Golestan University of Medical Sciences
Journal of Clinical and Basic Research
Online ISSN: 2538-3736

# Metavirome survey of eight ray-finned fishes: Domination of giant viral members from pandoravirus and megaviricetes

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## Abstract

**Background:** Fish are an essential source of food worldwide. Most microbial diversity in marine ecosystems remains uncharacterized, with viruses accounting for most of the remaining diversity. This poses a potential major threat to public health, making it critical to understand the variety of viruses in marine ecosystems. Our objective was to assess the virome of ray-finned fishes in terms of diversity and its potential relation to human diseases.

**Methods:** Metagenomic data from a BioProject on ray-finned fishes, with the accession number PRJNA493014 containing eight Sequence Read Archive (SRA) experiments, were retrieved from the SRA. Sequencing data were trimmed and assembled using Unicycler. Reads from each sequencing run were mapped to the reference using Kraken. Unassigned viral reads from the genomes of the eight ray-finned fish species were used to find giant viral fingerprints.

**Results:** We found 2,228,888 sequence reads among ray-finned fishes representing viral kingdom fingerprints. The pooled genome assay of ray-finned fishes revealed a significant abundance of viruses in three clades: the genus *Pandoravirus* (31%), the order *Herpesvirales* (23%), and the kingdom *Bamfordvirae* (10%). Additionally, 432,281 out of 2,031,445 reads (21%) remained unassigned as viruses. According to findings from GiantVirusFinder, 17,931 hits (4.15%) were mapped to the genomes of known giant viruses.

**Conclusion**: Considering that humans regularly consume seafood as a primary part of their diet, it is essential to note that ray-finned fishes serve as hosts to various giant DNA viruses with extensive evolutionary histories. As demonstrated here, there is a need to screen fish for viral infections that may be linked to human illnesses.

**Article History**

Received: 11 May 2024
Received in revised form: 4 August 2024
Accepted: 10 September 2024
Published online: 18 September 2024
DOI: 10.29252/JCBR.8.3.14

**Keywords**

Skates, Fish
Giant viruses
Virome
Metagenome

**Article Type:** Original Article

OPEN ACCESS
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## Highlights

**What is current knowledge?**

The virome of marine ecosystems, particularly in fish, remains largely unexplored, despite viruses being the most abundant organisms on Earth. Previous studies have shown the high viral diversity in aquatic environments, with viruses playing crucial roles in nutrient and carbon cycles. Marine viral communities, including those in ray-finned fishes, feature viral families such as *Pandoravirus, Herpesvirales*, and *Bamfordvirae*. Some fish species have been identified as hosts for viruses linked to human diseases, indicating potential for cross-species viral transmission.

**What is new here?**

This study presents a detailed metavirome analysis of eight ray-finned fish species, revealing a significant prevalence of giant viruses, including members of *Pandoravirus* and Megaviricetes. It highlights the discovery of extensive viral diversity in ray-finned fishes, with over 4% of viral reads mapped to known giant viruses. This suggests that the fish virome may act as a reservoir for potentially new viruses, with implications for human health, as these species are consumed regularly. The study introduces the need to investigate the impact of giant viruses on fish populations and their possible links to human diseases.

## Introduction

Fish represent one of the major sources of food worldwide. Aquatic animal species in marine ecosystems are exposed to a wide variety of microorganisms (1). Viruses, as the most abundant organisms on Earth (1,2), significantly influence the nutrient and carbon cycles in aquatic environments (3). A study conducted by Breitbart et al. in 2002 on two uncultured marine viral communities revealed that over 65% of the microbial diversity in aquatic communities is uncharacterized, with most of the remaining diversity comprising viruses. Furthermore, the most abundant viral genome constitutes only 2-3% of the total population in marine communities, indicating high viral diversity (4).

Phylogenetic classification has identified approximately 72 orders (And 79 suborders) and 514 families of bony fish (5). Calculations based on fish population density have shown that some fish shoals can consist of nearly three billion fish in a single school, making this an exceptionally dense population among vertebrates. Consequently, this large number of fish can harbor an increasing number of viruses compared to other vertebrates. Viruses are horizontally transmitted between fish, often through contaminated feces (6). A meta-transcriptomics study conducted by Geoghegan et al. in 2018 on four fish species demonstrated that densely shoaling fish had the highest number of viruses compared to less social fish species. Interestingly, identifying the number of potential novel viruses in fish, as previously detected in mammals and birds, may indicate cross-species transmission (7), which can occur through the horizontal transmission of viruses by birds or other fish predators.

*Actinopterygii*, known as ray-finned fishes, encompasses more than 32,000 species (5) and represents nearly half of the living vertebrate species within the Neopterygii (Modern ray-fins) sub-class. Neopterygii is the most species-rich clade of bony fishes (>99.9%) (8,9) and includes members such as Ginglymodi, Halecomorphi, and Teleostei. Teleostei, with the most species, is the dominant group of modern Actinopterygians. Non-teleost Actinopterygians are often called living fossils (8). Ray-finned fishes exhibit a remarkable ability to adapt to different aquatic environments, including deep ocean trenches, high mountain streams, and extreme habitats with conditions like acidity, subzero temperatures, hypersalinity, hypoxia, temporariness, and fast-flowing water, with a history dating back over 400 million years (10). It is worth noting that, in addition to their lengthy evolutionary history, ray-finned fishes are crucial for human food consumption. In 2010, it was estimated that 80.6% of fish catches were attributed to this group (11).

The extensive diversity of ray-finned fishes makes them a potential source of viral species. The presence of billions of viral particles per milliliter of ocean water, along with the significant role of viruses in causing mortality in aquatic environments, highlights their capacity to influence population structures in the ocean (12,13). Fish, being a primary source of human food, are in direct contact with ocean water and can carry various potentially new and emerging viruses (14-17). Viral populations can lead to numerous infections in marine environments, emphasizing the importance of studying the presence of viruses in fish (Suttle, 2007). A valuable approach for assessing the diversity and richness of environmental virus samples is metagenomics (2), which conducts a culture-independent analysis to survey the genetic content of all microbial organisms in an environmental sample using biotechnological and bioinformatics methods. Additionally, metavirome evaluation assesses the metagenome of the entire viral population in a single sample (18,19).

The primary aim of this study was to investigate the genome fingerprints of viral genomes that could potentially pose a threat to public health. Furthermore, we discuss the possible evolutionary role of viral diversity in marine ecosystems and its impact on the emergence of viral outbreaks.

[ DOI: 10.29252/JCBR.8.3.14 ]

## Methods

### Recovery of metagenomics resource

Metadata for the metagenome were retrieved from the Sequence Read Archive (SRA) (20) using the Galaxy server (21), as previously described (22). The data were obtained from a BioProject focused on ray-finned fishes with the accession number PRJNA493014, which included eight SRA experiments. The SRA accession numbers were as follows: SRR7903826 for *Acipenser schrenckii* (Amur sturgeon), SRR7903827 for *Odontobutis haifengensis*, SRR7903828 for *Odontobutis yaluensis* (Dark sleeper), SRR7903829 for *Rhinogobius giurinus*, SRR7903830 for *Hiodon tergisus* (Mooneye), SRR7903831 for *Polyodon spathula* (Paddlefish), SRR7903832 for *Denticeps clupeoides* (Denticle herring), and SRR7903833 for *Rutilus rutilus* (Common roach).

### Assembly and quality control of raw FASTQ files

Illumina sequencing files were processed to remove chimeric sequences, correct barcode errors, and eliminate duplications using the Trimmomatic tool (23). Single-ended SRA entries containing FASTQ files were assembled using Unicycler (24) with the default package settings. Contig quality was assessed using the Quality Assessment Tool (25).

### Metavirome classification and taxonomic affiliation

Reads from each sequencing run were aligned to genomic sequences in the viral database using the Kraken taxonomic assignment tool (26). The number of reads mapped to the reference was counted using the same Kraken tool. Additionally, classified data were visualized using Krona (27,28). The root of the chart was set as viral families, and the distribution of each viral taxon was estimated based on the depth of sequence reads.

### Mapping unidentified sequence reads to the giant viral database

A pooled set of unassigned reads from the genomes of all eight ray-finned fish species was used to identify giant viral fingerprints. The GiantVirusFinder package was employed to identify the number of sequence reads that matched the genomes of 288 known giant viruses (29). The package supports a fast-greedy alignment algorithm for identifying hits to giant viruses (30). The source code was modified to support blast version 2.8.1+, and the expected threshold was adjusted to 0.1.

## Results

### Metavirome survey

This study investigated metagenomic data from eight ray-finned fish species for their virome. Overall, 2,228,888 sequence reads were assigned to the viral kingdom. A pooled genome assay revealed a high abundance of *Pandoravirus* (31%), *Herpesvirales* (23%), and *Bamfordvirae* (10%) among all virus kingdoms. The following sections will present the virome of each ray-finned fish. The study results presented here are taxonomically assigned. For some groups of viruses that lack supergroup classifications like class, order, or family, we have defined them in their respective sections. Consequently, the genus *Pandoravirus*, the order *Herpesvirales*, the family *Polydnavirus*, and the kingdom *Bamfordvirae* are the root classifications for their respective clades, as shown in Figure 1.

### Diversity of viral species in *Acipenser Schrenckii*

According to viral taxonomy, the metagenomics data of *Acipenser schrenckii* comprised 464,973 classified reads and 91,977 unclassified reads. *A. schrenckii* included three main clades: *Pandoravirus*, *Herpesvirales*, and *Bamfordvirae*, accounting for 5% of the total virome. As illustrated in Figure 1, the genus *Pandoravirus* made up 34% of all viruses, followed by *Herpesvirales* at 28%. The most prevalent Pandoraviral species were *P. malecodensis* (28%), *P. dulcis* (24%), *P. neocaledonia* (10%), *P. salinus* (9%), *P. quercus* (4%), and *P. inopinatum* (2%). Unclassified *Pandoravirus* species constituted 24% of this lineage (Figure 1).

The order *Herpesvirales*, with 131,119 reads, accounted for 28% of *A. schrenckii*'s virome. In addition to *Alloherpesviridae*, the *Herpesviridae* family was represented by some members of human herpesviruses. Specifically, the subfamily *Betaherpesvirinae* (3% of all viruses) included Roseolovirus (41% of *Betaherpesvirinae*) and Cytomegalovirus (22% of *Betaherpesvirinae*), both responsible for known human-associated viral pathogens. Other human-associated viruses from the family *Herpesviridae* included *Human alphaherpesvirus 2* (1%) within the subfamily *Alphaherpesvirinae*, *Human betaherpesvirus 7* (5%), *Human betaherpesvirus 6B* (1%), and *Human betaherpesvirus 6A* (0.1%) within *Betaherpesvirinae*, and *Human gammaherpesvirus 8* (0.4%) belonging to the *Gammaherpesvirinae* subfamily.

The kingdom *Bamfordvirae* and its order *Nucleocytoviricota* comprised two major clades of large viral families: *Megaviricetes* (65%) and *Poxviridae* (26%). The class *Megaviricetes* included three families, namely *Phycodnaviridae* (71%), *Mimiviridae* (20%), and *Pimascovirales* (9%). The family *Mimiviridae* encompassed two genera, *Mimivirus* (24%) and *Cafeteriavirus* (11%), along with five different species: *Moumouvirus* (58%), *Megavirus chiliensis* (22%), *Acanthamoeba polyphaga mimivirus* (2%), *Cafeteria roenbergensis virus* (11%), and *Yellowstone lake mimivirus* (5%).

### Viral population of *Odontobutis haifengensis*

In the virome of *O. haifengnis*, the most abundant viral orders were *Herpesvirales* (19%), *Pandoravirus* (17%), *Orthornavirae* (10%), and *Bamfordvirae* (10%). The family *Herpesviridae* (14%) constituted 74% of the order *Herpesvirales*. Within the *Alphaherpesvirinae* subfamily, *Human alphaherpesvirus 2* accounted for a mere 0.05% of the viral population. The *Betaherpesvirinae* subfamily was represented by *Human betaherpesvirus 6B* (0.6% of all viral sequence reads) and *Human betaherpesvirus 7* (0.1%).

As depicted in Figure 2, the giant *Pandoravirus* was predominantly composed of *P. salinus* (37%), *P. macleodensis* (22%), *P. dulcis* (12%), *P. inopinatum* (6%), *P. neocaledonia* (5%), and *P. quercus* (3%). Additionally, 1,773 unassigned reads were attributed to other *Pandoravirus* species, accounting for 16% of this category.

The order *Orthornavirae*, with 6,542 reads, consisted of two main sub-orders: *Kitrinoviricota* (40%) and *Pisuviricota* (38%). The majority of viral species in these sub-orders were associated with plant and marine viral species. Notably, the *Flaviviridae* family (45%) within the *Kitrinoviricota* sub-order included major human viral pathogens, namely *Hepacivirus* (63%) and *Tick-borne encephalitis virus* (19%).

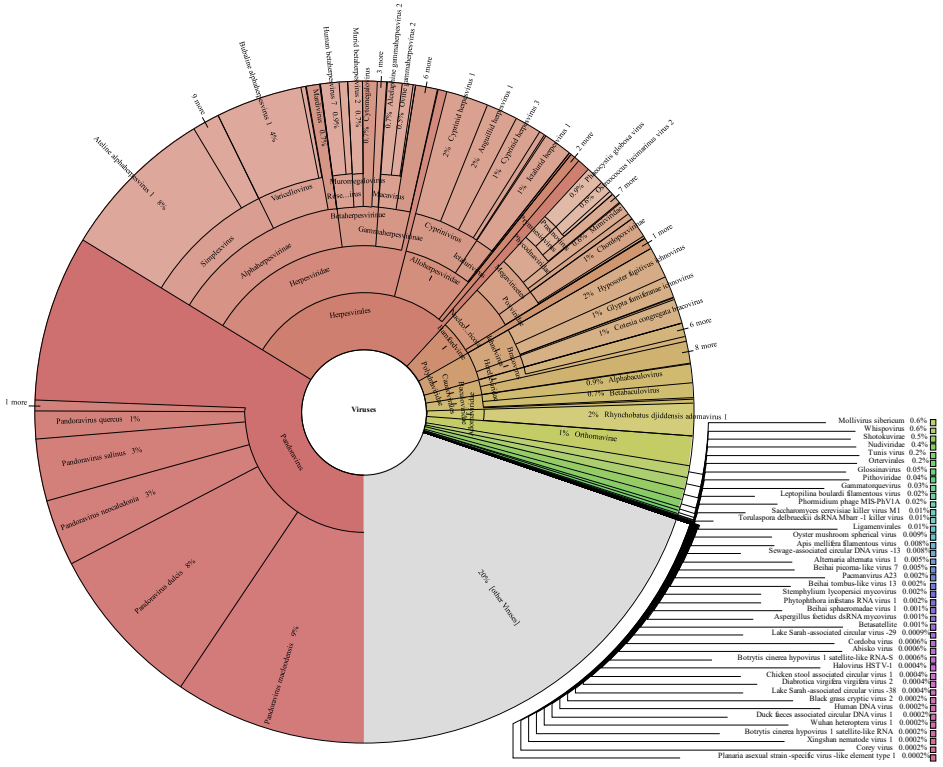


**Figure 1.** The taxonomic plot of acipenser schrenckii virome

[ DOI: 10.29252/JCBR.8.3.14 ]

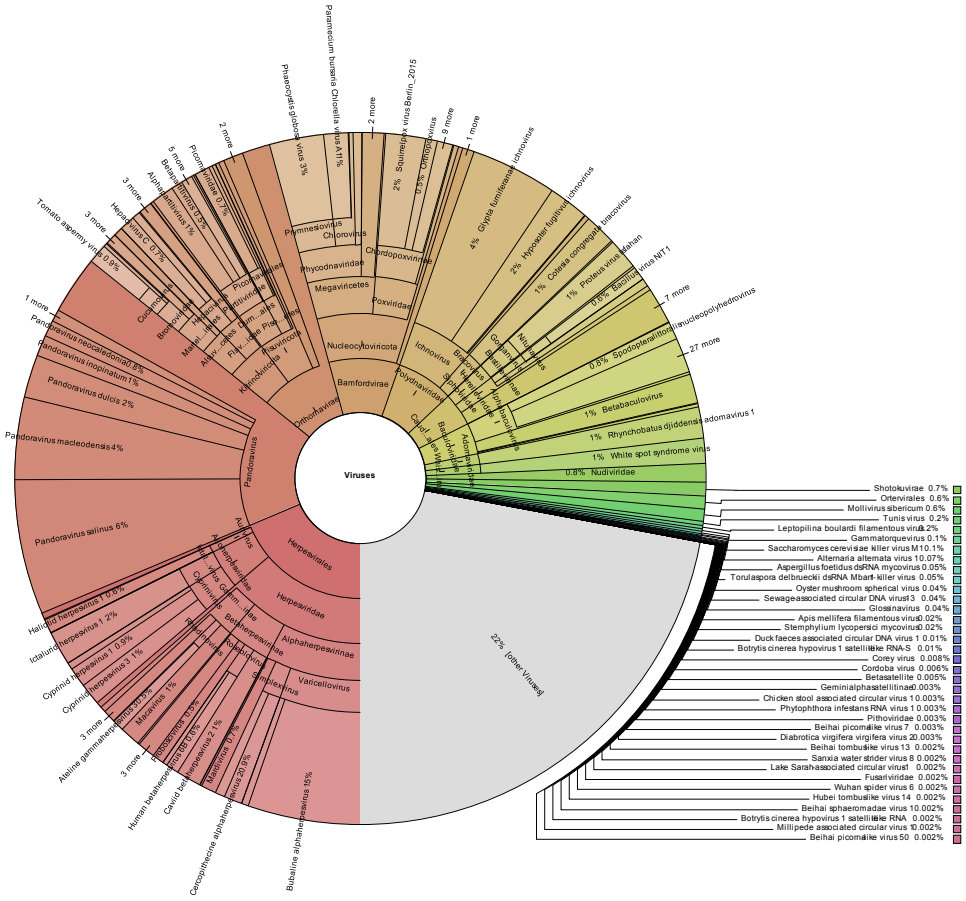


**Figure 2.** The taxonomic plot of odontobutis haifengensis virome

**Virome of *Odontobutis yaluensis***

The virome of *O. yaluensis* included three main viral orders: *Herpesvirales* (24%), *Bamfordvirae* (15%), and *Pandoravirus* (8%). Additional orders comprised *Baculoviridae* (6%) and *Orthornavirae* (5%). The *Herpesvirales* encompassed three major families: *Herpesviridae* (79%), *Alloherpesviridae* (15%), and *Malacoherpesviridae* (5%). Within the *Herpesviridae* family, the viruses *Human alphaherpesvirus 3* and *Human betaherpesvirus 6B* were found in the *Alphaherpesvirinae* and *Betaherpesvirinae* sub-families, respectively (Figure 3).

The sub-order *Nucleocytoviricota* within *Bamfordvirae* included two prominent families: avian *Poxviridae* and giant viruses of *Megaviricetes*. The *Mimiviridae* family accounted for 5% of *Bamfordvirae* and consisted of *Mimivirus* (59%), *Moumouvirus* (35%), and *Cafeteriavirus* (5%). The genus *Mimivirus* was represented solely by *Megavirus chiliensis*.

Further investigation into giant viruses was conducted within the *Pandoravirus* taxonomy. Recognized species included *P. macleodensis* (30%), *P. salinus* (27%), *P. dulcis* (14%), *P. inopinatum* (5%), *P. neocaledonia* (5%), and *P. quercus* (3%), while the remaining 16% of the taxonomy represented other Pandoraviruses (Figure 3).

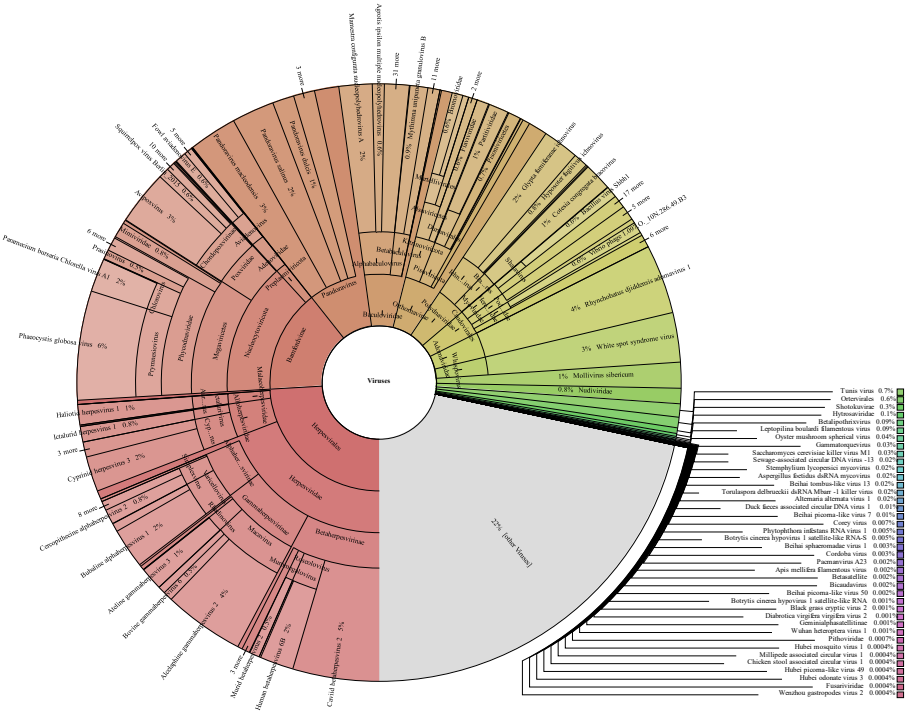


**Figure 3**. The taxonomic plot of odontobutis yaluensis virome

[ DOI: 10.29252/JCBR.8.3.14 ]

### Distribution of the viral population in *Rhinogobius giurinus*

In the virome of *R. giurinus*, 59% of the metagenomics data comprised three major orders: *Pandoravirus* (24%), *Herpesvirales* (20%), and *Bamfordvirae* (15%). Other viral taxa included *Polydnaviridae* (5%), *Baculoviridae* (3%), and *Caudovirales* (3%). Additional virus-associated sequence reads can be found in Figure 4.

The genus *Pandoravirus* consisted of *P. dulcis* (38%), *P. macleodensis* (20%), *P. salinus* (9%), *P. quercus* (9%), *P. neocaledonia* (7%), and *P. inopinatum* (3%). Furthermore, Pandoraviruses made up 15% of this giant virus category. *Herpesviridae* comprised 79% of the *Herpesvirales* order, with *Alphaherpesvirinae* containing *Human alphaherpesvirus two* and *Human alphaherpesvirus 3*. The *Betaherpesvirinae* subfamily included *Roseolovirus*, *Human betaherpesvirus 6B*, *Human betaherpesvirus 6A*, and *Human betaherpesvirus 7*. Reads associated with *Human gammaherpesvirus 4* and *Human gammaherpesvirus 8* were observed in the *Gammaherpesvirinae* subfamily (Figure 4).

*Bamfordvirae* contained two main superorders, *Nucleocytoviricota* (85%) and *Preplasmiviricota* (15%). Similar to *Preplasmiviricota*, *Poxviridae* within *Nucleocytoviricota* consisted of avian viruses. *Megaviricetes* within *Nucleocytoviricota* included three major groups of giant viruses, namely *Phycodnaviridae* (89%), *Mimiviridae* (5%), and *Pimascovirales* (5%). *Phycodnaviridae* consisted of several species, including *Phaeovirus* (23%), *Prymnesiovirus* (19%), *Prasinovirus* (9%), *Raphidovirus* (7%), *Chlorovirus* (1%), *Chrysochromulina ericina virus* (0.7%), *Aureococcus anophagefferens virus* (0.7%), and *Orpheovirus IHUMI-LCC2* (0.3%). The species in the *Mimiviridae* family were as follows: *Moumouvirus* (53%), *M. chiliensis* (24%), *Cafeteria roenbergensis virus* (21%), and *Yellowstone lake mimivirus* (1%). In addition, the order of *Pimascovirales* included the *Iridoviridae* (65%) and *Ascovirus* (35%) families.

### Diversity of the virome of *Hiodon tergisus*

The virome analysis of *H. tergisus* showed a distinct distribution of viral taxonomy compared to other ray-finned fishes. The sequence reads rate was significantly different, with *Pandoravirus* accounting for 41% of the viruses in *H. tergisus*. *Herpesvirales* constituted 18%, *Polydnaviridae* 11%, and *Bamfordvirae* 4% of the prevalent viral clades in the taxonomy assignments (Figure 5).

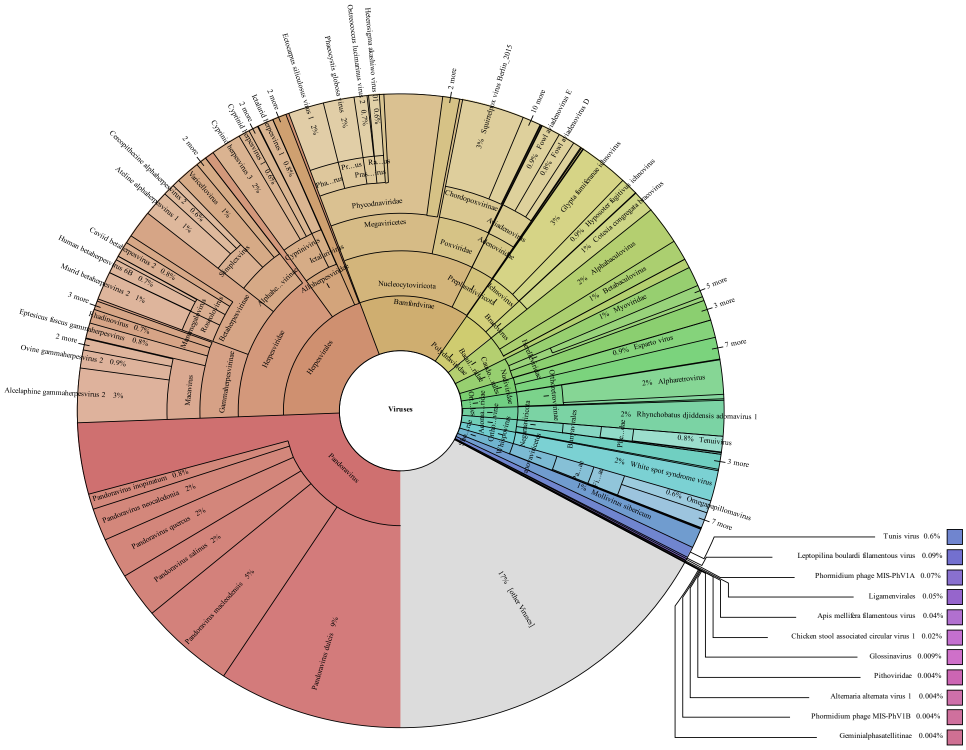


**Figure 4.** The taxonomic plot of rhinogobius giurinus virome

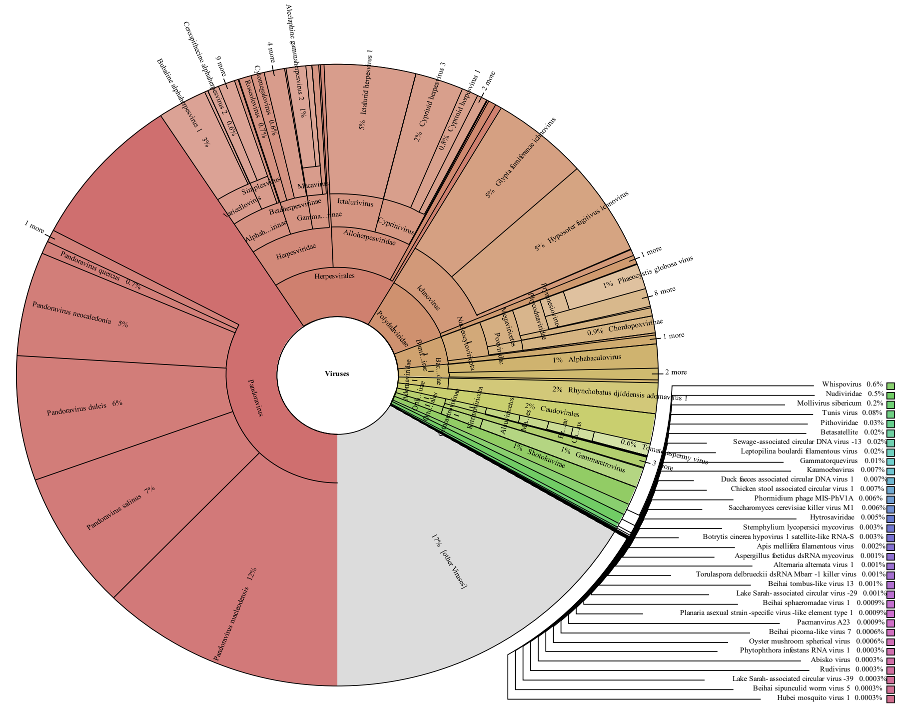


**Figure 5.** The taxonomic plot of hiodon tergisus virome

[ DOI: 10.29252/JCBR.8.3.14 ]

*Pandoravirus* was composed of *P. macleodensis* (30%), *P. salinus* (18%), *P. dulcis* (16%), *P. neocaledonia* (13%), *P. quercus* (2%), and *P. inopinatum* (2%). The remaining 20% of this taxonomy consisted of other Pandoraviruses. *Herpesvirales* included three prominent families: *Herpesviridae* (48%), *Alloherpesviridae* (48%), and *Malacoherpesviridae* (2%). *Alphaherpesvirinae* contained half of the *Herpesvirales*, while *Beta-* and *Gammaherpesvirinae* accounted for 20% and 28% of the other viruses in this family, respectively. Human-associated viruses within the *Herpesviridae* family included *Human alphaherpesvirus 2* (3%), *Human betaherpesvirus 6B* (4%), *Human betaherpesvirus 7* (2%), *Human betaherpesvirus 6A* (0.3%), and *Human gammaherpesvirus 8* (0.4%).

*Polydnaviridae* only contained insect viruses. The kingdom *Bamfordvirae* also included two main phyla: *Nucleocytoviricota* (89%) and *Preplasmiviricota* (11%). The class *Megaviricetes* included two families and one order of giant viruses, namely *Phycodnaviridae* (84%), *Mimiviridae* (4%), and *Pimascovirales* (12%). *Phaeocystis globosa virus* (56%), *Ectocarpus siliculosus virus 1* (21%), *Ostreococcus lucimarinus virus 2* (10%), *Bathycoccus sp. RCC1105 virus BpV* (0.8%), *Micromonas sp. RCC1109 virus MpV1* (0.6%), *Paramecium bursaria Chlorella virus A1* (1%), *Heterosigma akashiwo virus 01* (2%), *Aureococcus anophagefferens virus* (0.9%), *Orpheovirus IHUMI-LCC2* (0.7%), and *Chrysochromulina ericina virus* (0.7%) were the species within the *Phycodnaviridae* family. Four species, including *Moumouvirus* (51%), *M. chiliensis* (24%), *Cafeteria roenbergensis virus* (22%), and *Yellowstone lake mimivirus* (1%), were found in the *Mimiviridae* family. *Pimascovirales* was composed of seven species: *Ranavirus* (40%), *Chloriridovirus* (34%), *Iridovirus* (8%), *Trichoplusia ni ascovirus 2c* (7%), *Spodoptera frugiperda ascovirus 1a* (5%), *Heliothis virescens ascovirus 3a* (5%), and *Lymphocystivirus* (1%).

**Metavirome assay of *Polyodon spathula***

The results revealed a similar distribution of viral populations in *P. sapathula*. The genus *Pandoravirus* was the most prevalent, comprising 35% of all viruses, followed by *Herpesvirales* with 21% and *Bamfordvirae* with 6%. Other viral taxonomies are displayed in Figure 6. *Pandoravirus* comprised six species, including *P. macleodensis* (29%), *P. dulcis* (21%), *P. salinus* (13%), *P. neocaledonia* (9%), *P. quercus* (3%), and *P. inopinatum* (2%). Additionally, 25% of this genus consisted of other unassigned *Pandoravirus* species (19393 reads).

The taxonomy of *Herpesvirales* consisted of two families, including Herpesviridae (63%) and Alloherpesviridae (31%), and one genus, Aurivirus (5%). Similar to other fish, *Alphaherpesvirinae* contained *Human alphaherpesvirus 2* (4%) and *Human alphaherpesvirus 3* (2%). *Human betaherpesvirus 6B* (17%) and *Human betaherpesvirus 7* (11%) represented the human-associated Betaherpesvirinae. No human viruses were observed in the *Gammaherpesvirinae* subfamily.

The class *Megaviricetes* constituted 67% of the phylum *Nucleocytoviricota* and 53% of the kingdom *Bamfordvirae*. In *P. spathula*, the viral taxonomy of *Megaviricetes* consisted of two families, *Phycodnaviridae* (59%) and *Mimiviridae* (29%), along with one order, *Pimascovirales* (11%). The family *Phycodnaviridae* comprised several genera, including *Prymnesiovirus* (51%), *Prasinovirus* (20%), *Chlorovirus* (10%), *Phaeovirus* (5%), and *Raphidovirus* (3%). The *Mimiviridae* family included four species: *Moumouvirus* (77%), *M. chiliensis* (14%), *Cafeteria roenbergensis virus* (5%), and *Yellowstone lake mimivirus* (1%). *Pimascovirales* was composed of four genera, namely *Chloriridovirus* (65%), *Ascovirus* (16%), *Ranavirus* (14%), and *Lymphocystivirus* (5%).

**Analysis of viral species in *Denticeps clupeoides***

As shown in Figure 7, *Pandoravirus* (32%), *Herpesvirales* (29%), and *Bamfordvirae* (8%) were the most prevalent clades in the viral taxonomy of *D. clupeoides*. This result was relatively similar to that observed in other ray-finned fishes. The six prevalent *Pandoravirus* species were *P. macleodensis* (34%), *P. dulcis* (16%), *P. salinus* (15%), *P. neocaledonia* (12%), *P. quercus* (%), and *P. inopinatum* (2%).

Similarly to other ray-finned fishes, *Herpesviridae* constituted the majority (82%) of the *Herpesvirales* order, followed by *Alloherpesviridae* (18%) and *Aurivirus* (1%). As reported previously, only *Herpesviridae* contained human-associated herpes viruses. This included *Human alphaherpesvirus 2* (4%) in the *Alphaherpesvirinae* subfamily, *Human betaherpesvirus 6B* (28%) in *Betaherpesvirinae*, and *Human gammaherpesvirus 8* (1%) in *Gammaherpesvirinae*.

The third prevalent viral taxonomy, the kingdom *Bamfordvirae*, comprised two phyla: *Nucleocytoviricota* (90%) and *Preplasmiviricota* (10%). *Megaviricetes* was the main class of *Nucleocytoviricota*, consisting of two prominent families, *Phycodnaviridae* (90%) and *Mimiviridae* (4%), along with one order, *Pimascovirales* (6%). The family *Phycodnaviridae* contained six genera, including *Prymnesiovirus* (88%), *Chlorovirus* (4%), *Phaeovirus* (0.8%), *Prasinovirus* (0.8%), *Raphidovirus* (0.5%), and *Coccolithovirus* (0.08%). *Pimascovirales* included four genera, *Ranavirus* (73%), *Chloriridovirus* (16%), *Ascovirus* (10%), and *Marseillevirus* (0.1%), and three species, *Heliothis virescens ascovirus 3a* (4%), *Trichoplusia ni ascovirus 2c* (4%), and *Spodoptera frugiperda ascovirus 1a* (1%). *Mimiviridae* comprised two genera, *Mimivirus* (26%) and *Cafeteriavirus* (18%), and three species, *Moumouvirus* (55%), *Megavirus chiliensis* (26%), and *Cafeteria roenbergensis virus* (18%).

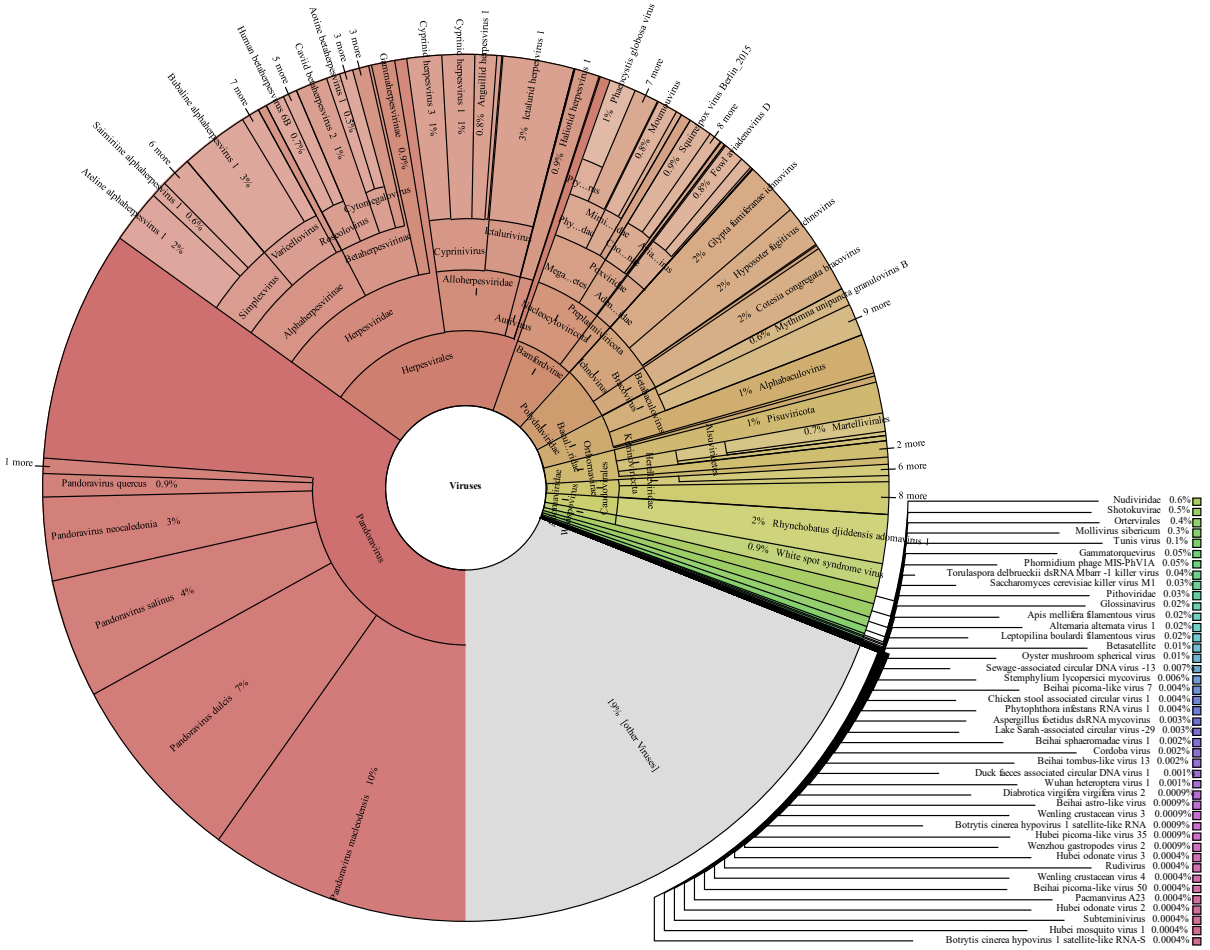


**Figure 6.** The taxonomic plot of polyodon spathula virome

[ DOI: 10.29252/JCBR.8.3.14 ]

### Virome structure of *Rutilus rutilus*

As a member of the ray-finned fishes, the metagenomic data of *R. rutilus* were analyzed for viral sequence reads. *Pandoravirus* (35%), *Herpesvirales* (25%), and *Bamfordvirae* (18%) were the dominant taxonomical groups of viruses in *R. rutilus*. Other viral members are depicted in Figure 8. Members of *Pandoravirus* were similar to those found in other ray-finned fishes mentioned earlier. In this regard, *P. macleodensis* (34%), *P. dulcis* (18%), *P. salinus* (11%), *P. neocaledonia* (10%), *P. quercus* (7%), and *P. inopinatum* (2%) were the *Pandoravirus* species observed in the virome of *R. rutilus*.

Furthermore, *Herpesviridae* (63%), *Alloherpesviridae* (33%), and *Malacoherpesviridae* (2%) constituted the *Herpesvirales* taxonomy in *R. rutilus*. *Human alphaherpesvirus 3* (0.7%) in the genus *Varicellovirus* and *Human alphaherpesvirus 2* (4%) in the genus *Simplexvirus* were observed in *Alphaherpesvirinae*. *Human betaherpesvirus 6B* (23%) was the only human-associated herpesvirus in *Betaherpesvirinae*. Moreover, *Human gammaherpesvirus 8* (5%) in the genus *Rhadinovirus* was observed solely in the *Gammaherpesvirinae* subfamily.

The Kingdom *Bamfordvirae* was taxonomically composed of two main phyla, *Nucleocytoviricota* (94%) and *Preplasmiviricota* (6%). *Megaviricetes* made up 36% of *Nucleocytoviricota*. *Phycodnaviridae* was the major family (93%) within the lineage of *Megaviricetes*, followed by *Pimascovirales* (4%) and *Mimiviridae* (3%). Six genera found in the virome of *R. rutilus* included *Prymnesiovirus* (71%), *Prasinovirus* (11%), *Chlorovirus* (5%), *Phaeovirus* (5%), *Raphidovirus* (0.9%), and *Coccolithovirus* (0.007%). The giant viral species within the family included *Phaeocystis globosa virus* (71%), *Ostreococcus lucimarinus virus 2* (11%), *Aureococcus anophagefferens virus* (6%), *Ectocarpus siliculosus virus 1* (5%), *Paramecium bursaria Chlorella virus A1* (4%), *Heterosigma akashiwo virus 01* (0.9%), *Chrysochromulina ericina virus* (0.4%), and *Orpheovirus IHUMI-LCC2* (0.1%). The order of *Pimascovirales* contained five genera, including *Chloriridovirus* (43%), *Ranavirus* (35%), *Ascovirus* (11%), *Iridovirus* (10%), and *Lymphocystivirus* (0.5%). Additionally, the family of *Mimiviridae* comprised two genera, namely *Cafeteriavirus* (43%) and *Mimivirus* (28%). It also included five species within *Mimiviridea*, including *Cafeteria roenbergensis virus* (43%), *Moumouvirus* (28%), *Acanthamoeba polyphaga mimivirus* (15%), *Megavirus chiliensis* (14%), and *Yellowstone lake mimivirus* (0.9%).

### Prevalence of giant virus signatures in ray-finned fishes' metagenomes

As a result, 432,281 out of 2,031,445 of the viral reads (21%) were unassigned to any known viruses. The GiantVirusFinder package was employed to assess the distribution of giant viruses. Accordingly, 17,931 hits (4.148%) were mapped to the genomes of 288 known giant viruses (e-value < 0.1). Interestingly, Figure 9 depicts the presence of unidentified giant viruses in marine environments.

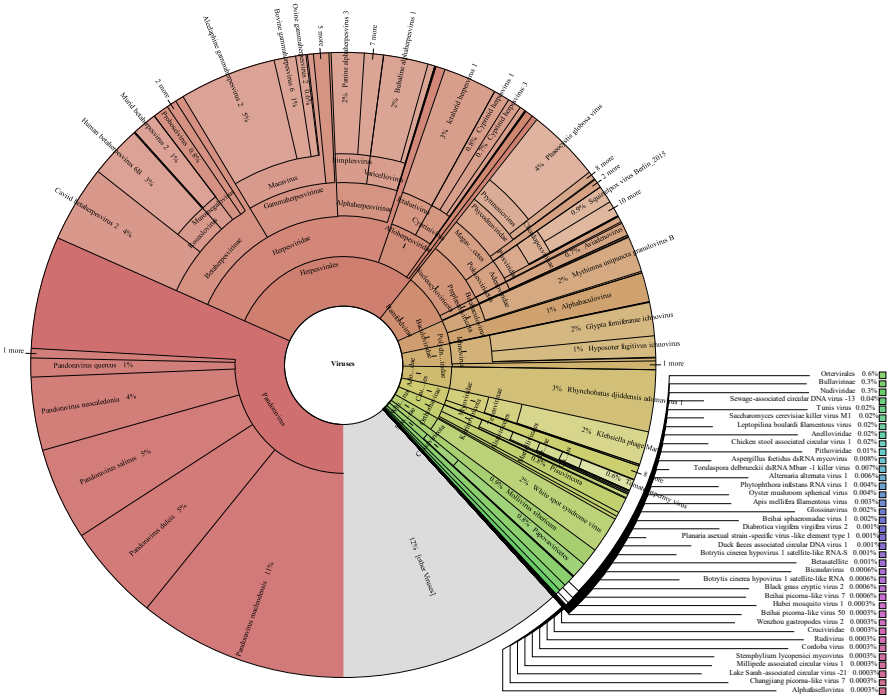


**Figure 7.** The taxonomic plot of denticeps clupeoides virome

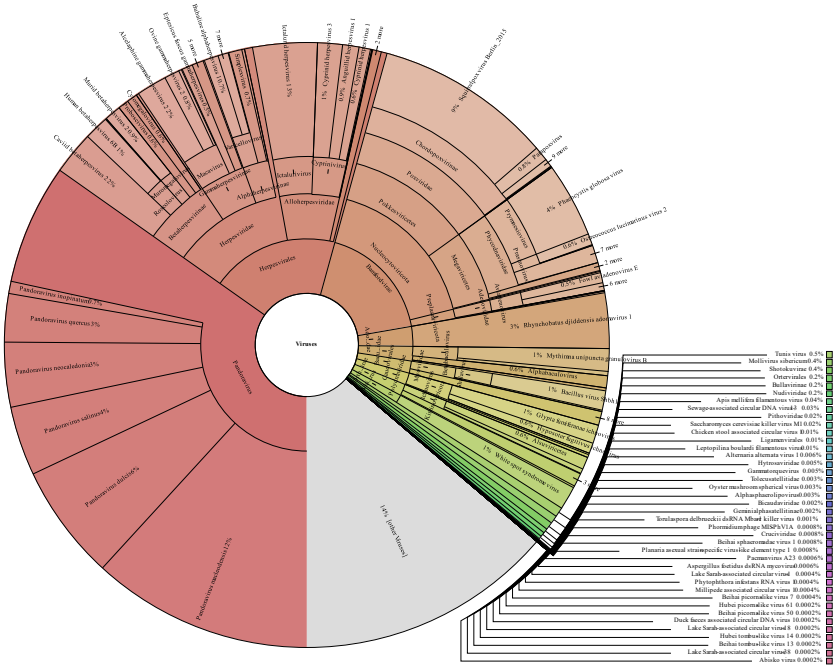


**Figure 8.** The taxonomic plot of rutilus rutilus virome

[ DOI: 10.29252/JCBR.8.3.14 ]

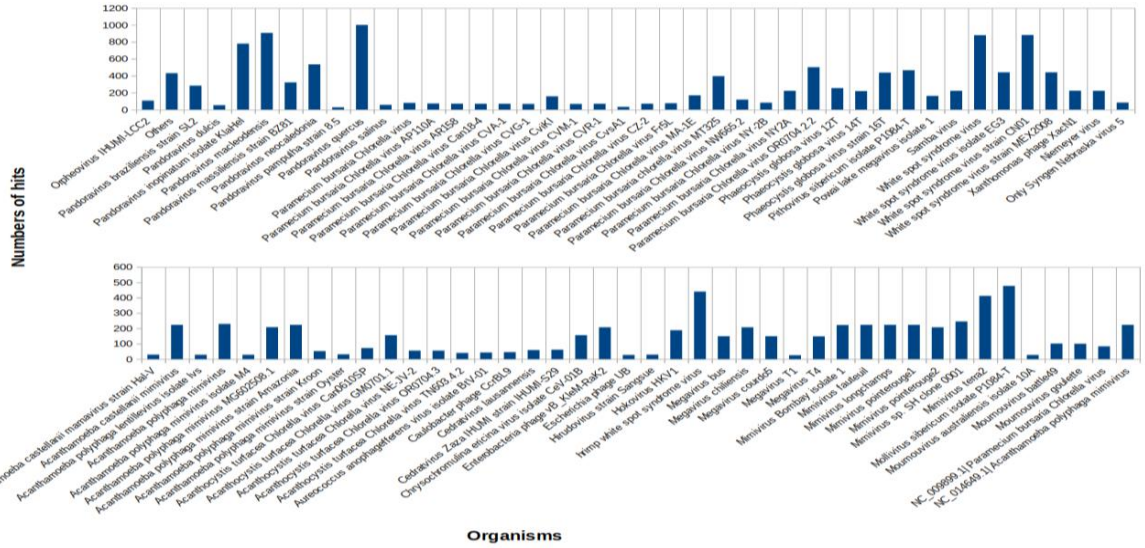


**Figure 9.** A bar chart representing the number of mapped hits to known giant viruses. Reads with fewer than 20 hits were categorized as "Others." The data comprise 17,931 total reads of unassigned viruses with significant alignment scores.

## Discussion

Numerous viruses remain unidentified by nature due to our inability to cultivate them in laboratory conditions. However, viral typing does not solely rely on cultivation. Thanks to continuous advancements in scientific fields and the discovery of various techniques and methods, obtaining essential information about microorganisms has become more feasible. Metagenomics employs techniques that utilize genetic information from microorganisms in their natural habitats, providing insights into their evolutionary history and other pertinent information without the need for laboratory cultivation (31). In this study, the metavirome of eight ray-finned fish species was investigated. The results revealed that *Pandoravirus* accounted for 31%, *Herpesvirales* for 23%, and *Bamfordvirae* for 10% of the total viral sequence fingerprints. Notably, these viruses were prevalent in ray-finned fish populations distributed across the world, primarily in freshwater and riverine environments. Below is a brief overview of the eight fish species under investigation.

*Acipenser schrenckii* (Amur sturgeon) is known as a living fossil with a history dating back approximately 200 million years (32,33). The Amur sturgeon is a large river species, capable of reaching up to 3 meters in length and weighing up to 100 kilograms. It displays strong adaptability and can thrive in temperatures ranging from 0°C to 33°C, becoming active within the range of 13°C to 26°C. The Amur sturgeon holds significance for genetic resource conservation and is a valuable food source due to its high nutritional value in freshwater aquaculture (34-36).

*Odontobutis haifengensis* is an endangered species native to China and inhabits river environments (37,38). The *Odontobutis* genus is distributed in freshwater regions of Eastern Asia and the Yalu River in China (39). *Rhinogobius giurinus* is an amphidromous goby species inhabiting East Asia's marine, brackish, and freshwater environments. This ornamental fish feeds on aquatic insects, invertebrates, small fishes, zooplankton, phytoplankton, and plant detritus (40).

*Hiodon tergisus* is a North American freshwater fish that prefers spawning in temperatures between 10-15°C. This species typically grows to about 130 mm and weighs approximately 24 grams. Its diet includes plankton, insects, mollusks, crayfish, and small fishes (41–44). Furthermore, *Polyodon spathula* (Paddlefish) is considered a living fossil, native to the Northern Hemisphere, and inhabits riverine streams, broad rivers, and backwaters. It can grow to weigh over 90 kg and live up to 60 years. Paddlefish is also known for its filter-feeding behavior and has applications in aquaculture (45-47). Notably, this fish is valued for its high nutritional content, particularly its caviar and meat (48).

*Denticeps clupeoides* is a small teleost species of fish found in the freshwater regions of Western Africa. It holds importance for understanding relationships among lower teleosts (49,50). Additionally, *Rutilus rutilus* (Common roach) primarily inhabits freshwater environments and can grow up to 500 mm long. These fish are endemic to various regions in Europe and western Asia (51,52). Common roaches have a diverse diet that includes various food sources in their natural environment (53,54), and they are also a significant food source for humans.

Viruses are pervasive and influential in aquatic environments, significantly affecting microbial community mortality and the marine food cycle. Moreover, viruses can control planktonic population composition through infection (55), and they can acquire genes from their hosts, which is a common mechanism observed in viruses, particularly giant viruses (56). Host-switching, a phenomenon identified in the evolutionary history of viruses, further demonstrates the intricate links between viruses and their hosts (57).

Accordingly, one fascinating outcome of this meta-virome study was the high prevalence of *Pandoravirus* and Megaviricetes, both of which belong to the group of viruses known as giant viruses. These double-stranded DNA viruses were first identified in *Acanthamoeba* in 2003 (58). Giant viruses are found in terrestrial and aquatic environments and often interact with humans. Some giant viruses have been linked to human-associated diseases (59-61). The results of this study diverge from previously published articles, as they indicate a higher abundance of RNA viruses in aquatic environments (57).

In a study by Costa et al. on the virome of ten freshwater ray-finned fish species across seven taxonomic orders and thirteen locations within the Murray-Darling Basin in Australia, the most abundant vertebrate-associated viruses in ray-finned fishes belonged to the *Arenaviridae* and *Astroviridae* families (62). Additionally, studies by Geoghegan et al. demonstrated that the *Astroviridae* family had the highest abundance compared to other viral families in fish viromes (7,63,64). Despite variations in results, the viruses identified in fish were related to vertebrates and, in some cases, human-associated diseases. The presence of RNA viruses, DNA viruses, and giant viruses in fish populations and their connection to humans suggest potential evolutionary links between these viruses.

While information about viruses that infect fish remains limited, the high diversity among fish species and their significant role in vertebrate evolution implies that they might be a source of emerging viruses. Moreover, the exchange of genetically adapted viruses or quasispecies between different environments can lead to cross-species transmission, particularly in DNA viruses that exhibit high recombination rates (65). Virus genetic changes can facilitate such transmissions, making it crucial to consider the potential threats posed by aquatic viromes to public health. Although this study did not assess the completeness and integrity of human-associated viral genomes and giant viruses, mapping reference genomes will address this in future studies.

## Conclusion

Population growth drives the need for more food resources, with seafood, especially fish, being one of the primary sources. The key point is that various viruses bolster food sources with a robust evolutionary history. As the findings have shown, there is a high abundance of giant DNA viruses in some ray-finned fish, many of which are consumed by humans. Therefore, further investigation into the virome of these aquatic species is crucial. This research would enhance our understanding of the potential human-associated illnesses these viruses might cause.

## Acknowledgement

Not applicable.

## Funding sources

Not applicable.

## Ethical statement

Not applicable.

## Conflicts of interest

The authors declare that they have no competing interests.

[ DOI: 10.29252/JCBR.8.3.14 ]

## Author contributions

FSA has been involved in data preparation, FASTQ file quality checks and assembly, and has written the first draft of the manuscript. AM has been working on viral taxonomy generation for ray-finned fish, data visualization, detection of antivirus signatures in unassigned viral taxa, and has served as the research manager.

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[ DOI: 10.29252/JCBR.8.3.14 ]

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**How to Cite:**
Askari FS, Mohebbi A. Metavirome survey of eight ray-finned fishes: Domination of giant viral members from pandoravirus and megaviricetes. *JCBR.* 2024;8(3):14-22.

[ DOI: 10.29252/JCBR.8.3.14 ]