

FishmiRNA: An Evolutionarily Supported MicroRNA Annotation and Expression Database for Ray-Finned Fishes

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Abstract

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression involved in countless biological processes and are widely studied across metazoans. Although miRNA research continues to grow, the large community of fish miRNA researchers lacks exhaustive resources consistent among species. To fill this gap, we developed FishmiRNA, an evolutionarily supported miRNA annotation and expression database for ray-finned fishes: www.fishmirna.org. The self-explanatory database contains detailed, manually curated miRNA annotations with orthology relationships rigorously established by sequence similarity and conserved syntenies, and expression data provided for each detected mature miRNA. In just few clicks, users can download the annotation and expression database in several convenient formats either in its entirety or a subset. Simple filters and Blast search options also permit the simultaneous exploration and visual comparison of expression data for up to any ten mature miRNAs across species and organs. FishmiRNA was specifically designed for ease of use to reach a wide audience.

Key words: teleost, Holostei, actinopterygian, noncoding RNA, whole-genome duplication, bowfin, *Amia calva*.

Introduction

MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators of gene expression that act by binding to the 3'-untranslated region of target messenger RNAs when incorporated into the RNA-induced silencing complex (Jonas and Izaurralde 2015; Bartel 2018). Regulatory functions of miRNAs have now been implicated in countless biological processes, including cell differentiation and proliferation, organ development and physiology, pathologies and diseases (Mendell and Olson 2012; Sun and Lai 2013), and genetic noise buffering, especially in stressful conditions (Schmiedel et al. 2015; Liufu et al. 2017). Furthermore, miRNAs evolve in lineage- and environment-specific manners and can modulate alternative developmental and physiological pathways that may influence adaptation, diversification, and speciation (Loh et al. 2011; Li and Zhang 2013; Quah et al. 2015).

Although several databases and bioinformatic tools facilitate the study of miRNAs in cellular, developmental, physiological, and pathological contexts, they cover mostly humans and main laboratory model organisms, so researchers studying other species often lack appropriate resources to accurately facilitate miRNA studies in their systems. This situation is exacerbated in ray-finned fishes, actinopterygians. Although several valuable miRNA annotation databases already exist, miRNA annotations in fish species are often unavailable,

incomplete, or inconsistent across fish phylogeny, inhibiting the study of miRNAs in half of all vertebrate species. The legacy database miRBase was created in 2006 but has not been updated since 2018 (Kozomara et al. 2019). miRBase contains partial miRNA annotations for several fish species, but none are exhaustive or curated and many new or improved annotations have not been incorporated. In 2015, MirGeneDB was created and covers a breadth of metazoan species, but this database provides miRNA annotation for only one fish species, zebrafish (Fromm et al. 2020). miRNAs, however, are of great interest in fish research with studies ranging from genomic evolution (Xiong et al. 2019; Desvignes et al. 2021), development (Giraldez et al. 2006; Kasper et al. 2017; Gay et al. 2018), medical models (Hsu et al. 2017), aquaculture (Herkenhoff et al. 2018; Blödorn et al. 2021; Cardona et al. 2021), pathology (Andreassen and Høyheim 2017; Wang et al. 2018), toxicology (Goodale et al. 2019; Ahkin Chin Tai and Freeman 2020), to adaptation, and speciation (Franchini et al. 2019; Kelley et al. 2021). In most fish miRNA studies, the lack of consistent annotation resources leads to using, by default, combinations of annotations existing in other, often distantly related species, or to de novo prediction of miRNAs using different algorithms in different research groups, leading to inconsistent results among species. Therefore, although miRNA research continues to

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grow rapidly, the large community of fish miRNA researchers is plagued by the lack of exhaustive and phylogenetically supported resources.

The complexity of studying miRNAs in fish emerges from two main sources. First, a whole-genome duplication, called the teleost genome duplication or TGD, initially duplicated every miRNA gene (Amores et al. 1998; Taylor et al. 2003; Braasch et al. 2016). This genome duplication was followed by lineage-specific gene resolution events that confound orthology assignment among species (Postlethwait 2007). Second, because fish represent more than half of living vertebrate species and inhabit virtually all aquatic habitats on the planet, they harbor dramatic variations in gene repertoires, which makes miRNA gene and mature miRNA computational predictions and annotations unreliable without expert manual curation.

To fill this resource gap, we developed FishmiRNA: An evolutionarily supported miRNA annotation and expression database for ray-finned fishes.

New Approaches

FishmiRNA integrates several new approaches that provide accurate, consistent, and exhaustive annotations of evolutionarily conserved miRNAs in ray-finned fishes, as well as the innovative ability to explore expression data sets across species and organs in a user-friendly interface.

Two novelties of FishmiRNA for achieving miRNA annotation consistency among species are the full integration of the TGD in gene orthology assignments and a broad phylogenetic context. For that purpose, we built on our recent establishment of genome-wide synteny-verified miRNA gene orthologies among several fish species, including the spotted gar *Lepisosteus oculatus* whose lineage, the Holostei, diverged before the TGD. This work allowed us to infer the miRNA gene repertoire of the hypothetical Teleost–Holostei last common ancestor (TH-LCA) (Desvignes et al. 2021). In addition, FishmiRNA annotations rely on small RNA sequencing expression data to first, identify miRNA gene loci and second, to detect the most abundantly expressed mature miRNAs, leading to data-supported annotation of both the 5p and 3p strands for the majority of genes (76% across the database). The novel annotation of four teleost species and of the bowfin, *Amia calva*, a second holostean outgroup to the teleosts (Thompson et al. 2021), further increased confidence in the inferred TH-LCA miRNA gene repertoire and in intermediate teleost ancestors. This ancestral reconstruction approach, a cornerstone of the FishmiRNA database, allows the retracing of gene evolution across lineages. Each miRNA gene annotated in FishmiRNA is thus linked to its orthologs among other teleosts and with the TH-LCA. So far, FishmiRNA contains miRNA gene and mature miRNA annotations for 10 actinopterygian species, including eight teleost species (zebrafish, catfish, panga, medaka, molly, perch, stickleback, and icefish) and two Holostei species that diverged before the TGD (gar and bowfin) (table 1). These species were selected based on their broad phylogenetic distribution within ray-finned fishes, on their importance in evolutionary,

aquacultural, and biomedical research, and on the availability of high-quality genome sequences.

A unique approach of FishmiRNA unavailable in any other annotation database is to provide expression data for mature miRNAs. The consistent re-analysis of expression data for each species using the smallRNA-seq software *Prost!* (Desvignes et al. 2019) coupled with a graphical module enables users to compare the expression patterns of up to ten miRNAs from any species in the database, thus offering an innovative opportunity to incorporate in a study the evolutionary conservation of expression of any miRNA across species.

Finally, an important novel approach of FishmiRNA is the simplicity and efficient design of its user-friendly website. Contrary to other databases that incorporate hundreds of webpages, FishmiRNA database relies on a single webpage and two spreadsheets: an annotation table and an expression table, both of which can be interactively and quickly filtered, searched, and exported, in full or in part. This novel approach eases access to miRNA annotations and miRNA expression data and significantly increases the diversity of users reached by providing accessibility to miRNA data to anyone, with or without bioinformatic skills.

Database Features

Graphic Information

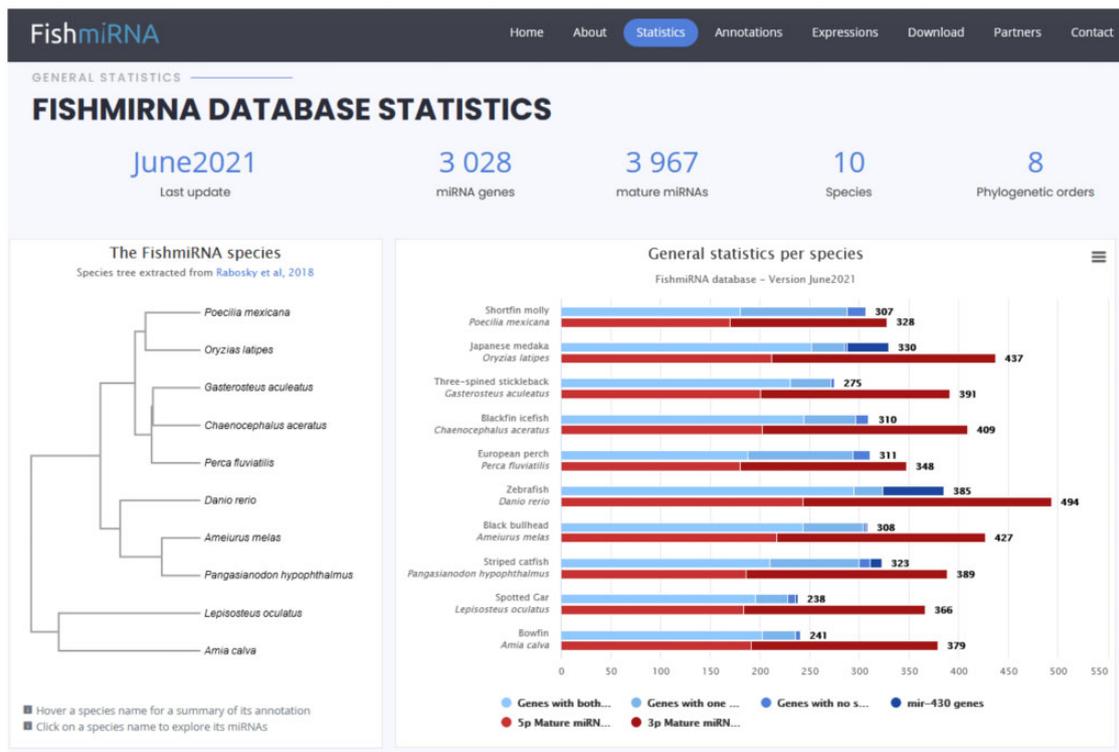
A summary of the annotation database is displayed at the top of the page in a phylogenetic context (Rabosky et al. 2018), including graphical representations of general statistics for each species (fig. 1). These immediate visual representations help users determine which species' annotation would be the most appropriate to apply to their experimental question or species of interest. For example, if FishmiRNA lacks a species, a user might select the phylogenetically most closely related species with the largest miRNA annotation. The phylogenetic coverage of FishmiRNA will grow as new species become annotated.

The FishmiRNA Annotation Database

The FishmiRNA annotation database consists of a single table containing 38 columns and as many lines as there are miRNA genes in the database, currently 3,028 genes. Four columns present the taxonomy of the species and its reference genome assembly; six columns describe the miRNA gene with a name, potential previous names, a unique gene identifier, links to miRBase, Ensembl, and to other databases when available; six columns describe the miRNA hairpin with a name, its sequence, position, and strand in the genome assembly; two columns report gene orthology among teleosts and with the TH-LCA; three columns inform on gene clustering; one column summarizes 5p and 3p strand annotations, each detailed in eight columns providing the mature strand name, a unique mature identifier, a reference sequence, its position in the genome, and whether this mature miRNA can be produced by more than one miRNA gene. All of this information can be simultaneously visualized for each gene in an “individual gene ID card” by clicking on the magnifier button associated with the miRNA gene name in the annotation table (fig. 2).

Table 1. Summary of Annotations and Sequencing Data Included in FishmiRNA.

Species	Genome Assembly	Annotation Citation	Sequencing Data NCBI Accession No.	Expression Data Citation
Spotted gar, <i>Lepisosteus oculatus</i>	LepOcu1 (GCA_000242695.1)	Braasch et al. (2016)	PRJNA296503	Braasch et al. (2016)
Bowfin, <i>Amia calva</i>	AmiCal1 (GCA_017591415.1)	Present study	PRJNA255850	Present study
Black bullhead, <i>Ameiurus melas</i>	AMELA_1.0 (GCA_012411365.1)	Present study	PRJNA730692	Present study
Striped catfish, <i>Pangasianodon hypophthalmus</i>	GENO_Phyp_1.0 (GCF_009078355.1)	Present study	PRJNA256963	Present study
Zebrafish, <i>Danio rerio</i>	GRCz11 (GCA_000002035.4)	Desvignes et al. (2014)	PRJNA240316	Desvignes et al. (2014)
Japanese medaka, <i>Oryzias latipes</i>	ASM223467v1 (GCA_002234675.1)	Desvignes et al. (2021)	PRJNA477647	Gay et al. (2018)
Molly, <i>Poecilia mexicana</i>	P_mexicana-1.0 (GCA_001443325.1)	Kelley et al. (2021)	PRJNA471100	Kelley et al. (2021)
European perch, <i>Perca flavescens</i>	GENO_Pfluv_1.0 (GCA_010015445.1)	Present study	PRJNA256973	Present study
Three-spined stickleback, <i>Gasterosteus aculeatus</i>	BROAD S1	Desvignes et al. (2019)	PRJNA486149	Desvignes et al. (2019)
Blasckfin icefish, <i>Chaenocephalus aceratus</i>	cace 20180227a pilon1	Kim et al. (2019)	PRJNA310135	Desvignes et al. (2016)

**Fig. 1.** General statistics of the FishmiRNA database. Screen shot of the home page. On the left, the species present in FishmiRNA database are displayed in their phylogenetic context ([Rabosky et al. 2018](#)). On the right, the miRNA gene (in blue) and mature miRNA (in red) annotation statistics are given for each species.

For rendering purposes, only nine columns are displayed by default, but users can select their own column preferences or display the entire table ([fig. 2](#)). Users can also filter the database by species, by FishmiRNA Gene ID, hairpin and mature miRNA names, and by orthology relationships among teleosts and with the TH-LCA. In addition, users can search the database by genomic location or

by a Blast search of sequences on hairpins or mature miRNA sequences ([fig. 2](#)). The entirety or filtered parts of the annotation database can be immediately exported as a spreadsheet or a GFF file. Hairpin and mature miRNA sequences can also be exported in a click in FASTA format for ready use with smallRNA-seq analysis software or other purposes ([fig. 2](#)).

EXPLORE FISHMIRNA DATABASE

Show/Hide columns
Click on the table below by selecting column name(s)

Search
Filter by source and/or by position
Search by BLAST against hairpin and/or mature sequences

Export
For the filtered row(s) export data, sequences or annotations

9 items selected
Number of visible columns: 9/38.
All | Reset
Remove all filters
Remove all filters in the search box.

By source | By position | By BLAST

Species: Nothing selected | Species: Nothing selected
Select species common name(s). | Select species scientific name(s).

FishmiRNA:
Filter by FishmiRNA Gene ID.

Hairpin: mir-144 | Mature:
Filter by hairpin name. | Filter by mature miRNA name.

Teleost: | TH-LCA*:
Filter by orthology among teleosts. | Filter by orthology with the TH LCA*.

Copy | Excel | CSV
The data.
Hairpin
A FASTA file of the hairpin sequence(s).
All | 5p | 3p
A FASTA file of the mature sequence(s).
GFF
A GFF file of the miRNA(s).

Show: 10 entries

	Species common name	Species scientific name	Hairpin name	Orthology among teleosts	Orthology with Teleost-Holostei LCA	5p miRNA name	5p miRNA sequence	3p miRNA name	3p miRNA sequence
<input type="checkbox"/>	Black bullhead	<i>Ameiurus melas</i>	ame-mir-144	mir144	TH-LCA-mir144	ame-miR-144-5p	GGATATCATATACTGTAAGT	ame-miR-144-3p	CTACAGTATAGATGATGACT
<input checked="" type="checkbox"/>	Blackfin icefish	<i>Chaenocephalus aceratus</i>	cac-mir-144	mir144	TH-LCA-mir144	cac-miR-144-5p	AGGATATCATCTTACTGTA	cac-miR-144-3p	CTACAGTATAGATGATGAC
<input type="checkbox"/>	Bowfin	<i>Amia calva</i>	aca-mir-144	mir144	TH-LCA-mir144	aca-miR-144-5p	GGATATCATATACTGTAAGT	aca-miR-144-3p	TACAGTATAGATGATGACT
<input type="checkbox"/>	European perch	<i>Perca fluviatilis</i>	pfl-mir-144	mir144	TH-LCA-mir144	pfl-miR-144-5p	GGATATCATCTTACTGTAAGT	pfl-miR-144-3p	CTACAGTATAGATGATGAC
<input checked="" type="checkbox"/>	Japanese medaka	<i>Oryzias latipes</i>	ola-mir-144	mir144	TH-LCA-mir144	ola-miR-144-5p	GGATATCATCTTACTGTAAGT	ola-miR-144-3p	CTACAGTATAGATGATGACT
<input type="checkbox"/>	Shortfin Molly	<i>Poecilia mexicana</i>	pme-mir-144	mir144	TH-LCA-mir144	pme-miR-144-5p	GGATATCATCTTACTGTAAG	pme-miR-144-3p	CTACAGTATAGATGATGACT
<input checked="" type="checkbox"/>	Spotted Gar	<i>Lepisosteus oculatus</i>	loc-mir-144	mir144	TH-LCA-mir144	loc-miR-144-5p	GGATATCATATACTGTAAGT	loc-miR-144-3p	CTACAGTATAGATGATGAC
<input type="checkbox"/>	Striped catfish	<i>Pangasianodon hypophthalmus</i>	phy-mir-144	mir144	TH-LCA-mir144	phy-miR-144-5p	GGATATCATATACTGTAAGT	phy-miR-144-3p	TACAGTATAGATGATGACT
<input type="checkbox"/>	Three-spined stickleback	<i>Gasterosteus aculeatus</i>	gac-mir-144	mir144	TH-LCA-mir144	gac-miR-144-5p	GGATATCATCTTACTGTAAGT	gac-miR-144-3p	CTACAGTATAGATGATGAC
<input checked="" type="checkbox"/>	Zebrafish	<i>Danio rerio</i>	dre-mir-144	mir144	TH-LCA-mir144	dre-miR-144-5p	GGATATCATCGTACTGTAAGT	dre-miR-144-3p	CTACAGTATAGATGATGAC

Showing 10 of 10 entries (filtered from 3,028 total entries) 4 rows selected

Select all | Deselect all | For the selected row(s): Explore expression

Display the gene ID Card

Select miRNA genes and explore the expression of their mature products

Fig. 2. Exploring the FishmiRNA Annotation database. Screen shot of the Annotation section of the database. Users can choose to add or remove columns in the display and can filter and search the database by species, miRNA gene and mature miRNA names, and orthology among species. The entire annotation database, or subsets of it, can be exported in various convenient formats. The magnifier icon opens the corresponding gene identification card that contains all the FishmiRNA information related to this gene. Selecting genes using tick boxes on the left allows the filtering of the FishmiRNA expression database for mature products of selected genes.

The filtering of miRNA genes reduces the portion of the annotation table displayed to only genes of interest, which can then be individually selected using tick boxes to conveniently filter their respective mature miRNAs and explore their expression patterns in a single click (fig. 2).

The FishmiRNA Expression Database

The FishmiRNA expression database consists of a single table containing 23 columns and as many lines as mature miRNAs detected in the smallRNA-seq libraries analyzed. Three columns provide information on the mature miRNA name, its

unique mature identifier, and the sequence of the most highly expressed isoform; and ten columns that contain the raw counts and ten columns that contain normalized read counts (reads per million, RPM) for each mature miRNA in a selection of major organs (brain, gills, heart ventricle, skeletal muscle, intestine, liver, ovary, testis, hematopoietic kidney, and spleen). Not all species have expression data for all of the selected organs; the entry “No_Data” signals these cases. For rendering purposes, only the mature miRNA name, its sequence, and normalized read counts are displayed by default. Similar to the annotation table, users can configure

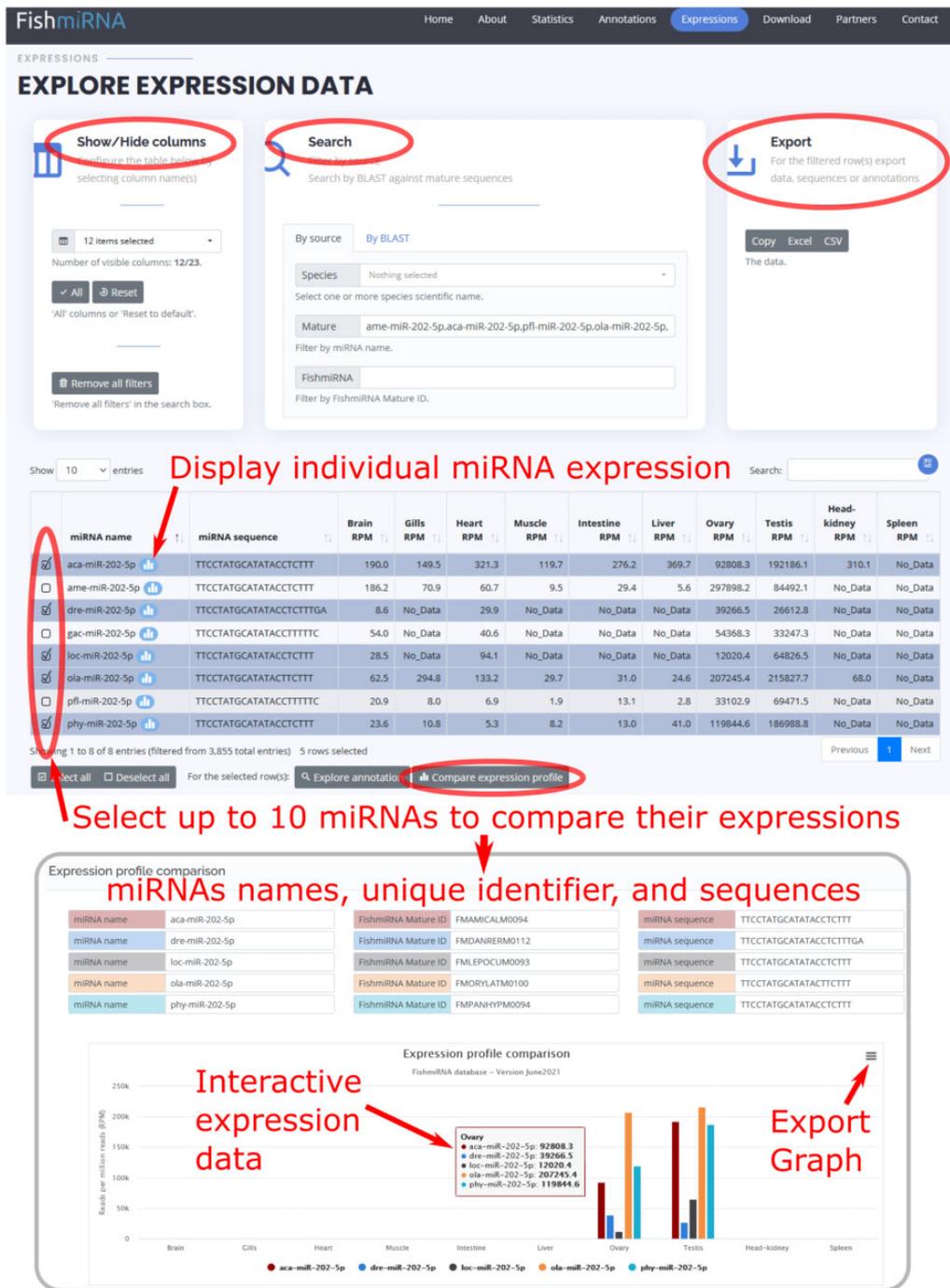


Fig. 3. Exploring the FishmiRNA Expression database. Screenshot of the Expression section of the database. Like the Annotation section, users can choose to add or remove columns in the display and can filter and search the database by species, mature miRNA name, and unique identifier. The entire expression database, or subsets of it, can also be exported in convenient table formats. The histogram icon displays the corresponding mature miRNA expression pattern in a variety of organs. Users can also select up to any 10 mature miRNAs using tick boxes on the left to display their expression patterns on the same graph.

columns shown in the expression table, filter by species, mature miRNA name, unique identifier, or explore expression data by Blast. The entire expression database or filtered parts of it can also be immediately exported (fig. 3).

One of the most useful features of the FishmiRNA expression database is its graphical module. The expression profile of each mature miRNA can be visualized by a click on the blue

histogram button associated with its name (fig. 3). This link opens a modal box displaying expression data in a histogram with the organ of highest expression in red and the mean expression across organs marked by a horizontal red line. Furthermore, by selecting mature miRNAs using tick boxes next to the mature miRNA name, users can instantly plot on the same graph the expression profiles of up to any ten

Fig. 4. Downloads and links. Screenshot of the Download section of the database. Quick links for downloading, per species or for all species, annotations files in FASTA format and raw output files from the smallRNA-seq software *Prost!* used for the annotation and expression analyses. Links to the publication of each original annotation are also provided along with links to the NCBI Project of the expression data analyzed and the article that published these original data.

mature miRNAs in the database. All graphs generated can also be exported in various image formats using the drop-down menu located at the top-right corner of the graph (fig. 3).

Quick Download Links and Origin of Analyzed Data

To facilitate the dissemination, re-use, and transparency of data provided in FishmiRNA, the download section of the FishmiRNA database provides quick links for downloading, per species or for all species, annotation files in FASTA format, and raw output files from the smallRNA-seq software *Prost!* used for the annotation and expression analyses (Desvignes et al. 2019) (fig. 4). Furthermore, links to the publication of each original annotation are provided along with links to the NCBI BioProject of the expression data analyzed and the article that published these original data (fig. 4).

Materials and Methods

Small RNA sequencing data presented in FishmiRNA are all publicly available in NCBI (table 1). Organ sampling and library preparation protocols as well as sequencing platforms may differ between species and therefore expression patterns may not be fully comparable. All species were, however, re-analyzed the same way and for each species, Illumina sequencing libraries were simultaneously analyzed using *Prost!* (Desvignes et al. 2019), selecting for read length from 17 to 25 nucleotides with a minimum of five identical reads. Publicly available genome assemblies were used for each species (table 1). Gene and mature miRNA annotations were performed as described previously (Desvignes et al. 2019), using orthology and ohnology relationships established across species (Desvignes et al. 2021), and following nomenclature rules established for zebrafish (Desvignes et al. 2015, 2020;

Ruzicka et al. 2019). FishmiRNA was developed based on the RumimiR web interface (Bourdon et al. 2019).

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Author Contributions

Study concept and design: T.D., J.H.P., and J.B. Acquisition of data: T.D., S.G., and J.B. Analysis and interpretation of data: T.D., J.M., J.S., C.G., J.H.P., and J.B. Web interface development: P.B. Wrote the manuscript: T.D. and J.B. Critical revision of the manuscript: T.D., P.B., C.G., J.H.P., and J.B. Obtained funding: J.H.P., T.D., P.B., and J.B. Study supervision: T.D., J.H.P., and J.B.

Data Availability

All data generated or analyzed during this study are included in the published article, in the FishmiRNA database, and/or deposited in NCBI.

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