Near-chromosome level genome assembly of devil firefish, *Pterois miles*

Christos V. Kitsoulis[1,2], Vasileios Papadogiannis[2], Jon B. Kristoffersen[2], Elisavet Kaitetzidou[2], Aspasia Sterioti[2,3], Costas S. Tsigenopoulos[2], Tereza Manousaki[2,*]

1. Faculty of Medicine, University of Crete, Heraklion, Greece
2. Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion, Greece
3. Cretaquarium, Hellenic Centre for Marine Research, Heraklion, Greece

*Correspondence: tereza@hcmr.gr

ABSTRACT

Devil firefish (*Pterois miles*), a member of Scorpaenidae family, is one of the most successful marine non-native species, dominating around the world, that was rapidly spread into the Mediterranean Sea, through the Suez Canal, originating from the Indian Ocean. Even though lionfishes (Scorpaenidae) are identified among the most prosperous marine invaders, within this taxonomic group, the genomic resources are scant, while reference genome assemblies are totally absent. Here, we built and analyzed the first reference genome assembly of *P. miles* and explored its evolutionary background. The resulting genome assembly consisted of 660 contigs and scaffolds (N50 = 14,5 Mb) with a total size of about 902 Mb, while delivering 98% BUSCO completeness. We identified and described the large amount of transposable elements present in the genome and based on genomic data we constructed the first teleost phylogeny which includes a member of genus *Pterois*. The high-quality and contiguity *de novo* genome assembly built herein provides a valuable resource for future studies in species biology and ecology, lionfish phylogeny and the influence of transposable elements on the evolution of vertebrate genomes.

INTRODUCTION

The devil firefish, *Pterois miles* (Bennett, 1828), is a venomous species of the Scorpaenidae family native to the Western Indo-Pacific region, from South Africa to Red Sea and East to Sumatra (Schultz, 1986). The first occurrence of *P. miles*, as a single specimen, in the Mediterranean Sea was recorded off the Levantine coast in 1991 (Golani and Sonin, 1992), while the second, of two individuals, was almost twenty years later in the same area (Bariche et al., 2013). Soon after, the frequency of appearances, along the eastern Mediterranean Sea, rapidly increased (Crocetta et al., 2015; Kletou et al., 2016; Bilge et al., 2017; Mabruk and Rizgalla, 2019; Katsanevakis et al., 2020; Vavasis et al., 2020). Although the origin of species colonization in the Mediterranean Sea followed the invasion pattern of other Lessepsian migrants introduced from the Red Sea, through Suez Canal (Bariche et al., 2013; Dailianis et al., 2016; Kletou et al., 2016; Bariche et al., 2017; Chiesa et al., 2019;

Dimitriou et al., 2019), the contribution of long-distance dispersal via aquarium trading remains a possibility (Bariche et al., 2017; Dimitriou et al., 2019). Lionfishes (genus: *Pterois*) are considered among the most thriving invaders in the history of marine invasions (Albins and Hixon, 2008) because of their rapid expansion worldwide (Azzurro et al., 2017). Indeed, the introduction in the western Atlantic of *P. miles* and of its con-generic *P. volitans*, together referred as the invasive lionfish complex (Lyons et al., 2019), is one of the fastest and most dominant marine fish introductions to date (Kletou et al., 2016, and references therein). For the Mediterranean Sea, the Suez Canal is the major pathway responsible for the spread of most of the non-indigenous species that constantly reshape its biodiversity and fishery resources (Kleitou et al., 2022). Invasive non-indigenous marine species, in general, are considered to have major impact on local biodiversity while threatening marine industries and frequently human health (Bax et al., 2003; Arim et al., 2005; Blakeslee et al., 2019). Furthermore, they are commonly studied in evolutionary biology as models or "natural experiments" in order to explore invasion's dynamics and adaptations to new niches (Barrett, 2015). Data derived from Whole Genome Sequencing (WGS) could provide promising opportunities in the exploration of potential adaptations that shape fitness of invaders, as well as the dynamics of colonization.

Biological characteristics of *P. miles* such as rapid somatic growth, signature anti-predatory defenses (Côté and Smith, 2018), reproductive success, discernible predatory behavior, low parasitism and ecological flexibility might explain its rapid distribution in the Mediterranean Sea (Dailianis et al., 2016), whereas the species population is rising rapidly along the coastlines (Kletou et al., 2016). Yet, few genomic resources of the species are available, including the mitochondrial genome (Dray et al., 2016) and DNA barcoding data (Chiesa et al., 2019, and references therein).

The aim of this study was to construct, annotate and analyze the first high-quality genome assembly of *P. miles*. Through a combination of Oxford Nanopore Technologies (ONT), Pacific Biosciences (PacBio) and Illumina reads, we explored the genomic background of such a successful and unique invader, the devil firefish. Being the first top-quality genome sequenced within the Scorpaenidae family, this valuable resource could provide a critical conveyance to unveil and highlight the insights of species' biology, ecology and phylogeny in further investigations of invasive traits across the Mediterranean Sea.

MATERIALS & METHODS

Sample collection, DNA and RNA libraries construction and sequencing

Animal care and handling were carried out following well established guidelines (Degrazia and Beauchamp, 2019).

A specimen was caught alive in Bali (North coast), Crete, Greece and was transferred in the indoor facilities of Cretaquarium (part of the Hellenic Centre for Marine Research, HCMR). The specimen was kept alive for three weeks in a 150 L tank with semi-closed circuit with airlift, mechanical and biological filtration, water recirculation rates of 30 and 100%/h.

Temperature was adjusted at 19 ºC +/- 1,0 ºC and the photoperiod was set to 12hL:12hD. The specimen was anesthetized using clove oil, and blood was collected with a sterilized and heparinized syringe from the caudal vein of the fish. Blood was kept in sterilized and heparinized 1.5 ml tubes on ice, until DNA extraction.

DNA was extracted, for the purpose of ONT sequencing, from 4ul of blood with the Qiagen Genomic Tip 100/G kit. DNA integrity was assessed by electrophoresis in 0.4 % w/v megabase agarose gel. Three ligation libraries (SQK-LSK109) were constructed using 2.3 microgram DNA as input for each library. The libraries were sequenced on two R9.4.1 flow cells, on the in-house MinION Mk1B and MinION Mk1C sequencers, respectively. The resulting fast5-files were basecalled with Guppy v5.0.11, using the "sup" (super-accuracy) configuration and a minimum quality score of 7.

For the Illumina sequencing, the same procedure and protocol were used for DNA extraction. The template DNA for Illumina sequencing was sheared by ultrasonication in a Covaris instrument. A PCR-free library was prepared with the Kapa Hyper Prep DNA kit with TruSeq Unique Dual Indexing.

For transcriptomic data, total RNA was extracted from seven tissues (brain, gonad, gills, heart, liver, muscle and spleen). Tissue grinding in liquid nitrogen with pestle and mortar was followed by the sample homogenization and lysis in TRIzol buffer (Invitrogen) according to the manufacturer's guidelines. The quantity and the quality of the extracted RNA was estimated with NanoDrop ND-1000 spectrophotometer and with agarose gel (1.5%) electrophoresis, as well as with Agilent 2100 Bioanalyzer using RNA 6000 pico kit. The library preparation was conducted using Pacific Biosciences protocol for Iso-Seq™ Express Template Preparation for Sequel and Sequel II Systems. The seven libraries (one for each tissue) were sequenced on a PacBio sequel II instrument, on one SMRT cell.

Illumina and Pacific Biosciences library preparation and sequencing were carried out by the Norwegian Sequencing Centre (www.sequencing.uio.no), hosted in the University of Oslo.

Genomic data pre-processing

The length filtering and adapter trimming of basecalled ONT reads were carried out with Porechop v0.2.4 (https://github.com/rrwick/Porechop) using default parameters, adding the extra parameter "–discard_middle" to prune reads with potential inner adapters. The quality control was performed using Nanoplot v.1.20 (De Coster et al., 2018). The quality assessment of Illumina reads was performed with FastQC v0.11.9 (Andrews, 2010) while both filtering of low quality reads and adapter trimming, using Trimmomatic v0.39 (Bolger et al., 2014). The reads were processed by Trimmomatic on a 4-base sliding window with an average cutting-off threshold score lower than 15 Phred score. Leading and trailing bases with a quality score less than 10 Phred were trimmed out, while reads shorter than 75 bp and average score lower than 30 Phred were removed.

De novo genome assembly

For the *de novo* genome assembly, using a hybrid approach, the long reads from ONT were combined with short and highly accurate Illumina reads. At first, the ONT reads were used for the construction of the initial *de novo* assembly and the first rounds of polishing, while the Illumina reads were used for the later rounds of polishing. The draft assembly was built from ONT reads using the *de novo* assembler Flye v2.9. (Kolmogorov et al., 2019), which uses a repeat graph as core data structure, with default parameters and a genome size estimation of 900Mb. Then, the draft assembly was polished in two rounds with RACON v1.4.3 (Vaser et al., 2017) using the filtered long reads, mapped against the draft assembly by Minimap v2.22 (Li, 2018). Further polishing was performed by Medaka v1.4.4 (https://github.com/nanoporetech/medaka) and consequently with Pilon v1.23 (Walker et al., 2014) for which the preprocessed Illumina reads were used, after being mapped against the resulting assembly from Medaka, using Minimap v2.22 (Li, 2018).

The resultant assemblies (e.g., draft, intermediate and final) were evaluated all through the above procedure by two commonly used criteria: (i) the N50 statistic from contigs' size, using QUAST v.5.0.2 (Gurevich et al., 2013), and (ii) the completeness score based on the presence of universal single copy ortholog genes, using BUSCO v.5.3 (Manni et al., 2021) against Actinopterygii ortholog dataset 10 (actinopterygii_odb10). BUSCO was run with default parameters adding the extra parameter "--augustus" to enable species-specific training for gene prediction by AUGUSTUS v.3.4 (Stanke et al., 2008). Alternative values (e.g. L90) were calculated and visualized (Table 3, Figure 3) with a custom python tool, ELDAR (https://github.com/ckitsoulis/ELDAR).

The whole genome assembly pipeline which was used in the present study was previously designed by Danis et al. (2021), containerized by Angelova et al. (2022) (https://github.com/genomenerds/SnakeCube) and ran in the IMBBC High performance computing (HPC) facility "Zorbas" (Zafeiropoulos et al., 2021).

Genome annotation

Transposable elements annotation

A *de novo* transposable elements (TEs) library was generated from the constructed genome assembly of *P. miles*, using the Extensive *de novo* TE Annotator (EDTA) package (Ou et al., 2019), an automated whole-genome TE annotation pipeline, with default parameters. In our case, the RepeatModeler2 (Flynn et al., 2020) was utilized to additionally support the identification of TE families inside the EDTA algorithm, using the extra parameter "--sensitive 1". The non-redundant TE library was then separated into three sub-libraries based on its TEs classification, so far, using a custom python script "library_split.py": i) Classified TE sequences, ii) Unclassified TE sequences in the level of superfamily (partially classified), and iii) Unclassified - Unknown TE sequences. The two latter sub-libraries were classified again using DeepTE (Yan et al., 2020), a transposon classification tool which depends on

convolutional neural networks (CNN). The annotation probability threshold was strictly set to 0.8 ("-prop_thr 0.8"). A step of headers' correction and reformation, using bash commands, in every sub-library occurred before their concatenation to the final TE annotated library, in order to achieve a compatible format for the next steps. Finally, RepeatMasker v4.1.2 (Tarailo-Graovac and Chen, 2009) performed the initial TE annotation and genome soft-masking, utilizing the NCBI/RMblast search engine, based on the previously-described library. To achieve a more accurate and detailed annotation/categorisation of TE (Table 4), based on an up-to-date TE classification system (Makalowski et al., 2019), a python-based parser "RM_parser.py" was developed and used for the output files of RepeatMasker. The designed workflow is presented schematically in Figure 1.

Structural annotation - gene prediction

For transcriptome data, circular consensus sequencing (CCS) reads were generated using the CCS application on SMRT Link v10.2 and then Iso-Seq analysis was performed on them, with default parameters, using the Iso-Seq pipeline, IsoSeq v3.4 (https://github.com/PacificBiosciences/IsoSeq), until the production of high quality (HQ) consensus full-length transcripts. The IsoSeq pipeline included three basic steps: i) generation of CCS reads, ii) classification of full-length (FL) reads, and iii) clustering of full-length non-contatamer (FLNC) reads to obtain high-quality consensus transcripts. The number of resulting intermediate reads to final isoforms in the IsoSeq pipeline are presented in Table 5. The HQ transcripts were aligned (spliced-wise) against the soft-masked genome assembly of *P. miles*, using GMAP v2021.08.25 (Wu and Watanabe, 2005). SAM files were sorted and converted to BAM using samtools v1.15.1 (Danecek et al., 2021), and the redundant transcripts were finally collapsed to generate a non-redundant HQ full-length transcripts set, using cDNA Cupcake v28.0 (https://github.com/Magdoll/cDNA_Cupcake) with a minimum alignment coverage equal to 0.99 and a minimum alignment identity of 0.95.

Gene prediction was conducted based on a hybrid strategy of transcriptome-based (non-redundant high quality transcripts), homology-based (curated protein sets) and *ab initio* methods, using a semi-automated workflow consisting of 12 individual tools and intermediate custom python and bash scripts (Figure 2). In the first step, HQ isoforms and curated proteomes of 20 actinopterygian species were aligned (spliced-wise) to the soft-masked genome assembly. The non-redundant HQ trascriptome set was previously aligned using GMAP v2021.08.25 (Wu and Watanabe, 2005). For protein homology evidence, a BLAST database was generated from protein sequences of 20 species (Table 1), being downloaded from UniProtKB/Swiss-Prot (https://www.uniprot.org/), using DIAMOND v2.0.14 (Buchfink et al., 2015). In the second step, Mikado v2.3.3 (Venturini et al., 2018) was used, a python-based pipeline which identifies the "best" set of transcripts from multiple sources, in order to return potential gene models from the transcriptome and protein homology evidence. Homology evidence for each of the predicted transcripts provided to Mikado were generated based on the BLAST DB, using DIAMOND v2.0.14 (Buchfink et al., 2015) while open reading frame (ORF) predictions of Mikado-selected transcripts were produced by

Transdecoder v5.5 (https://github.com/TransDecoder/TransDecoder). All information and evidence were merged afterwards to generate the most accurate evidence-based gene models, using Mikado steps "serialise" and "pick ". These gene models had been used in later steps of gene prediction and annotation update. In the third step, Augustus v3.4 (Stanke et al., 2008) was trained with two optimization rounds on a subset of gene models (generated in step 2) that fulfilled specific criteria: i) full length, ii) non-redundant over, iii) a blast hit score of 0.5, and iv) with at least 2 exons. The training set was selected using a custom python script "select_training.py". To take advantage of Augustus ability to incorporate hints (gene, protein, intron etc) for generating high confident gene models, species-specific exon hints and spliced protein alignments were generated and merged, secondarily. For exon hints, the exons coordinates were extracted from the previously produced annotation file using python scripts. For the spliced protein alignments, three well annotated protein sets of species *Oryzias latipes* (downloaded from UniProtKB), *Gasterosteus aculeatus* (downloaded from Ensembl) and *Argyrosomous regius* (Papadogiannis et al., 2022) were aligned to genome assembly, using Exonerate v2.4 (https://github.com/nathanweeks/exonerate). The annotation files were merged, sorted and then filtered for exonic evidence extraction using python. *Ab initio* prediction on *P. miles* genome assembly, alongside the generated hints, was performed by Augustus v3.4 (Stanke et al., 2008) with extra parameters "--allow_hinted_splicesites=atac" and "--alternatives-from-evidence=false". In the fourth step, gene models, generated in the previous steps (from Mikado and Augustus), were merged into a consensus gene set, after two updating rounds, using PASA v2.4.1 (Haas et al., 2003), an eukaryotic genome annotation pipeline. For this reason, Mikado-predicted protein coding gene models were loaded into PASA to create the initial MySQL DB of transcripts, the Augustus predictions were loaded to the DB and it was updated later on with the Mikado-predicted genes. The same procedure was followed, as a second updating round, starting this time from the resulting annotation of the first round. In the last step, genes were filtered to remove predictions with in-frame STOP codons and those that overlap with TEs. For the first case, the gene models were cleaned for potential identical isoforms using Agat (https://github.com/NBISweden/AGAT), the artifacts were recognised using gffread (https://github.com/gpertea/gffread), while they were removed with bash commands. For the second case, candidate models were found using bedtools v2.30 (Quinlan and Hall, 2010) "intersection" command, with a minimum overlapping score of 0.5 "-f 0.50 " and filtered out with bash commands as well. The completeness evaluation of transcripts and genes, in each step, was performed using BUSCO v5.3 (Manni et al., 2021) against the Actinopterygii ortholog dataset 10 (Table 6).

Table 1. Species included in protein homology BLAST database.

Scientific name	Common name	UniProt ID	Number of proteins	Reference
Amphilophus citrinellus	Midas cichlid	UP000261340	31,742	Yunyun et al. (2022)
Amphiprion ocellaris	Clown anemonefish	UP000257160	31,745	Ryu et al. (2022)
Astyanax mexicanus	Blind cave fish	UP000018467	39,383	McGaugh et al. (2014)
Betta splendens	Siamese fighting fish	UP000515150	41,617	Fan et al. (2018)
Carassius auratus	Goldfish	UP000515129	82,968	Chen et al. (2019)
Clupea harengus	Atlantic herring	UP000515152	37,255	Kongsstovu et al. (2019)
Danio rerio	Zebrafish	UP000000437	46,841	Howe et al. (2013)
Esox lucius	Northern pike	UP000265140	71,519	Rondeau et al. (2014)
Gymnodraco acuticeps	Ploughfish	UP000515161	39,915	Bista et al. (2022)
Haplochromis burtoni	Burton's mouthbrooder	UP000264840	34,332	Brawand et al. (2014)
Hippocampus comes	Tiger tail seahorse	UP000264820	27,735	Lin et al. (2016)
Ictalurus punctatus	Channel catfish	UP000221080	40,203	Wang et al. (2022)
Lepisosteus oculatus	Spotted gar	UP000018468	22,463	Braasch et al. (2016)
Oreochromis niloticus	Nile tilapia	UP000005207	74,622	Conte et al. (2017)
Oryzias latipes	Japanese rice fish	UP000001038	36,128	Kasahara et al. (2007)
Perca flavescens	American yellow perch	UP000295070	21,644	Feron et al. (2020)
Salmo salar	Atlantic salmon	UP000087266	82,390	Lien et al. (2016)
Sparus aurata	Gilthead sea bream	UP000472265	69,200	Perez-Sanchez et al. (2019)
Takifugu rubripes	Japanese pufferfish	UP000005226	51,078	Kai et al. (2011)
Xiphophorus maculatus	Southern platyfish	UP000002852	35,279	Schartl et al. (2013)

Functional annotation

The functional annotation of *P. miles* predicted gene set was performed using three different strategies and tools. The first approach was based on similarity search (reciprocal hits) against the annotated genes of zebrafish (*D. rerio*) using BLASTp v2.12+ (Altschul et al., 1990) with parameters: "-evalue 1e-6 ", "-max_target_seqs 1" and "-hdps 1". In the second one, results were fetched with EggNOG-mapper v2.1.7 (Cantalapiedra et al., 2021) based on fast orthology assignments using pre-computed clusters and phylogenies from eggNOG v5.0 database (Huerta-Cepas et al., 2019). For the last approach, annotations were retrieved using PANNZER2 (Törönen and Holm, 2022), a weighted k-nearest neighbour classifier which is based on SANSparallel (Koskinen and Holm, 2012) for homology similarity against UniProt and enrichment statistics, using various user-defined scoring functions. Prediction of gene names, Gene Ontology (GO) annotations, KEGG pathway IDs, Pfam domains and descriptions from all aforementioned strategies were filtered and assigned to gene models using a custom python script "FUNfilter.py". Gene names, in each case, were selected based on the most frequent occurrence, while KEGG pathway IDs and Pfam domains derived directly from EggNOG-mapper. An additional step was performed for GO terms (biological process) being mapped to gene models, by using the assigned gene names as queries and retrieving terms from UniProtKB (https://www.uniprot.org/) with "Retrieve/ID mapping tool", for human, mouse and zebrafish. Finally, GO terms resulted as a set of terms between those predicted by EggNOG-mapper v2.1.7 and UniProtKB.

Phylogenomic analyses

Orthology assignment

To identify orthologous and paralogous genes, 46 whole-genome protein coding gene sets (longest isoforms) from teleost species (Supplementary Table 1), along with *P. miles*, were compared using OrthoFinder v2.5.2 (Emms and Kelly, 2019), with default parameters. The initial dataset was collected from Genomes - NCBI Datasets (https://www.ncbi.nlm.nih.gov/datasets/genomes/) and Ensembl DB (https://www.ensembl.org/) based on the following criteria: i) Scaffold level and greater, and ii) N50 > 10Mb. The longest isoform per gene was selected initially (in GFF format) using Agat (https://github.com/NBISweden/AGAT) and then extracted as FASTA file with gffread (https://github.com/gpertea/gffread). Each proteome set was assessed for completeness using BUSCO v.5.3 (Manni et al., 2021) against Actinopterygii ortholog dataset 10. For the final set, only proteomes which exceeded a predefined completeness threshold (90%) were included and only one species per genus was kept for the final analysis.

Species tree inference

The phylogenetic hierarchical orthogroups (HOGs) produced by OrthoFinder were filtered, at first, to select those with complete representation from *P. miles* and then, only those

containing a single gene copy per species, to exclude potential paralogs. Afterwards, orthogroups which had a representation from at least 43 out of 47 species (> 91.4%) were selected, using a custom python script (aragorn_orthoX.py). The protein sequences of each HOG were aligned using MAFFT v7.505 (Katoh and Standley, 2013). In the case of missing taxa, the aligned orthogroup sequences were corrected using a custom python script (gimli_clean.py) and then concatenated to a superalignment matrix with bash commands. The initial matrix was trimmed to remove spurious sequences and poorly aligned regions using trimAl v1.4.1 (Capella-Gutiérrez et al., 2009), with default parameters and strict mode. ModelTest-NG v0.1.7 (Darriba et al., 2019) was used for the selection of the best-fit model and IQTREE v2.2.0.3 (Minh et al., 2020) for the maximum-likelihood phylogenetic tree inference. To assess the confidence of branches, IQ-TREE was run for 1000 bootstrap replicates (ultrafast bootstrap mode). The phylogenetic tree was finally visualized using R/RStudio Team (2022) and package "ggtree" (Yu, 2020) within a custom R script (tree.R), selecting *Lepisosteus oculatus* and *Polyodon spathula*, as an outgroup clade.

Comparative genomic analysis

Synteny analysis

Synteny analysis was performed on gene level between *P. miles* and *G. aculeatus*. For this purpose, one-to-one orthologues were selected from the HOGs produced earlier by OrthoFinder, to compare the physical localization of genetic loci within species. The 42 longest contigs of *P. miles* genome assembly (representing ~73.5% of genome size) were selected for visualization against the 21 chromosomes of *G. aculeatus*, using Circos (Krzywinski et al., 2009).

Gene families expansion and contraction

Changes in gene families size (expansions and contractions) were estimated using CAFE v5 (Mendes et al., 2020). The HOGs summary table of all species from OrthoFinder was retrieved and modified earlier by a custom python script "aragorn_orthoX.py", resulting in a count matrix of genes per species and family, in order to be used by CAFE. Following CAFE's developers instructions, gene families absent in more than 10 species out of 47 and the ones in which the difference between the maximum and minimum number of genes was greater than 70 ($max_i(n_{genes}) - min_i(n_{genes}) > 70$), were filtered out from the analysis. An ultrametric binary tree was produced with R package "ape" (Paradis and Schliep, 2019) in a custom R script "tree_calibration.R". For that, we used the phylogenetic tree, produced earlier, and the divergence times taken from TIME-TREE (http://www.timetree.org/) between 4 different species' combinations, *Polyodon spathula* - *Danio rerio*, *Danio rerio* - *Takifugu rubripes*, *Oryzias latipes* - *Mola mola* and *Dicentrarchus labrax* - *Mola mola*. CAFE was finally run using 3 different gamma function categories (-k) to estimate λ parameter (corresponding to the rate of change of families), 400 iterations (-I) and a p-value equal to

0.05 (-pvalue). After the analysis, we selected for visualization only the Perciformes clade, as a subset of the phylogenetic tree.

Duplication events estimation

To infer gene duplication events in *P. miles* from gene family trees and the estimated phylogenetic tree, we used GeneRax v2.0.4 (Morel et al., 2020). Initially, protein sequences from each HOG, produced by OrthoFinder, were aligned to each other using MAFFT v7.505 (Katoh and Standley, 2013) and trimmed with trimAl v1.4.1 (Capella-Gutierrez et al., 2009), in strict mode. Families with less than three sequences were excluded from the following procedure. From each gene family was estimated the best-fit model and later was used for the inference of a single maximum-likelihood gene tree, using IQTREE v2.2.0.3 (Minh et al., 2020). After substitution model correction in some cases, gene trees and their models along with the phylogenetic species tree were used to estimate duplication events with GeneRax.

Gene ontology terms descriptive analysis

For GO terms descriptive analysis, firstly we downloaded the core ontology (OBO format) from Gene Ontology DB (http://geneontology.org/docs/download-ontology/), and then the predicted gene set of *P. miles* and their assigned GO terms were mapped with GO biological process descriptions provided by the core ontology, using a custom python script "obo_mapper.py". Then, these GO terms and their descriptions were grouped/mapped into the gene families of their genes (HOGs from OrthoFinder), which were previously identified as rapidly expanding from CAFE and involved in duplication events by GeneRax.

RESULTS

Genomic sequencing results

Sequencing yielded a total of 38.66 Gb of raw genomic ONT reads, from which 36.16 Gb had a Phred quality score above Q7, as well as 3.75 Gb of raw Illumina reads. After the pre-processing steps of both trimming and quality filtering, 35.72 Gb of ONT, for the initial assembly, and 3.16 Gb of Illumina reads, for later polishing, were maintained for the downstream process (Table 2).

Table 2. Summary of genomic sequencing throughput.

Sequencing technology	Raw reads	Quality-controlled reads	Coverage
Illumina	24,836,074	20,980,358	3.5 x
MinION	2,245,868	2,224,738	39.5 x

Genome size and assembly completeness

The final genome assembly contained 660 contigs with a total length of about 902.3 Mb. The longest contig was sized at 36.5 Mb and the N50 statistic value at 14.5 Mb (Table 3). At least, 90% of the genome size was represented in the 83 longest contigs of the produced assembly (Figure 3). The GC content of the genome was calculated at 40.78% (GC-rich regions at the 42 longest contigs are presented in Supplementary Figure 1). For genome completeness assessment, 3,566 out of 3,640 BUSCO genes were present (98%), against the Actinopterigyian ortholog dataset (v.10). Of those, 3,551 genes (97.6%) were complete, while only 74 (2.0%) were missing (Table 3), suggesting a high level of contiguity and completeness of the *de novo* genome assembly.

Genome annotation

Transposable elements annotation

About 46.5% of the genome assembly (~ 416.7 Mb) in *P. miles*, consisted of transposable elements (Figure 4). Class I Retroelements make up 4.65% of the genome assembly and LTR order is the most dominant with a representation of at least 4.23%, with its superfamily Gypsy of 1.47%. Class II of TEs (DNA transposons) represents a high amount (28.6%) of the whole genome, while elements of the TIR order and its superfamily CACTA were mostly found, with 17.46% and 4.83% respectively, among the high-confident and completely classified DNA TEs. Additionally, 9.8% of the masked genome are regions of complex composition of overlapping TEs, not clearly defined as discrete elements during masking (Table 4). The distribution of TE content in the 42 longest contigs is presented in Supplementary Figure 1.

Table 3. Polished genome assembly statistics and completeness.

Number of contigs	660
Total length	902,353,306 bp
GC (%)	40.78
Longest contig	36,477,432 bp
N50	14,490,642 bp
N75	5,565,202 bp
L90	83
BUSCO completeness score	
Complete	3,551 (97.6%)
Single	3,515 (96.6%)

Duplicated	36 (1.0%)
Fragmented	15 (0.4%)
Missing	74 (2.0%)
Total number of orthologs (Actinopterygii)	3,640

Transcriptome analysis

From 6,245,243 initial CCS reads, Iso-seq analysis yielded a total of 124,307 HQ consensus isoforms. The samples with the higher number of transcripts were the heart, spleen and liver (Table 5), and they shared almost 6,500 of them (Supplementary Figure 2). However, the total amount of HQ transcripts shared between all sampled tissues was notably low (~1,000). From the total number of HQ transcripts, 91,666 were aligned properly to the assembled genome (representing 73.74%) and used as evidence for the gene prediction.

Table 4. Transposable elements annotation statistics.

Transposable elements	number of elements	length occupied (bp)	% of genome size
Retroelements (Class I)	173,376	41,925,013	4.65 (8.99)
LTR	165,075	38,160,626	4.23 (8.61)
BEL-Pao	635	141,992	0.02 (0)
Copia	2,380	432,776	0.05 (0.06)
Gypsy	31,595	13,241,124	1.47 (1.92*)
ERV	2,667	529,295	0.06 (0.1)
DIRS	239	123,175	0.01
DIRS1	239	123,175	0.01
Ngaro	0	0	0
Penelope	1,268	407,091	0.05 (0.09)
LINE	3,602	1,523,385	0.17 (0.36)
R2	100	88,857	0.01 (0.02)
RTE	438	169,256	0.02 (0.03)
Jockey	77	60,944	0.01 (0.01)
L1	982	524,323	0.06 (0.1)
SINE	817	132,603	0.01 (0.02)

tRNA	817	132,603	0.01 (0.02)
7L	0	0	0
5S	0	0	0
Overlapping Retroelements	2,375	1,578,133	0.17
DNA transposons (Class II)	1,036,247	258,303,777	28.63 (33.8)
TIR	759,014	157,567,545	17.46
Tc1-Mariner	20,095	3,271,776	0.36 (0.92)
hAT	116,270	24,446,997	2.71 (7.06)
Mutator	109,966	16,578,613	1.84
Merlin	0	0	0
Transib	0	0	0
P	23,489	3,918,909	0.43 (0.01)
PiggyBac	176	17,979	0 (0)
PIF-Harbinger	23,232	3,871,691	0.43 (1.2)
CACTA	311,697	43,611,897	4.83
Helitron	82,240	13,621,796	1.51
Maverick	146	206,087	0.02
Overlapping DNA transposons	177,832	83,009,131	9.2
Unclassified	32,965	7,246,688	0.8 (1.1)
Simple repeats	359,730	20,717,298	2.3 (2.3)
Low complexity	37,555	2,860,435	0.32 (0.32)
Complex overlapping regions	149,699	88,617,633	9.82

*grouped as Gypsy/DIRS1 by RepeatMasker
in parenthesis are presented the percentages calculated by RepeatMasker

Structural and Functional annotation

The hybrid approach of HQ full-length transcripts-based, homology-based and *ab initio*-based methods resulted in a total of 25,410 candidate protein-coding gene models. We filtered out genes with in-frame stop codons (266 putative genes) and those overlapping with TEs (505 gene models). We ended up with 24,639 potential gene models (Supplementary_file1_annotation.gff), representing in size 382.3 Mb of the genome assembly (Table 6). A subset of 22,473 genes were assigned with gene names and 23,521 matched at

least one functional description, accounting 89.7% and 95.4% of the total number of genes, respectively. In terms of GO, KEGG pathway IDs and PFAM domains, 22,115 (88.3%), 15,071 (61.1%) and 20,003 (81.1%) genes were annotated, in each case (Supplementary_file2_functional.tsv).

From a core set of 3,640 single-copy orthologous genes (Actinopterigyii lineage, odb 10), 3,414 (93.8%) were found to be present in the predicted gene set (Table 7), with 3,233 (88.8%) identified as complete (3,082 as single-copy and 151 as duplicated) and 181 (5.0%) as fragmented, while 224 (6.2%) of them were not present, using BUSCO v5.3 (Manni et al., 2021).

Table 5. From CCS reads to high-quality isoforms.

Tissue	CCS	HiFi	FLNC (polyA)	HQ isoforms
muscle	682,522	651,026	679,844	34,862
liver	863,535	814,531	861,396	50,651
heart	747,994	704,226	743,228	73,273
brain 1	16,449	15,739	15,986	2,467
brain 2	33,032	32,232	32,810	7,042
gonad	396,510	377,750	395,080	17,804
spleen	596,858	568,143	594,852	66,103
gills	193,697	184,914	192,470	25,831
consensus isoforms				124,307

Table 6. Basic statistics of predicted gene models.

Type	Number	Mean size (bp)	Longest (bp)	Shortest (bp)	Genome size (%)
gene	24,645	15,518	445,933	201	42.37
transcript	25,040	15,440	445,933	201	42.84
5' UTR	13,119	228	10,216	1	0.24
exon	231,331	207	14,732	3	5.33
CDS	227,256	159	6,853	16	4
intron	206,291	1,609	188,322	21	36.05
3' UTR	11,114	914	14,608	5	1.07

Orthology assignment and Phylogenomic analysis

The total number of genes included in the proteomes of all 47 teleost fish species (Supplementary Table 1) and analyzed by OrthoFinder, was 1,108,753 and 97.8% of them were assigned to 28,397 phylogenetic HOGS. After the filtering step, 1,193 HOGs were selected to construct the superalignment matrix. Before trimming, the matrix consisted of 1,018,881 alignment positions, while after filtering it contained 473,254 (46.4%) positions which were used for the phylogenomic analysis. JTT + I + G4 + F was identified as the best-fit model and used for the phylogenetic tree inference (Figure 5). At the resulting maximum-likelihood phylogenetic tree, almost all branches were supported with 100 bootstraps. Based on the constructed phylogeny, *P. miles* is placed within the Perciformes clade.

Table 7. Completeness assessment in each step of structural annotation workflow.

Type (Source)	Complete (%)	Single (%)	Duplicate (%)	Fragmented (%)	Missing (%)	Final (%)
HQ isoforms (PacBio)	77.3	19.9	57.4	1.9	20.8	79.2
Consensus transcripts (Mikado)	74.7	69	5.7	1.6	23.7	76.7
Genes (Augustus)	82	80.7	1.3	5.4	12.6	87.4
Consensus genes (PASA-filtered)	88.8	84.7	4.1	5	6.2	93.8

Synteny analysis

Synteny analysis on gene level unveiled high conserved syntenic coding regions between the 42 longest contigs of *P. miles* and the 21 chromosomes of *G. aculeatus*, sharing at total 8,035 one-to-one orthologous genes (Figure 6).

Gene repertoire evolution

Gene family evolution analysis in *P. miles* estimated 228 rapidly evolving gene families (out of 15,405 included families), at a significance level of 0.01 (p-value). From these rapidly evolving families, 136 were identified as expanding and 92 as contracting. The total number of genes included in these rapidly expanding families was 373. The corresponding state of the number of estimated gene families' gains-losses inside the Perciformes species is presented in Figure 7, as a subset of Figure 5.

The number of families included in the duplication events estimation analysis was 23,775 with an average of 43 genes per family. The largest family included 1,638 genes. The total number of genes included in the gene duplication events estimation was 1,036,460. In *P.*

miles, 728 gene families were identified with duplication events, including an amount of 2,263 individual genes.

The descriptive analyses for rapidly expanding gene families of CAFE and those involved in duplication events in GeneRax are presented in Figure 8. GO terms were classified into eight categories, associated with metabolism, immune system, development, growth, antimicrobial response, toxin transport, reproduction and locomotion, and the numbers of gene families, involved genes and unique terms were calculated for both results from CAFE and GeneRax. The top terms in both analyses were included in gene families associated with "metabolism", "development", "immune" and "growth".

DISCUSSION

Here, we presented and analyzed the first high-quality genome assembly for the lessepsian migrant species *P. miles*, which is, also, the first assembly for the whole Scorpaenidae family. We positioned the species in the teleost tree for the first time and studied its complex repeat and gene content. In this study, a lionfish near-chromosome genome assembly of high-quality and contiguity was constructed from genomic data derived from three MinION flow cells and an Illumina Hiseq4000 platform, finally ending in a total size of 902.3 Mb and distributed in 660 contigs. To our knowledge, this is the first reference genome in Pteroinae and so far the only available genome of the Scorpaenidae family.

Repeat content, gene prediction & functional annotation

The representation of TEs in *P. miles* genome (46.5% of genome assembly) is notably higher than in other species inside Perciformes, such as *G. aculeatus*, 13.02% (Shao et al., 2019), *S. lucioperca*, 39.0% (Nguinkal et al., 2019) and *E. lanceolatus*, 45.1% (Wang et al., 2019). Furthermore, its repetitive content is higher compared to species of similar genome size (0.9-1 Gb) such as *O. niloticus* (21.34%), *A. mexicanus* (25.21%), *O. latipes* (26.74%), *C. idella* (40.08%) and *L. oculatus* (16.06%), as presented by (Shao et al., 2019, and references therein). The percentage of DNA transposons (Class II) in the assembled genome (28.63-33.8%) is only comparable to the corresponding ones in *D. rerio* (46.27% with ~1.3 Gb genome size, Shao et al. (2019)) and *C. idella* (25.57% with ~900 Mb genome size, Shao et al., 2019). Despite the positive correlation between genome size and the abundance of TEs in fish genomes, also confirmed here, it would be extremely interesting to investigate further the relationship between the TE heterogeneity, in terms of copy number and composition, and genomes' evolution (Sotero-Caio et al., 2017; Shao et al., 2019). For example, 20 distinct superfamilies were recognised in the genome of *P. miles*, from a minimum of 77 Jockey elements to about 311,700 CACTA (Table 4), taking advantage both of the thorough classification resulting from the designed pipeline and the detailed annotation from "RM parser.py" (Figure 4). Additionally, studies have revealed the multi-functional role of TEs on the evolution of vertebrate genomes, from genomic architecture (Sotero-Caio et al., 2017) to their relationship with non-coding RNAs (Bourque et al., 2018) and confluence to

transcription regulation (Drongitis et al., 2019; Fueyo et al., 2022). Albeit, it would be noteworthy in the superfamily level to explore the patterns in the accumulation of TEs, , their roles and consequently their contribution to gene duplication events, and genome dynamics in general.

Table 7. Fish genome size and TE content comparison.

Species	Genome size (Mb)	TE content (%)	Reference
Pterois miles	902.5	46.51	present study
Gasterosteus aculeatus	461.5	13.02	Shao et al., 2019
Sander lucioperca	1014	39.0	Nguinkal et al., 2019
Epinephelus lanceolatus	1128	45.1	Wang et al., 2019
Oreochromis niloticus	927.3	21.34	Shao et al., 2019
Astyanax mexicanus	1191.2	25.21	Shao et al., 2019
Oryzias latipes	868.9	26.74	Shao et al., 2019
Ctenopharyngodon idella	900.5	40.08	Shao et al., 2019
Lepisosteus oculatus	945.8	16.06	Shao et al., 2019

Phylogenomic analysis, synteny and gene repertoire

Scorpaenidae (order: Perciformes) is a taxonomically widespread family which includes by now 370 marine species (Smith et al., 2018, and references therein), known to be venomous. Despite their worldwide distribution and diversity, this group's biology is clearly understudied, as well as their unexplored phylogeny. Here, we presented the first phylogenetic tree that includes a representative of this family, devil firefish *P. miles*, based on whole genome data (Figure 5). This effort could be an origin for further genomic and evolutionary studies inside this family.

One-to-one orthologous genes between *P. miles* and *G. aculeatu*s, exhibited high conserved synteny (Figure 6), which confirms the high quality and completeness of constructed genome assembly. Indeed, between contigs 2, 3, 4, 6 and 7 of devil firefish and chromosomes 16, 10, 11, 18 and 5 of three-spined stickleback, there was high pairwise conservation, respectively. Additionally, an interesting fact arose, which revealed the fusion of coding regions from more than one contigs of *P. miles* to single chromosomes of *G. aculeatus* (e.g. contigs 1 and 12 to chromosome 7, contigs 5, 29, 41 to chromosome 1, contigs 9, 16, 17 to chromosome 4) and their later rearrangements (e.g. contigs 8, 23 to chromosome 19).

Duplication events estimation and descriptive functional analysis unveiled the extended presence of gene families, being involved in major biological processes, such as metabolism,

somatic growth, immunity and reproduction (Figure 8). These families may potentially contribute to species morphology, anti-predatory tactics, rapid spread and adaptation in new marine habitats. Noteworthy, a sufficient number of immune-related gene families were identified, including immunoglobulins (Ig heavy-chain variable, light-chain variable genes), interleukins (interleukin 10 receptor), lysozymes (antimicrobial response), genes contributing to the regulation of antiviral innate immunity (e.g. TRIM35) and transcription factors that regulate the expression of MHC class II genes. An interesting finding was a detected duplication in the gene family of meprins (meprin-F in fish, Marın (2015)), proteins that are involved in toxins transport. Based on the results, it could worth additional studies on genes responsible for the unique morphology (e.g. spines development) of devil firefish and its successful adaptation to new habitats, with the contribution of more genomic data inside the family of Scorpaenidae, that would become available in the future.

CONCLUSION

In this study, we provide the first near-chromosome and high-quality genome assembly of devil firefish, its complex repeat and gene content, and we construct the first phylogeny including a member of genus *Pterois*, based on whole genome sequencing data. All the analyses performed here, highlighted the importance of *P. miles* genome as a valuable resource for further studies regarding the influence of transposable elements on genome evolution, the correlation between gene duplications and adaptation to new niches, lionfish rapid spread worldwide and its dominance, and fish toxins evolution.

CODE AVAILABILITY

All customs scripts, designed workflows and used software commands that have been used during this study are available at the following GitHub repositories:

- https://github.com/ckitsoulis/Pterois-miles-Genome
- https://github.com/ckitsoulis/ELDAR
- https://github.com/genomenerds/SnakeCube

DATA AVAILABILITY

Genomic data from Illumina and Oxford Nanopore Technologies, and transcriptomic data from Pacific Biosciences can be accessed in the ENA database under the IDS *ERR10286272*, *ERR10286273* and *ERR10286274-81* respectively. The genome assembly is available under the ENA ID *GCA_947000775*. Gene and functional annotations can be found in the supplementary data files.

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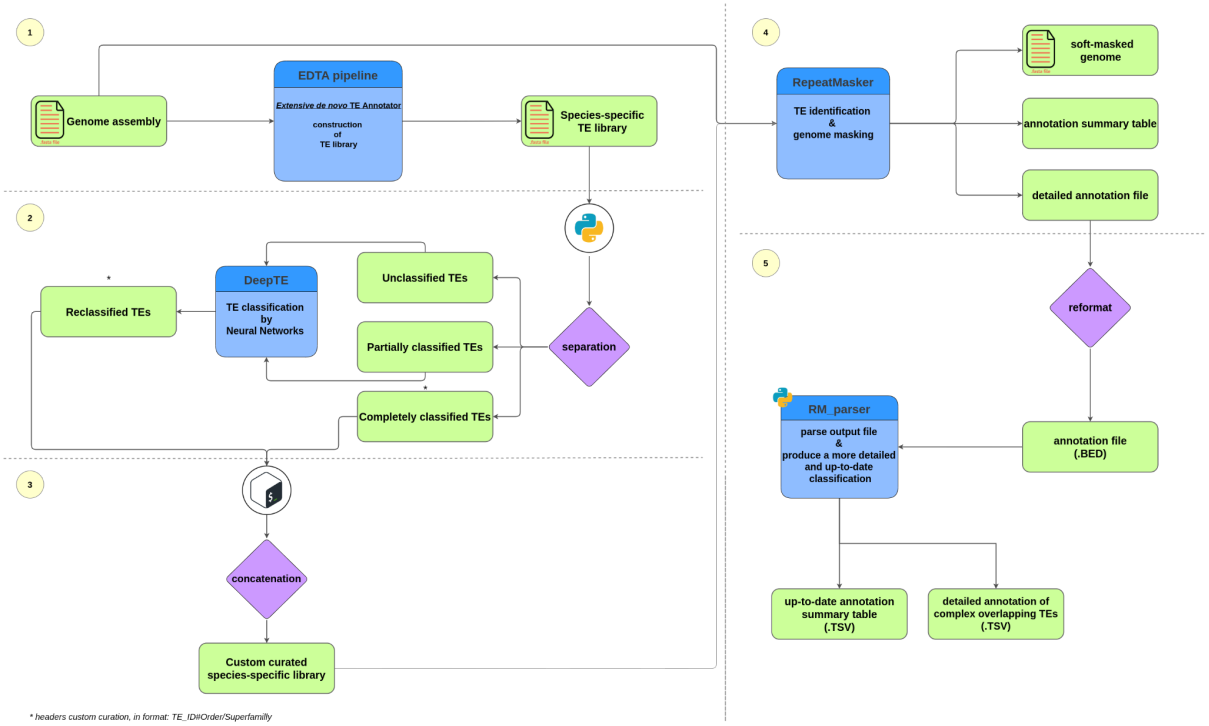


Figure 1. Transposable elements annotation workflow.

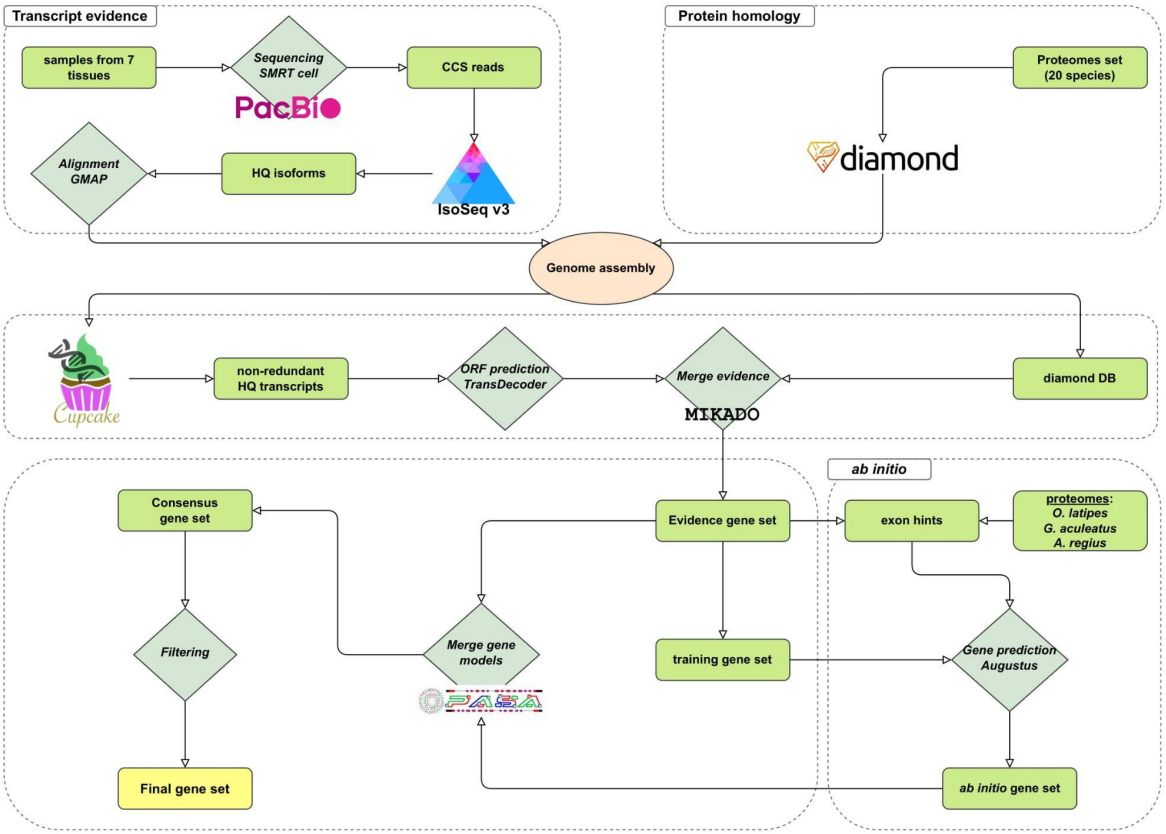


Figure 2. Structural annotation workflow.

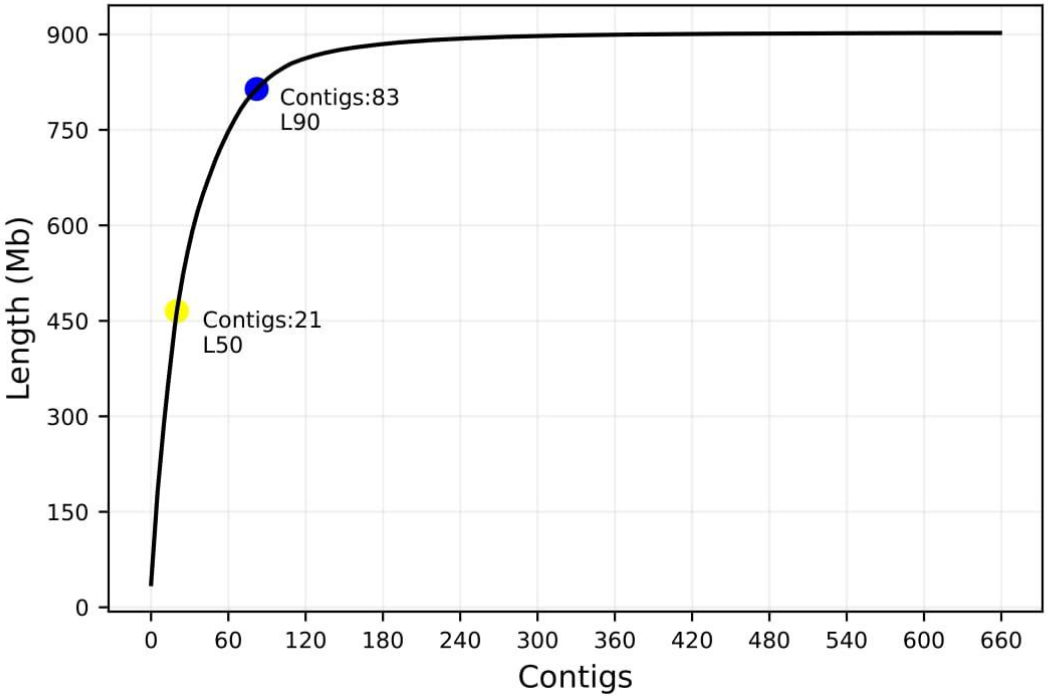


Figure 3. Cumulative sum of contigs' lengths of *P. miles* genome assembly. The yellow dot shows the number of contigs that represent at least 50% (L50) and the blue one at least 90% (L90) of the genome size, respectively.

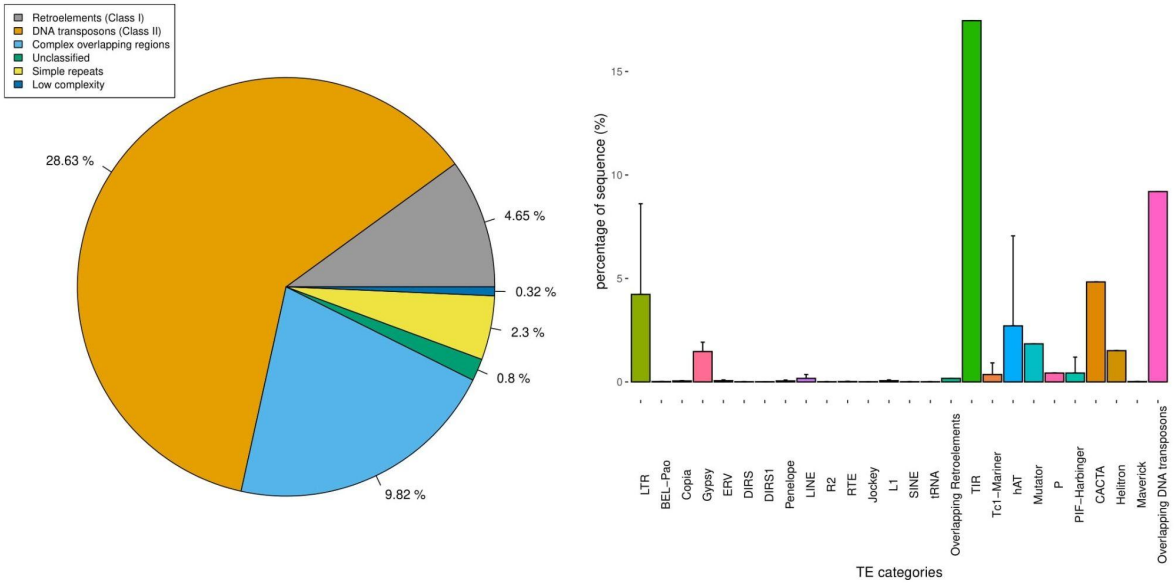


Figure 4. Percentage of TE categories representation in the genome of *P. miles*.

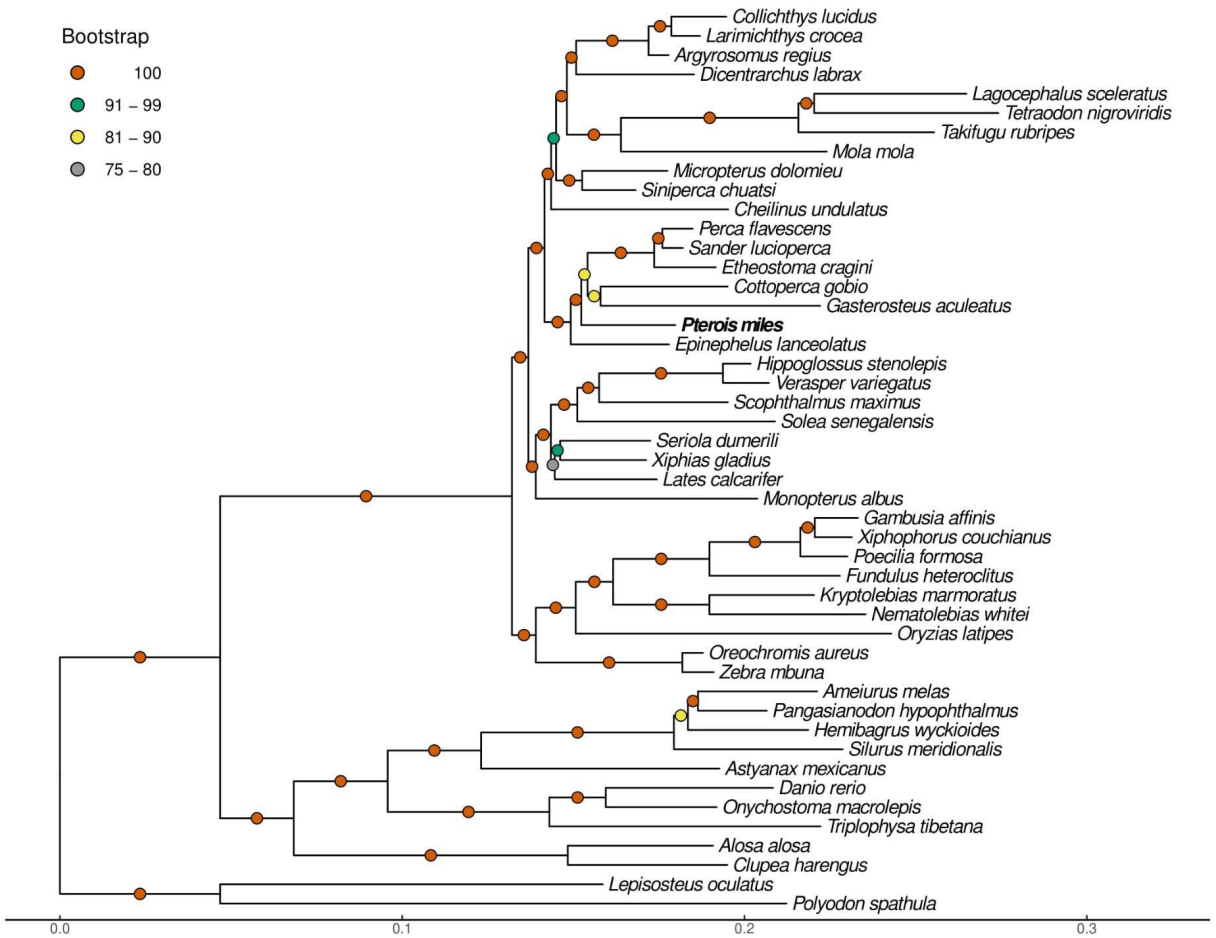


Figure 5. Maximum-likelihood phylogenetic tree using JTT + I + G4 + F substitution model and *P. spathula* - *L. oculatus* clade as an outgroup.

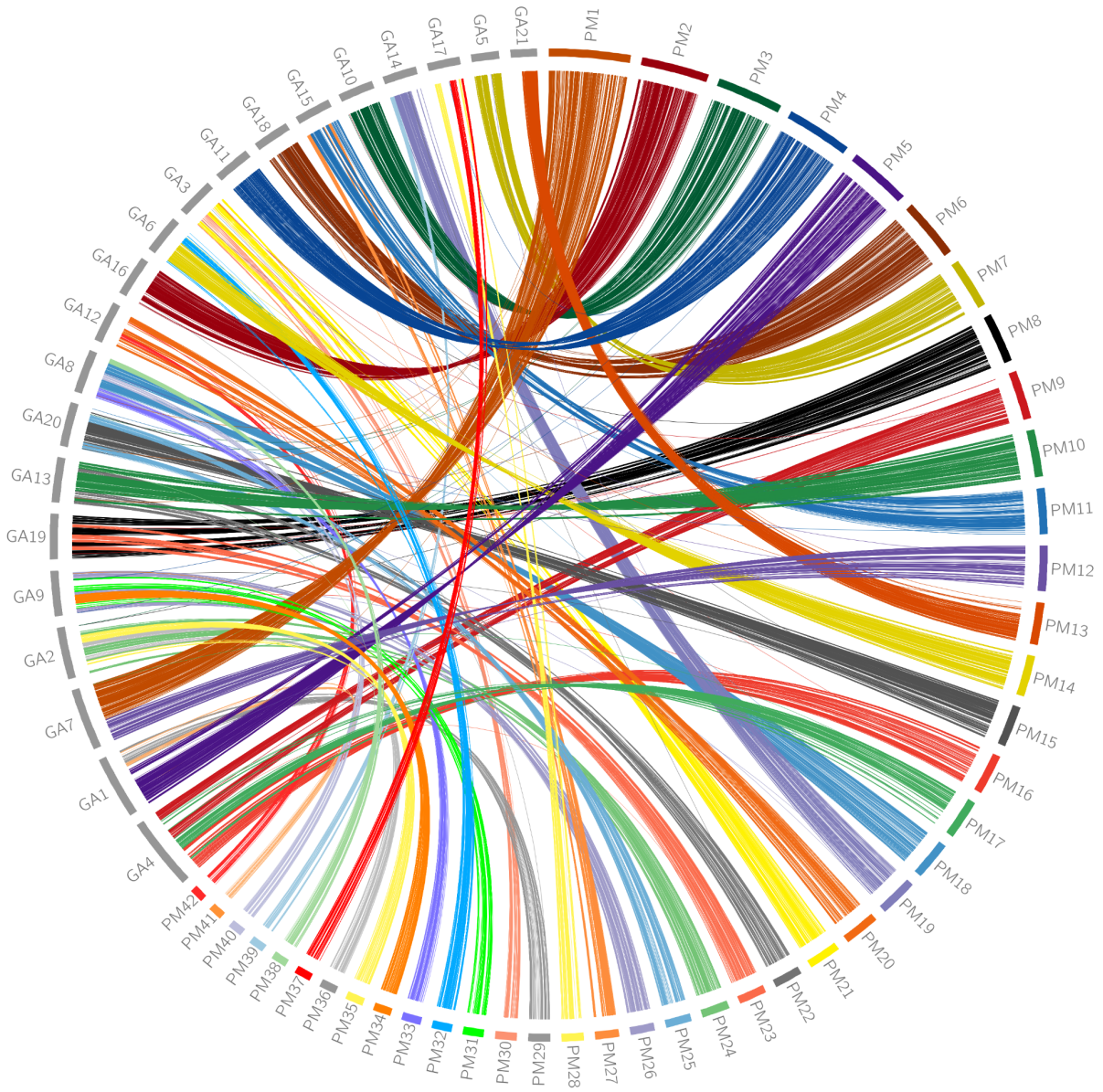


Figure 6. Circos plot which presents the syntenic locations of orthologous genes between the 42 longest contigs of *P. miles* (right, PM) and the 21 chromosomes of *G. aculeatus* (left, GA). Ribbons link orthologous genes between the two species, and colors represent the different contigs of *P. miles*.

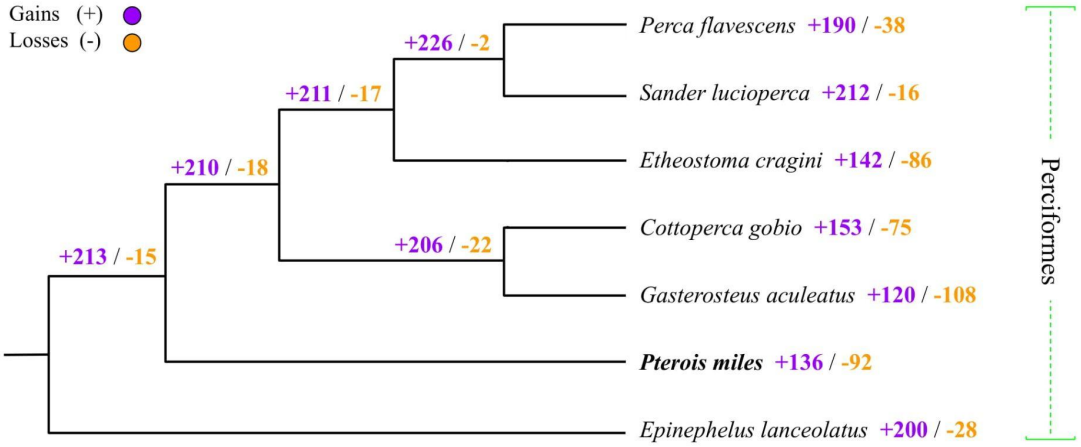
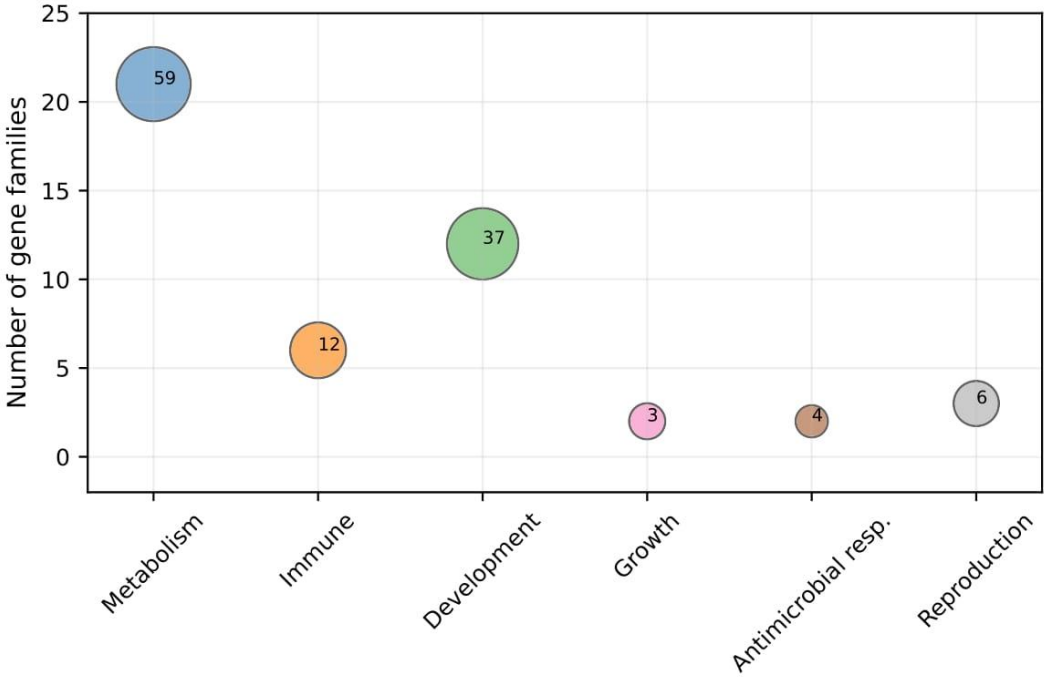


Figure 7. Gene family evolution analysis, including the number of gained (purple) and lost gene families (orange) for the Perciformes clade.

(a)



(b)

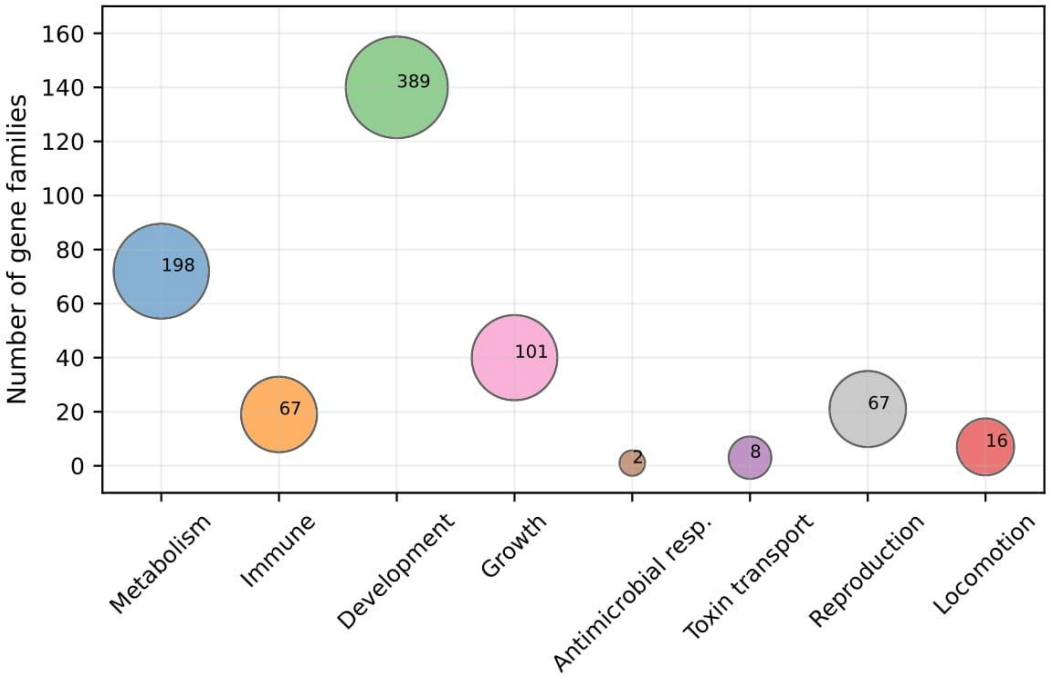


Figure 8. Number of gene families associated with specific biological processes for (a) rapidly expanding from CAFE, and (b) with duplications from GeneRax. The size of each circle is defined by the binary logarithm ($\log_2$) of the number of genes multiplied by the number of unique terms and then adding a scalar factor. The visualization of figures was performed with a custom python script "GO_plots.py".