

ANALYSIS OF COMPOSITION AND ABUNDANCE OF VIRAL COMMUNITIES ASSOCIATED WITH CORAL *Acropora formosa*

Doan Thi Nhung¹, Bui Van Ngoc^{1,2,*}

¹Institute of Biotechnology, VAST, Vietnam

²Graduate University of Science and Technology, VAST, Vietnam

Received 13 October 2020, accepted 8 August 2021

ABSTRACT

Coral reefs harbor the extraordinary biodiversity and not only provide livelihoods for coastal communities but also play a crucial role in economic development generally. Unfortunately, they are in decline in Vietnam and around the world because mass coral bleaching events have become more common worldwide. However, little is discovered, about viruses that infect corals and their symbionts. Herein, we present metagenomic analyses of the viral communities in coral mucus associated with healthy and bleached coral *Acropora formosa* which was collected at Con Dao Island, Vietnam. Interestingly, the number of viral species in bleached specimens are higher than those in healthy status. Viruses similar to those that infect humans and some marine animals also appeared in the coral viral assemblage. The results indicated that the proportion of shared viruses were quite small, and represented extremely abundance. Among the phage identified, vibriophage and cyanophage were only presented in healthy and bleached coral, respectively. Therefore, coral-associated viruses could prospectively infect all constituents of the holobiont - coral, microalgal and microbial. Thus, we expect viruses to be illustrated prominently in the preservation and breakdown of coral health.

Keywords: *Acropora formosa*, DADA2, Kaiju, Krona, metagenomics, R programming language, virus, whole genome sequencing.

Citation: Doan Thi Nhung, Bui Van Ngoc, 2021. Analysis of composition and abundance of viral communities associated with coral *Acropora formosa*. *Academia Journal of Biology*, 43(3): 113–123. <https://doi.org/10.15625/2615-9023/15595>

*Corresponding author email: bui@ibt.ac.vn

©2021 Vietnam Academy of Science and Technology (VAST)

INTRODUCTION

Corals are covered by a gel-forming mucus layer, containing plentiful nutrients for a great of symbiotic microorganisms, such as archaea, viruses, bacteria, algae and fungi (Bythella & Wild, 2011; Vollmer et al., 2013). These organisms form a community living in close relationship with coral called coral holobiont, play an important role in providing corals nutrition by photosynthesis process (Kim, 2006), for examples, coral-associated bacteria supply a food source for corals such as the Cyanobacteria symbiotic on the coral provide large amounts of nitrogen through nitrogenase enzyme and beneficial metabolic capabilities (Lesser et al., 2004); and protection by synthesizing antibiotics to fight pathogens (Kvennefors et al., 2012). Some hypotheses supposed that the changes in coral holobiont composition will enable coral survival under future environmental changes (Reshef et al., 2006). In recent years, the degradation of coral reefs along the coastal area in Vietnam has been investigated. According to Dung (2020), bleaching corals are observed widely along Vietnam's coastal zones. Bleaching etiology is induced by the loss of coral endosymbiotic zooxanthellae (genus *Symbiodinium*). Aside from environmental factors, some argue that bacterial infection is considered as the associated impact on disrupting algal photosynthesis (Rosenberg et al., 2009). For instance, *Vibrio* spp. are pathogens that cause the bleaching of the coral *Oculina patagonica* (Kushmaro et al., 2001) and *Pocillopora damicornis* (Ben-Haim et al., 2003). Consequently, the relation of bacteria with other microorganisms in the coral holobiont is concerned.

Recently, viruses have been proved to infect all the microorganisms, containing the prokaryotic and eukaryotic species (Marhaver et al., 2008; Soffer et al., 2014). Through observations of virus-like particles in corals, it seems that their presence is an indicator of coral stress or disease (Davy et al., 2006). The high correlation between viruses and bacteria in mucus layer has been demonstrated in some

studies in Vietnam's coral habitats (Nguyen-Kim et al., 2015; Pham The Thu, 2014). Since viruses are ubiquitous in the ocean and represent a notable factor of bacterial control (Fuhrman & Schwalbach, 2003; Suttle, 2007), it is expected that coral virus populations will include abundant bacteriophages. Therefore, in the direction of coral microbiological research, studies of viral composition and abundance are highly potential and meaningful, contributing to the explorations of microorganism community interactions in the coral mucus layer.

Previously, studies on marine microorganisms in general and coral viruses in particular were limited because most of them can be cultured using traditional methods, which made it difficult to analyze the composition of a whole microbial community. The recent advances in DNA sequencing technologies have supported scientists to access the function of coral reefs and microbial diversity, improving knowledge about the mechanisms of microorganism impactation to the stability of the coral reef ecosystem. Indeed, metagenomics and next-generation sequencing (NGS) technology have been developed in recent years for the determination and nomenclature of both culturable and unculturable microorganisms. These technologies investigate the complex microbial communities and their response mechanisms to environmental stresses, access to the taxonomic compositions, relative abundances and metabolic potential of all microbes within the coral holobiont (Kysela et al., 2005). Metagenomics is also a powerful tool for identifying the coral pathogens and disease-forming mechanisms, for example the work of Sato et al. (2017) elucidating black band disease pathogenesis. Cooperating with bioinformatic tools enhances metagenomic results analysis effectively, especially for a huge amount of data in this field of microbiome. Moreover, there are powerful tools owned the ability to display results into graphs that allow users to make visual assessments. Application of this approach has been used in several aspects of marine biology

such as fish identification by DNA barcoding (Nguyen Manh Linh et al., 2019), gene screening from sponge-associated microorganisms (Tran Thi Hong et al., 2019) or whole genome sequencing of microalgae (Nguyen Van Lam et al., 2015), but rare of viral association with corals.

The bleaching event in Con Dao Island had continued since 1998 (Vo, 2000), to which *Acropora* is the most susceptible coral genus after 20 years (Vo et al., 2020). In this study, we will analyze the composition of the viral community living in the mucus layer of coral *Acropora formosa* (Dana, 1846) in healthy and bleached samples by classifying and assigning taxonomy to them went inferring obtained metagenomic sequences from the public database. We will also access the viral diversity and relative abundance to evaluate the dominant species in each community of health status and find the differences. This study is a premise for further studies of viral and microorganism communities living in coral and their effects on coral health to guide future long-term protection measures.

MATERIALS AND METHODS

Data used in this study was the metagenomic data of viral community in the mucus layer of coral *A. formosa*, provided by the Department of Bioinformatics (Institute of Biotechnology, Vietnam Academy of Science and Technology, Vietnam). Viral samples were collected twice, once in the dry season and once in the wet season, at Con Dao Island, Vietnam. Their genomes were extracted by viral nucleic acids extraction kit (Roche, Diagnostics, Meylan, France) and sequenced using Miseq short-read sequencing platform (Illumina Inc., San Diego, USA).

First, the metagenome database collected above was cleaned up to trim low-quality reads (QC < 30) for downstream and further

analyses by the *filterandtrim* function of DADA2 package in R programming language (Callahan et al., 2016). Then, the clean database was processed and using the Kaiju program to classify and assign taxonomy to the viral community. Kaiju is a program in which high-throughput sequencing reads (Illumina, Roche/454) from metagenomic whole genome sequencing or metatranscriptomics experiments are classified taxonomy sensitively (Menzel et al., 2016). Sequencing reads are assigned to taxa in the NCBI taxonomy and a reference database including microbial and viral protein sequences. As Kaiju uses protein-level classification, the process reaches a higher sensitivity compared with nucleotide methods. Kaiju is a free software allowing users to modify and redistribute it under the terms of the GNU General Public License. Subsequently, we use the taxonomic results along with their frequency data from kaiju to analyze the composition and abundance of the viral communities. At the same time, we also compare and find out the differences between the two communities of two health status and visualize the results by Krona (Ondov et al., 2011), thereby, we expect to explore some viruses which just appear in either healthy or bleached coral mucus sample. These results will contribute and support further studies of coral microbiome in the future.

RESULTS AND DISCUSSION

After filtering out low-quality sequencing reads and trimming the reads to a consistent length of the two samples, the results in Table 1 showed that we acquired 42599 clean reads from 52486 reads in the bleached coral sample, meaning approximately 19% low-quality reads were removed. Besides, with the healthy coral sample, we also filtered and retained over 82% reads (37786 reads in, 31047 reads out).

Table 1. Filterandtrim statistic result

	Reads.in	Reads. out	Frequency (%)
Bleached	52486	42599	81,16
Heathy	37786	31047	82,16

Table 2. The first 20 viral species identified in the mucus layer of coral *A. formosa*

	Kingdom	Phylum	Class	Order	Family	Genus	Species
Bleached	<i>Heunggongvirae</i>	<i>Uroviricota</i>	<i>Caudoviricetes</i>	<i>Caudovirales</i>	<i>Podoviridae</i>	unclassified <i>Podoviridae</i>	<i>Puniceispirillum phage HMO-2011</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	unclassified <i>Circoviridae</i>	<i>Avon-Heathcote Estuary associated circular virus 19</i>
	<i>Sangervirae</i>	<i>Phixviricota</i>	<i>Malgrandaviricetes</i>	<i>Petitvirales</i>	<i>Microviridae</i>	unclassified <i>Microviridae</i>	<i>Microviridae Fen7940_21</i>
	<i>Sangervirae</i>	<i>Phixviricota</i>	<i>Malgrandaviricetes</i>	<i>Petitvirales</i>	<i>Microviridae</i>	unclassified <i>Microviridae</i>	<i>Microviridae Fen7918_21</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Repensiviricetes</i>	<i>Geplafuvirales</i>	<i>Geminiviridae</i>	<i>Begomovirus</i>	<i>Lycianthes yellow mosaic virus</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	<i>environmental samples</i>	<i>Circoviridae 6 LDMD-2013</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	unclassified <i>Circoviridae</i>	<i>Palaemonetes kadiakensis Mississippi grass shrimp associated circular virus</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	unclassified <i>Circoviridae</i>	<i>Pleurochrysis carterae circular virus</i>
	<i>Shotokuvirae</i>	<i>Cressnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	unclassified <i>Circoviridae</i>	<i>Hermit crab associated circular virus</i>

	<i>Shotokuvirae</i>	<i>Cressdnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	<i>Cyclovirus</i>	<i>Dragonfly associated cyclovirus 3</i>
Healthy	<i>Bamfordvirae</i>	<i>Nucleocytoviricota</i>	<i>Megaviricetes</i>	<i>Algavirales</i>	<i>Phycodnaviridae</i>	<i>Prymnesiovirus</i>	<i>Phaeocystis globosa virus</i>
	<i>Loebvirae</i>	<i>Hofneiviricota</i>	<i>Faserviricetes</i>	<i>Tubulavirales</i>	<i>Inoviridae</i>	<i>Vicialiavirus</i>	<i>Vibrio phage VCY-phi</i>
	<i>Sangervirae</i>	<i>Phixviricota</i>	<i>Malgrandaviricetes</i>	<i>Petitvirales</i>	<i>Microviridae</i>	unclassified <i>Microviridae</i>	<i>Alces alces faeces associated-microvirus MP21 4718</i>
	<i>Shotokuvirae</i>	<i>Cressdnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	<i>Cyclovirus</i>	<i>Dragonfly associated cyclovirus 4</i>
	<i>Shotokuvirae</i>	<i>Cossaviricota</i>	<i>Quintoviricetes</i>	<i>Piccovirales</i>	<i>Parvoviridae</i>	<i>Dependoparvovirus</i>	<i>Avian dependoparvovirus 1</i>
	<i>Shotokuvirae</i>	<i>Cressdnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	<i>Circovirus</i>	<i>Canine circovirus</i>
	<i>Shotokuvirae</i>	<i>Cressdnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	<i>Circovirus</i>	<i>Starling circovirus</i>
	<i>Sangervirae</i>	<i>Phixviricota</i>	<i>Malgrandaviricetes</i>	<i>Petitvirales</i>	<i>Microviridae</i>	unclassified <i>Microviridae</i>	<i>Microviridae Fen7895_21</i>
	<i>Shotokuvirae</i>	<i>Cressdnaviricota</i>	<i>Arfiviricetes</i>	<i>Cirlivirales</i>	<i>Circoviridae</i>	unclassified <i>Circoviridae</i>	<i>Callinectes ornatus blue crab associated circular virus</i>
	<i>Sangervirae</i>	<i>Phixviricota</i>	<i>Malgrandaviricetes</i>	<i>Petitvirales</i>	<i>Microviridae</i>	unclassified <i>Microviridae</i>	<i>Parabacteroides phage YZ-2015a</i>

When analyzing viral metagenomic sequence data by Kaiju program, we acquired the taxonomic assignments for 202 viral species (118 species in bleached coral and 84 species in healthy one) living in the mucus layer of coral *A. formosa* (Table 2). Overall, most of the species were commonly found in the sea water and around coral reefs. The change in the composition of the viral community in bleached corals compared to healthy ones was acceptable with previous studies (Nguyen-Kim et al., 2015). Simultaneously, their compositions and abundances in relation to each coral health were acquired based on the frequencies of specific coding sequences appeared in the processed data. Thereby, we analyzed the fluctuations of viral community at different levels, which were shown in the Krona pie charts below to visualize the results.

In Figure 1a, the results represented the average relative abundance of viral association in bleached coral at the phylum level. Phylum *Cressdnaviricota*, belonging to kingdom *Shotokuvirae*, dominated the community, accounting for about 91% of total identified viruses. In addition, the

second in terms of quantity was phylum *Uroviricota* with 3%, followed by the phyla *Phixviricota* and *Hofneiviricota* at 2%. Regarding healthy corals (Fig. 1b), phylum *Uroviricota* was dominant with the highest proportion of 40%. Subsequently, phylum *Cressdnaviricota* was accounted for 27% and ranked second, followed by phylum *Hofneiviricota* with 26%. There were some remarkable differences in the viral composition between two status of coral health. For instance, two phyla *Cressdnaviricota* and *Uroviricota* witnessed obvious fluctuations between panels (phylum *Cressdnaviricota* with 91% dominated in bleached coral but rapidly decreased to 27% in healthy coral). A similar trend was applied to other phyla, which suggested that viral composition and coral health status might affect each other. In sum, the results in Figure 1 indicated the dominance of the four phyla *Cressdnaviricota*, *Uroviricota*, *Phixviricota* and *Hofneiviricota* in both two coral samples, accounted for over 98% of total viruses in the bleached sample and 94% in the healthy one.

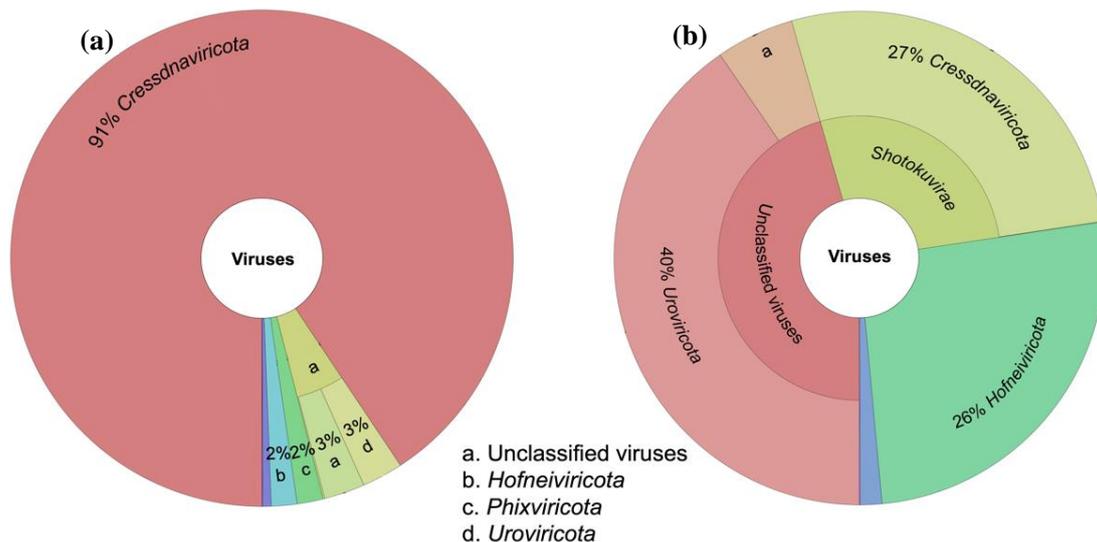


Figure 1. Composition and abundance of viral communities in bleached (a) and healthy (b) coral at phylum level. The pie charts showed taxonomic classification of viral communities in different status of coral health at phylum level using Kaiju program along with the percentage of each phylum compared to the total viruses

At class level, the viral class with the highest amount in bleached coral was *Repensiviricetes* (phylum *Cressdnaviricota*; kingdom *Shotokuvirae*) and in healthy coral was *Caudoviricetes* (phylum *Uroviricota*; kingdom *Heunggongvirae*), accounting for 82% and 40% of total viruses, respectively. The shift of viral composition at the class level was similar to the one at the phylum

level, shown in Figure 2. Class *Arfiviricetes* and class *Faserviricetes* had higher percentages in healthy coral (15% and 26%), compared to that of bleached corals (8% and 2%). Class *Repensiviricetes* was accounted for a small proportion in the healthy community (13%). Only the percentage of class *Malgrandaviricetes* showed an insignificant change in two health status.

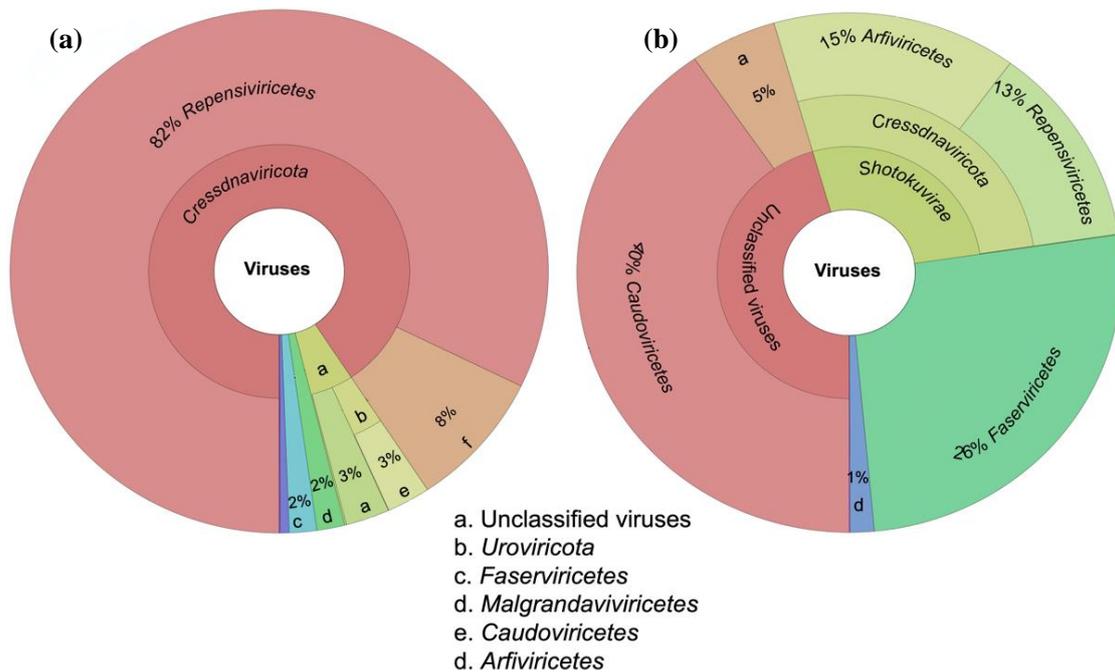


Figure 2. Composition and abundance of viral communities in bleached (a) and healthy (b) corals at class level. The pie charts illustrated taxonomic classification of viral communities in different status of coral health at class level using Kaiju program along with the percentage of each class compared to the total viruses

In Figure 3, coral-associated viral metagenomic sequences were analyzed to identify the mucus microbiome and the diversity of the viral community at genus (Figs. 3a, 3b) and species (Figs. 4a, 4b) levels. Five genera (*Gemykrogvirus*, *Circovirus*, unclassified *Circoviridae*, unclassified *Siphoviridae* and unclassified *Microviridae*) were fairly abundant in both healthy and bleached corals. There were some genera (Figs. 3a, 3b), which had significant shifts

such as *Gemykrogvirus* (0.09–2%), unclassified *Circoviridae* (7–12%), *Circovirus* (0.12–1.06%) and unclassified *Siphoviridae* (0.07–37%). Besides, genera *Dependoparvovirus*, *Escherichia virus If1*, *Fibrovirus*, *Alphabaculovirus* and *Prymnesiovirus* only appeared in healthy coral while the other genera *Chivirus*, *Uetakevirus*, *Chlamydiamicrovirus*, *Autographivirinae*, *Gokushovirinae* and *Ralstonia virus RSS1* were found in bleached coral mucus sample.

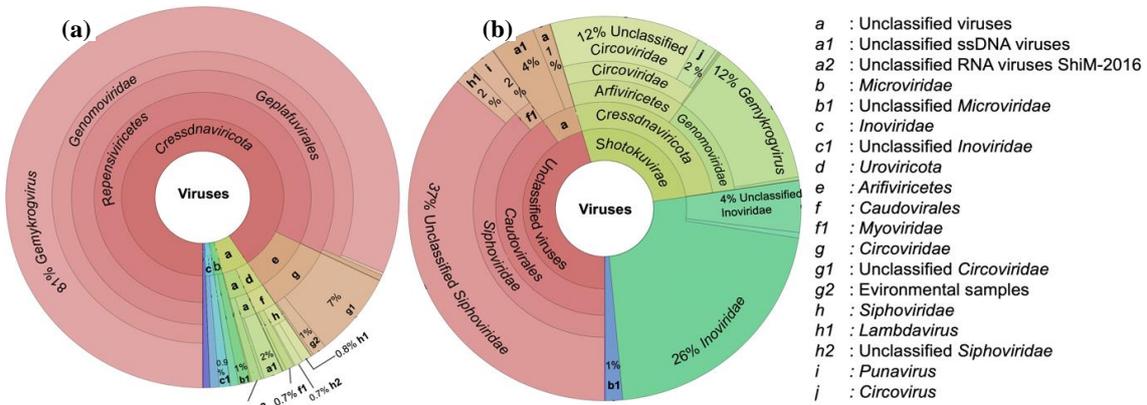


Figure 3. Composition and abundance of viral communities in bleached (a) and healthy (b) corals at genus level. The pie charts demonstrated taxonomic classification of viral communities in different status of coral health at genus and species level using Kaiju program along with the percentage of each genus and species compared to the total viruses

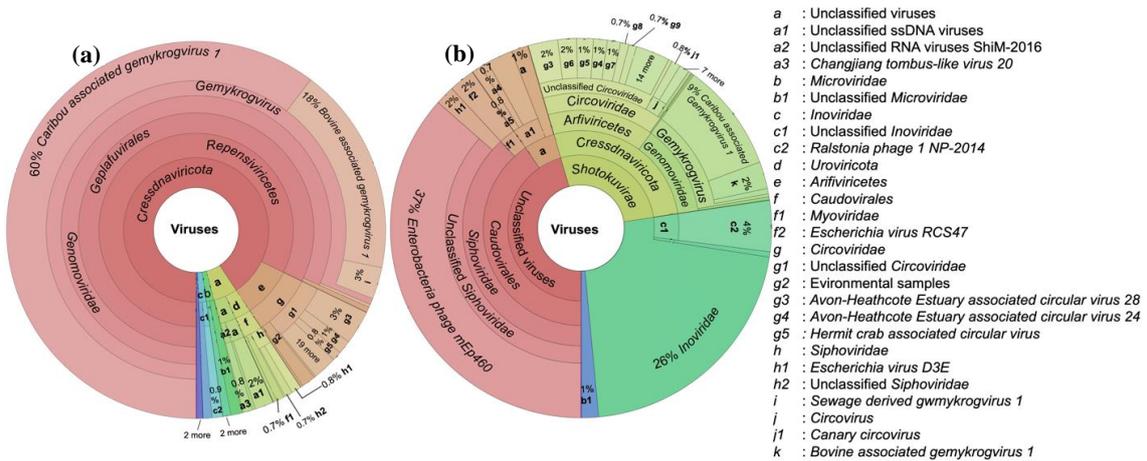


Figure 4. Composition and abundance of viral communities in bleached (a) and healthy (b) corals at specie level. The pie charts demonstrated taxonomic classification of viral communities in different status of coral health at genus and species level using Kaiju program along with the percentage of each genus and species compared to the total viruses

At the species level (Figs. 4a, 4b), there were a notable fluctuation inconsiderate species indicated the differences between the two communities. The viral species *Human associated circovirus 1* (genus *Circovirus*; family *Circoviridae*), *Microviridae Fen7895_21* (genus *unclassified Microviridae*; family *Microviridae*), *Human fecal virus Jorvi4* (genus *unclassified Circoviridae*; family *Circoviridae*), *Stenotrophomonas phage SMA7* (genus

Subteminivirus; family *Inoviridae*) and *Vibrio virus VCY* (genus *Vicialiavirrus*; family *Inoviridae*) were found in healthy coral samples. Otherwise, some only appeared in bleached coral like species *Microviridae Fen7940_21* (genus *unclassified Microviridae*; family *Microviridae*), *Microviridae Fen7918_21* (genus *unclassified Microviridae*; family *Microviridae*), *Bovine faeces associated circular DNA virus 1*, *Synechococcus phage S-SM2* (genus

unclassified *Bellamyvirus*; family *Bellamyvirus*), *Ralstonia phage RSK1* (genus unclassified *Podoviridae*; family *Podoviridae*) and *Prochlorococcus virus PTIM40* (genus *Libanvirus*; family *Myoviridae*). The viral associations of two coral health status were almost separated and the diversity of each community were great. The appearances of the coral-associated phages, especially vibriophage (*Vibrio* phage VCY-phi) in healthy coral and cyanophage (*Cyanophage* P-TIM40) in bleached coral were proved involving in coral health. *Vibrio* bacteria are common in the seawater environment as well as the coral mucus, causing adverse effects on the health of coral by diseases such as yellow band/blotch disease and tissue necrosis in temperate gorgonian corals. Therefore, the presence of vibriophage in coral holobiont can suggest infection to bacteria pathogens and therefore benefit the coral (Marhaver et al., 2008).

CONCLUSION

After analyzing the metagenomic sequence data of viruses living in the mucus layer of coral *A. formosa*, we obtain their composition and abundance. In which, the four phyla *Cressdnaviricota*, *Uroviricota*, *Phixviricota* and *Hofneiviricota* dominate the communities in both two coral samples, account for over 98% and 94% of total viruses in bleached and healthy samples, respectively. At the same time, they also have massive diversity due to a large number of virus species. Besides, at the genus level, we find a notable shift in some considerate genera such as *Gemykrogvirus*, unclassified *Circoviridae*, *Circovirus* and unclassified *Siphoviridae*. At the specie level, interesting viruses, which only live in one coral status, are found; especially the coral-associated phage *Vibrio* phage VCY-phi (specie *Vibrio virus* VCY; genus *Vicialiavirus*) in healthy coral and Cyanophage P-TIM40 (specie *Prochlorococcus virus* PTIM40; genus *Libanvirus*) in bleached coral. Based on the results of the diversity analysis, the viral associations in each coral health status are

highly diverse and concomitantly distinct from one another due to a variety of species. Nevertheless, the differences are not only affected by health status but also by other factors such as temperature or environmental conditions which can be studied in further researches on analyzing microbiome, the composition and function of microbial communities living on the mucus layer of coral and their influences to coral health.

Acknowledgements: This research is funded by DALIDA project under grant number NDT.37.FRA/18 and supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.04-2018.309.

REFERENCES

- Bak R., Joenje M. I. J., Lambrechts D., Nieuwland G., 1998. Bacterial suspension feeding by coral reef benthic organisms. *Mar. Ecol. Progr. Ser.*, 175: 285–288.
- Ben-Haim Y., Zicherman-Keren M., Rosenberg E., 2003. Temperature-regulated bleaching and lysis of the coral *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. *Appl. Environ. Microbiol.*, 69(7): 4236–4242.
- Bythella C. J., Wild C., 2011. Biology and ecology of coral mucus release. *J. Exp. Mar. Biol. Ecol.*, 408: 88–93.
- Callahan B.J., McMurdie P.J., Rosen M.J., Han A.W., Johnson A.J.A., Holmes S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods.*, 13(7): 581–583.
- Davy S. K., Burchett S. G., Dale A. L., Davies P., Davy J. E., Muncke C., Hoegh-Guldberg O., Wilson W.H., 2006. Viruses: agents of coral disease? *Dis. Aquat. Organ.*, 69(1): 101–110.
- Dana J. D., 1846–1849. Zoophytes. United States Exploring Expedition during the years 1838–1842. *Lea and Blanchard, Philadelphia.*, 7: 1–740.
- Dung L. D., 2020. The status of coral reefs in central Vietnam's coastal water under

- climate change. *Aquat. Ecosyst. Health. Manag.*, 23(3): 323–331.
- Fuhrman J. A., Schwalbach M., 2003. Viral influence on aquatic bacterial communities. *Biol. Bull.*, 204(2): 192–195.
- Kim B. R., 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar. Ecol. Progr. Ser.*, 322: 929–933.
- Kushmaro A., Banin E., Loya Y., Stackebrandt E., Rosenberg E., 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int. J. Syst. Evol. Microbiol.*, 51(4): 1383–1388.
- Kvennefors E. C. E., Sampayo E., Kerr C., Vieira G., Roff G., Barnes A. C., 2012. Regulation of bacterial communities through antimicrobial activity by the coral holobiont. *Microb. Ecol.*, 63(3): 605–618.
- Kysela D. T., Palacios C., Sogin M. L., 2005. Serial analysis of V6 ribosomal sequence tags (SARST-V6): a method for efficient, high-throughput analysis of microbial community composition. *Environ. Microbiol.*, 7(3): 356–364.
- Lesser M. P., Mazel C. H., Gorbunov M. Y., Falkowski P. G., 2004. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science (New York, N.Y.)*, 305(5686): 997–1000.
- Marhaver K. L., Edwards R. A., Rohwer F., 2008. Viral communities associated with healthy and bleaching corals. *Environ. Microbiol.*, 10(9): 2277–2286.
- Menzel P., Ng K. L., Krogh A., 2016. Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat. Commun.*, 7: 11257.
- Nguyen-Kim H., Bettarel Y., Bouvier T., Bouvier C., Doan-Nhu H., Nguyen-Ngoc L., Nguyen-Thanh T., Tran-Quang H., Brune J., 2015. Coral mucus is a hot spot for viral infections. *Appl. Environ. Microbiol.*, 81(17): 5773–5783.
- Nguyen-Kim H., Bouvier T., Bouvier C., Bui V. N., Le-Lan H., Bettarel Y., 2015. Viral and bacterial epibionts in thermally-stressed corals. *J. Mar. Sci. Eng.*, 3(4): 1272–1286.
- Nguyen Manh Linh, Pham The Thu, Nguyen Van Quan, Pham Van Chien, Dao Huong Ly, Dinh Van Nhan, Dam Thi Len, 2019. DNA barcoding application of mitochondrial COI gene to identify some fish species of family Gobiidae in Vietnam. *Vietnam Journal of Marine Science and Technology*, 18(4): 443–451.
- Nguyen Van Lam, Pham Quang Huy, Nguyen Quoc Dai, Hoang Minh Hien, Dang Diem Hong, Le Van Son, Chu Hoang Ha, Truong Nam Hai, Nguyen Cuong, 2015. Genome assembly and annotation of the heterotrophic microalga *Schizochytrium Mangrovei* PQ6 in Viet Nam. *Vietnam. J. Sci. Technol.*, 2(6).
- Ondov B., Bergman N., Phillippy A., 2011. Krona: Interactive Metagenomic Visualization. *BMC. Bioinform.*, 12: 385.
- Pham The Thu, 2014. Correlation between viruses and bacteria in coral mucus at Cat Ba and Long Chau areas, Vietnam. *Academia Journal of Biology*, 36(3): 301–308.
- Reshef L., Koren O., Loya Y., Zilber-Rosenberg I., Rosenberg E., 2006. The coral probiotic hypothesis. *Environ. Microbiol.*, 8(12): 2068–2073.
- Rosenberg E., Kushmaro A., Kramarsky-Winter E., Banin E., Yossi L., 2009. The role of microorganisms in coral bleaching. *ISME. J.*, 3(2): 139–146.
- Sato Y., Ling E., Turaev D., Laffy P., Weynberg K., Rattei T., Willis B., Bourne D., 2017. Unraveling the microbial processes of black band disease in corals through integrated genomics. *Sci. Rep.*, 7(1): 1–14.
- Soffer N., Brandt M. E., Correa A. M. S., Smith T. B., Thurber R. V., 2014. Potential role of viruses in white plague coral disease. *ISME. J.*, 8(2): 271–283.

- Suttle C. A., 2007. Marine viruses—major players in the global ecosystem. *Nat. Rev. Microbiol.*, 5(10): 801–812.
- Tran Thi Hong, Pham Viet Cuong, Nguyen Thi Kim Cuc, 2019. Screening genes encoding protein protease inhibitor from metagenome of sponge-associated microorganisms in Quang Tri sea, Vietnam. *Academia Journal of Biology*, 41(2): 49–60.
- Vo S. T. 2000. The corals at Con Dao Archipelago (South Vietnam): Before, during and after the bleaching event in 1998. *International Coral Reef Symposium Indonesia*, 9: 23–27.
- Vo S. T., Phan K. H., Hua T. T., Thai M. Q., Hoang X. B., 2020. Genus-specific bleaching at Con Dao Islands, Southern Vietnam, June 2019. *Galaxea, J. C. R. S.*, 22(1): 27–28.
- Vollmer V. S., Baker C. A., Coffroth M-A., Harvell C. D., Medina M., 2013. Understanding the coral holobiont through science and scuba. *Smithson. Contrib. Mar. Sci.*, 173–186.