kmer-SSR>) (Pickett et al., 2017) on the processed reads of the *T. tambroides* genome. Dinucleotides, trinucleotides, tetranucleotides, pentanucleotides and hexanucleotides were included in the SSRs analysis. Only SSRs with at least four repeats were selected for the study.

Functional Annotation of Protein-Coding Genes

The predicted protein sequences were functionally annotated to EggNOG mapper (evolutionary genealogy of genes: Non-supervised Orthologous Groups) with a minimum E-value of 0.001. Functional annotation of genes was performed by mapping against three public databases, GO (Gene ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), and COG (the Clusters of Orthologous Groups).

In addition, predicted protein sequences were mapped against several previously published growth- and immune-related genes based on literature review (Overturf et al., 2010; Palti, 2011; Vagner & Santigosa, 2011; Zhenzhen et al., 2014; Hu et al., 2016; Ma et al., 2016; Chandhini & Rejish, 2019; Lin et al., 2019; Dam et al., 2020; Damzamn et al., 2020; Guan & Qiu, 2020). 82 growth- and 31 immune-related genes (113 genes in total) were downloaded from the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>) and used as references. Subsequently, the genes were further filtered based on a stringent E-value cutoff of 10^{-10} .

Ortholog Inference and Phylogenetic Construction

The *Tor tambroides* genome was assembled in this study, while other genome sequences used were obtained from NCBI (National Center for Biotechnology Information) and summarised in Table 1. A total of 50 species genomes together with 2 outgroups (additional species from Coelacanthiformes and Acipenseriformes) were included.

We had identified single-copy orthologous genes in each genome using BUSCO v5.2.2 (Benchmarking Universal Single-Copy Orthologs) (Manni et al., 2021) across all 53 Actinopterygii species. BUSCO was run with orthologs in actinopterygii_odb10 (updated 2021-02-19) using default parameters. All BUSCOs found in a single-copy and multi-copy for each species were used for phylogenetic analysis. Subsequently, BUSCO sequences were individually aligned with MUSCLE (Multiple Sequence Comparison by Log-Expectation) across all 53 species (Edgar, 2004). Gaps and unmatched sites were removed from the resulting multiple sequence alignments (MSAs) using trimAl (Capella-Gutiérrez et al., 2009). These trimmed MSAs were concatenated into a supermatrix. A maximum-likelihood (ML) phylogenetic tree was inferred from the supermatrix using IQ-TREE (Nguyen et al., 2015). Molecular Evolutionary Genetic Analysis (MEGA) v11.0.10 was used to analyse and view the generated phylogenetic tree (Kumar et al., 2018).

The complete genome sequence of *T. tambroides* reported in this study is available under NCBI Bioproject PRJNA708136 (<https://submit.ncbi.nlm.nih.gov/subs/bioproject/SUB9225867/overview>). The sequencing reads in this study are also available under NCBI SRA entry SUB10492101 (<https://submit.ncbi.nlm.nih.gov/subs/sra/SUB10492101>).

Ethics Statement

All experiments comply with ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Table 1. Species found within class Actinopterygii obtained from NCBI were used in this study to build the Maximum-likelihood (ML; IQ-TREE) tree to infer the phylogenetic relationship of these fishes with *T. tambroides*. A total of 50 species with two outgroups were reported and classified into their respective residing environment, including freshwater, marine, or diadromous.

	Species	Order	Translated CDS size (Mb)	BUSCO5 completeness	No. of chromosomes	Accession Numbers
Freshwater	<i>Archocentrus centrarchus</i>	Cichliformes	13.4	99.6	24	GCF_007364275.2
	<i>Astyanax mexicanus</i>	Characiformes	13.7	98.6	25	GCF_000372685.2
	<i>Austrofundulus limnaeus</i>	Cyprinodontiformes	11.7	89.6	0	GCF_001266775.1
	<i>Betta splendens</i>	Anabantiformes	13.8	99.4	22	GCF_900634795.3
	<i>Carassius auratus</i>	Cypriniformes	20.8	99.3	60	GCF_003368295.1
	<i>Colossoma macropomum</i>	Characiformes	14.1	99.7	0	GCF_904425465.1
	<i>Coregonus clupeaformis</i>	Salmoniformes	21.1	98.6	41	GCF_018398675.1
	<i>Cyprinodon tularosa</i>	Cyprinodontiformes	13.3	99.0	0	GCF_016077235.1
	<i>Danio rerio</i>	Cypriniformes	17.3	97.3	25	GCF_000002035.6
	<i>Esox lucius</i>	Esociformes	14.9	99.7	26	GCF_011004845.1
	<i>Etheostoma cragini</i>	Perciformes	12.8	96.5	24	GCF_013103735.1
	<i>Etheostoma spectabile</i>	Perciformes	13.1	96.0	25	GCF_008692095.1
	<i>Haplochromis burtoni</i>	Cichliformes	14.7	98.1	1	GCF_018398535.1
	<i>Lepisosteus oculatus</i>	Lepisosteiformes	10.8	98.5	30	GCF_000242695.1
	<i>Melanotaenia boesemani</i>	Atheriniformes	13.6	99.8	25	GCF_017639745.1
	<i>Oncorhynchus mykiss</i>	Salmoniformes	24.3	99.7	33	GCF_013265735.2
	<i>Oreochromis niloticus</i>	Cichliformes	16.3	99.8	23	GCF_001858045.2
	<i>Oryzias latipes</i>	Beloniformes	13.5	98.5	25	GCF_002234675.1
	<i>Oryzias melastigma</i>	Beloniformes	13.1	98.2	25	GCF_002922805.2
	<i>Paramormyrops kingsleyae</i>	Osteoglossiformes	13.9	98.7	0	GCF_002872115.1
	<i>Poecilia reticulata</i>	Cyprinodontiformes	13.0	97.7	24	GCF_000633615.1
	<i>Perca flavescens</i>	Perciformes	13.1	99.6	25	GCF_004354835.1
	<i>Perca fluviatilis</i>	Perciformes	14.1	98.5	24	GCF_010015445.1

	<i>Pimephales promelas</i>	Cypriniformes	14.2	98.3	1	GCF_016745375.1
	<i>Poecilia mexicana</i>	Cyprinodontiformes	13.6	98.2	0	GCF_001443325.1
	<i>Polyodon spathula</i>	Acipenseriformes	17.4	96.4	61	GCF_017654505.1
	<i>Pungitius pungitius</i>	Perciformes	13.7	99.6	1	GCF_902500615.1
	<i>Pygocentrus nattereri</i>	Characiformes	14.5	99.9	30	GCF_015220715.1
	<i>Salvelinus namaycush</i>	Salmoniformes	19.7	95.3	43	GCF_016432855.1
	<i>Sander lucioperca</i>	Perciformes	14.6	99.6	25	GCF_008315115.2
	<i>Scleropages formosus</i>	Osteoglossiformes	13.8	99.2	26	GCF_900964775.1
	<i>Simochromis diagramma</i>	Cichliformes	15.3	98.9	0	GCF_900408965.1
	<i>Sinocyclocheilus rhinoceros</i>	Cyprinoformes	22.2	95.8	1	GCF_001515625.1
	<i>Sinocyclocheilus anshuiensis</i>	Cyprinoformes	22.1	99.8	1	GCF_001515605.1
	<i>Sinocyclocheilus grahami</i>	Cyprinoformes	21.9	99.9	1	GCF_001515645.1
	<i>Thalassophryne amazonica</i>	Batrachoidiformes	12.3	97.6	23	GCF_902500255.1
	<i>Xiphophorus maculatus</i>	Cyprinodontiformes	13.4	99.1	25	GCF_002775205.1
	<i>Xiphophorus couchianus</i>	Cyprinodontiformes	13.7	99.8	25	GCF_001444195.1
	<i>Xiphophorus hellerii</i>	Cyprinodontiformes	13.9	99.6	25	GCF_003331165.1
Marine	<i>Chelmon rostratus</i>	Acanthuriformes	12.4	99.8	25	GCF_017976325.1
	<i>Epinephelus lanceolatus</i>	Perciformes	13.7	99.2	25	GCF_005281545.1
	<i>Gadus morhua</i>	Gadiformes	13.2	96.9	24	GCF_902167405.1
	<i>Hippoglossus hippoglossus</i>	Pleuronectiformes	13.3	99.6	25	GCF_009819705.1
	<i>Plectropomus leopardus</i>	Perciformes	12.7	95.8	25	GCF_008729295.1
	<i>Thunnus maccoyii</i>	Scombriformes	14.0	99.8	25	GCF_910596095.1
	<i>Toxotes jaculatrix</i>	Carangaria	12.8	99.9	24	GCF_017976425.1
	<i>Latimeria chalumnae</i>	Coelacanthiformes	10.6	87.2	1	GCF_000225785.1
Diadromous	<i>Acanthopagrus latus</i>	Perciformes	14.3	99.9	25	GCF_904848185.1
	<i>Alosa sapidissima</i>	Clupeiformes	15.0	98.8	25	GCF_018492685.1
	<i>Kryptolebias marmoratus</i>	Cyprinodontiformes	13.0	99.1	25	GCF_001649575.2
	<i>Lates calcarifer</i>	Carangaria	13.7	99.8	1	GCF_001640805.1
	<i>Oreochromis aureus</i>	Cichliformes	14.4	99.6	23	GCF_013358895.1

Results & Discussion

Characterisation of *T. tambroides* and its genome sequencing

The genomic sequencing and assembly statistics of the target *T. tambroides* in this study were summarized in Table 2. The total contig length is 1,235,136,976 bp with 44,726 contigs. The longest contig length recorded is 445,922 bp. The contig GC content documented in this study is 36.55%.

Table 2. Genomic sequencing and assembly statistics

Total contig length	1,235,136,976 bp
Number of contigs	44,726
Longest contig length	445,922 bp
Contig N50 length	44,262
Contig GC (%) content	36.55
BUSCO Completeness (Actinopterygii_odb10)	
Actinopterygii_odb10: Complete BUSCOs	84.3% (3069)
Complete and single copy BUSCOs	39.2% (1427)
Complete and duplicated BUSCOs	45.1% (1642)
Missing BUSCOs	8.3% (301)

The assembled clean reads of the *T. tambroides* genome were subjected to K-mer analysis using Jellyfish software and visualised using GenomeScope (K-mer:31), as shown in Figure 1. The y-axis had demonstrated the amount of K-mers found at each corresponding depth on the x-axis. There is no heterozygous peak generated at 23, and a low heterozygosity level of 0.194% was recorded. The depth of the homozygous peak can be observed at 46, which accounts for the identical 31-mers from both strands of DNA. The kmer-based statistical approach had revealed that 24.675% of the genomic content is repeated whereas 75.325% of the content is unique. The genome size of *T. tambroides* can be predicted by division of K-mer number over K-mer depth. The k-mer number detected in this study is 69 Gb. Therefore, the expected genome size is predicted as 1.5 Gb.

The *T. tambroides* genome size of 1.5 Gb was found to locate within the range of *Cyprinidae* genome sizes, including grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) at 1.07 Gb (Wang et al., 2015) and 1.7 Gb (Xu et al., 2014), respectively. Besides, its GC% content reported (36.55%) was slightly lower than the 37.4% and 37.3% seen in *C. idella* and *C. carpio*. In general, a more outstanding GC content was found in seawater (usually above 40%) than freshwater fish (less than 40%), and also in migratory than a non-migratory species (Lu & Luo, 2020). Thus, it is suggested that the genomic GC content may be influenced by different living environments (Lu & Luo, 2020). Besides, the contrary relationship between genomic size (1.5 Gb) and GC content (36.55%) was shown in genome of *T. tambroides*. However, such assertion was insignificant based on a study reviewing 14 species, thus suggesting the collection of more genomic data for further validation (Lu & Luo, 2020).

Repetitive elements accounted for 39.79% in *T. tambroides* genome, which is likely to be associated with its genome duplication. It is found to be in accordance to the repetitive elements found across order Cypriniformes species reporting to be around 35% to 40%,

including *Danio rerio*, *Cyprinus carpio*, *Sinocycloheilus graham*, *Sinocycloheilus rhinoceros*, *Sinocycloheilus anshuiensis* and *Pimephales promelas* (Yuan et al., 2018). Within teleost fish, researchers had found out that the expansion of repetitive elements may be the factor for the expansion of fish genome size as observed across 52 teleost species (Yuan et al., 2018). Having a high repetitive element content within the genome could speed up the generation of novel genes for adaptation purposes. However, an excessive amount of it would bring to abnormal combination and splicing, resulting in unstable genomes (Hong, 1998). In short, it is discouraged for unlimited growth of repetitive elements with its genomic size but limited to certain levels and shaped under specific natural selection (Yuan et al., 2018).

Figure 1. Estimation of genome size, repeat content and heterozygosity by GenomeScope, based on 31-mers (read length = 150 bp; kmer max coverage at 1000).

SSR Analysis

A total of 392,346 SSRs had been successfully identified from the genome of *T. tambroides*. Four types of SSRs had been tabulated with their length, total counts, average length and

distribution (Table 3) while Figure 2 is showing the composition of each type of SSR within the genome of *T. tambroides*. The dinucleotide repeats had covered up to 70% of the entire SSR population, encompassing 275,715 SSRs. The trinucleotide and tetranucleotide repeats counts are 59,978 (15%) and 49,089 (13%), and they are ranked second and third out of all the four SSRs. The remaining 2% SSRs were 7,388 pentanucleotide repeats found within the genome of *T. tambroides*. The top three highest frequency dinucleotide repeats SSRs are AT/TA (1,058,829), AC/GT (71,569) and CA/TG (70,249) composing up to 89% of the entire dinucleotide SSRs (Figure 3). The top three trinucleotide repeats covered up to one-third of the trinucleotide SSR repeats, namely AAT/ATT, TTA/TAA and TAT/ATA. As for the tetranucleotide repeats SSRs, the AGAT/ATCT, GATA/TATC and TCTA/TCGA counts were 6,209, 5,386 and 3,826 respectively, covering up to 30% of the entire tetranucleotide SSRs. Correspondingly, 18% of the pentanucleotide repeat SSRs were AAAAT/ATTTT (6.42%), ATAAT/ATTAT (6.13%) and TTTTA/TAAAA (5.96%) respectively. Overall, the dinucleotides SSRs, namely AT/TA (26.97%), AC/GT (25.96%) and CA/TG (17.90%) had occupied more than half of the SSR population found within the genome of *T. tambroides*.

SSRs allow faster adaptation to environmental stress through increment of DNA quantity and raw material for adaptative evolution during genome evolution. Thus, it can be said the mutation rate of microsatellite is dependent on the repeated unit length with a more common observation of mono- and dinucleotide repeats as compared to other repeats with longer lengths due to respective stability (Schlotterer, 2000). The frequency of tetranucleotide was higher than trinucleotide within the genome of *T. tambroides*, which was shown in other ray-finned fishes as well (Lei et al., 2021). Less occurrence of trinucleotide SSRs repeats can be due to its attribution as a triplet code to form part of the gene and also the presence of a mismatch repair system in the exonic region to maintain greater trinucleotide repeats (Lei et al., 2021).

SSR repeats with poly (A/T) tracts were found in a greater abundance than repeats with poly (G/C) tracts in other ray-finned fishes across all types of SSRs including dinucleotide, trinucleotide, tetranucleotide and pentanucleotide (Lei et al., 2021). The higher frequency of poly (A) can be due to the re-integration of the processed genes from mRNA back into the genome with an attached poly (A) tail, while poly (G/C) is not included in this integrative mechanism (Lei et al., 2021). In addition, greater poly (A) occurrence can be explained through the formation of pseudogenes and its necessity in the universal retrotransposon (Toth et al., 2000; Borodulina et al., 2016). The (GC) repeats are more stable than (AT) repeats thus increasing the difficulty to be slipped during replication (Gur-Arie et al., 2000).

Dinucleotides AT/TA is the common microsatellites repeat found across the fish genomes (Lei et al., 2021) which is observed within the genome of *T. tambroides* as well. Furthermore, for trinucleotide repeats, the occurrence of (CCG) n (16 counts) and (ACG) n (2572 counts; 5%) repeats were rare in *T. tambroides* as well. This phenomenon can be explained by the presence of the highly mutable CpG dinucleotide within the motif due to methylation (Lei et al., 2021). While in tetranucleotide repeats, the G+C content of SSRs was observed in a lower frequency because of its influence on the mutation rate as there is no statistical significance between 25% G+C content but each was significant difference from the 50% G+C repeat content (Eckert et al., 2002).

The repetitive element found within the genome of *T. tambroides* was reported as 4.15%. It had been reported previously that the expansion of repetitive elements would cause a further expansion in the fish genome. Therefore, it can be said that the fish genome size is positively correlated with the repetitive elements (Yuan et al., 2018). However, a study reviewing the SSR across 14 fish species contradicts the statement (Lei et al., 2021). The generation of novel genes for adaptation can be accelerated with the presence of high repetitive element content, for instance in salmon, which is likely to be associated with genome duplication (Lien et al., 2016). Besides, the variation observed within microsatellites can be due to differential selective constraints causing accumulated preference for different microsatellite types (Lei et al., 2021). However, overburden could cause abnormal recombination and splicing, resulting in genome instability (Yuan et al., 2018). In short, it can be concluded that the repetitive elements must be limited to shape under specific natural selection by the environment. Its unambiguous role in genomic function still remains to be explored.

Figure 2. The composition of dinucleotide, trinucleotide, tetranucleotide, pentanucleotide and hexanucleotide SSRs identified from the genome of *T. tambroides*.

Figure 3. SSRs percentage graph with selected top six frequency SSRs from each group: (A) dinucleotide repeats, (B) trinucleotide repeats, (C) tetranucleotide repeats, (D) pentatetranucleotide repeats.

Table 3. The number, total length and average length of five different types of SSRs found within the genome of *T. tambroides*.

Nucleotide Repeats	Total counts	Total length (bp)	Average length (bp)	Percentage found in the whole genome (%)
Dinucleotide	173425	1387400	8.000	0.112
Trinucleotide	21201	133850	6.313	0.011
Tetranucleotide	78303	332947	4.252	0.027
Pentanucleotide	35009	147053	4.200	0.012
Hexanucleotide	19077	79193	4.151	0.006

Functional Annotation of *T. tambroides*

For functional annotation of *T. tambroides* genome, coding region was extracted using Interproscan (Jones et al., 2014), generating 96,736 predicted non-redundant protein sequences. Subsequently, the sequences were annotated using eggNOG mapper to map the predicted protein sequences to GO, KEGG, and COG databases. The sequence length of each

unigene ranged from < 100 bp to > 2000 bp (Figure 4), which showed a decreasing trend as the length increases. Table 4 shows the number of unigenes annotated to either GO, KEGG or COG, unigenes annotated to at least one of the databases and all the databases. A total of 42,694 (75.19%), 25,560 (45.01%), and 55,981 (98.58%) of unigenes were annotated to GO, KEGG, and COG databases respectively. Out of a total of 56,785 annotated unigenes, 56,094 (98.78%) of the unigenes had been found to have a significant match to at least one of the databases while 21,878 (38.53%) unigenes portrayed a notable match to all the three databases. Figure 5 illustrates the distribution of unigenes across GO, KEGG, and COG databases.

Figure 4. Length distribution of unigenes of *T. tambroides*.

Figure 5. Venn diagram showing differences and similarity of unigenes of *T. tambroides* annotated to GO, KEGG, and COG databases.

Table 4. Functional annotation of unigenes to the various database.

Database	Number of Unigenes	Percentage (%)
GO	42,694	75.19
KEGG	25,560	45.01
COG	55,981	98.58
Annotated in at least one database	56,094	98.78
Annotated in all database	21,878	38.53
All unigenes	56,785	100.00

Annotation of *T. tambroides* genome to each main ontology of GO database was shown in Figure 6, including biological process, molecular functions, and cellular components. Under biological process, metabolism (4475; 6.56%) had the greatest count, followed by development (3337; 4.89%) and catalytic activity (2149; 3.15%). On the other hand, a total of 1239 counts (1.82%) were responsible for binding, 761 (1.12%) accounted for transferase activity and 688 (1.01%) accounted for protein binding, under the molecular function category. Furthermore, under the cellular components category, 2139 (3.14%) were accounted for cell organization and biogenesis while 1672 (2.45%) and 1284 (1.88%) were categorized as cell and intracellular respectively.

Another annotation was performed towards a widely-used reference database KEGG equipped with multiple pathways for better integration and interpretation of large-scale datasets. *T. tambroides* genome had successfully mapped towards 304 known KEGG pathways (Figure 7), including organismal system, cellular processes, environmental information processing, genetic information processing and metabolism. Out of the five main aforementioned categories, the largest count (36340; 39.4%) is from organismal system whilst genetic information processing (4324; 4.69%) had the lowest count. The categories reported on the greatest number of counts were signal transduction from environmental information processing (19577; 21.22%), endocrine system from organismal system (9415;

8.79%) and immune system (8110; 10.21%) from organismal system. Figure 8 depicts the top ten KEGG cluster components found in each main aforementioned category. The top three largest count can be observed in metabolic pathway (4302; 4.66%), PI3K-Akt signaling pathway (1500; 1.63%) and MAPK signaling pathway (1464; 1.59%) from signal transduction.

Figure 9 illustrates the classification of 96,736 predicted protein sequences towards COG database consisting of clusters of orthologous groups. There were altogether 25 COG classifications and can be grouped under four main clusters: information storage and processing (8906; 15.17%), cellular processes and signaling (25248; 43.01%), metabolism (8402; 14.31%) and poorly characterized (16152; 27.51%). Among all the clusters, greater counts were originated from function unknown (16152; 27.51%), followed by signal transduction mechanism (14284; 24.33%) and transcription (4455; 7.59%). Overall, the genome of *T. tambroides* is enriched with gene families in the categories of signal transduction, endocrine system, immune system and metabolic pathways. It is found to be consistent with the common carp genome (Xu et al., 2014) and Javan mahseer transcriptome (Lau et al., 2021b).

Figure 6. GO classifications

Figure 7. KEGG classifications

Figure 8. Top 10 KEGG classification

Figure 9. COG classifications

R: General function prediction only
S: Function unknown
C: Energy production and conversion
E: Amino acid transport and metabolism
F: Nucleotide transport and metabolism
G: Carbohydrate transport and metabolism
H: Coenzyme transport and metabolism
I: Lipid transport and metabolism
P: Inorganic ion transport and metabolism
Q: Secondary metabolites biosynthesis, transport and metabolism
D: Cell cycle control, cell division, chromosome partitioning
M: Cell wall/membrane/envelope biogenesis
N: Cell motility
O: Posttranslational modification, protein turnover, chaperones
T: Signal transduction mechanism
U: Intracellular trafficking, secretion and vesicular transport
V: Defence mechanisms
W: Extracellular structures
Y: Nuclear structures
Z: Cytoskeleton
A: RNA processing and modification
B: Chromatin structure and dynamics
J: Translation, ribosomal structure and biogenesis
K: Transcription
L: Replication, recombination and repair

Immunity-related Annotation

To characterize a comprehensive defense landscape of *T. tambroides* against pathogenic infections, the genomic sequences of *T. tambroides* had been functionally annotated to identify pathways and genes associated with the fish immunity. GO classification had revealed on 14,489 unigenes (63.15%) were functionally annotated to immune-related GO terms. Among the annotated categories, metabolism (4475; 30.89%), stress response (1824; 12.59%) and protein metabolism (1135; 7.83%) had reported on the greatest counts. KEGG pathway analysis had revealed 41 immune-related pathways, including MAPK signaling pathway, Toll-like receptor (TLR) signaling pathway, Wnt signaling pathway, NOD-like receptor signaling pathway and so on (Figure 10) (Zhenzhen et al., 2014).

Among all the components in the fish immune system, TLR family is an essential type of pattern-recognition receptors expressing on antigen-presenting cells, involved in innate immune response and the subsequent promotion of adaptive immunity (Akira et al., 2006). We had successfully identified 11 different *TLRs* and 12 *TLR* genes matched to the genome of *T. tambroides* (Table 5). The major histocompatibility complex (MHC) are the important molecules for the recognition of foreign substances via binding peptide fragments from pathogens and presenting them for T cell elimination (Neefjes et al., 2011). Both MHC class I and II had been identified in the genomic dataset as well. MHC genes serve as a candidate disease resistance marker due to their highly polymorphic characteristics in teleost (Lanfords et al., 2001). Both TLR and MHC were also found within the transcriptomic dataset of *Trachinotus ovatus* (Zhenzhen et al., 2014). Thus, it is believed that further analysis of both genes will be able to provide insights into the immune system of *T. tambroides* as well as other teleosts.

Figure 10. Top 20 annotated KEGG immune pathway of *T. tambroides* genome.

Table 5. Immune-related genes. Genes marked with * asterisk sign were genes selected after e-value cutoff while best parameters were inputted for genes that did not pass the cutoff filter.

Gene Location	Gene	Accession	Bit Score	Aligned Length	Similarity (%)	Reference
g81977.t1	*60S ribosomal protein L8	XP_034741439.1	469	234	97.863	Chandhini & Rejish, 2019
g19500.t1	*C-C motif chemokine ligand 4	AAT76448.1	99	100	47.000	Chandhini & Rejish, 2019
g44664.t1	*C-X-C motif chemokine receptor 4	BAA32797.1	650	338	93.491	Chandhini & Rejish, 2019
g10856.t1	*Interleukin 15	NP_001034654.1	231	195	58.974	Zhenzhen et al., 2014
g54821.t1	*Interleukin 17a/f3 precursor	NP_001018628.1	181	162	62.963	Zhenzhen et al., 2014
g88203.t1	*Interleukin 17C	BAV01243.1	251	162	83.333	Zhenzhen et al., 2014
g8088.t1	*Interleukin 21	NP_001122046.1	179	132	69.697	Zhenzhen et al., 2014
g36229.t1	*Interleukin 22	NP_001018628.1	177	170	53.529	Zhenzhen et al., 2014
g49276.t1	*Interleukin 34	QCX35396.1	365	209	85.646	Ma et al., 2016
g58362.t1	*Interleukin 8 precursor	ACI66507.1	137	95	67.368	Zhenzhen et al., 2014
g27467.t1	*Interleukin-10 precursor	NP_001018621.2	295	180	80.556	Zhenzhen et al., 2014
g7879.t1	*Interleukin-11	XP_018980434.1	343	190	88.421	Zhenzhen et al., 2014
g52117.t1	*Interleukin-12 subunit alpha-like	XP_023261134.1	95.5	124	41.935	Zhenzhen et al., 2014
g42556.t1	*Myeloid differentiation primary response protein 88	XP_018923074.1	544	284	92.254	Zhenzhen et al., 2014
g48515.t1	*Non classical MHC class I antigen	ABI21842.1	260	360	40.833	Zhenzhen et al., 2014
g5772.t1	*Toll-like receptor 1	NP_001124065.1	659	402	79.104	Ma et al., 2016
g41359.t1	*Toll-like receptor 13	NP_001133860.1	736	915	45.027	Zhenzhen et al., 2014
g91903.t1	*Toll-like receptor 14	AXL48518.1	970	807	59.108	Zhenzhen et al., 2014
g60188.t1	*Toll-like receptor 2 precursor	NP_997977.1	1191	787	75.222	Zhenzhen et al., 2014

g7235.t1	*Toll-like receptor 21	AVX48323.1	1352	832	84.014	Ma et al., 2016
g41359.t1	*Toll-like receptor 22 precursor	NP_001117884.1	827	927	47.249	Ma et al., 2016
g42203.t1	*Toll-like receptor 3	ABL11473.1	1404	769	90.117	Palti, 2011
g26383.t1	*Toll-like receptor 4ba	AHH85806.1	355	204	85.784	Palti, 2011
g26383.t1	*Toll-like receptor 4bb	AHH85807.1	367	240	76.25	Palti, 2011
g29022.t1	*Toll-like receptor 5b precursor	NP_001124067.2	496	347	71.758	Zhenzhen et al., 2014
g59438.t1	*Toll-like receptor 7	AIS23537.1	1360	1038	66.956	Zhenzhen et al., 2014
g48547.t1	*Toll-like receptor 9	ADE20130.1	1561	977	83.419	Chandhini & Rejish, 2019
g54821.t1	Interleukin 17N	BAV01251.1	44.7	80	35.000	Zhenzhen et al., 2014

Growth-related Annotation

To tap into the growth-related aspect of the *T. tambroides* genomic landscape, we functionally annotated the genome based on previously characterized growth-related genes. Table 6 shows the mapped BLAST results of immune-related genes on the genome of *T. tambroides* where there were 74 genes labelled with asterisk signs that had passed the E-value cutoff filter of 10^{-10} . KEGG pathway analysis revealed 19 pathways associated with growth, including pathways in cancer, insulin signaling pathway, endocytosis, focal adhesion, and mTOR signaling pathway (Zhenzhen et al., 2014) (Figure 11).

Thus, it can be said that a significant similarity had been exhibited with previously published immune-related genes. For instance, growth hormone (GH) and insulin-like growth factor-binding protein (IGFBP) which are responsible for the regulation of GH and IGF (Zhenzhen et al., 2014) were detected in high similarity in the genome of *T. tambroides* (Table 6). Somatostatins were found to have an inhibitory role in promoting the release of GH (Li & Lin, 2010).

It was found out that regulation of appetite, protein and lipid metabolism, weight gain and muscle growth are also part of the complex growth process (Zhenzhen et al., 2014). A few genes related to appetite and muscle were detected in the genome of *T. tambroides*, for instance, neuropeptide Y (*NPY*), pro-opiomelanocortin (*POMC*), leptin and myostatin (Table 6). These genes play various roles where *NPY* and *POMC* are exerting antagonistic roles in stimulating or inhibiting feeding (Zhenzhen et al., 2014). In addition, myostatin is a negative regulator of muscle growth and its polymorphism is associated with growth traits (Nadjar-Boger & Funkenstein, 2011). In addition, leptin is responsible for the regulation of energy intake and usage (Zhenzhen et al., 2014).

Moreover, genes that encode for proteolytic digestive enzymes (chymotrypsin-like elastase, *cela*) and are related to protein metabolism (cathepsin L, *clsL* and cathepsin K, *clsK*) were detected in the genome of *T. tambroides* as well. In addition, a number of lipid metabolism regulation genes were found in the genome of *T. tambroides*, including lipase C (*lipC*), phospholipase A2 (*pla2*), elongation of very long chain fatty acid family 6 (*elovl6*), apolipoprotein B (*apob*), acetyl-CoA carboxylase (*ACACA*, *ACACB*) and fatty acid synthase (*FASN*). This can be due the fact that *T. tambroides* is a semi-fatty fish and it contains 4.6-5.2% of muscle crude lipid (Özogul and Özogul, 2007), indicating the importance of lipid metabolism in this fish. Lipase is a key enzyme involved in lipid hydrolysis while apolipoprotein is a lipid-associated protein that regulates lipid homeostasis through the transport of triacylglycerol and phospholipid from the liver to other tissues (Infante & Cahu, 2007). *ACACB* was found not only to be associated with fat yield and percentage, but it also plays role in protein yield as well (Han et al., 2018). *FASN* is an important element in lipid metabolism and its expression could vary due to fatty acid content in both fat and meat (Renaville et al., 2018). Furthermore, glucose-6-phosphatase (*g6pc*) which regulates carbohydrate metabolism were detected in *T. tambroides* as well. These growth-related genes may serve as the possible molecular growth-related markers for further marker-assisted breeding. Further studies are required to confirm the roles of these genes in the growth of *T. tambroides*.

Figure 11. Annotation to growth-related KEGG pathway.

Table 6. Growth-related genes. Genes marked with * asterisk sign were proteins selected after e-value cutoff while best parameters were input for the gene that did not pass the cutoff filter.

Gene Location	Gene	Accession	Bit Score	Aligned Length	Similarity (%)	Reference
g79685.t1	*Acetyl-CoA carboxylase alpha (Haplotype 1)	ADT82650.1	4772	2390	95.314	Guan & Qiu, 2020
g79685.t1	*Acetyl-CoA carboxylase alpha (Haplotype 2)	ADX43925.1	4772	2390	95.816	Guan & Qiu, 2020
g44363.t1	*Acetyl-CoA carboxylase 2	XP_018962132.1	3695	1845	96.585	Guan & Qiu, 2020
g54099.t1	*Acetyl-CoA Acetyltransferase 2	AAD34966.1	751	395	92.152	Hu et al., 2016
g1109.t1	*Alpha actinin 3	XP_031588180.1	1574	897	82.051	Damzamn et al., 2020
g59012.t1	*Adiponectin	NP_001373470.1	1574	897	82.051	Hu et al., 2016
g16898.t1	*Alpha-1-Microglobulin/Bikunin Precursor	XP_005451429.1	189	189	44.444	Dam et al., 2020
g82874.t1	*Apolipoprotein B	NP_001013332.1	407	259	78.764	Dam et al., 2020
g83831.t1	*ATP synthase	BAA82837.1	961	518	93.822	Dam et al., 2020
g40123.t1	*ATPase H ⁺ transporting V1 subunit E1	AAM34666.1	440	226	96.460	Guan & Qiu, 2020
g8813.t1	*G2/mitotic-specific cyclin-B1	NP_571588.1	112	215	91.071	Guan & Qiu, 2020
g31308.t1	*Cell division cycle protein 20	NP_998245.2	919	496	90.726	Guan & Qiu, 2020
g3538.t1	*Conserved Edge Expressed	ABK35126.2	548	326	90.184	Overturf et al., 2010
g81872.t1	*Chymotrypsin-like elastase family member 1	XP_018937349.1	461	264	93.561	Dam et al., 2020
g49416.t1	*Creatine kinase M-type	XP_028660838.1	616	366	80.874	Damzamn et al., 2020
g66522.t1	*Peroxisomal carnitine O-octanoyltransferase	XP_018953213.1	1228	612	94.935	Lin et al., 2019
g50598.t1	*Cathepsin K precursor	NP_001017778.1	634	333	89.189	Dam et al., 2020
g2135.t1	*Cathepsin L	AAI08032.1	568	336	76.786	Dam et al., 2020

g46341.t1	*25-hydroxycholesterol_7-alpha-hydroxylase	XP_699028.2	878	506	82.016	Lin et al., 2019
g16441.t1	*Delta-6_desaturase	AZL94116.1	926	444	100.00	Vagner & Santiagosa, 2011
g39490.t1	*Elongation of very long chain fatty acids protein 6	XP_018957993.1	438	266	82.707	Dam et al., 2020
g4692.t1	*Fatty acid-binding protein 10-A	NP_694492.1_	240	126	90.476	Hu et al., 2016
g23845.t1	*Fatty acid synthase	ARO92273.1	4788	2486	94.328	Lin et al., 2019
g76435.t1	*Farnesyl pyrophosphate synthase isoform X1	XP_005472704.1	608	359	81.058	Lin et al., 2019
g19569.t1	*Forkhead box protein K1 isoform X1	XP_025764132.1	790	695	64.173	Lin et al., 2019
g33446.t1	*G0/G1 switch protein 2	AVM4087.1	215	112	91.071	Damzamn et al., 2020
g51734.t1	*Glucose-6-phosphatase	AVP32214.1	649	351	95.157	Dam et al., 2020
g87856.t1	*Growth hormone	CAA43007.1	385	210	95.238	Ma et al., 2016
g40036.t1	*Growth hormone receptor	ADZ13484.1	490	616	46.753	Ma et al., 2016
g40036.t1	*Growth hormone receptor 1	AAY86769.1	490	616	46.753	Hu et al., 2016
g80039.t1	*Glutathione synthetase	XP_018970229.1	883	475	90.105	Lin et al., 2019
g71468.t1	*70-kDa heat shock proteins	AAF70445.1	1125	634	86.278	Hu et al., 2016
g74743.t1	*Insulin-like growth factor-binding protein 1	ACV72066.1	524	262	96.947	Guan & Qiu, 2020
g58943.t1	*Insulin-like growth factor-binding protein 2	ACM47497.1	548	274	97.080	Guan & Qiu, 2020
g74742.t1	*Insulin-like growth factor-binding protein 3	ACM47527.1	313	173	88.439	Guan & Qiu, 2020
g38084.t1	*Inositol Monophosphatase 1	XP_018975133.1	571	282	97.518	Lin et al., 2019
g85602.t1	*Insulin-induced_gene_1_protein	NP_956163.1	481	242	97.107	Hu et al., 2016
g59760.t1	*Leptin b1	QEN91938.1	337	168	95.238	Zhenzhen et al., 2014

g72992.t1	*Leptin A2	AGK24956.1	309	171	88.889	Zhenzhen et al., 2014
g83000.t1	*Tyrosine-protein kinase JAK2-like	XP_022620925.1	1602	967	78.904	Zhenzhen et al., 2014
g61842.t1	*Lipopolysaccharide binding protein	NP_001118057.1	672	469	69.936	Hu et al., 2016
g55290.t1	*Hepatic triacylglycerol lipase	XP_018956861.1	964	498	92.169	Dam et al., 2020
g49598.t1	*Myocyte Enhancer Factor 2	BAA33567.1	372	475	51.158	Overturf et al., 2010
g21088.t1	*Myostatin 1	AJF48833.1	515	351	68.661	Overturf et al., 2010
g21088.t1	*Myostatin 2	AJF48834.1	726	366	94.536	Overturf et al., 2010
g94911.t1	*Myogenic factor 5	AWG41988.1	486	240	97.083	Ma et al., 2016
g28564.t1	*Neuropeptide Y receptor Y7	NP_001007219.1	650	372	88.710	Zhenzhen et al., 2014
g34597.t1	*Cytochrome P450	AAK37960.1	711	505	66.535	Hu et al., 2016
g21266.t1	*Paired box protein 7 isoform X1	XP_013988550.1	580	496	62.903	Overturf et al., 2010
g37657.t1	*Cytosolic phospholipase A2 gamma-like	XP_018952444.1	660	558	60.394	Dam et al., 2020
g45170.t1	*Proopiomelanocortin	AAM93491.2	387	222	83.333	Zhenzhen et al., 2014
g68580.t1	*Peroxisome proliferator-activated receptor alpha	CAJ76702.1	698	468	73.077	Hu et al., 2016
g41063.t1	*Peroxisome proliferator-activated receptor beta	ACR15760.1	690	430	76.279	Hu et al., 2016
g76564.t1	*Preprosomatostatin I	AJB44559.1	218	114	96.491	Zhenzhen et al., 2014
g79784.t1	*Preprosomatostatin II	AJB44560.1	125	94	73.404	Zhenzhen et al., 2014
g56581.t1	*Preprosomatostatin III	AJB44561.1	221	12	95.833	Zhenzhen et al., 2014
g42530.t1	*Prolactin	CAA31060.1	382	210	95.714	Ma et al., 2016
g75666.t1	*Prolactin receptor	QIB98245.1	1089	611	86.579	Ma et al., 2016
g7570.t1	*60S ribosomal protein L10	XP_018921966.1	439	214	98.131	Damzamn et al., 2020
g29499.t1	*Antithrombin-III	XP_018920986.1	845	450	92.444	Dam et al 2020
g81491.t1	*SMAD family member 3	ABI94729.1	877	423	99.527	Lin et al., 2019
g94343.t1	*Secreted Protein Acidic And Cysteine Rich	XP_003447656.1	519	300	83.667	Lin et al., 2019

g17664.t1	*Squalene monooxygenase	NP_001103509.1	970	528	89.394	Ma et al., 2016
g27484.t1	*Somatostatin Receptor type 1-like	XP_018943223.1	749	367	98.365	Ma et al., 2016
g16955.t1	*Somatostatin Receptor type 2-like	XP_018946514.1	597	337	87.834	Zhenzhen et al., 2014
g82792.t1	*Somatostatin Receptor type 3-like	XP_018963641.1	514	261	98.084	Zhenzhen et al., 2014
g12909.t1	*Somatostatin Receptor type 5-like	XP_018961210.1	661	443	81.49	Zhenzhen et al., 2014
g32515.t1	*Signal transducer and activator of transcription 1b	NP_956385.2	1161	695	79.712	Zhenzhen et al., 2014
g1399.t1	*Signal transducer and activator of transcription 2	NP_001258730.1	1359	852	78.873	Zhenzhen et al., 2014
g23419.t1	*Signal transducer and activator of transcription 3	BAH47263.1	1675	806	99.256	Zhenzhen et al., 2014
g28531.t1	*Signal transducer and activator of transcription 4	NP_001004510.1	1324	679	95.582	Zhenzhen et al., 2014
g48083.t1	*Signal transducer and activator of transcription 5	BAH47264.1	1549	768	97.266	Zhenzhen et al., 2014
g4785.t1	*Ubiquitin carboxyl-terminal hydrolase 38	XP_003449754.1	1415	1013	70.582	Lin et al., 2019

Orthologs and Phylogenetic Inferences

The phylogenetic relationship of *T. tambroides* with other ray-finned fishes was inferred through the BUSCO supermatrix approach through single-copy orthologs. The BUSCO completeness of each Actinopterygii species was summarized in Table 7. MUSCLE was used to align all the genomic sequences of all 53 species (Edgar, 2004). The phylogenetic tree was plotted using the maximum-likelihood model (ML; IQ-TREE) based on the single-copy and multi-copy orthologs (Figure 12). All the species fall under class Actinopterygii but belong to 18 different orders, including Cypriniformes, Perciformes, Clupeiformes, Cichliformes, Characiformes, Cyprinodontiformes, Anabantiformes, Salmoniformes, Esociformes, Gadiformes, Pleuronectiformes, Lepisosteiformes, Atheriniformes, Beloniformes, Osteoglossiformes, Batrachoidiformes with Coelacanthiformes and Acipenseriformes rooted as outgroup. As a member of the family Cyprinidae, *T. tambroides* formed a monophyletic cluster with the species within order Cypriniformes, namely species from genus *Sinocyclocheilus*, *Carassius auratus*, *Pimephales promelas* and *Danio rerio*.

Table 7. BUSCO completeness of 52 ray-finned fishes for the inference of phylogenetic relationship with *T. tambroides* using BUSCO supermatrix approach.

	Species	BUSCO5 Completeness (%)				
		Complete	Complete and single-copy	Complete and duplicated-copy	Fragmented	Missing
Freshwater	<i>Archocentrus centrarchus</i>	99.6	65.9	33.7	0.1	0.3
	<i>Astyanax mexicanus</i>	98.6	68.6	30.0	0.7	0.7
	<i>Austrofundulus limnaeus</i>	89.6	65.6	24.0	1.3	6.1
	<i>Betta splendens</i>	99.4	58.6	40.8	0.2	0.4
	<i>Carassius auratus</i>	99.3	24.6	74.7	0.2	0.5
	<i>Colossoma macropomum</i>	99.7	70.3	29.4	0.1	0.2
	<i>Coregonus clupeaformis</i>	98.6	38.2	60.4	0.7	0.7
	<i>Cyprinodon tularosa</i>	99.0	71.0	28.0	0.5	0.5
	<i>Danio rerio</i>	97.3	51.8	45.5	1.4	1.3
	<i>Esox lucius</i>	99.7	53.6	46.1	0.1	0.2
	<i>Etheostoma cragini</i>	96.5	58.0	38.5	1.1	2.4
	<i>Etheostoma spectabile</i>	96.0	56.5	39.5	0.6	3.4
	<i>Haplochromis burtoni</i>	98.1	61.4	36.7	0.8	1.1
	<i>Lepisosteus oculatus</i>	94.7	54.4	40.3	3.4	1.9
	<i>Melanotaenia boesemani</i>	99.8	61.4	38.4	0.0	0.2
	<i>Oncorhynchus mykiss</i>	99.7	29.1	70.6	0.1	0.2
	<i>Oreochromis niloticus</i>	99.8	58.1	41.7	0.1	0.1
	<i>Oryzias latipes</i>	98.5	59.8	38.7	0.0	1.5
	<i>Oryzias melastigma</i>	98.2	63.1	35.1	0.7	1.1
	<i>Paramormyrops kingsleyae</i>	98.7	51.6	47.1	0.8	0.5
	<i>Poecilia reticulata</i>	97.7	61.9	35.8	1.1	1.2
	<i>Perca flavescens</i>	99.6	63.6	36.0	0.2	0.2
	<i>Perca fluviatilis</i>	98.5	62.2	36.3	0.8	0.7

	<i>Pimephales promelas</i>	98.3	63.1	35.2	0.7	1.0
	<i>Poecilia mexicana</i>	98.2	57.2	41.0	1.1	0.7
	<i>Polyodon spathula</i>	96.4	34.3	62.1	1.0	2.6
	<i>Pungitius pungitius</i>	99.6	62.2	37.4	0.1	0.3
	<i>Pygocentrus nattereri</i>	99.9	62.3	37.6	0.0	0.1
	<i>Salvelinus namaycush</i>	95.3	40.5	54.8	1.4	3.3
	<i>Sander lucioperca</i>	99.6	53.0	46.6	0.2	0.2
	<i>Scleropages formosus</i>	99.2	58.0	41.2	0.2	0.6
	<i>Simochromis diagramma</i>	98.9	58.0	40.9	0.3	0.8
	<i>Sinocyclocheilus rhinoceros</i>	97.5	27.6	69.9	1.5	1.0
	<i>Sinocyclocheilus anshuiensis</i>	98.4	27.9	70.5	0.9	0.7
	<i>Sinocyclocheilus grahami</i>	94.8	35.4	59.4	2.8	2.4
	<i>Thalassophryne amazonica</i>	97.6	66.0	31.6	0.7	1.7
	<i>Xiphophorus maculatus</i>	99.9	65.7	34.2	0.1	0.0
	<i>Xiphophorus couchianus</i>	99.6	59.6	40.0	0.2	0.3
	<i>Xiphophorus hellerii</i>	99.6	63.7	35.9	0.2	0.2
Marine	<i>Chelmon rostratus</i>	99.8	74.5	25.3	0.1	0.1
	<i>Epinephelus lanceolatus</i>	99.2	67.7	31.5	0.6	0.2
	<i>Gadus morhua</i>	96.9	61.2	35.7	0.4	2.7
	<i>Hippoglossus hippoglossus</i>	99.6	59.8	39.8	0.1	0.3
	<i>Plectropomus leopardus</i>	95.8	71.7	24.1	2.0	2.2
	<i>Thunnus maccoyii</i>	99.8	63.2	36.6	0.0	0.2
	<i>Toxotes jaculatrix</i>	99.9	68.9	31.6	0.0	0.1
	<i>Latimeria chalumnae</i>	87.2	56.3	30.9	3.0	9.8
Diadromous	<i>Acanthopagrus latus</i>	99.9	58.7	41.2	0.0	0.1
	<i>Alosa sapidissima</i>	98.8	57.1	41.7	0.4	0.8
	<i>Kryptolebias marmoratus</i>	99.1	64.1	35.0	0.5	0.4
	<i>Lates calcarifer</i>	99.8	66.0	33.8	0.1	0.1
	<i>Oreochromis aureus</i>	99.6	63.2	36.4	0.2	0.2

Figure 12. Maximum-likelihood tree plotted on 53 Actinopterygii species inferred from a supermatrix of 3,640 BUSCO. The species were classified based on their respective orders.

Conclusion

We present the first Malaysian mahseer (*T. tambroides*) genome assembled with low-coverage Nanopore long reads and high-coverage Illumina short reads. *De novo* genomic assembly had generated a draft genome with an estimated genome size of 1.5 Gb [84.3% BUSCO completeness (Actinopterygii_odb10)]. 392,346 SSRs had been identified from the genome with dinucleotide repeats AT/TA as the most common SSR. A total of 96,736 non-redundant protein-coding sequences was predicted and used for later functional annotation. Predicted protein sequences had mapped to 304 known KEGG pathways with signal transduction as the highest representation. Furthermore, genes showing significant similarity to published growth- and immune-related genes were identified, hoping to serve as a potential marker for future molecular breeding of *T. tambroides*. In addition, the first genome-based evolutionary relationship of *T. tambroides* between other ray-finned fishes had been inferred using a Maximum-likelihood tree. It is hoped that this genomic data of *T. tambroides* could be a more powerful tool that continues to resolve questions of species identification, evolutionary biology, morphological variations, sequences related to sex differentiation, growth, reproduction, and immune, which are helpful for further conservation of *Tor* species.

CrediT author statement

Lau Melinda Mei Lin: Writing-Original Draft, Data curation, Conceptualization. **Lim Leonard Whye Kit:** Data Curation, Writing-Original Draft, Conceptualization. **Chung Hung Hui:** Conceptualization, Funding acquisition, Writing-Review and Editing. **Han Ming Gan:** Methodology, Conceptualization, Writing-Review and Editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

References

- Abram, Q. H., Dixon, B., & Katzenback, B. A. (2017). Impacts of low temperature on the teleost immune system. *Biology*, 6(4), 39.
- Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, 124(4), 783-801.

- Ashfaq, H., Soliman, H., Saleh, M., & El-Matbouli, M. (2019). CD4: A vital player in the teleost fish immune system. *Veterinary research*, 50(1), 1-11.
- Austin, C. M., Tan, M. H., Harrisson, K. A., Lee, Y. P., Croft, L. J., Sunnucks, P., ... & Gan, H. M. (2017). *De novo* genome assembly and annotation of Australia's largest freshwater fish, the Murray cod (*Maccullochella peelii*), from Illumina and Nanopore sequencing read. *GigaScience*, 6(8), gix063.
- Borodulina, O. R., Golubchikova, J. S., Ustyantsev, I. G., & Kramerov, D. A. (2016). Polyadenylation of RNA transcribed from mammalian SINEs by RNA polymerase III: Complex requirements for nucleotide sequences. *Biochimica et Biophysica Acta (BBA)- Gene Regulatory Mechanisms*, 1859(2), 355-365.
- Bruna, T., Hoff, K.J., Lomsadze, A., Stanke, M., & Borodovsky, M. (2021). BRAKER2: Automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS Supported by a Protein Database. *NAR Genomics and Bioinformatics*, 3(1).
- Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, 25(15), 1972-1973.
- Chandhini, S., & Rejish Kumar, V. J. (2019). Transcriptomics in aquaculture: Current status and applications. *Reviews in Aquaculture*, 11(4), 1379-1397.
- Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, 34(17), i884-i890.
- Dam, C. T. M., Ventura, T., Booth, M., Pirozzi, I., Salini, M., Smullen, R., & Elizur, A. (2020). Intestinal transcriptome analysis highlights key differentially expressed genes involved in nutrient metabolism and digestion in yellowtail kingfish (*Seriola lalandi*) fed terrestrial animal and plant proteins. *Genes*, 11(6), 621.
- Danzmann, R. G., Kocmarek, A. L., Norman, J. D., Rexroad, C. E., & Palti, Y. (2016). Transcriptome profiling in fast versus slow-growing rainbow trout across seasonal gradients. *BMC Genomics*, 17(1), 1-18.
- Di Genova, A., Buena-Atienza, E., Ossowski, S., & Sagot, M. F. (2021). Efficient hybrid *de novo* assembly of human genomes with WENGAN. *Nature Biotechnology*, 39(4), 422-430.
- Eckert, K. A., Yan, G., & Hile, S. E. (2002). Mutation rate and specificity analysis of tetranucleotide microsatellite DNA alleles in somatic human cells. *Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center*, 34(3), 140-150.
- Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*, 32(5), 1792-1797.
- Firdaus-Nawi, M., and Zamri-Saad, M. (2016). Major components of fish immunity: A review. *Pertanika Journal of Tropical Agricultural Science*, 39, 393–420.

- Guan, W. Z., & Qiu, G. F. (2020). Transcriptome analysis of the growth performance of hybrid mandarin fish after food conversion. *PloS One*, 15(10), e0240308.
- Han, B., Liang, W., Liu, L., Li, Y., & Sun, D. (2018). Genetic association of the ACACB gene with milk yield and composition traits in dairy cattle. *Animal genetics*, 49(3), 169-177.
- Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics*, 107(1), 1-8.
- Hoang, H. D., Pham, H. M., Durand, J. D., Tran, N. T., & Phan, P. D. (2015). Mahseers genera *Tor* and *Neolissochilus* (Teleostei: cyprinidae) from southern Vietnam. *Zootaxa*, 4006(3), 551-568.
- Hong, J. (1998). The distribution trends in simple repetitive stretches of DNA. *Chinese Journal of Biochemistry and Molecular Biology*, 14(1), 65-70.
- Hu, G., Gu, W., Sun, P., Bai, Q., & Wang, B. (2016). Transcriptome analyses reveal lipid metabolic process in liver related to the difference of carcass fat content in rainbow trout (*Oncorhynchus mykiss*). *International Journal of Genomics*, 2016.
- Infante, J. Z., & Cahu, C. L. (2007). Dietary modulation of some digestive enzymes and metabolic processes in developing marine fish: Applications to diet formulation. *Aquaculture*, 268(1-4), 98-105.
- Ingram, B., Sungan, S., Gooley, G., Sim, S. Y., Tinggi, D., & De Silva, S. S. (2005). Induced spawning, larval development and rearing of two indigenous Malaysian mahseer, *Tor tambroides* and *T. douronensis*. *Aquaculture research*, 36(10), 983-995.
- Jaafar, F., Na-Nakorn, U., Srisapoome, P., Amornsakun, T., Duong, T. Y., Gonzales-Plasus, M. M., ... & Parhar, I. S. (2021). A current update on the distribution, morphological features, and genetic identity of the Southeast Asian Mahseers, *Tor* species. *Biology*, 10(4), 286.
- Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., ... & Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, 30(9), 1236-1240.
- Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, 12(4), 357-360.
- Kottelat, M. (2000). Notes on the taxonomy, nomenclature and distribution of some fishes of Laos. *Journal of South Asian Natural History*, 5(1), 83-90.
- Kottelat, M., Pinder, A. C., & Harrison, A. (2018). *Tor tambra*. The IUCN Red List of Threatened Species, (2018-2).
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35(6), 1547.
- Langefors, Å., Lohm, J., Grahn, M., Andersen, Ø., & Schantz, T. V. (2001). Association between major histocompatibility complex class IIB alleles and resistance to

- Aeromonas salmonicida* in Atlantic salmon. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268(1466), 479-485.
- Lau, M. M. L., Kho, C. J. Y., Lim, L. W. K., Sia, S. C., Chung, H. H., Lihan, S., & Apun, K. (2021c). Microbiome analysis of gut bacterial communities of healthy and diseased Malaysian mahseer (*Tor tambroides*). bioRxiv doi: 10.1101/2021.12.08.471852
- Lau, M. M. L., Lim, L. W. K., Chung, H. H., & Gan, H. M. (2021b). The first transcriptome sequencing and data analysis of the Javan mahseer (*Tor tambra*). *Data in Brief*, 39, 107481.
- Lau, M. M. L., Lim, L. W. K., Ishak, S. D., Abol-Munafi, A. B., & Chung, H. H. (2021a). A review on the emerging Asian aquaculture fish, the Malaysian mahseer (*Tor tambroides*): Current status and the way forward. In *Proceedings of the Zoological Society*, 1-11. Springer India.
- Lei, Y., Zhou, Y., Price, M., & Song, Z. (2021). Genome-wide characterization of microsatellite DNA in fishes: survey and analysis of their abundance and frequency in genome-specific regions. *BMC genomics*, 22(1), 1-22.
- Li, W., & Lin, H. (2010). The endocrine regulation network of growth hormone synthesis and secretion in fish: Emphasis on the signal integration in somatotropes. *Science China Life Sciences*, 53(4), 462-470.
- Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Kent, M. P., Nome, T., ... & Davidson, W. S. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature*, 533(7602), 200-205.
- Lim, L. W. K., Tan, H. Y., Aminan, A. W., Jumaan, A. Q., Moktar, M. Z., Tan, S. Y., Balinu, C. P., Robert, A. V., Chung, H. H., & Sulaiman, B. (2018). Phylogenetic and expression of ATP-binding cassette transporter genes in *Rasbora sarawakensis*. *Pertanika Journal of Tropical Agricultural Science*, 41(3), 1341-1354.
- Lim, L. W. K., Chung, H. H., Lau, M. M. L., Aziz, F., & Gan, H. M. (2021a). Improving the phylogenetic resolution of Malaysian and Javan mahseer (*Cyprinidae*), *Tor tambroides* and *Tor tambra*: Whole mitogenomes sequencing, phylogeny and potential mitogenome markers. *Gene*, 791, 145708.
- Lim, L. W. K., Chung, H. H., Ishak, S. D., & Waiho, K. (2021b). Zebrafish (*Danio rerio*) ecotoxicological ABCB4, ABCC1 and ABCG2a gene promoters depict spatiotemporal xenobiotic multidrug resistance properties against environmental pollutants. *Gene Reports*, 23, 101110.
- Lim, L. W. K., Chung, H. H., Hussain, H., & Gan, H. M. (2021c). Genome survey of sago palm (*Metroxylon sagu* Rottboll). *Plant Gene*, 28, 100341.
- Lim, L. W. K., Lau, M. M. L., Chung, H. H., Hussain, H., & Gan, H. M. (2022). First high-quality genome assembly data of sago palm (*Metroxylon sagu* Rottboll). bioRxiv doi: 10.1101/2022.1.1.474478

- Lin, G., Thevasagayam, N. M., Wan, Z. Y., Ye, B. Q., & Yue, G. H. (2019). Transcriptome analysis identified genes for growth and omega-3/-6 ratio in saline tilapia. *Frontiers in Genetics*, 10, 244.
- Lu, G., & Luo, M. (2020). Genomes of major fishes in world fisheries and aquaculture: Status, application and perspective. *Aquaculture and Fisheries*, 5(4), 163-173.
- Ma, D., Ma, A., Huang, Z., Wang, G., Wang, T., Xia, D., & Ma, B. (2016). Transcriptome analysis for identification of genes related to gonad differentiation, growth, immune response and marker discovery in the turbot (*Scophthalmus maximus*). *PLoS One*, 11(2), e0149414.
- Manni M, Berkeley MR, Seppely M, Simão FA, Zdobnov EM. 2021. BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Molecular Biology and Evolution*.
- Marçais, G., & Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of kmers. *Bioinformatics Methods and Protocols*, 27(6), 764-770.
- Mikheenko, A., Prijibelski, A., Saveliev, V., Antipov, D., & Gurevich, A. (2018). Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics*, 34(13), i142-i150.
- Nadjar-Boger, E., & Funkenstein, B. (2011). Myostatin-2 gene structure and polymorphism of the promoter and first intron in the marine fish *Sparus aurata*: Evidence for DNA duplications and/or translocations. *BMC genetics*, 12(1), 1-19.
- Natnan, M. E., Low, C. F., Chong, C. M., Bunawan, H., & Baharum, S. N. (2021). Integration of omics tools for understanding the fish immune response due to microbial challenge. *Frontiers in Marine Science*, 8, 751.
- Neefjes, J., Jongsmā, M. L., Paul, P., & Bakke, O. (2011). Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nature Reviews Immunology*, 11(12), 823-836.
- Ng, C. K. (2004). Kings of the river—Mahseer in Malaysia and the region. Kuala Lumpur, Malaysia: Inter Sea Fishery.
- Nguyen, L. T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, 32(1), 268-274.
- Overturf, K., Sakhrani, D., & Devlin, R. H. (2010). Expression profile for metabolic and growth-related genes in domesticated and transgenic coho salmon (*Oncorhynchus kisutch*) modified for increased growth hormone production. *Aquaculture*, 307(1-2), 111-122.
- Özogul, Y., & Özogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, 100(4), 1634-1638.

- Palti, Y. (2011). Toll-like receptors in bony fish: From genomics to function. *Developmental & Comparative Immunology*, 35(12), 1263-1272.
- Pickett, B. D., Miller, J. B., & Ridge, P. G. (2017). Kmer-SSR: A fast and exhaustive SSR search algorithm. *Bioinformatics*, 33(24), 3922-3928.
- Renaville, B., Bacciu, N., Lanzoni, M., Mossa, F., & Piasentier, E. (2018). Association of single nucleotide polymorphisms in fat metabolism candidate genes with fatty acid profiles of muscle and subcutaneous fat in heavy pigs. *Meat Science*, 139, 220–227.
- Riera Romo, M., Pérez-Martínez, D., & Castillo Ferrer, C. (2016). Innate immunity in vertebrates: An overview. *Immunology*, 148(2), 125-139.
- Roberts, T. R. (1999). Fishes of the cyprinid genus *Tor* in the Nam Theun watershed (Mekong basin) of Laos, with description of a new species. *Raffles Bulletin of Zoology*, 47, 225-236.
- Roberts, T. R. and M. Z. Khaironizam (2008). Trophic polymorphism in the Malaysian fish *Neolissochilus sorooides* and other old world barbs (Teleostei, Cyprinidae). *Natural History Bulletin of the Siam Society*, 56, 25–53.
- Sales, C. F., Silva, R. F., Amaral, M. G., Domingos, F. F., Ribeiro, R. I., Thomé, R. G., & Santos, H. B. (2017). Comparative histology in the liver and spleen of three species of freshwater teleost. *Neotropical Ichthyology*, 15(1).
- Salinas, I., & Magadán, S. (2017). Omics in fish mucosal immunity. *Developmental & Comparative Immunology*, 75, 99-108.
- Schlötterer, C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosoma*, 109(6), 365-371.
- Shreshtha, T. K. (1997). The Mahseer in the rivers of nepal disrupted by dams and ranching strategies (p. 271). Kathmandu, Nepal: Mrs. Bimala Shrestha.
- Smith, N. C., Rise, M. L., & Christian, S. L. (2019). A comparison of the innate and adaptive immune systems in cartilaginous fish, ray-finned fish, and lobe-finned fish. *Frontiers in immunology*, 10, 2292.
- Tan, M. H., Austin, C. M., Hammer, M. P., Lee, Y. P., Croft, L. J., & Gan, H. M. (2018). Finding Nemo: Hybrid assembly with Oxford Nanopore and Illumina reads greatly improves the clownfish (*Amphiprion ocellaris*) genome assembly. *GigaScience*, 7(3), gix137.
- Tóth, G., Gáspári, Z., & Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. *Genome research*, 10(7), 967-981.
- Vagner, M., & Santigosa, E. (2011). Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: A review. *Aquaculture*, 315(1-2), 131-143.
- Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., & Schatz, M. C. (2017). GenomeScope: Fast reference-free genome profiling from short reads. *Bioinformatics*, 33(14), 2202-2204.

- Wang, Y., Lu, Y., Zhang, Y., Ning, Z., Li, Y., Zhao, Q., ... & Zhu, Z. (2015). The draft genome of the grass carp (*Ctenopharyngodon idellus*) provides insights into its evolution and vegetarian adaptation. *Nature genetics*, 47(6), 625-631.
- Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Completing bacterial genome assemblies with multiplex MinION sequencing. *Microbial Genomics*, 3(10).
- Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y., ... & Sun, X. (2014). Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. *Nature genetics*, 46(11), 1212-1219.
- Yuan, Z., Liu, S., Zhou, T., Tian, C., Bao, L., Dunham, R., & Liu, Z. (2018). Comparative genome analysis of 52 fish species suggests differential associations of repetitive elements with their living aquatic environments. *BMC genomics*, 19(1), 1-10.
- Zhenzhen, X., Ling, X., Dengdong, W., Chao, F., Qiongyu, L., Zihao, L., ... & Haoran, L. (2014). Transcriptome analysis of the *Trachinotus ovatus*: Identification of reproduction, growth and immune-related genes and microsatellite markers. *PloS one*, 9(10), e109419.