

Metavirome Survey of Eight Ray-Finned Fishes Revealed Domination of Giant Viral Members of Pandoravirus and Megaviricetes

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Research

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Abstract

Background

Fishes are an essential source of food all around the world. The bulk of the microbial diversity in marine ecosystems is uncharacterized, with viruses accounting for most of the remaining diversity. This may be a major danger to public health, therefore knowing the variety of viruses in the marine ecosystem is critical. The goal was to assess the virome of ray-finned fishes in terms of variety and relation to human diseases.

Results

It was revealed that the viral kingdom was allocated 2,228,888 sequence reads. The pooled genome assay of the ray-finned fishes indicated a significant abundance of viruses in three clades of the genus *Pandoravirus* (31%), the order of *Herepesvirales* (23%), and the kingdom of *Bamfordvirae* (10%). Furthermore, 432281/2031445 reads (21%) of mapped reads were unassigned viruses. According to the GiantVirusFinder findings, 17931 hits (4.15%) were mapped to the genomes of known giantviruses.

Conclusion

Humans eat seafood regularly as one of their primary diets. Ray-finned fishes have a long evolutionary history and, as demonstrated here, are hosts to a variety of large DNA viruses. Fish must be screened for viral infections that are linked to human illnesses.

Background

Fishes are one of the major sources of food worldwide. Aquatic animal species in marine ecosystems are exposed to a wide variety of microorganisms [1]. Viruses are the most abundant organisms on earth [1, 2] and affect the nutrient and carbon cycle in aquatic environments [3]. According to the first study conducted on two uncultured marine viral communities by Breitbart et al. in 2002, it was found that over 65% of microorganism diversity in marine communities is uncharacterized, and the majority of remaining diversity are viruses. On the other hand, the most abundant viral genome comprises 2–3% of the total population in marine communities, indicating high viral diversity [4].

Phylogenetic classification has shown approximately 72 orders (and 79 suborders), 514 families of bony fish have been identified [5]. According to calculations made on the population density of fish, it is determined that some fish shoal can occupy nearly three billion fish in a single school [6]. This rate represents a very dense population among vertebrates. On the other hand, this number of fishes can harbor an increasing number of viruses compared to other vertebrates. Viruses are transmitted horizontally between fishes, which may occur through contaminated feces [7]. A study with a meta-

transcriptomics approach on four fish species conducted by Geoghegan et al. in 2018 showed densely shoaling fish had the highest number of viruses compared to the most unsocial fish species. Interestingly, identifying the number of potential novel viruses in fishes as previously detected in mammals and birds may indicate cross-species transmission [8]. This can also occur by the horizontal transmission of viruses by birds or other fish predators.

Actinopterygii, known as ray-finned fishes, with more than 32,000 species (9), contains nearly half of the living vertebrate species belonging to the *Neopterygii* (modern ray-fins) sub-class. *Neopterygii* is the most species-rich clade of bony fishes (> 99.9%) [9, 10]. *Neopterygii* comprises members, including *Ginglymodi*, *Halecomorphi*, and *Teleostei*. *Teleostei* is the dominant species of modern Actinopterygian with the highest number of fishes. Also, non-teleost Actinopterygians are known as living fossils [9]. Ray-finned fishes, which can live in different aquatic environments such as deep ocean trenches, high mountain streams, grow in extreme habitats with acidic, subzero, hypersaline, hypoxic, temporary, and fast-flowing water environments, dating back to more than 400 million years ago [11]. It is necessary to say that in addition to their long history, ray fishes are very important in providing human food. According to an estimate made in 2010, 80.6% of the catches were related to this fish species [12].

The vast diversity of ray-finned fishes makes them a source of different viral species. The existence of billions of viral particles per milliliter of the ocean and as the most important cause of death in those waters make them capable of altering populations' structures in the ocean [13–15]. On the other hand, fish is the most important source of consumption in human food and in direct contact with ocean water that can carry various and even new emerging viruses [16–19]. A population of viruses can cause many infections occurring in marine environments. Therefore, it is necessary to study the viral presence in a fish [2]. A new approach that can be used to survey the diversity and richness of the environmental virus samples is metagenomics [2]. In a culture-independent analysis, metagenomics surveys the genetic content of all microbial organisms in an environmental sample using biotechnological and bioinformatics methods. Also, Metavirome evaluates the metagenome of the entire viral population in one sample [20, 21].

The purpose of the current study was to investigate the genome fingerprints of the viral genome that could potentially pose a threat to public health. We also discuss the possibility of the evolutionary role of viral diversity in marine ecosystems and their impact on the emerging viral outbreak.

Methods

Recovery of metagenomics resource

Metadata for metagenome were retrieved from the Sequence Read Archive (SRA) [22] by using the Galaxy server [23]. Data were from a Bioproject on ray-finned fishes with the accession number of PRJNA493014 containing eight SRA experiments. The SRA accession numbers were SRR7903826 *Acipenser schrenckii* (Amur sturgeon), SRR7903827 (*Odontobutis haifengensis*), SRR7903828 *Odontobutis yaluensis* (dark

sleeper), SRR7903829 (*Rhinogobius giurinus*), SRR7903830 *Hiodon tergisus* (Mooneye), SRR7903831 *Polyodon spathula* (paddlefish), SRR7903832 *Denticeps clupeoides* (denticle herring), and SRR7903833 *Rutilus rutilus* (common roach).

Assembly and quality control of raw FASTQ files

Illumina sequencing files were polished for chimeric sequences, barcode errors, and duplication using the Trimmomatic tool [24]. Single-ended SRA entries containing FASTQ files were assembled by using Unicycler [25]. Assemblies are performed with the package's default settings. Contigs were checked for their quality through Quality Assessment Tool (QUAST) [26].

Metavirome classification and taxonomic affiliation

Reads from each sequencing run were mapped to the viral database's genomic sequences using the Kraken taxonomic assignment tool [27]. The number of reads mapped to reference was counted using the same Kraken tool. Furthermore, classified data were plotted by using Krona [28, 29]. The root of the chart was set as viral families. The distribution of each viral taxa was estimated through the depth of sequence reads.

Mapping unidentified sequence reads to Giant viral database

A pooled unassigned read of the genome of all eight ray-finned fish species was used to find giant viral fingerprints. GiantVirusFinder package was used to identify the number of sequences reads to the genome of 288 known giantviruses [30]. The packaged supports a fast-greedy alignment algorithm to identify hits to the giantviruses [31]. However, the source code was modified to support blast version 2.8.1+. Furthermore, the expected threshold was changed to 0.1.

Results

Metavirome survey

In this study, metagenomics data of eight ray-finned fish species were investigated for their virome. Overall, 2228888 sequence reads were assigned to the virus kingdom. Pooled genome assay revealed a high abundance of *Pandoravirus* (31%), *Herpesvirales* (23%), and *Bamfordvirae* (10%) of all kingdom of viruses. In the following virome of each ray-finned fish will be presented. The presented study results are taxonomically assigned, and as some groups of viruses may not have some super group classifications like class, order, or family, we defined them in their related sections. Accordingly, the genus of *Pandoravirus*, the order of *Herpesvirales*, the family of *Polydnavirus*, and the kingdom of *Bamfordvirae* are the roots of their clades, as shown in Fig. 1.

Diversity of viral species in *Acipenser schrenckii*

According to the viral taxonomy, *Acipenser schrenckii* metagenomics data was comprised of 464,973 classified and 91,977 reads. *A. schrenckii* comprised three main clades of *Pandoravirus*, the order of *Herpesvirales*, and the kingdom of *Bamfordvirae* (5%). As illustrated in Fig. 1, the genus *Pandoravirus* encompassed 34% of all viruses, followed by *Herpesvirales* with 28%. The most prevalence *Pandoraviral* species were *P. malecodensis* (28%), *P. dulcis* (24%), *P. neocaledonia* (10%), *P. salinus* (9%), *P. quercus* (4%) and *P. inopinatum* (2%). Other unclassified *Pandoravirus* species were composed of 24% of this lineage (Fig. 1A).

The order of *Herpesvirales* with 131,119 reads composed 28% of all the virome of *A. schrenckii*. In addition to *Alloherpesviridae*, the *Herpesviridae* family was contained in some members of human herpesviruses. Accordingly, the subfamily of *Betaherpesvirinae* (3% of all viruses) was comprised of *Roseolovirus* (41% of *Betaherpesvirinae*) and *Cytomegalovirus* (22% of *Betaherpesvirinae*), which was responsible for known human-associated viral pathogens. Accordingly, *Human alphaherpesvirus 2* (1%) within the subfamily of *Alphaherpesvirinae*, *Human betaherpesvirus 7* (5%), *Human betaherpesvirus 6B* (1%), and *Human betaherpesvirus 6A* (0.1%) within *Betaherpesvirinae* and *Human gammaherpesvirus 8* (0.4%) belong to the *Gammaherpesvirinae* subfamily were constituted the human-associated viruses of the family of *Herpesviridae*.

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

Viral population of *Odontobutis haifengensis*

Herpesvirales (19%), *Pandoravirus* (17%), *Orthornavirae* (10%), and *Bamfordvirae* (10%) were the most abundant orders of the viruses in the virome of *O. haifengensis*. Family *Herpesviridae* (14%) comprised 74% of the order of *Herpesvirales*. *Alphaherpesvirinae* subfamily consisted of *Human alphaherpesvirus 2* (0.05% of viruses). The subfamily of *Betaherpesvirinae* was comprised of *Human betaherpesvirus 6b* (0.6% of viruses) and *Human betaherpesvirus 7* (0.1% of viruses).

As shown in Fig. 1B, the giant *Pandoravirus* was made by *P. salinus* (37%), *P. malecodensis* (22%), *P. dulcis* (12%), *P. inopinatum* (6%), *P. neocaledonia* (5%), and *P. quercus* (3%). Furthermore, 1773 unassigned reads were also formed by other *Pandoravirus* species (16%).

The order of *Orthornavirae* with 6542 reads comprised two main sub-order of *Kitrinoviricota* (40%) and *Pisuviricota* (38%). The majority of viral species in these two sub-orders were those associated with vegetable and marine viral species. However, the *Flaviviridae* family (45%) within the *Kitrinoviricota* sub-order consisted of major human viral pathogens, namely *Hepacivirus* (63%) and *Tick-borne encephalitis virus* (19%).

Virome of *Odontobutis yaluensis*

The virome of *O. yaluensis* consisted of three main orders of *Herpesvirales* (24%), *Bamfordvirae* (15%), and *Pandoravirus* (8%). Further orders were including, *Baculoviridae* (6%) and *Orthornavirae* (5%). Three major prevalent families of *Herpesvirales* were *Herpesviridae* (79%), *Alloherpesviridae* (15%) and *Malacoherpesviridae* (5%). The *Herpesviridae* viruses comprised *Human alphaherpesvirus 3* and *Human betaherpesvirus 6B* in alpha- and *Betaherpesvirinae* sub-families, respectively (Fig. 1C).

Sub-order of *Nucleocytoviricota* within *Bamfordvirae* was comprised of two prominent families of avian *Poxviridae* and giantviruses of *Megaviricetes*. *Mimiviridae* family was encompassed 5% of *Bamfordvirae*, and it has consisted of *Mimivirus* (59%), *Moumouvirus* (35%), and *Cafeteriavirus* (5%). *Megavirus chiliensis* was the only member of the genus *Mimivirus*.

Further giantviruses were investigated in the *Pandoravirus* taxonomy. *P. macleodensis* (30%), *P. salinus* (27%), *P. dulcis* (14%), *P. inopinatum* (5%), *P. neocaledonia* (5%), and *P. quercus* (3%) were the only recognized species, and 16% of the taxonomy were other *Pandoraviruses* (Fig. 1D).

Distribution of the viral population in *Rhinogobius giurinus*

59% of the metagenomics data of *R. giurinus* was comprised of three major orders of *Pandoravirus* (24%), *Herpesvirales* (20%), and *Bamfordvirae* (15%). Other viral taxa were including, *Polydnviridae* (5%), *Baculoviridae* (3%), and *Caudovirales* (3%). Further virus-associated sequence reads can be found in Fig. 1D.

The order of *Pandoravirus* consisted of *P. dulcis* (38%), *P. macleodensis* (20%), *P. salinus* (9%), *P. quercus* (9%), *P. neocaledonia* (7%), and *P. inopinatum* (3%). Further, *Pandoraviruses* formed 15% of this giantvirus category.

Herpesviridae was comprised 79% of the *Herpesvirales* order, of which *Alphaherpesvirinae* was composed of *Human alphaherpesvirus 2* and *Human alphaherpesvirus 3*. *Roseolovirus* within *Betaherpesvirinae* was contained *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.

Bamfordvirae was contained two main super-orders, namely *Nucleocytoviricota* (85%) and *Preplasmiviricota* (15%). Similar to *Preplasmiviricota*, *Poxviridae* within *Nucleocytoviricota* was composed of avian viruses. In addition, *Megaviricetes* within *Nucleocytoviricota* consisted of three major groups of giantviruses, including, *Phycodnaviridae* (89%), *Mimiviridae* (5%), and *Pimascovirales* (5%). *Phycodnaviridae* was comprised 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 of the *Mimiviridae* family were as follows; *Moumouvirus* (53%), *M. chiliensis* (24%), *Cafeteria roenbergensis*

virus (21%), and *Yellowstone lake mimivirus* (1%). The order of *Pimascovirales* has included *Iridoviridae* (65%) and *Ascovirus* (35%) families.

Diversity of virome of *Hiodon tergisus*

Virome analysis of *H. tergisus* showed an almost different distribution of viral taxonomic among the ray-finned fishes. However, the sequence reads rate was significantly different from others as *Pandoravirus* with 142901 sequence reads comprised 41% of viruses in *H. tergisus*. *Herpesvirales* with 18%, *Polydnaviridae* with 11%, and *Bamfordvirae* with 4% were the other prevalent viral clades in the taxonomy assignments (Fig. 1E).

Pandoravirus was composed of *P. macleodensis* (30%), *P. salinus* (18%), *P. dulcis* (16%), *P. neocaledonia* (13%), *P. quercus* (2%), and *P. inopinatum* (2%). Other *Pandoraviruses* formed the last 20% of this taxonomy. *Herpesvirales* was composed of three main families including, *Herpesviridae* (48%), *Alloherpesviridae* (48%), and *Malacoherpesviridae* (2%). *Alphaherpesvirinae* contained half of the *Herpesvirales* while *Beta-* and *Gammaherpesvirinae* had 20% and 28% of other viruses of this family, respectively. *Human alphaherpesvirus 2* (3%), *Human betaherpesvirus 6B* (4%), *Human betaherpesvirus 7* (2%), *Human betaherpesvirus 6A* (0.3%), *Human gammaherpesvirus 8* (0.4%) were the human-associated viruses of *Herpesviridae* family.

Polydnaviridae has only contained insect viruses. Additionally, the kingdom of *Bamfordvirae* has consisted of two main phyla of *Nucleocytoviricota* (89%) and *Preplasmiviricota* (11%). The class of *Megaviricetes* has contained two families and one order of giantviruses, including *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 comprised of seven species including, *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. spathula*. Accordingly, genus *Pandoravirus* with 35% of all viruses was most prevalent, and the order of *Herpesvirales* followed it with 21% and *Bamfordvirae* with 6%. Other viral taxonomies are shown in Fig. 1F.

Pandoravirus was comprised of six species, including *P. macleodensis* (29%), *P. dulcis* (21%), *P. salinus* (13%), *P. neocaledonia* (9%), *P. quercus* (3%), and *P. inopinatum* (2%). Further, 25% of this genus consisted

of other unassigned (19393 reads) *Pandoravirus* species.

The taxonomy of *Herpesvirales* was composed of two families of *Herpesviridae* (63%), *Alloherpesviridae* (31%), and one genus of *Aurivirus* (5%). Relatively similar to what was observed in other fishes, *Alphaherpesvirinae* consisted of *Human alphaherpesvirus 2* (4%) and *Human alphaherpesvirus 3* (2%). *Human betaherpesvirus 6B* (17%) and *Human betaherpesvirus 7* (11%) were the human-associated *Betaherpesvirinae*. Additionally, no human virus was observed in the *Gammaherpesvirinae* subfamily.

The class of *Megaviricetes* was formed 67% of the phylum of *Nucleocytoviricota* and 53% of the kingdom of *Bamfordvirae*. Two families of *Phycodnaviridae* (59%) and *Mimiviridae* (29%) along with one order of *Pimascovirales* (11%) were formed viral taxonomy of *Megaviricetes* in *P. spathula*. Family *Phycodnaviridae* was comprised of a few genera including, *Prymnesiovirus* (51%), *Prasinovirus* (20%), *Chlorovirus* (10%), *Phaeovirus* (5%), and *Raphidovirus* (3%). *Mimiviridae* has also contained four species, including *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 clupeioides*

As shown in Fig. 1G, *Pandoravirus* with 32%, *Herpesvirales* with 29%, and *Bamfordvirae* with 8% were the most prevalent clades of the viral taxonomy of *D. clupeioides*. This result was relatively similar to those in other ray-finned fishes.

Six prevalent *Pandoravirus* species were *P. macleodensis* (34%), *P. dulcis* (16%), *P. salinus* (15%), *P. neocaledonia* (12%), *P. quercus*, and *P. inopinatum* (2%).

Like what was observed in other ray-finned fishes, *Herpesviridae* had the most proportion (82%) of *Herpesvirales* order, and it was followed by *Alloherpesviridae* (18%) and *Aurivirus* (1%). As reported above, only *Herpesviridae* contained human-associated herpes viruses. Accordingly, *Human alphaherpesvirus 2* (4%) in *Alphaherpesvirinae* subfamily, *Human betaherpesvirus 6B* (28%) in *Betaherpesvirinae* and *Human gammaherpesvirus 8* (1%) in *Gammaherpesvirinae* have formed the human-associated herpes viral species.

The third prevalent viral taxonomy, the kingdom of *Bamfordvirae*, comprised two phyla of *Nucleocytoviricota* (90%) and *Preplasmiviricota* (10%). *Megaviricetes* was the main class of *Nucleocytoviricota*. It was also contained two main families, including *Phycodnaviridae* (90%) and *Mimiviridae* (4%) and one order, namely *Pimascovirales* (6%). *Phycodnaviridae* was contained six genera including, *Prymnesiovirus* (88%), *Chlorovirus* (4%), *Phaeovirus* (0.8%), *Prasinovirus* (0.8%), *Raphidovirus* (0.5%) and *Coccolithovirus* (0.08%). *Pimascovirales* has contained four genera and three species including, *Ranavirus* (73%), *Chloriridovirus* (16%), *Ascovirus* (10%) and *Marseillevirus* (0.1%) as genera and *Heliothis virescens ascovirus 3a* (4%), *Trichoplusia ni ascovirus 2c* (4%) and *Spodoptera frugiperda ascovirus 1a* (1%). *Mimiviridae* was comprised of two genera of *Mimivirus* (26%) and *Cafeteriavirus*

(18%) and three species including, *Moumouvirus* (55%), *Megavirus chiliensis* (26%), and *Cafeteria roenbergensis virus* (18%).

Virome structure of *Rutilus rutilus*

As one member of ray-finned fishes, the metagenomic data of *R. rutilus* were assessed 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 shown in Fig. 1H.

Members of *Pandoravirus* were similar to other ray-finned fishes reported above. 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%) were established *Herpesvirales* taxonomy in *R. rutilus*. *Human alphaherpesvirus 3* (0.7%) in the genus of *Varicellovirus* and *Human alphaherpesvirus 2* (4%) in the genus *Simplexvirus* 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 in the *Gammaherpesvirinae* subfamily.

The Kingdom of *Bamfordvirae* was taxonomically comprised of two main phyla of *Nucleocytoviricota* (94%) and *Preplasmiviricota* (6%). *Megaviricetes* was made 36% of *Nucleocytoviricota*. *Phycodnaviridae* was the major family (93%) within the lineage of *Megaviricetes*, and it was followed by *Pimascovirales* (4%) and *Mimiviridae* (3%). Six genera found in the virome of *R. rutilus* were including, *Prymnesiovirus* (71%), *Prasinovirus* (11%), *Chlorovirus* (5%), *Phaeovirus* (5%), *Raphidovirus* (0.9%), and *Coccolithovirus* (0.007%). The giant viral species within the family were *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* has contained five genera as follows; *Chloriridovirus* (43%), *Ranavirus* (35%), *Ascovirus* (11%), *Iridovirus* (10%), and *Lymphocystivirus* (0.5%). In addition, the family of *Mimiviridae* was composed of two genera, namely *Cafeteriavirus* (43%) and *Mimivirus* (28%). It was also observed five species within *Mimiviridae* including, *Cafeteria roenbergensis virus* (43%), *Moumouvirus* (28%), *Acanthamoeba polyphaga mimivirus* (15%), *Megavirus chiliensis* (14%), and *Yellowstone lake mimivirus* (0.9%).

Prevalence of giantvirus signatures in Ray-finned fishes' metagenomes

As a result, 432281/2031445 reads (21%) of mapped reads were unassigned viruses. To assess the distribution of giantviruses, the GiantVirusFinder package was employed. Accordingly, 17931 hits (4.148%) were mapped to the genome of 288 known giantviruses (e-value < 0.1). Interestingly, Fig. 2 shows the circulation of yet unidentified giantviruses in marine environments.

Discussion

There are so many different types of viruses in nature that we do not know them since we cannot cultivate them in laboratory conditions. Cultivation is not the only solution for viral typing. Due to day-to-day advances in scientific fields and the discovery of various techniques and methods, it has become more accessible to achieve the desired information about microorganisms. Metagenomics utilizes some techniques using genetic information of any microorganism in its natural habitat, evolutionary history, and any other information required without the need for laboratory process [32].

In the present study, the metavirome of eight ray-finned fish species was investigated. According to the results, *Pandoravirus* with 31%, *Herepesvirales* with 23%, and *Bamfordvirae* with 10% had the highest abundance of viruses. It is noteworthy that these viruses were abundant in the ray-finned fishes that were geographically belonged to the different parts of the world but mostly in freshwater and riverine. Where there is more connection with humans, we will give a brief review about these 8 species of fish in the following.

Acipenser schrenckii (Amur sturgeon), with a history of ~ 200 million years known as a living fossil [33, 34]. Also, it is a large river species with a maximum length of 3 meters and a weight of 100 kilograms, strong adaptability, having mild temperament, tolerating in the range of 0–33°C but activating in the range of 13–26°C that inhabits the Amur River. This species is important in terms of genetic resource conservation and food source because it has high nutritional value in freshwater aquaculture [35–37].

The following ray-finned fish species was *Odontobutis haifengensis*, an endangered species native in China and inhabited by rivers [38, 39]. The *Odontobutis* genus is distributed in freshwaters of Eastern-Asia and Yalu River in China [40, 41].

An amphidromous goby species that live in marine, brackish and, fresh waters environments in Eastern-Asia is *Rhinogobius giurinus*. An ornamental fish (43) consumes aquatic insects, invertebrates, small fishes, zooplankton, phytoplankton, and plant detritus [42].

Hiodon tergisus, is a North American freshwater fish and lives in the waters with 10–15 OC temperature during spawning. Its height and weight are about 130 mm and 24 gr, respectively. They feed on plankton, insects, mollusks, crayfish, and small fishes [43–46].

Polyodon spathula (Paddlefish) is considered a living fossil (49) and native of the Northern Hemisphere. It homes in the riverine streams, broad rivers, and backwaters. It weighs more than 90 kg and can live up to 60 years. Also, it can act as a filter feeder into aquaculture [47–49]. The remarkable thing about this fish is its high nutritional value, provided through its caviar and meat [50].

Denticeps clupeioides is a small teleost species of fish that inhabits the freshwater of Western Africa. This species is a key taxon for find relationships among lower teleosts [51–53].

Rutilus rutilus (common roach), as one of the studied ray-finned fishes, live primarily in freshwater and has a length up to 500 mm, which is endemic in most parts of Europe and western Asia [54, 55]. Common roach feeds on a variety of food sources in its natural environment [56, 57]. It is also an important food source for humans.

The action of viruses in aquatic environments is pervasive and influential. As a crucial factor in the mortality of microbial communities, Viruses can affect the food cycle of the marine environment. On the other hand, by causing infection, it can effectively control the composition of planktonic populations [58]. In addition to the effects of viruses on their environment, these intelligent entities can also acquire abilities from the same environment. An examination of the evolutionary history of viruses showed that they could receive genes from the hosts that they infect (61), especially it is a common mechanism in viruses like giantviruses [59]. Also, Other studies have suggested a phenomenon called host-switching that has occurred during the evolutionary history of viruses [60]. This information could indicate broader links between viruses and their hosts.

An exciting result obtained in the present metavirome study was the high frequency of Pandoravirus and Megaviricetes; both belong to a group of viruses called giantviruses. They are double-stranded DNA viruses that were first identified in *Acanthamoeba* in 2003 [61]. Giantviruses exist in most terrestrial and aquatic environments, which are in close interacts with humans. These viruses have been identified in several cases in human-associated diseases [62–64]. The results of this study are substantially differed from other previously published articles, as it was shown that RNA viruses were more abundant in aquatic environments [60]. According to Costa et al.'s study on the virome of ten freshwater ray-finned fish species across seven taxonomic orders at thirteen locations across the Murray–Darling Basin in Australia, it was found that the viruses of Arenaviridae and Astroviridae families were the most abundant vertebrate-associated viruses in ray-finned fishes [65].

Furthermore, studies conducted by Geoghegan et al. shown that Astroviridae had the highest abundance compared to other viral families in the virome of fishes [8, 66, 67]. A noteworthy point in these studies is that despite the differences in the results, viruses that infect fish were vertebrate-related viruses associated with different types of human-associated diseases. The presence of RNA viruses, DNA viruses, and giantviruses in fish schools or shoaling and humans may indicate an evolutionary link between them.

Despite having the greatest diversity among vertebrates and having a variety of viruses that have played an important role in the evolution of vertebrates, there is little information about fish viruses and their association with human viruses. Most of the information is about viruses that infect humans [60]. Nevertheless, the information obtained suggests a possible link between marine caliciviruses and disease in humans. Also, the presence of avian influenza in marine birds [58, 68] implicates a risk of aquatic virome posed to public health. Since fishes are the most prosperous species among terrestrial and aquatic vertebrates, they can be a source for spreading new emerging viruses.

Furthermore, a small portion of genetically adapted viruses or quasispecies from the different environments can jump between species and cause infection [69]. Given that among viruses, DNA viruses have the highest recombination (genomic rearrangements) rate [69], and genetic changes in viruses can lead to cross-species transmission [70]. Furthermore, based on the present study results, which is also supported in a previous study [71], it can be implicated that viruses can jump from aquatic vertebrates to terrestrial vertebrates and pose a threat to the human population.

Conclusion

Population growth is a factor that leads people to use more food resources. One of these food sources is seafood, especially fish. The point is that food sources with the strong evolutionary background are supported by living many different viruses. As the findings demonstrated a high abundance of different giant DNA viruses in some ray-finned fishes, most of which are human seafood, it is necessary to investigate these aquatic species' virome further. This would help to enhance our knowledge of the human-associated illnesses these viruses may cause in the future.

Declarations

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Figures

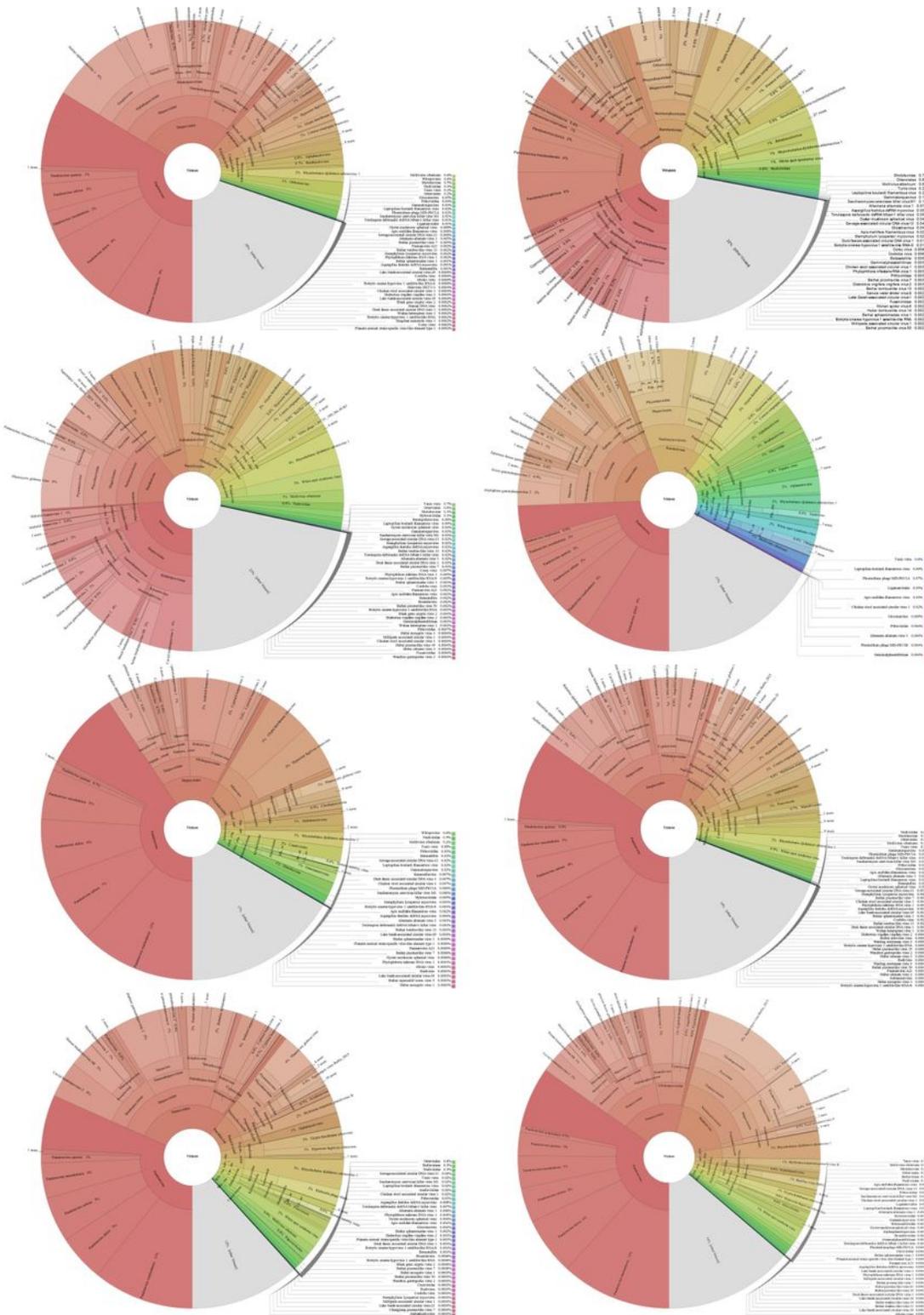


Figure 1

Accordingly, the genus of Pandoravirus, the order of Herpesvirales, the family of Polydnavirus, and the kingdom of Bamfordvirae are the roots of their clades, as shown in Figure 1.

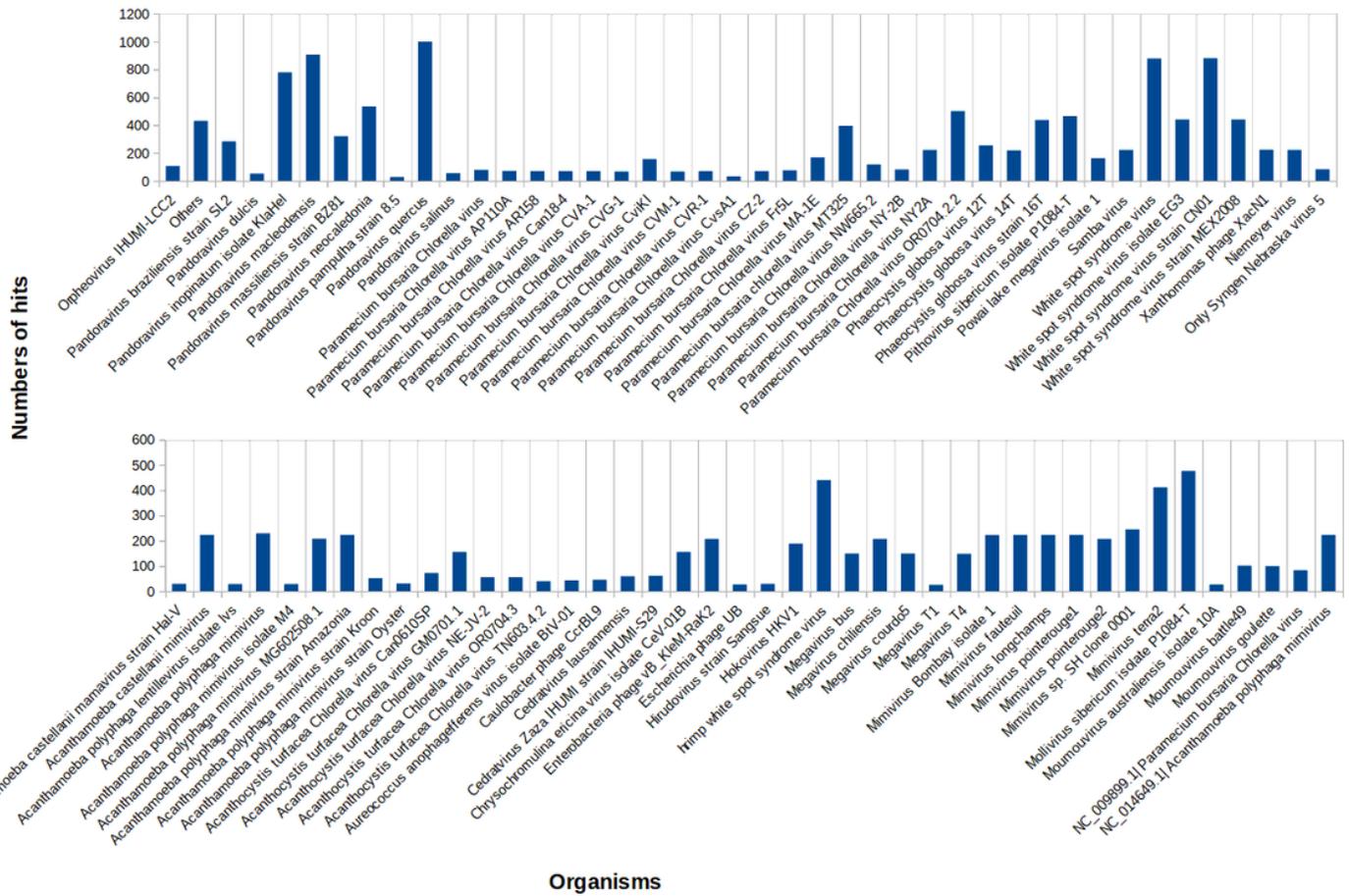


Figure 2

Figure 2 shows the circulation of yet unidentified giantviruses in marine environments.