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Viral Composition in Metagenomes of Rivers Located in the Amazon Mangrove Coast, Northeast of Pará, Brazil

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A B S T R A C T

In aquatic ecosystems there is a wide variety of viruses, including bacteriophages and others infecting plants, humans and animals. This study aimed to evaluate the viral diversity in surface water samples from three rivers located in the Amazonian mangrove, in higher and lower rainfall period. After concentration by organic flocculation, the viral nucleic acid was extracted by a commercial kit and subjected to the *multiplex* RT-qPCR and construction of *NGS* libraries. A total of 47 samples were analyzed and sixty-one different viral families were identified, with the *Caudovirales* order (*Myoviridae*, *Autographiviridae*, *Podoviridae* and *Siphoviridae*) being the most representative among the ten most abundant. An increase in the number of viral reads was observed during the lowest rainfall period in the analyzed rivers, with the exception of the Mocajuba river. The families composition was peculiar among the rivers evaluated during the different climatic seasons. Human enteric viruses were not found by both approaches. This study showed the great viral diversity in three different mangroves rivers and the absence of human enteric viruses, a common waterborne-viruses. More studies are needed to elucidate the virus role on the ecology of aquatic ecosystems and the biotic and abiotic impacts on viral diversity and resistance.

Introduction

Viruses are very diverse agents present in aquatic environments and can overcome even the bacterial abundance in these ecosystems (Wommack and Colwell, 2000). The majority of them constitute the class of bacteriophages and it has an ecological function into these environments. On the other hand, another part of this diversity encompasses exogenous viruses that infect plants, humans,

aquatic and terrestrial animals and that are introduced in the aquatic ecosystems in different ways (Wommack and Colwell, 2000 and Suttle, 2005).

Among the important functions that bacteriophages play in aquatic environments are nutrient cycling, generated by the lysis of host microorganisms, which increases the rate of movement of nutrient particles into dissolved organic matter, diverting nutrients from higher trophic levels back to the base of aquatic food web; and the host diversity promotion through population control of the dominants (Lang *et al.,* 2009). In addition, they are eliminated in feces and do not replicate in the natural environment, besides to having morphological and biological properties similar to enteric viruses which can predict their transport to the aquatic environments. For this reason, they are currently considered good environmental indicators as surrogates to the use of faecal indicator bacteria (FIB) (McMinn *et al.,* 2017; Toribio-Avedillo *et al.,* 2021).

The release of raw sewage, without prior treatment, is the main factor for the occurrence of human, animal, plant, bacterial and fungal viruses in aquatic ecosystems, constituting the sewage viroma, a complex and varied matrix of pathogenic and commensal viruses (Martínez-Puchol *et al.,* 2020). Enteric viruses are examples of rivers water contaminants through wastewater. They are the main etiological agents of waterborne disease, mainly gastroenteritis. These viruses have characteristics of high and fast spread, with high stability and environmental resistance, high viral excretion in feces (10^{11} particles/g) and low infective dose (approximately 10^{1} particles) (Cadamuro *et al.,* 2021). Norovirus (NoV), Sapovirus (SaV), Astrovirus (AstV) and Adenovirus (AdV) stand out among this class of viruses responsible for diarrheal diseases in humans (Grabow, 2007; Bányai *et al.,* 2018).

The Amazonian mangrove is recognized for its role in the biological diversity of marine and coastal environments, for its natural richness and high productivity, which supports important socio-economic activities of riverside populations, such as oyster farming (Tenorio *et al.,* 2015). The rainfall variability is very peculiar in the state of Pará, as there is precipitation throughout the year, with two seasons well-defined regionally as winter (very rainy) and summer Amazonian (less rainy).

In this study we evaluated the viral genomic diversity and occurrence of enteric viruses by next-generation sequencing (*NGS*) and multiplex RT-qPCR, respectively, in surface water samples collected from three rivers located in the Amazonian mangrove coast, during periods of higher and lower rainfall.

Materials and Methods

Sampling Points and Collections

The study was carried out in three communities located in the northeast region of Pará, Northern Brazil, known by their aquaculture activities along the mangrove coast, including the oyster culture. The surface water river samples were collected in the years of 2017 and 2018 in the lower (December to June) and higher rainy amazon season (July to November) directly in the areas dedicated to the cultivation of oysters in the Emboraí river (1°03'52.3"S 46°28'32.3"W), Urindeua river (0°41'54.5"S 47°21'42.6"W) and Mocajuba river (0°51'06.2"S 47°53'25.8"W). All collection points can be seen in the figure 1. After collections, the water samples were placed in sterilized polypropylene bottles and kept under refrigeration (2-8°C) until arrival at the laboratory for processing.

Processing of River Water Samples

One liter of river water samples was concentrated by the organic flocculation with skimmed milk method (Calgua *et al.,* 2008). Briefly, the samples were acidified (pH 3.5) using a 6N HCl solution and 10 mL of flocculated skim milk solution were added. After homogenization for 8 hours and sedimentation

for another 8 hours, 900 mL was aspirated and the remaining 100 mL were transferred to centrifuge tubes and centrifuged at 3,500 rpm for 20 minutes at 4°C. The pellet was neutralized with 1 mL of phosphate buffer pH 7.5 (0.2M sodium phosphate monobasic-0.2M sodium phosphate dibasic 1:2 v/v). An aliquot was set aside to perform total nucleic acid extraction.

Extraction of Total Nucleic Acid

Two different nucleic acid extractions were performed: one for the detection of human gastroenteric viruses and another one for the *NGS* approach. For the first purpose, the commercial QIAamp RNA viral kit (QIAGEN) was used, following the manufacturer's protocol with the addition of Brome Mosaic Virus (BMV) as an internal control (IC) in the viral lysis step. For the second one, the nucleic acids were purified using commercial kit (ReliaPrep Viral TNA MiniPrep Custom, Promega) according manufacturer instructions.

The nucleic acid masses (ng/µL) were quantified by fluorometry on the Qubit 2.0, using RNA and dsDNA High Sensitivity Assay (Invitrogen, USA).

Enteric viruses detection by Multiplex RT-qPCR

An initial screening for human gastroenteric viruses (Adenovirus-AdV, Norovirus-NoV GI/GII, Sapovirus-SaV and Astrovirus-AstV) was carried out by RT-qPCR in the individual samples using the XGEN Gastroviral Multiplex kit, which has specific primers and probes for detection of a partial region of the genes Hexon (AdV), ORF1-ORF2 Junction (NoV GI/GII), ORF1 Polymerase-Capsid junction (SaV) and Outer Capsid Protein (AstV). Briefly, three 25µL reactions were performed for each sample containing the following targets: PSGV1 (NoV GI, GII and IC); PSGV2 (AdV, AstV) and PSGV3 (SaV). A volume of 10 µL of the extracted nucleic acid was added to 15 µL of reagent mix containing: 12.5 µL of Mastermix; 1.5 µL of specific primer and probe pool (PSGV1, PSGV2 and PSGV3) and 1.0 µL of enzyme mix. The reactions were performed in the QuantStudio5 (Applied Biosystems) under the following conditions: 15 minutes at 42°C; 3 minutes at 94°C; 40 cycles of 94° for 08 seconds and 60°C for 34 seconds. The preparation of reagents mixture and reactions were carried out in separate rooms to avoid cross contamination. All procedures were carried out under the biosafety standards for laboratories (BSL-2).

Next Generation Sequencing

Total nucleic acids were separated and pooled on equimolar masses according to two criteria: a) Amazon season of the period in which they were collected: high (December to June) and low (July to November) rainy season; b) by sampling point. The pools were submitted to the double stranded cDNA synthesis (cDNA Synthesis System, Roche Diagnostics, Germany) and sequencing libraries were constructed using Illumina Nextera XT DNA Library Prep kit. The libraries quality was evaluated by high-resolution automated electrophoresis on Agilent BioAnalyzer 2100 using High Sensitivity DNA Reagents (Agilent Technologies). The libraries were pooled, denatured, diluted and sequenced on Illumina HiSeq 2500 instrument with the high-output V4 2x100-bp sequencing kit.

Bioinformatic Analysis

The raw sequencing data were pre-processed to perform adapters trimming and quality control using Trim Galore and PRINSEQ (PReprocessing and INformation of SEQuence) lite tools. A Phred 30 quality score was applied. The IDBA-UD algorithm (a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth) was used to the contigs assembles. The reads and contigs screening was carried out on Diamond v.0.9.19.120, with National Center for Biotechnology Information (NCBI) non-redundant protein database (NR database). Viral taxonomic classification from the reads and identification of contigs that had similarity to viruses was performed

by the Krona tool and the Geneious program v. 9.1.8, respectively. The richness and diversity data were performed in the EstimateS software, using one set of replicated sampling units with 100 randomizations for estimators and indexes (S obs Mao Tau, Jack Knife, Chao 1) (Colwell, 2013).

Climatic Data

The climatic data, such as precipitation (mm) and temperature (°C), were obtained from the INMET Brazilian Meteorological Database (https://portal.inmet.gov.br/) by searching the nearest automatic stations to the collection sites.

Results and Discussion

During this study, 47 water samples from river located on the coast of Amazon mangrove were analyzed individually (RT-qPCR) or grouped (*NGS* approach). A diverse assortment of viral sequences was identified in the metagenomes of these samples.

The sequencing generated a total of 305,919,726 reads and 1,828,970 contigs were obtained after assembly (Table 1). Double-stranded DNA (dsDNA) and positive single-stranded RNA (ssRNA+) viruses represented more than 99% (n=213,506) of the mapped reads while the others together (ssDNA, ssRNA-, RNA RT, dsRNA) accounted for approximately 0.03% (n=70) (Table 2).

Sixty-one different viral families were identified in the six pooled set of river water samples. It was possible to observe two different clusters regarding the similarity in the composition of viral families based on the number of viral reads: the first cluster (Figure 2, marked in purple) included the samples from the Mocajuba river (high and less rainy); and the second one (Figure 2, marked in green) grouped the samples from the Urindeua and Emboraí rivers, showing similarity of viral composition among these samples. The Chao1 and Jack Knife diversity estimators indicated that among 70 to 73 viral families could be sampled in the set analyzed. The rarefaction curve generated by the Mao Tau estimator plotted the average number of species found on each sample and indicated that the viral families sampled in the same rivers are within the mean expected among the 95% upper and low confidence interval (Figure 3).

Bacteriophages belonging to the order *Caudovirales* (families *Myoviridae*, *Autographiviridae*, *Podoviridae* and *Siphoviridae*) were the most frequent among the ten most abundant viral families (Figure 4).

The composition of families was peculiar among the rivers evaluated during the different climatic seasons (Figure 5). *Nimaviridae* and *Tobaniviridae* were the only families found in the period of highest rainfall between the Emboraí/Mocajuba and Urindeua/ Mocajuba rivers, respectively.

As for the period of lower precipitation, it was possible to observe a heterogeneity composition, where there were families found only between the Emboraí/ Urindeua (*Geminiviridae*), Emboraí/ Mocajuba (*Rhabdoviridae*), Urindeua/Mocajuba rivers (*Asfarviridae*, *Tectiviridae* and *Tospoviridae*). Analyzing the three mangrove rivers together, the most families were shared on all sampling points with families exclusively detected in the period of lowest (*Geminiviridae*, *Rhabdoviridae*, *Tectiviridae*, *Tristromaviridae*, *Reoviridae*, *Microviridae*, *Potyviridae*, *Phasmaviridae*, *Flaviviridae*, *Anelloviridae*, *Lipothrixviridae*) and highest (*Nimaviridae*, *Coronaviridae*, *Alphaflexiviridae*, *Lispiviridae*, *Totiviridae*, *Hantaviridae* and *Paramyxoviridae*) rainfall, respectively.

There were also families that were identified in both climatic seasons: n=13 in all three rivers analyzed (*Ackermannviridae*, *Alloherpesviridae*, *Arteriviridae*, *Autographiviridae*, *Baculoviridae*, *Demerecviridae*, *Herelleviridae*, *Herpesviridae*, *Mimiviridae*, *Myoviridae*, *Podoviridae*, *Poxviridae*, *Siphoviridae*); *n*=3 among Emboraí and Urindeua rivers (*Adenoviridae*, *Nudiviridae*, *Papillomaviridae*); *n*=3 among Urindeua and Mocajuba rivers

(*Bromoviridae*, *Iridoviridae*, *Phycodnaviridae*); *n*=3 among Emboraí and Mocajuba rivers (*Closteroviridae*, *Drexlerviridae*, *Marseilleviridae*); *n*=7 on the Emboraí river (*Bacilladnaviridae*, *Hytrosaviridae*, *Peribunya-viridae*, *Phycodna-viridae*, *Polydna-viridae*, *Polyomaviridae*, *Retroviridae*); *n*=2 on the Urindeua river (*Inoviridae*, *Mitoviridae*); *n*=6 on the Mocajuba river (*Arenaviridae*, *Caulimoviridae*, *Iflaviridae*, *Phenuiviridae*, *Picornaviridae*, *Sphaerolipoviridae*).

The precipitation rates varied from 0 to 56.50 mm and 179.40 to 328.40 mm among samples collected during dry and wet season, respectively. The rain did not influence the number of viral families found, but there was an increase in the number of reads during the period of lowest rainfall in the analyzed rivers, with the exception of the Mocajuba River in the rainy season, which remained similar to that found in the period of lowest rainfall (Figure 6).

Viral RNA/DNA of enteric viruses was not detected by RT-qPCR in tested river samples. However, the nucleic acid extraction was validated by amplification of the Brome Mosaic Virus (BMV) RNA2 gene used in all assays as an internal control (*Ct* mean 27.73 to 33.79).

Viruses are ubiquitous worldwide and in aquatic ecosystems this is no different. A diversity of viruses can be found, be it virioplankton viruses or others infecting animals, plants and humans (Lang *et al.*, 2009).

Viruses that cause gastroenteritis in humans were not detected in the samples analyzed. The absence of human enteric viruses by the RT-qPCR method was confirmed by sequencing, as no contig belonging to these viruses was mapped. Sequences belonging to the *Adenoviridae* family were found, however, they belonged to a bat adenovirus (Bat mastadenovirus C). Unlike the findings obtained in the present study, it is common to detect human enteric viruses in urban river waters, in both developed and developing countries (Lin & Singh 2015; Hata *et al.*, 2014; Vieira *et al.*, 2016; Pang *et al.*, 2019). On the other hand, the high presence of the *Myoviridae* family in all sampled sites indicate that there is fecal contamination, which can includes domestic animal waste from small poultry/swine farms or even from wild animals.

This family includes members of somatic coliphages commonly described in fecally contaminated waters and the findings demonstrate that the risk of other infections cannot be ruled out if there is contact with these environments (Jofre *et al.*, 2016).

Most of the viral families mapped belonged to double-stranded DNA and positive single-stranded RNA viruses. These findings show the high abundance of these viruses in aquatic environments, since most viruses that infect bacteria and archaeal have a DNA genome, furthermore, it is known that the most of the characterized prokaryotic viruses belong to the order *Caudovirales*, dsDNA bacteriophages with tails, which accounted for more than 90% of the viruses found in the set of river water analyzed (Krupovic *et al.*, 2011, 2018; Adriaenssens *et al.*, 2018).

Within the order *Caudovirales*, the *Myoviridae*, *Autographiviridae*, *Podoviridae* and *Siphoviridae* families were the most abundant. With the exception of the *Autographiviridae* family, all others are commonly identified in metagenomic studies as component of the river and wastewater virome (Cai *et al.*, 2016; Silva *et al.*, 2017; Rusinol *et al.*, 2020; Toribio-Avedillo *et al.*, 2021). This group comprises the somatic coliphages, the most environmental abundant indicator bacteriophages and whose infect *Escherichia coli* and other coliforms through the cell wall by binding to specific receptors on the outer membrane (Toribio-Avedillo *et al.*, 2021). The *Autographiviridae* family was recently proposed and is quite diverse, comprising nine subfamilies and 132 genera whose members are recognized to infect bacteria of the *Betaproteobacteria* and *Gammaproteobacteria* classes, in addition to the *Cyanobacteria* phylum (Adriaenssens *et al.*, 2020).

Table.1 Metrics obtained after sequencing of set of water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).

Sampling Point	Samples pooled	Amazon Season	Months of collection	No of raw reads	No of reads trimmed	No of contigs	Minimum size (bp)	Maximum size (bp)	Average size (bp)
Emboraí River	01HC	High rainy	2017 (Mar, Jun, Dec) 2018 (Feb to Jun, Dec)	31,825,078	31,688,512	155,614	200	87,273	729
	01HS	Less rainy	2017 (Sep, Nov) 2018 (Jul to Oct)	56,246,072	56,028,914	351,257	200	212,755	756
Urindeua River	02HC	High rainy	2017 (Mar, Jun, Dec) 2018 (Feb to Jun)	33,147,410	33,008,764	162,812	200	141,363	681
	02HS	Less rainy	2017 (Aug to Oct) 2018 (Jul to Nov)	55,039,720	54,830,312	312,347	200	106,826	717
Mocajuba River	05HC	High rainy	2017 (Apr, Jun, Dec) 2018 (Feb to Jun)	58,149,554	57,741,036	362,900	200	187,537	734
	05HS	Less rainy	2017 (Aug to Oct) 2018 (Jul to Nov)	71,511,892	71,216,560	484,040	200	161,184	749
Total				305,919,726	304,514,098	1,828,970			

Table.2 Values of frequency (outside parentheses), absolute and relative abundance (inside parentheses) of viral families identified in the set of water samples from rivers on the Amazon mangrove coast, Northern Brazil, according to genome and Amazon season. (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).

Baltimore Classification	Viruses genome	High Rainy % (abs/relat)	Less Rainy % (abs/relat)	Total % (abs/relat)
I	Double-strand DNA	99.9 (84,126/0,39389)	99.9 (129,312/0,60546)	99.9 (213,438/0,99935)
II	Single-strand DNA	0.02 (17/0,00008)	0.01 (18/0,00008)	0.01 (35/0,00016)
III	Double-strand RNA	0.001 (1/0,00000)	0.001 (2/0,00001)	0.001 (3/0,00001)
IV	Positive single-strand RNA	0.03 (32/0,00015)	0.02 (36/0,00017)	0.03 (68/0,00032)
V	Negative single-strand RNA	0.008 (7/0,00003)	0.01 (14/0,00007)	0.009 (21/0,00010)
VI	Reverse transcribing RNA	0.007 (6/0,00003)	0.003 (5/0,00002)	0.005 (11/0,00005)
Total		(84,189/0,39419)	(129,387/0,60581)	213,576

Fig.1 Map showing the sampling points of collection of surface water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil.

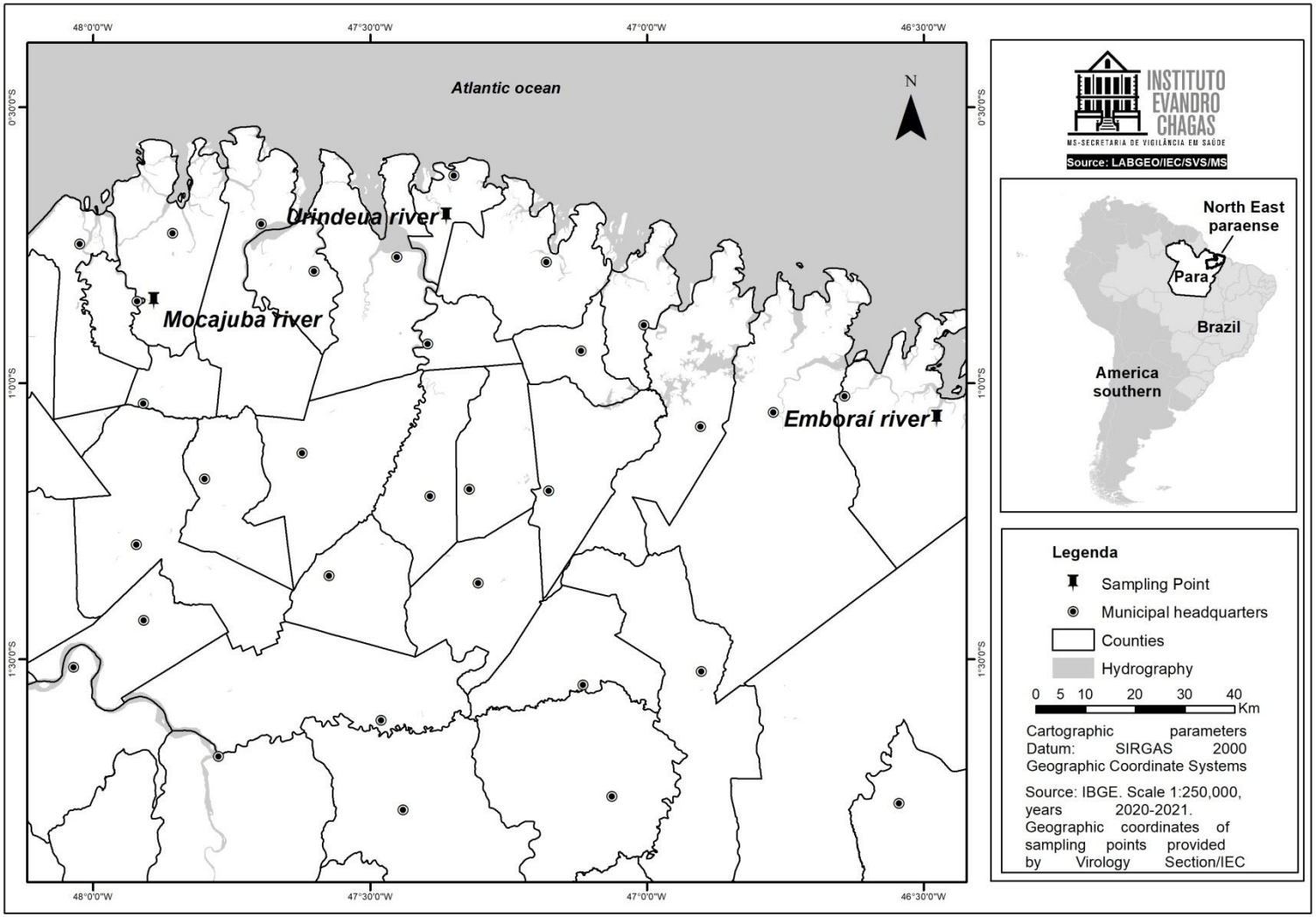


Fig.2 Heatmap showing the viral families abundance based on the number of reads mapped, in the water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil. The color scale represents the scale of values in ascending order: Yellow indicates lower values; Orange indicates average values (>260); Wine-colored indicates higher values. (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).

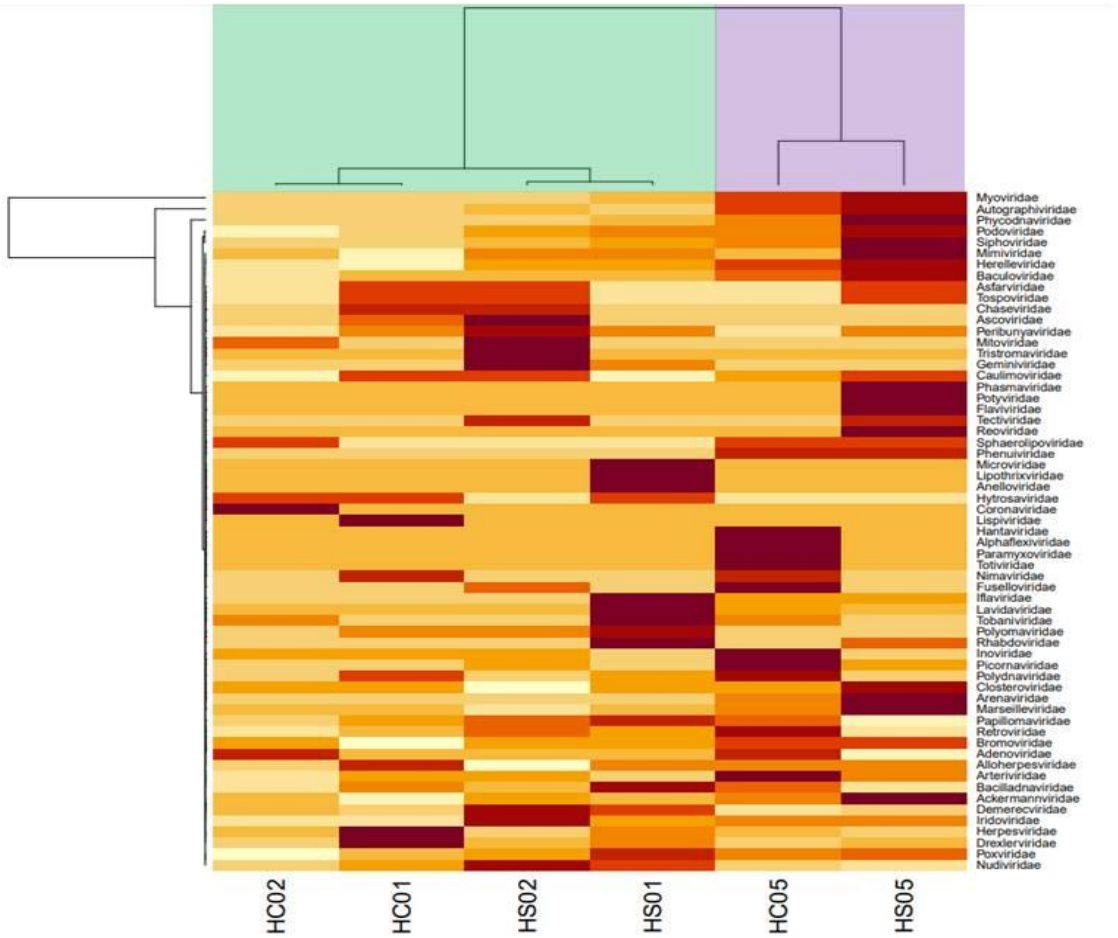


Fig.3 Rarefaction curves obtained in the water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil, according to three richness estimators: S obs Mao Tau (A), Chao1 (B) and Jack Knife (C).

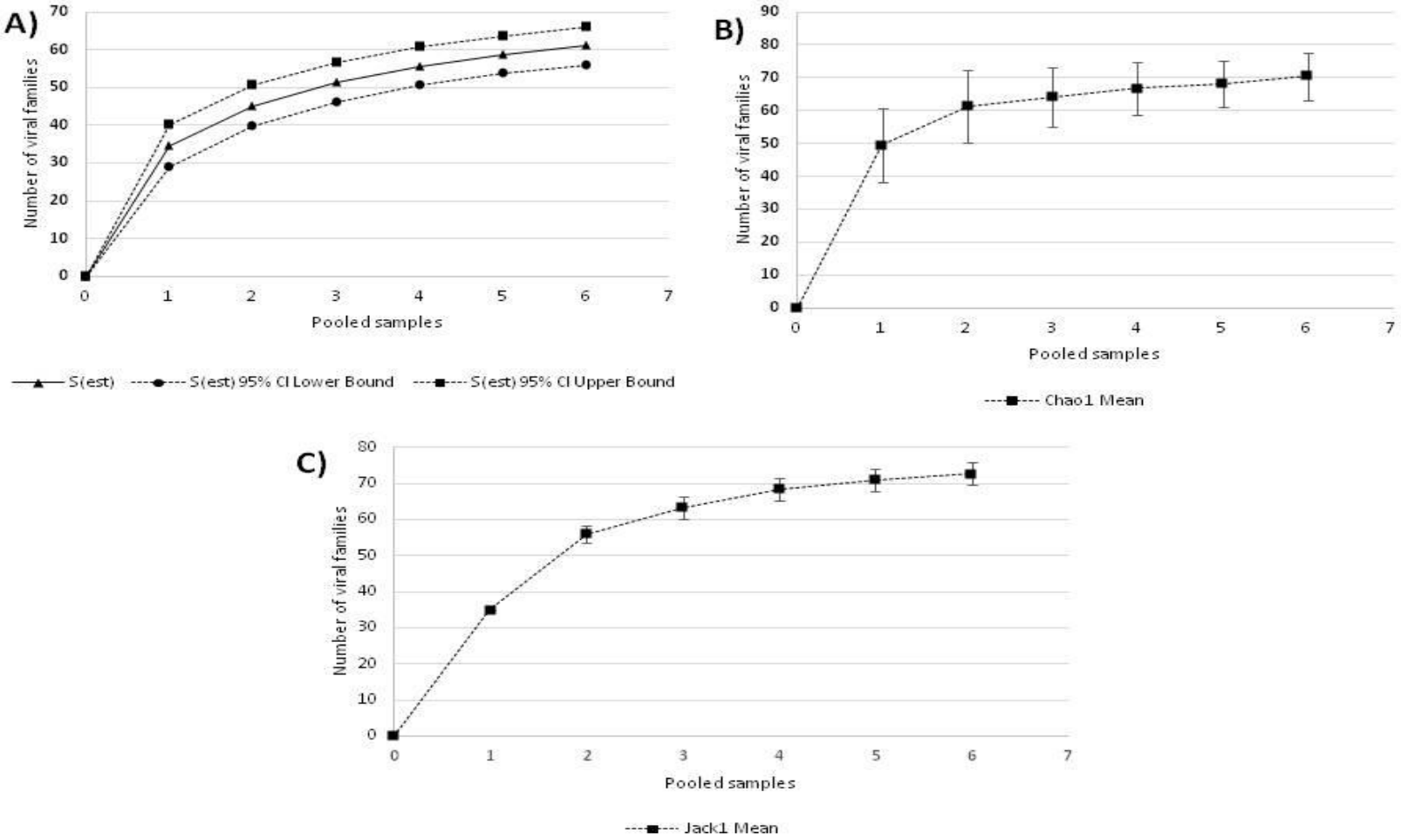


Fig.4 The ten most abundant viral families in the water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).

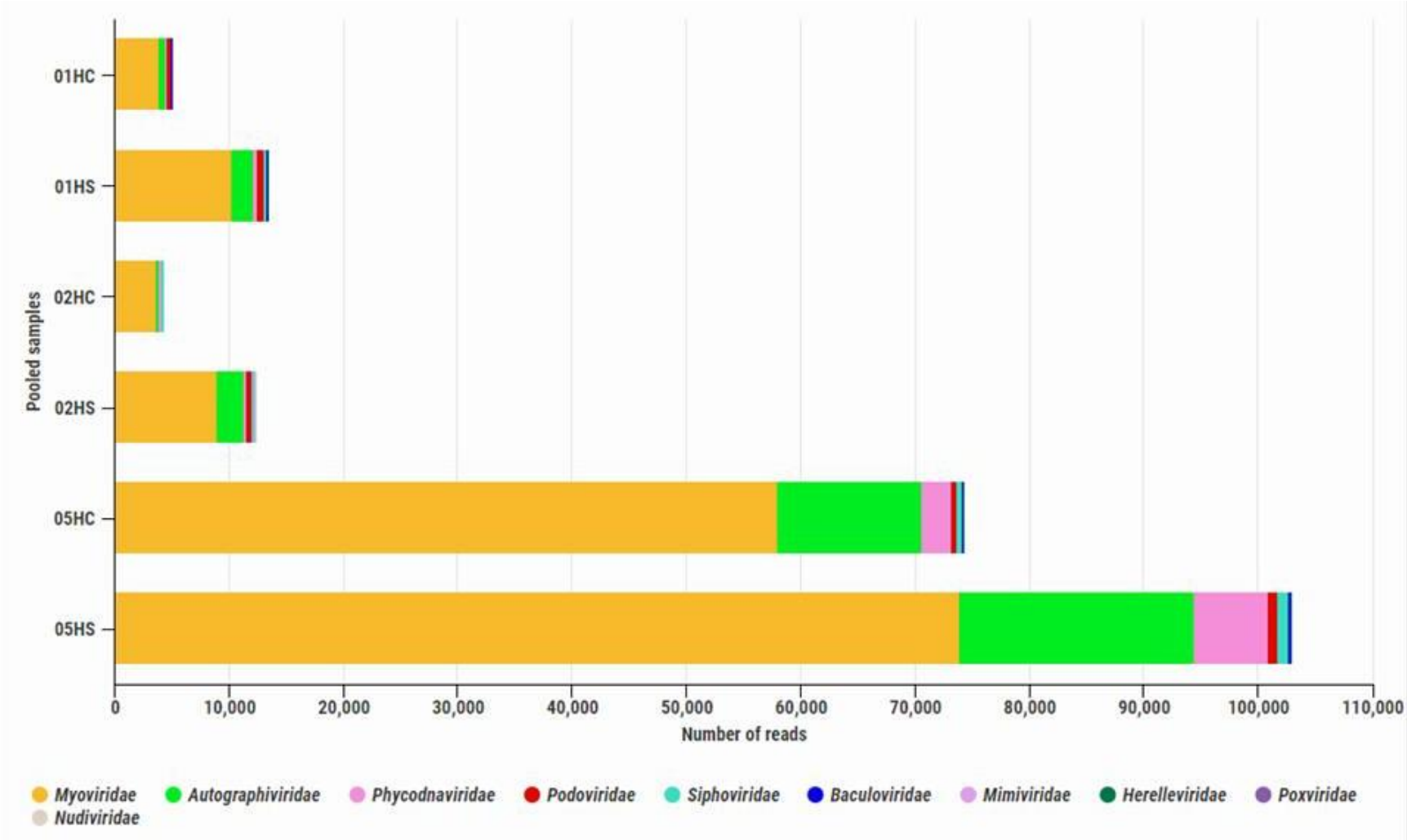


Fig.5 Venn diagram showing the list of families found in the periods of higher and lower precipitation in the water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil. The values presented between the intersections of the sets represent the number of families found in both Amazonian climatic seasons (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).

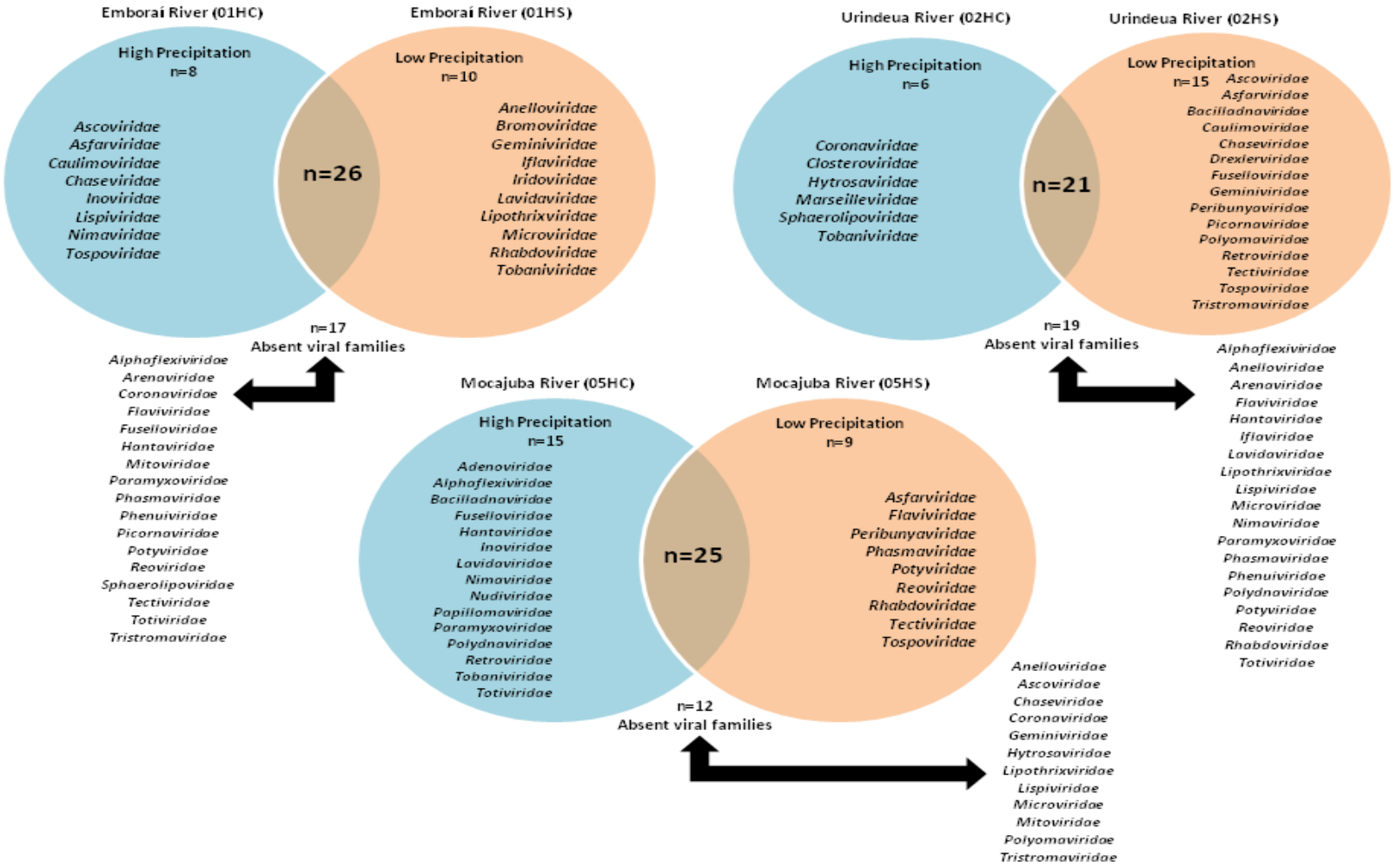
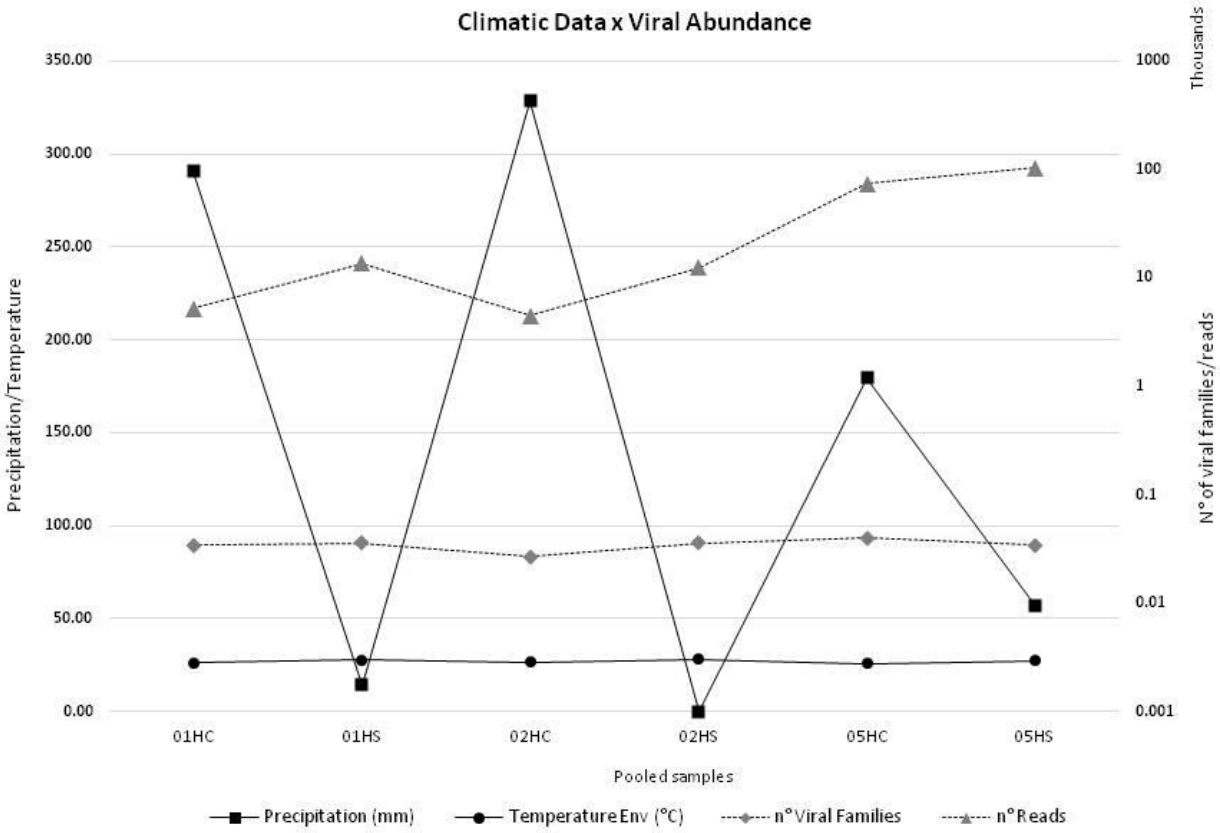


Fig.6 Climatic data and viral abundance in the water samples from rivers located on the Amazon mangrove coast, Northeast of Pará, Brazil (C=High rainy; S=Less Rainy; 01H Emboraí river; 02H Urindeua River, 05H Mocajuba river).



Other families known to include viruses that infect algae, protozoa, fungi, invertebrates, vertebrate or multiple hosts, even if in low abundance, were also found showing the diversity of the viral community in the evaluated ecosystems. In fact, studies show that environments with marine influence, such as mangroves, are more diversified, mainly due to the mixing of fresh and marine water and the sediment resuspension (Cai *et al.*, 2016). The *Herpesviridae* family, a very diverse one with three sub-families divided into more than 15 genera, was the most abundant (mainly in Emboraí river) among the viruses that infect vertebrate hosts, whose current members have hosts that include reptiles, birds and mammals (Gatherer *et al.*, 2021).

Positive single-stranded RNA viruses are the most diverse group of viruses with more than 20 described families (ICTV, 2011), of which at least eight families are known to infect marine animals (Lang *et al.*, 2009). In the present study, the families belonging to this group were: *Arteriviridae*, *Coronaviridae*, *Picornaviridae*, *Iflaviridae*, *Tobaniviridae*, *Flaviviridae*, *Potyviridae*, *Bromoviridae*, *Closteroviridae*, *Alphaflexiviridae* and *Mitoviridae*.

In this study, it was possible to view an increase in the number of viral reads in the period of lower rainfall for the rivers Emboraí and Urindeua, which was not noticeable for the Mocajuba River. Although rains facilitate the flow of pathogens from the land to the rivers, it increases and dilutes the water level; in addition, in periods of high rainfall, the water in Amazonian rivers becomes muddy, bringing inhibitors to concentration methods. In the less rainy period, the waters of the rivers are less diluted, which may have favored the binding of viruses to suspended solids that are in higher concentration, providing greater environmental persistence and protecting the viral particle from enzymes and other degrading agents, such as inactivation by solar radiation (Gerba, 2007). Studies have already been carried out in order to associate rainfall events with the viral profile in aquatic matrices. In Japan, there has been a trend towards increased concentrations of enteric viruses and faecal bacteria during and after rainfall events, due to the combined sewer system, that discharges wastewater into receiving water bodies along with the rain (Inoue *et al.*, 2020). In another study, a greater diversity and relative abundance of viruses was evidenced during summer compared to the winter in a subtropical freshwater reservoir in Taiwan, which is due to disturbances caused by typhoons during this period (Tseng *et al.*, 2013). Rusinol *et al.*, (2020) analyzing river water, showed greater viral diversity, with a high prevalence of bacteriophages, during autumn and winter.

The *Nimaviridae* family, found here exclusively in the Amazonian summer in the Emboraí and Mocajuba rivers, encompasses a single circular dsDNA virus species (White Spot Syndrome Virus) that infects a wide range of aquatic crustaceans causing a great economic impact on the productions (Wang *et al.*, 2019).

During the Amazonian winter, the only families detected in the rivers were *Geminiviridae* (Emboraí/ Urindeua), *Rhabdoviridae* (Emboraí/Mocajuba) and *Asfarviridae*, *Tectiviridae* and *Tospoviridae* (Urindeua/Mocajuba).

Geminiviridae are circular ssDNA plant viruses transmitted by insects (whiteflies, leafhoppers, treehoppers and aphids) with substantial economic impact in most tropical and subtropical regions of the world (Zerbini *et al.*, 2017).

Rhabdoviridae are eukaryotic viruses ssRNA(-) infecting different kingdom such as plants and animals (mammals, birds, reptiles and fish) in addition to arthropods that act as a host or vector in transmission to animals and plants (Walker *et al.*, 2018). *Tectiviridae* and *Tospoviridae* are viruses infecting bacteria and plants. *Asfarviridae* includes a dsDNA virus of African swine fever which infects domestic pigs and wild boar causing acute hemorrhagic fever and is transmitted by contact or by ticks (Alonso *et al.*, 2018). It is not possible to establish the origin of the viruses in the river waters

analyzed since they are estuaries with great influence of oceanic waters. Thus, further studies with a one-health approach are needed to carry out more effective environmental surveillance.

Although there were viral families identified exclusively in one of the Amazon climatic seasons, most of them were common to both seasons and in geographically distant sampling points, demonstrating that Amazonian Mangrove Rivers may have a typical virome, regardless of the impact exerted by the rains. In this sense, new studies should be developed aiming to characterize viromes in other rivers, including those located in mangroves.

Many years ago, the study of viral diversity in aquatic environments was a difficult task to perform, as the available methodologies depended on the viruses being present in a large number to be detectable by electron microscopy or molecular approaches. Today, the knowledge about the diversity analysis of complete viral genomes on complex samples, such as environmental ones, has been stimulated with the advance of next-generation sequencing. This study showed the diversity of the viral community in three different mangroves rivers by *NGS* approach and the absence of common human waterborne-viruses, such as the enteric viruses. More studies should be carried out in order to elucidate the role of the natural virus population in the ecology of aquatic ecosystems and the impacts caused by biotic and abiotic factors on viral diversity and resistance, including the effect of rainfall and water physical chemical parameters.

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References

Adriaenssens E M, Farkas K, Harrison C, Jones D L, Allison H E, McCarthy A J. 2018. Viromic analysis of wastewater input to a river catchment reveals a diverse assemblage of RNA viruses. mSystems, 3(3):e00025-15. doi: 10.1128/mSystems.00025-18.

Adriaenssens E M, Sullivan M B, Knezevic P, van Zyl L V, Sarkar B L, Dutilh B E, Alfenas-Zerbini P, Loboka M, Tong Y, Brister J R, Switt A I M, Klumpp J, Aziz R K, Barylski J, Uchiyama J, Edwards RA, Kropinski AM, Petty N K, Clokie M R J, Kushkina A I, Morozova V V, Duffy S, Gillis A, Rumnieks J, Kurtboke I, Chanishvili N, Goodridge L, Wittmann J, Lavigne R, Bin Jang H, Prangishvili D, Enault F, Turner D, Poranen M M, Oksanen H M, Krupovic M. 2020. Taxonomy of prokaryotic viruses: 2018-2019 update from the ICTV bacterial and archaeal viruses subcommittee. Arch Virol, 165(5): 1253-1260. doi: 10.1007/s00705-020-04577-8.

Alonso C, Borca M, Dixon L, Revilla Y, Rodriguez F, Escribano J M, ICTV Report Consortium. 2018. ICTV Virus Taxonomy Profile: Asfarviridae. J Gen Virol, 99(5): 613-614. doi: 10.1099/jgv.0.001049.

Bányai k, Estes M K, Martella V, Parashar U D. 2018. Viral gastroenteritis. Lancet, 14;392(10142):175-186. doi: 10.1016/S0140-6736(18)31128-0.

Cadamuro RD, Viancelli A, Michelon W, Fonseca TG, Mass AP, Krohn DMA, Peter NRW, Fongaro G. 2021. Enteric viroses in lentic and lotic freshwater habitats from Brazil's Midwest and South regions in the Guarani Aquifer area. Environ Sci Pollut Res Int, 28(24):31653-31658. doi: 10.1007/s11356-021-13029-y.

Cai L, Zhang R, He Y, Feng X, Jiao N. 2016. Metagenomic analysis of virioplankton of the subtropical Jiulong river estuary, China. Viruses, 8(2): 35. doi: 10.3390/v8020035.

Calgua B, Mengewein A, Grunert A, Boffil-Mas S, Clemente-Casares P, Hundesa A, Wyn-Jones A P, Lopez-Pila J M, Girones R. 2008. Development and aplication of a one-step low cost procedure to concentrate viruses from seawater samples. J Virol Methods, 153(2): 79-83. doi: 10.1016/j.jviromet.2008.08.003.

Colwell R K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Persistent URL <purl.oclc.org/estimates>.

Gatherer D, Depledge D P, Hartley C A, Szpara M L, Vaz P K, Benko M, Brandt C R, Bryant N A, Dastjerdi A, Doszpoly A, Gompels U A, Inoue N, Jarosinski K W, Kaul R, Lacoste V, Norberg P, Origgi F C, Orton R J, Pellett P E, Schmid D S, Spatz S J, Stewart J P, Trimpert J, Waltzek T B, Davison A J. 2021. ICTV virus taxonomy profile: Herpesviridae 2021. 2021. J Gen Virol, 102(10): 001673. doi: 10.1099/jgv.0.001673.

Gerba, C. P. Virus occurrence and survival in the environmental waters. In: Bosch, A.; Zuckerman, A. J.; Mushahwar, I. K. Human viruses in water. Oxford: Elsevier, 2007, p. 91-108.

Grabow W O K. Overview of health-related water virology. In: Human viruses in water. Albert Bosch editor. 2007. doi 10.1016/S0168-7069(07)17001-4. P. 1-25. Perspectives in medical virology, 17. 299 p.

Hata A, Katayama H, Kojima K, Sano S, Kasuga I, Kitajima M, Furumai H. 2014. Effects of rainfall events on the occurrence and detection efficiency of viruses in river water impacted by combined sewer overflows. Sci Total Environ, 468-469:757-63. doi: 10.1016/j.scitotenv.2013.08.093.

ICTV 2011. https://talk.ictvonline.org/ictv-reports/ictv_9th_report/. Virus Taxonomy: The Classification and Nomenclature of Viruses The 9th Report of the ICTV (2011).

Inoue K, Asami T, Shibata T, Furumai H, Katayama H. 2020. Spatial and temporal profiles of enteric viruses in the coastal waters of Tokyo Bay during and after a series of rainfall events. Sci Total Environ, 727:138502. doi: 10.1016/j.scitotenv.2020.138502.

Jofre J, Lucena F, Blanch A, Muniesa M. 2016. Coliphages as model organisms in the characterization and management of watrer resources. Water, 8(5): 199. doi: 10.3390/w8050199.

Krupovic M, Cvirkaite-Krupovic V, Iranzo J, Prangishvili D, Koonin E V. 2018. Viruses of archaea: Structural, functional, environmental and evolutionary genomics. Virus Res, 244:181-193. doi: 10.1016/j.virusres.2017.11.025.

Krupovic M, Prangishvili D, Hendrix R W, Bamford DH. 2011. Genomics of bacterial and archaeal viruses: dynamics within the prokaryotic virosphere. Microbiol Mol Biol Rev, 75(4):610-35. doi: 10.1128/MMBR.00011-11.

Lang A S, Rise M L, Culley A I, Steward G F. 2009. RNA viruses in the sea. FEMS Microbiol Rev, 33(2): 295-323. doi: 10.1111/j.1574-6976.2008.00132.x.

Lin J, Singh A. 2015. Detection of human enteric viruses in Umgeni River, Durban, South Africa. J Water Health, 13(4):1098-112. doi: 10.2166/wh.2015.238.

McMinn B R, Ashbolt N J, Korajkic A. 2017. Bacteriophages as indicators of faecal pollution and enteric virus removal. Lett Appl Microbiol, 65(1): 11-26. doi: 10.1111/lam.12736.

Pang X, Qiu Y, Gao T, Zurawell R, Neumann N F, Craik S, Lee B E. 2019. Prevalence, levels and seasonal variations of human enteric viruses in six major rivers in Alberta, Canada. Water Res, 153:349-356. doi: 10.1016/j.watres.2019.01.034.

Rusinol M, Martinez-Puchol S, Timoneda N, Fernandez-Cassi X, Perez-Cataluna A, Fernandez-Bravo A, Moreno-Mesonero L,

Moreno Y, Alonso J L, Figueras M J, Abril J F, Boffil-Mas S, Girones R. 2020. Metagenomic analysis of viruses, bactéria and protozoa in irrigation water. Int J Hyg Environ Health, 224:113440. doi: 10.1016/j.ijheh.2019.113440.

Silva B S O, Coutinho F H, Gregoracci G B, Leomil L, Oliveira L S, Froes A, Tschoeke D, Soares A C, Cabral A S, Ward N D, Richey J E, Krusche A V, Yager P L, Rezende C E, Thompson C C, Thompson F L. Virioplankton assemblage structure in the lower river and ocean continuum of the Amazon. 2017. mSphere, 2(5): e00366-17. doi: 10.1128/mSphere.00366-17.

Suttle C A. 2005. Viruses in the sea. Nature, 437(7057):356-61. doi: 10.1038/nature04160.

Tenorio G S, Souza-Filho P W M, Ramos E M L S, Alves P J O. 2015. Mangrove shrimp farm mapping and productivity on the Brazilian Amazon coast: Environmental and economic reasons for coastal conservation. Ocean & Coastal Management, 104:65-77. doi: 10.1016/j.ocecoaman.2014.12.006.

Toribio-Avedillo D, Blanch A R, Muniesa M, Rodríguez-Rubio L. 2021. Bacteriophages as fecal pollution indicators. Viruses, 13(6): 1089. doi: 10.3390/v13061089.

Tseng C, Chiang P, Shiah F, Chen Y, Liou J, Hsu T, Maheswararajah S, Saeed I, Halgamuge S, Tang S. 2013. Microbial and viral metagenomes of a subtropical freshwater reservoir subject to climatic disturbances. ISMEJ, 7(12): 2374-2386. doi: 10.1038/ismej.2013.118.

Vieira C B, Correa A A, Jesus M S, Luz S L B, Wyn-Jones P, Kay D, Vargha M, Miagostovich M P. 2016. Viruses Surveillance under Different Season Scenarios of the Negro River Basin, Amazonia, Brazil. Food Environ Virol, 8(1):57-69. doi: 10.1007/s12560-016-9226-8.

Walker P J, Blasdell K R, Calisher C H, Dietzgen RG, Kondo H, Kurath G, Longdon B, Stone D M, Tesh R B, Tordo N, Vasilakis N, Whitfield A E, ICTV Report Consortium. 2018. ICTV Virus Taxonomy Profile: Rhabdoviridae.J Gen Virol, 99(4): 447-448. doi: 10.1099/jgv.0.001020.

Wang H, Hirono I, Maningas M B B, Somboonwiwat K, Stentiford G, ICTV Report Consortium. 2019. ICTV Virus Taxonomy Profile: Nimaviridae. J Gen Virol, 100(7):1053-1054. doi: 10.1099/jgv.0.001248.

Wommack K E, Colwell R R. 2000. Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev, 64(1):69-114. doi: 10.1128/MMBR.64.1.69-114.2000.

Zerbini F M, Briddon R W, Idris A, Martin D P, Moriones E, Navas-Castillo J, Rivera-Bustamante R, Roumagnac P, Varsani A, ICTV Report Consortium. 2017. ICTV Virus Taxonomy Profile: Geminiviridae. J Gen Virol, 98(2): 131-133. doi: 10.1099/jgv.0.000738.

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