

# Coral-associated viruses and bacteria in the Ha Long Bay, Vietnam

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**ABSTRACT:** Viruses inhabiting the surface mucus layer of scleractinian corals have received little ecological attention so far. Yet they have recently been shown to be highly abundant and could even play a pivotal role in coral health. A fundamental aspect that remains unresolved is whether their abundance and diversity change with the trophic state of their environment. The present study examined the variability in the abundance of viral and bacterial epibionts on 13 coral species collected from 2 different sites in the Ha Long Bay, Vietnam: one station heavily affected by anthropogenic activity (Cat Ba Island) and one protected offshore station (Long Chau Island). In general, viral abundance was significantly higher in coral mucus (mean =  $10.6 \pm 2.0 \times 10^7$  virus-like particles ml<sup>-1</sup>) than in the surrounding water ( $5.2 \pm 1.3 \times 10^7$  virus-like particles ml<sup>-1</sup>). Concomitantly, the abundance and community diversity (inferred from phylogenetic and morphological analyses) of their mucosal bacterial hosts strongly differed from their planktonic counterparts. Surprisingly, despite large differences in water quality and nutrient concentrations between Cat Ba and Long Chau, there were no significant differences in the concentrations of epibiotic viruses and bacteria measured in the only 2 coral species (i.e. *Pavona decussata* and *Lobophyllia flabelliformis*) that were common at both sites. The ability of corals to shed bacteria to compensate for their fast growth in nutrient-rich mucus is questioned here.

**KEY WORDS:** Viruses · Coral-associated bacteria · Mucus · Symbionts · Coral reefs

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## INTRODUCTION

Coral reefs are among the most fragile marine habitats (Pandolfi et al. 2011), and they have experienced a rapid and strong decline over the past 3 decades (Hughes et al. 2003, Pandolfi et al. 2003, Bourne et al. 2009). Beside the destructive effects of hurricanes and predation (e.g. by corallivorous fish, snails and starfish) (Cole et al. 2011, Kayal et al. 2012, Hoeksema et al. 2013), microbial diseases are among the major causes for such decline of coral reefs worldwide (Rosenberg et al. 2009, Pollock et al. 2014). Their occurrence and intensity have consider-

ably increased in recent years, probably favored by climate change and the expanding anthropization and subsequent contamination of coastal waters (Harvell et al. 2002, Lesser et al. 2007). Efforts have been made to better identify the agents responsible for these coral diseases, and knowledge on the underlying ecological and physiological processes has greatly improved in the past few years. For example, we now have a much clearer vision of the role of prokaryotes in the development, progress and collapse of coral diseases such as the black-band disease (Bourne et al. 2011), white-band disease (Lentz et al. 2011), white plague (Cárdenas et al. 2012) and

white pox (Alagely et al. 2011). Several diseases have been shown to be caused by pathogens, such as members of the *Vibrionaceae* family (Kushmaro et al. 2001, Ben-Haim et al. 2003, Gomez-Gil et al. 2004, Cervino et al. 2008, Arotsker et al. 2009). Paradoxically, prokaryotes are also recognized for their symbiotic and species-specific association with corals (Rohwer et al. 2002, Goulet 2006, Apprill et al. 2012). For example, their ability to protect against invasive pathogens by the production of antibiotic compounds has long been described (Ritchie & Smith 2004, Reshef et al. 2006, Rypien et al. 2010, Shnit-Orland et al. 2012).

In the water column, prokaryotes are strongly subjected to lytic viral pressure, which usually accounts for 10 to 50% of bacterial mortality (Jardillier et al. 2005, Suttle 2007). There is increasing interest from marine microbiologists to study viruses inhabiting the superficial microlayer of corals, where they have been found to be highly abundant (Davy & Patten 2007, Leruste et al. 2012, Nguyen-Kim et al. 2014, 2015) and genetically diverse (Marhaver et al. 2008, Vega Thurber et al. 2009). Preliminary investigations on viral morphotypes and viral metagenomes in coral mucus have revealed that viruses can potentially infect all the prokaryotic and eukaryotic components of the holobiont (Marhaver et al. 2008). Not surprisingly then, viruses infecting bacteria and the symbiotic dinoflagellates *Symbiodinium* spp. are now considered integrative members of the viral assemblage (Wilson et al. 2005, Lohr et al. 2007, Vega Thurber et al. 2009, Correa et al. 2013). Many microbiologists even suspect that they could play a decisive role for coral viability by a strategic and environmentally driven control on both pathogenic and symbiotic microorganisms (Van Oppen et al. 2009, Vega Thurber & Correa 2011, Bettarel et al. 2014). Indeed, if viruses could represent a lytic barrier against colonization of surrounding pathogens (Barr et al. 2013a), they could also, via lysogenic infection, paradoxically protect bacterial symbionts from other viruses through lytic and lysogenic infection (Bettarel et al. 2014, Nguyen-Kim et al. 2015). However, still little is known about the factors that govern the distribution of such epibiotic viruses. For example, we lack information on whether global warming, nutrient enrichment of coastal waters, terrigenous sediment run-off, or anthropogenic environmental pollutants can alter viral community structure and therefore may influence their ecological role within the coral holobiont (Vega Thurber et al. 2008). Such information is crucial to elucidate the effective contributions of viruses to coral health.

To address this gap, our general objective was to examine the ecological traits of planktonic and epibiotic viruses and bacteria from 14 scleractinian coral species at 2 sites of different trophic status in the Ha Long Bay (Vietnam). Specifically, we first investigated the potential links between viral distribution and the abundance and morphological and phylogenetic diversity of their bacterial hosts. The second objective was to explore whether these viral and bacterial traits were influenced by the water quality and nutritive environment.

## MATERIALS AND METHODS

### Description of study sites and sampling strategy

The water and coral mucus samples were collected on 29 and 30 May 2012, between 07:00 h and 15:00 h during neap tide, in the vicinity of the United Nations Educational, Scientific and Cultural Organization World Heritage Site of Ha Long Bay (northern Vietnam) (Fig. 1). Two contrasting stations were sampled (see Faxneld et al. 2011). One is located in the Cat Ba archipelago (20° 47' 19.31" N, 107° 5' 42.87" E) and is subject to intense touristic and aquacultural activities and high industrial sediment loads. This disturbed (i.e. nearshore) reef area is situated close to the coast, in a semi-enclosed area with limited water exchange, and receives run-off water from several rivers. The other station, at Long Chau Island (20° 37' 57.45" N, 107° 8' 46.41" E), is not affected by anthropogenic activities, given its nature as a de facto marine protected area due to its military status (Thanh et al. 2004). This offshore area is located approximately 30 km south of the nearshore reef area and is an open zone with good water exchange; it is less affected by land run-off water (Faxneld et al. 2011).

The mucus from a total of 13 coral species was sampled according to the recommendation from Leruste et al. (2012) at Cat Ba Island (*Pavona* spp., *Pavona decussata*, *Fungia fungites*, *Sandolitha robusta*, *Goniastrea pectinata*, *Lobophyllia flabelliformis*, *Lobophyllia hemprichii*) and Long Chau Island (*Pavona frondifera*, *P. decussata*, *L. flabelliformis*, *Acropora hyacinthus*, *Acropora pulchra*, *Echinopora lamellosa*, *Favites pentagona* and *Platygyra carnosus*). Thus, 2 coral species (i.e. *P. decussata* and *L. flabelliformis*) were common to both sites. Briefly, duplicate biological samples of each coral species were collected by SCUBA diving from depths of 3 to 10 m. Mucus was collected using the desiccation method described in

detail elsewhere (Wild et al. 2005, Naumann et al. 2009). All coral samples were taken out of the water and exposed to air for 1 to 3 min, depending on the time for mucus secretion, which was variable among coral species. This stress caused the mucus to be secreted, forming long gel-like threads dripping from the coral surface. As recommended by Wild et al. (2005), the first 20 s of mucus production was discarded to prevent contamination and dilution by seawater. The fresh mucus (3 to 6 ml) was then distributed in polycarbonate tubes and immediately processed for DNA extraction and DGGE analyses, cell respiring activity and metabolic capacities, as well as concentration of culturable bacteria. One milliliter of mucus was transferred into 2 ml cryotubes, immediately fixed with formaldehyde (final concentration 3% v/v), flash-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until staining for viral and bacterial abundance analyses. Fifty milliliter duplicate seawater samples were also collected at approximately 1 m above the coral species, fixed and stored for the various analyses, as described for mucus samples.

#### Physicochemical parameters

Duplicate seawater samples were analyzed for nutrient and chl *a* contents, as well as for the different bacterial and viral parameters. Samples for nutrient measurements ( $\text{N-NO}_2$ ,  $\text{N-NO}_3$ ,  $\text{N-NH}_4$ ,  $\text{P-PO}_4$ ) were filtered through precombusted Whatman GF/F fiberglass filters, stored at  $-20^{\circ}\text{C}$  and analyzed according to Eaton et al. (1995). Chl *a* concentrations were determined by fluorometry (excitation wave length: 470 nm) after filtration onto Whatman GF/F filters and methanol extraction (Holm-Hansen et al. 1965). The chemical oxygen demand (COD) was estimated using potassium permanganate as oxidizing agent (Hossain et al. 2013). Salinity and temperature were measured *in situ*, 1 m above the corals species, using a CTD probe (SBE 19+, Sea-Bird Electronics).

#### Bacterial and viral concentrations

At each site and for each coral species, duplicate subsamples of 100  $\mu\text{l}$  of fixed mucus were eluted into 900  $\mu\text{l}$  of a solution of 0.02  $\mu\text{m}$  pore-size-filtered, pH 7 solution of 1% citrate potassium (made with 10 g potassium citrate,  $1.44\text{ g l}^{-1}\text{ Na}_2\text{HPO}_4\cdot 7\text{H}_2\text{O}$  and  $0.24\text{ g l}^{-1}\text{ KH}_2\text{PO}_4$ ) (Nguyen-Kim et al. 2014, adapted from Williamson et al. 2003). Samples were then vortexed at moderate speed for 5 min, and the number of

viruses and bacteria contained in 200 to 500  $\mu\text{l}$  of mucus solution was estimated after retention of the particles onto 0.02  $\mu\text{m}$  pore size membranes (Whatman Anodisc), rinsing with 500  $\mu\text{l}$  TE buffer and staining with the nucleic acid dye, SYBR Gold (Invitrogen) for 15 min. The different microorganisms were then counted using an epifluorescence microscope (Olympus BX51), under blue light (excitation wave length: 450 nm), as described in detail by Patel et al. (2007). The whole procedure is detailed in Leruste et al. (2012). The average proportion of the main bacterial morphotypes (rods, cocci, curved cells and filaments) was also evaluated for each sample. For the planktonic free-living viruses and bacteria, the above standard staining procedure was applied to 500  $\mu\text{l}$  of seawater, but without the potassium citrate extraction step, which was unnecessary.

#### Enumeration of culturable heterotrophic bacteria and vibrio species

Culturable heterotrophic bacteria (C-BAC) and culturable *Vibrionaceae* (C-VIB) were counted (one replicate) by plating 50  $\mu\text{l}$  of serial dilutions (1 and 100%) of both mucus and seawater samples, respectively, on (1) the non-selective artificial seawater (ASW) medium (Smith & Hayasaka 1982) and (2) the vibrio-selective medium thiosulphate citrate bile salts-sucrose agar (TCBS) (Uchiyama 2000). After 48 h incubation at *in situ* temperature, colony-forming units were counted in all the different plates. Counts did not increase after prolonged incubation.

#### DGGE bacterial community composition

The community structure of mucosal and planktonic bacteria was determined by denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA gene fragments (Morrow et al. 2012). Briefly, 50 ml of seawater and 2 ml of coral mucus of each species were filtered onto 0.2  $\mu\text{m}$  polycarbonate filters (Whatman) for total DNA extraction and stored at  $-20^{\circ}\text{C}$  until analysis. The PowerSoil DNA Isolation Kit was used to extract DNA from both water and mucus samples. The DNA sequences were then subjected to touchdown PCR using the primers 341F-GC and 518R (Ovreås et al. 1997), which target bacterial 16S rRNA genes (178 bp). PCR was carried out using 10 ng of extracted DNA and PuRe Taq Ready-To-Go PCR beads (GE Healthcare) using the PCR touchdown program (Muyzer et al. 1993). PCR products

were verified in 1.5% (wt/vol) agarose gel using SYBR Gold I nucleic acid gel stain (1:10 000 dilution; Molecular Probes). PCR samples were loaded onto 8% (wt/vol) polyacrylamide gels made with a denaturing gradient ranging from 35 to 65% (100% denaturant contains 7 M urea and 40% formamide). The DGGE was performed with an Ingeny Phor-U system in 0.5× tris-acetate-EDTA (TAE) buffer (Euromedex) at 60°C with a constant voltage of 80 V for 18 h. The DNA was then stained with the SYBR Gold nucleic acid dye. DNA bands were visualized on a UV trans-illumination table with the imaging system GelDoc XR (Bio-Rad) and analyzed using fingerprint and gel analysis Quantity One software (Bio-Rad). Band matching was performed with 1.00% position tolerance and 1.00% optimization. A band-matching table was generated to obtain the binary presence/absence matrix. Each DGGE band refers to operational taxonomic units (OTUs) representative of predominant bacterial taxa (Reche et al. 2005). The total number of OTUs was used to compare the richness between prokaryotic communities of all the samples. Similarity between DGGE profiles was obtained with an agglomerative hierarchical clustering analysis, which is based on the relative intensity matrix.

### Data analysis

Data were log transformed to satisfy requirements of normality and homogeneity of variance necessary for parametric analyses. A 1-way ANOVA was used to compare the different bacterial and viral parameters between habitats (mucus and seawater) and geographical sampling sites (Cat Ba and Long Chau) for the 2 common species (*P. decussata* and *L. flabelliformis*). The variability of bacterial community compositions between all samples and between the 2 common species (site effect) was assessed using a non-parametric statistical test. Briefly, we first computed the Jaccard dissimilarity index of the DGGE profiles (based on the presence/absence of OTUs) both between all pairs of corals and between the 2 common species. Variance of dissimilarity was computed according to Anderson (2001, 2006) (R functions `permutest` and `betadisper` from the library `vegan`, `permutational MANOVA [PERMANOVA]`) and based on permutations of actual dissimilarity values. Simple relationships between original data sets were also tested using Pearson correlation analysis. All statistical analyses were performed using XLSTAT software.

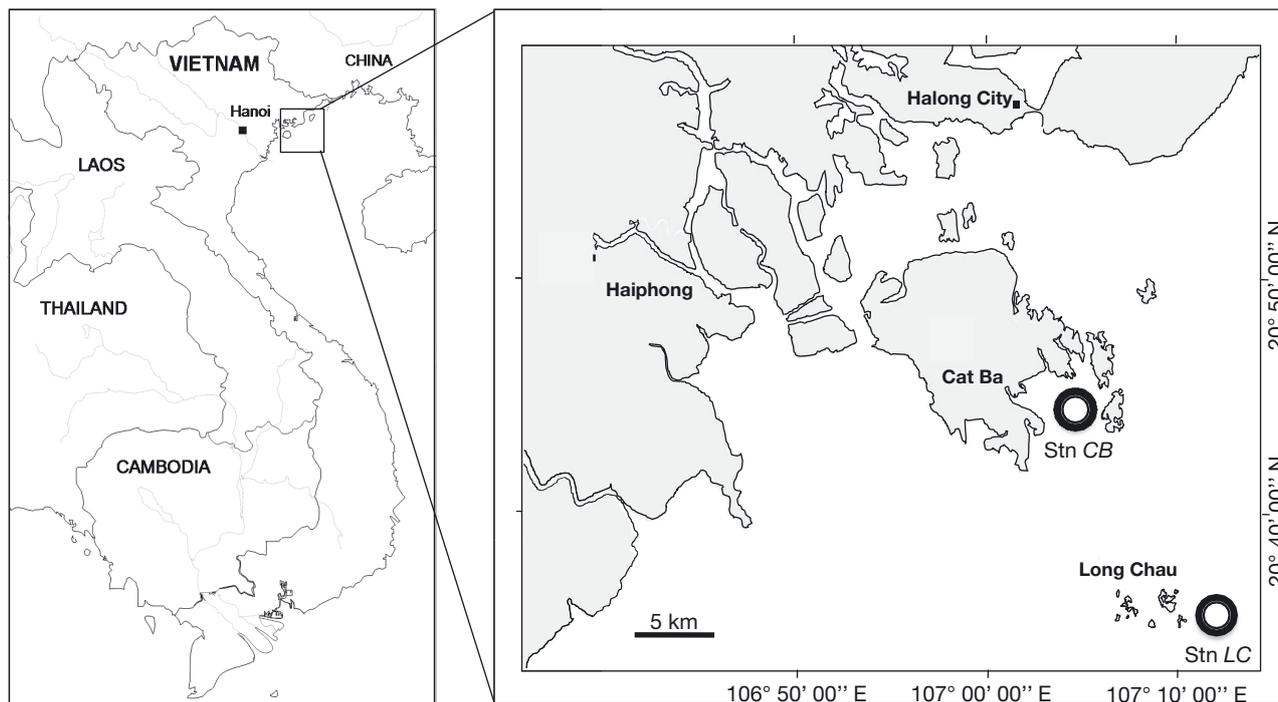


Fig. 1. Location of the 2 sampling sites, Cat Ba and Long Chau Island stations, in Ha Long Bay, northern Vietnam, Southeast Asia. CB: Cat Ba; LC: Long Chau

## RESULTS

### Environmental variables

During the sampling period, the 2 sites were highly contrasted in their physicochemical characteristics. Cat Ba, the site most heavily affected by anthropogenic activities, exhibited a higher nutrient concentration, water turbidity and COD, compared with the remote Long Chau Island (Table 1). For example, chl *a*, nitrite, nitrate, ammonium and phosphate concentrations were 71, 114, 147, 28 and 49% higher, respectively, in Cat Ba than in Long Chau (Table 1). During

the sampling, no trace of coral bleaching or injuries was observed in any of the sampled coral species.

### Viral and bacterial abundances

Viral abundance was consistently and significantly higher in coral mucus than in the surrounding seawater, being 1.4 and 2.8× higher, respectively, in Cat Ba and Long Chau. With the exception of *Goniastrea pectinata* in Cat Ba and *Acropora hyacinthus* in Long Chau, values generally comprised between  $10 \times 10^7$  and  $14 \times 10^7$  viruses  $\text{ml}^{-1}$  mucus (Fig. 2). In the 2 coral

Table 1. Geographical coordinates and physicochemical parameters of seawater in the 2 sampling stations. FTU: formazin turbidity unit; COD: chemical oxygen demand

Site	Latitude, Longitude	Temp. (°C)	Salinity (‰)	Chl <i>a</i> ( $\text{mg l}^{-1}$ )	Turbidity (FTU)	COD ( $\text{mg l}^{-1}$ )	N-NO <sub>2</sub> ( $\mu\text{g l}^{-1}$ )	N-NO <sub>3</sub> ( $\mu\text{g l}^{-1}$ )	N-NH <sub>4</sub> ( $\mu\text{g l}^{-1}$ )	P-PO <sub>4</sub> ( $\mu\text{g l}^{-1}$ )
Cat Ba	20°47'19.31"N, 107°5'42.87"E	30.1±0.1	29.1	1.2±0.2	1.5±0.3	2.5±0.1	7.9±0.8	166.7±14.5	39.3±1.7	20.2±0.9
Long Chau	20°37'57.45"N, 107°8'46.41"E	29.0±0.2	31.5	0.7±0.1	0.7±0.1	1.9±0.2	3.7±1.0	67.5±9.3	30.7±0.8	13.6±2.2

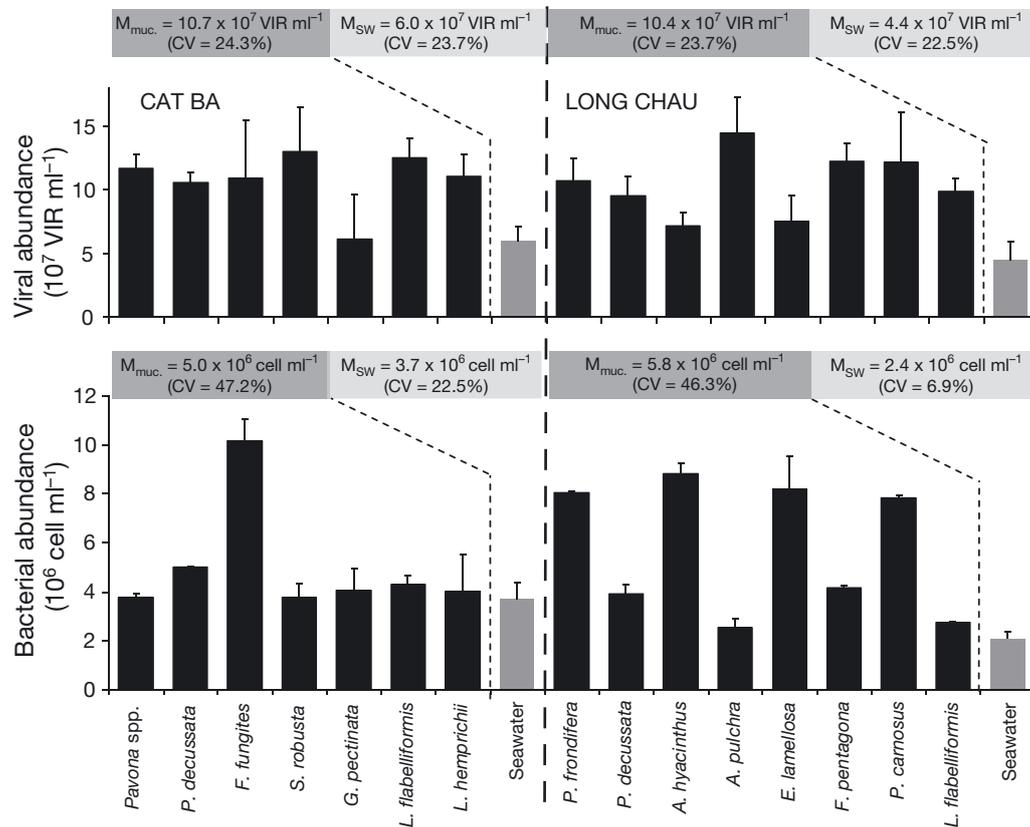


Fig. 2. Viral and bacterial abundances in coral mucus and seawater samples in Cat Ba and Long Chau Islands.  $M_{\text{muc}}$ : mean value obtained for the mucus samples;  $M_{\text{SW}}$ : mean values obtained for the seawater samples; VIR: viral abundance. See 'Materials and methods' for full genus names

Table 2. One-way ANOVA of the different viral and bacterial parameters measured in the coral mucus and seawater samples at Cat Ba and Long Chau stations. The inter-site comparison could only be realized from the results obtained for the 2 species that were common to both sites (i.e. *Lobophyllia flabelliformis* and *Pavona decussata*). BAC: bacterial abundance; VIR: viral abundance; VBR: virus-to-bacteria ratio; OTU: operational taxonomic unit. **Bold**: significantly different at  $p < 0.05$

Parameter	Mucus/ seawater (p-value)	Cat Ba/Long Chau (p-value)		
		Mucus ( <i>L. flabelliformis</i> )	Mucus ( <i>P. decussata</i> )	Seawater
BAC	<b><math>1.92 \times 10^{-9}</math></b>	0.106	0.059	<b><math>5.12 \times 10^{-6}</math></b>
VIR	<b><math>3.05 \times 10^{-9}</math></b>	0.285	0.459	<b>0.023</b>
VBR	<b>&lt;0.0005</b>	0.376	0.860	<b>0.042</b>
OTU	<b>0.014</b>	0.309	0.492	<b>0.047</b>
Cocci (%)	0.452	<b>0.023</b>	0.143	0.174
Rod (%)	<b>0.002</b>	0.693	<b>&lt;0.01</b>	<b>0.010</b>
Curved (%)	0.283	0.823	0.323	0.781
Filaments (%)	<b>0.007</b>	0.173	0.588	0.429

species that were common at both sites (ie *Pavona decussata* and *Lobophyllia flabelliformis*), the concentrations of viral epibionts did not show any significant differences between Cat Ba and Long Chau. On the contrary, the abundance of planktonic viruses was significantly higher in Cat Ba (mean =  $6.0 \times 10^7$  viruses  $\text{ml}^{-1}$ ,  $p < 0.05$ ) than in preserved Long Chau waters (mean =  $4.4 \times 10^7$  viruses  $\text{ml}^{-1}$ ,  $p < 0.05$ ) (Fig. 2, Table 2).

As for viruses, the abundance of bacterial communities was, on average, also higher in the coral mucus samples than in the surrounding seawater (Fig. 2, Table 2); although the differences were lower than with viruses, and mostly resulting from the high concentrations measured in *Fungia fungites* in Cat Ba or *A. hyacinthus* in Long Chau (Fig. 2). The inter-species variability in the abundance of mucosal bacteria (coefficient of variation

[CV] = 46.7%) was much higher than for their planktonic counterparts (CV = 14.7%) and for the mucosal viruses (CV = 23.0%) (Fig. 2). As was the case for viruses, the abundance of epibiotic bacteria in *P. decussata* and *L. flabelliformis* did not significantly differ between the 2 sampled sites. Conversely, planktonic bacterial cells were significantly more abundant in Cat Ba (mean =  $3.7 \times 10^6$  cells  $\text{ml}^{-1}$ ,  $p < 0.05$ ) than Long Chau (mean =  $2.4 \times 10^6$  cells  $\text{ml}^{-1}$ ,  $p < 0.05$ ) (Fig. 2, Table 2). Finally, regardless of the site, a significant and positive correlation was found between viral and bacterial abundances in coral mucus samples (Table 3).

At both sites, the virus-to-bacteria ratio (VBR) was also consistently and significantly higher in the mucus (mean at Cat Ba [ $m_{CB}$ ] =  $24.2 \pm 40.1\%$ ; mean at Long Chau [ $m_{LC}$ ] =  $24.1 \pm 68.8\%$ ) than seawater samples ( $m_{CB}$  =  $15.4 \pm 10.5\%$ ;  $m_{LC}$  =  $16.4 \pm 32.8\%$ ) (ANOVA,  $p < 0.05$ ). The inter-site comparison of the VBR in *P. decussata* and *L. flabelliformis* revealed higher values in the seawater in Long Chau than Cat Ba, whereas no significant difference could be found for the mucosal communities (Table 2).

### Bacterial morphotypes

Among the 4 main cell morphotypes studied, only rods and filamentous forms were significantly more abundant in mucus than in seawater samples (Fig. 3, Table 2). The respective proportions of cocci and rod-

Table 3. Pearson correlation coefficients between viral and bacterial parameters for the totality of coral mucus samples (Cat Ba and Long Chau). BAC: bacterial abundance; VIR: viral abundance; VBR: virus-to-bacteria ratio; OTU: operational taxonomic unit; C-VIB: culturable *Vibrionaceae*; C-BAC: culturable heterotrophic bacteria. **Bold**: Significant at  $p < 0.05$

Variable	BAC	VIR	VBR	OTU	C-VIB	C-BAC	Cocci	Rods	Curved	Filaments
BAC	<b>1</b>									
VIR	<b>0.500</b>	<b>1</b>								
VBR	0.049	-0.129	<b>1</b>							
OTU	0.169	0.187	-0.133	<b>1</b>						
C-VIB	-0.442	-0.374	-0.401	-0.366	<b>1</b>					
C-BAC	-0.328	-0.063	0.275	<b>-0.555</b>	0.111	<b>1</b>				
Cocci	<b>0.623</b>	0.032	-0.035	0.381	-0.311	-0.423	<b>1</b>			
Rods	-0.016	-0.246	0.092	0.162	-0.084	-0.211	0.000	<b>1</b>		
Curved	0.025	-0.238	-0.439	0.060	0.230	-0.473	0.285	0.433	<b>1</b>	
Filaments	-0.459	-0.441	-0.400	0.072	0.326	-0.205	0.223	0.125	<b>0.687</b>	<b>1</b>

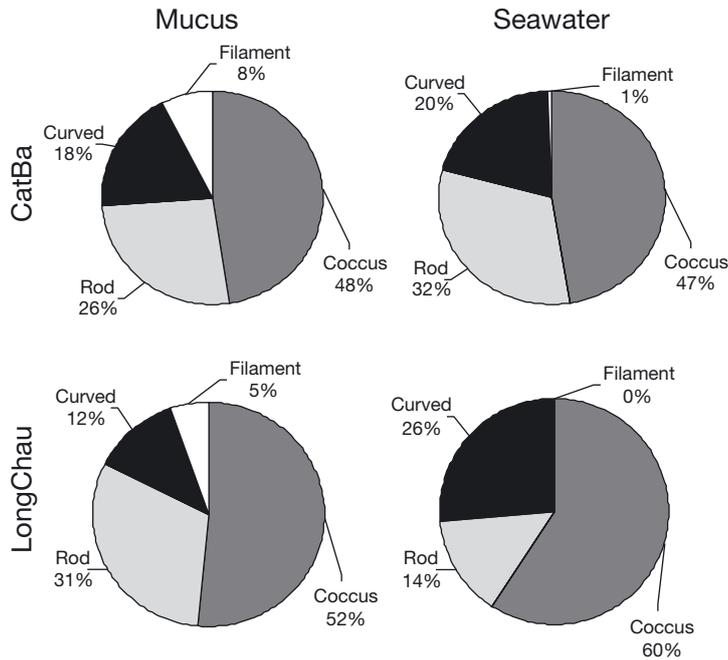


Fig. 3. Distribution of the main bacterial morphotypes in coral mucus and seawater samples in Cat Ba and Long Chau Islands

like bacteria in the mucus of *L. flabelliformis* and *P. decussata* exhibited significant differences between Cat Ba and Long Chau (Table 2).

### Culturable prokaryotes

The average concentration of C-BAC was 5.9- and 12.5-fold more elevated in the mucus than in seawater samples in Cat Ba and Long Chau, respectively (Fig. 4). For C-VIB, the difference between mucus and seawater was even greater, reaching 90- and 170-fold higher in mucus in Cat Ba and Long Chau, respectively (Fig. 4). A significant correlation was found between the abundance of C-BAC and the number of OTUs in the different coral species (Table 3). In contrast, C-VIB concentrations were not correlated with any of the other measured parameters.

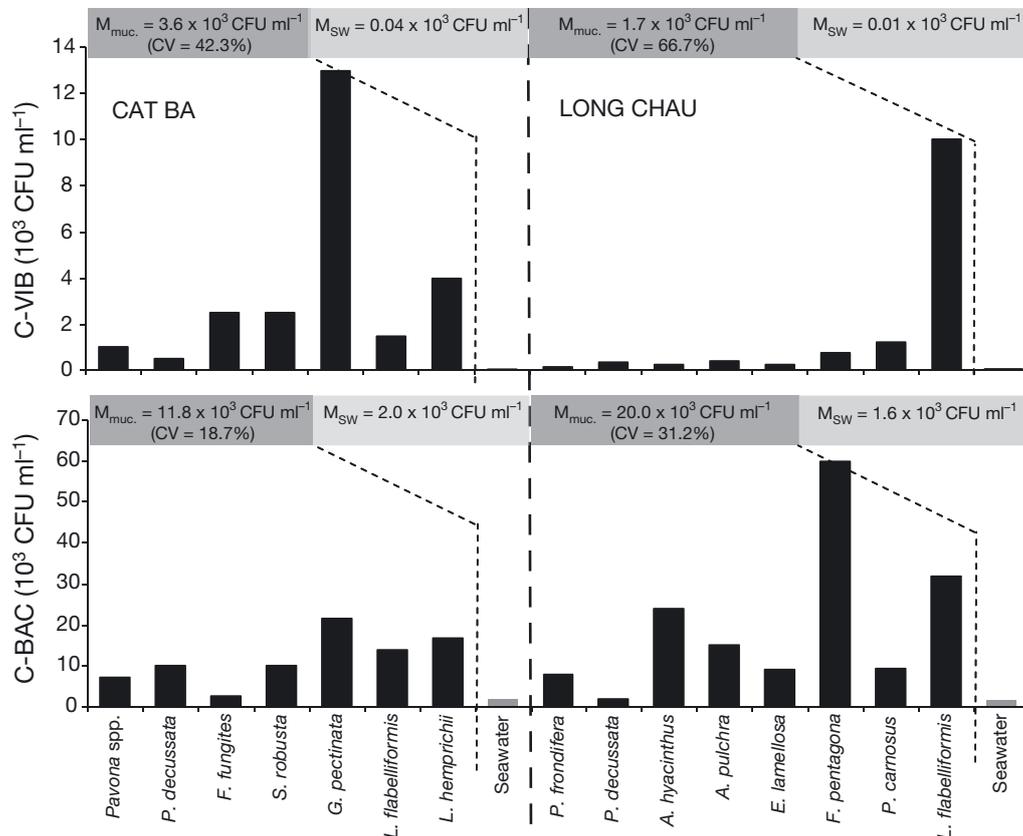


Fig. 4. Abundance of culturable heterotrophic bacteria (C-BAC) and culturable *Vibrionaceae* (C-VIB) in coral mucus and seawater samples in Cat Ba and Long Chau Islands. CFU: colony-forming units

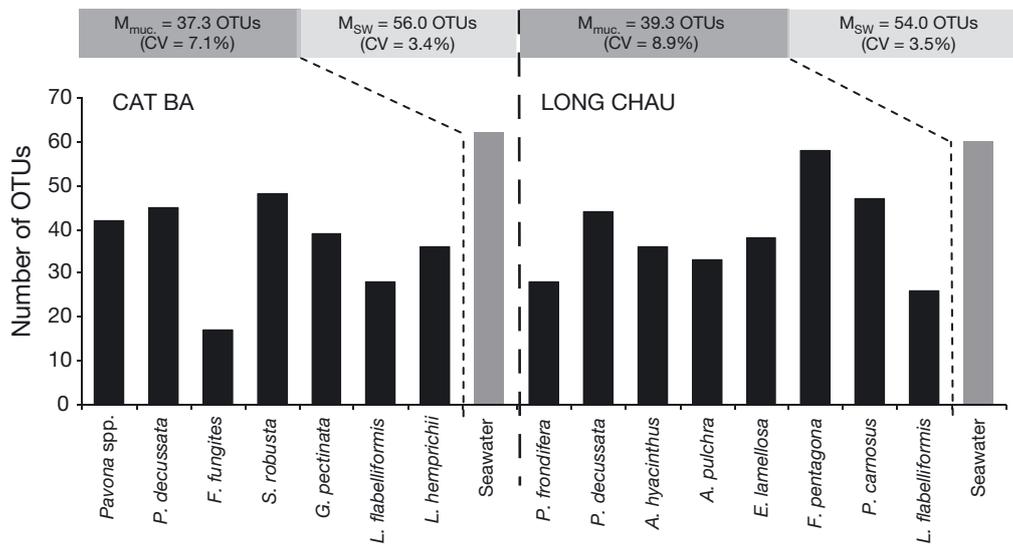


Fig. 5. Number of operational taxonomic units (OTUs) measured in coral mucus and seawater samples in Cat Ba and Long Chau Islands

**DGGE-based estimates of prokaryotic community genetic diversity**

Unlike the majority of the other parameters, the number of OTUs obtained by DGGE was consistently and significantly lower in mucus ( $m_{CB} = 37.3$ ;  $m_{LC} = 39.3$ ) than in seawater ( $m_{CB} = 56.0$ ;  $m_{LC} = 54.0$ ) (Fig. 5, Table 2). Nonetheless, there was no significant difference between the 2 studied sites for both *L. flabelliformis* and *P. decussata* (Table 2). The cluster analysis of DGGE profiles revealed a clear root discrimination of the community composition between planktonic and epibiotic bacteria (Fig. 6). Surprisingly, *P. decussata* exhibited the longest distance with seawater samples in Cat Ba and the shortest in Long Chau, suggesting that the intraspecies variability in OTU composition can be relatively high among coral species

(Fig. 6). The PERMANOVA revealed a higher level of variability in bacterial community composition between all the different coral species than between the 2 sites (PERMANOVA,  $p = 0.098$ ). Regarding the 2 common species (*P. decussata* and *L. flabelliformis*), their bacterial community composition was not significantly different between the 2 sites (PERMANOVA,  $p = 0.950$ ).

**DISCUSSION**

**Planktonic versus epibiotic abundance of viruses and bacteria**

In the present study, viral abundance was more than twice as high in the mucus of the different coral

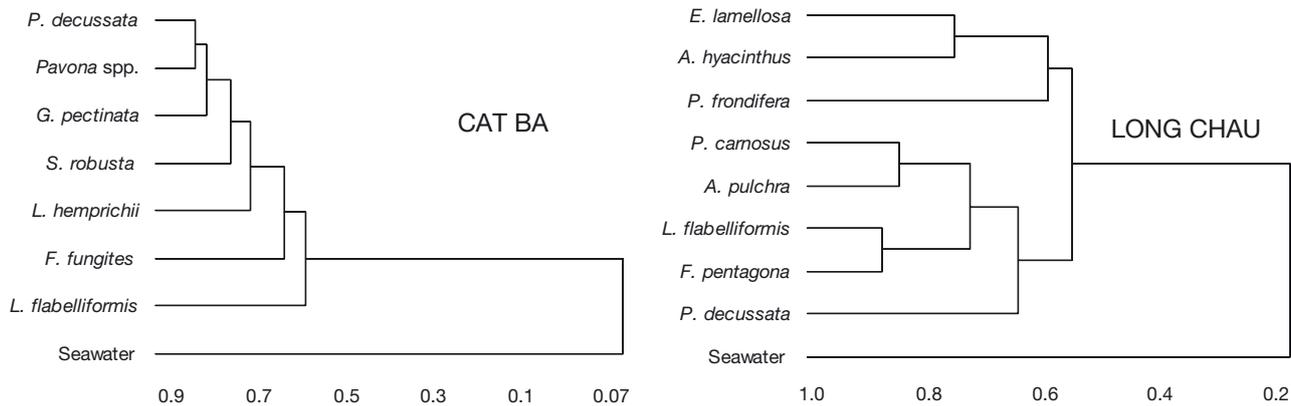


Fig. 6. Similarity dendrograms of the DGGE band patterns obtained with an agglomerative hierarchical clustering analysis from the mucus and seawater samples of Cat Ba and Long Chau

species than in the surrounding water. Similar observations have been previously reported from cultured (Leruste et al. 2012) or *in situ* corals (Davy et al. 2006, Patten et al. 2008, Nguyen-Kim et al. 2015). There are several explanations for such levels of abundance, such as the highly adhesive property of coral mucus. From the recent report of Barr et al. (2013b), we know that phage capsids and their Ig-like protein domains have strong chemical affinities with the mucin-glycoproteins of the mucus, resulting in viral enrichment in this organic layer. Viral proliferation could also be stimulated by the high nutritive quality of mucus promoting the fast growth of their bacteria hosts. The positive and significant correlation found between viral and bacterial epibionts supports the idea that most of the viral hosts were bacteria, which is in line with previous reports (Vega Thurber et al. 2009, Nguyen-Kim et al. 2014). Mucus is a biogel composed primarily of carbohydrates, which contribute to around 80% of the chemical composition (Ducklow & Mitchell 1979, Bansil & Turner 2006). Glucose is considered the most common carbohydrate component in coral mucus (Wild et al. 2010) and is recognized as a crucial energy source for most bacterial cells, which helps to explain why coral mucus is populated by active and fast-growing bacteria (Ritchie & Smith 2004, Brown & Bythell 2005). In the aquatic environment, viral activity and abundance are generally tightly coupled with the physiological state and abundance of their hosts (Weinbauer 2004, Maurice et al. 2010). Highly active cells typically allow a rapid and efficient completion of viral lytic cycles (Maurice et al. 2013), and this was the case in coral mucus, where bacterial respiring activity (as measured with the 5-cyano-2,3-ditoyl tetrazolium chloride [CTC] approach) was found to be much higher than in the water column (Nguyen-Kim et al. 2014). Levels of abundance were also much higher for epibiotic total bacteria, cultivable bacteria and vibrio, compared to their planktonic counterparts, which corroborates previous findings (Ritchie & Smith 2004) and helps explain the large occurrence of phages in mucus.

The bacterial community diversity revealed by microscopic observations and phylogenetic analysis also showed large differences between coral epibionts and planktonic cells, as reported on several occasions (Rohwer et al. 2002, Ritchie & Smith 2004, Kvennefors et al. 2010, Carlos et al. 2013). On average, rods and filamentous cells were more abundant in mucus. Prokaryotes are typically attracted by hot spots of high nutritive values, and specific shapes also give cells greater access to nutrients (Young

2006). With similar volumes, filament and rod morphotypes show a higher total surface area compared to cocci. As hypothesized by Steinberger et al. (2002), filamentation may benefit cells attached to a surface, because it increases that specific surface area in direct contact with the medium (coral mucus in our case). The DGGE analyses also confirmed that coral mucus represents a selective medium that harbors a unique consortium of bacteria, which is structurally different from that of the surrounding water (Rohwer et al. 2001, Koren & Rosenberg 2006, Carlos et al. 2013). Contrary to previous findings for most of the microbial parameters, the number of OTUs was higher in the seawater (mean = 55) than in the mucus (mean = 38.3). In the latter, these numbers were comparable to those reported in the literature by other studies: 41 bands for *Montastraea faveolata* (Guppy & Bythell 2006); 44 bands for *Acropora millepora* (Kvennefors et al. 2010); and 25 bands on average for *Madracis decactis*, *Mussismilia hispida*, *Palythoa caribaeorum* and *Tubastraea coccinea* (Carlos et al. 2013). Such discrepancies between mucus and seawater may be naturally attributed to the specific chemical composition of mucus, which is highly selective (Brown & Bythell 2005), but also to the antimicrobial properties of the former, which can typically inhibit the bacterial growth of certain phylogenetic groups or species and ensure the selection and maintenance of a limited number of active bacterial symbionts (Kvennefors et al. 2012).

#### **Coral inter-species variability of bacterial and viral communities**

In our study, all of the measured parameters exhibited large variations between the different coral species. Coral-associated bacterial community composition has long been shown to be species specific (Rohwer et al. 2002, Tremblay et al. 2011, Morrow et al. 2012), but viral and bacterial abundances can also strongly differ between coral species (Leruste et al. 2012, Nguyen-Kim et al. 2014, 2015). Such differences have been partly linked to the species-specific chemical composition of coral mucus (Ducklow & Mitchell 1979, Meikle et al. 1988, Krediet et al. 2013). Another potential explanation is the existence of large variations in mucus production, both within and between species, which could also be linked to the type and intensity of stress imposed on corals, and which may result in the dilution/concentration of the particles in the gel (Naumann et al. 2010, Cod-

deville et al. 2011). Alternatively, the substantial antimicrobial activities measured in coral mucus (Kvennefors et al. 2012) represent another strong biotic regulator of bacterial proliferation, which may differ from one species to the other (Shnit-Orland & Kushmaro 2008, Krediet et al. 2013). Finally, all these intrinsic determinants of bacterial abundance are suspected to indirectly impact the production and distribution of their viral parasites. The species-specific viscosity of this biogel (Brown & Bythell 2005) could also potentially influence the movement of viruses and their chance to encounter and infect bacteria within coral mucus.

### Inter-site comparison of viral and bacterial traits in coral mucus

In the ocean's water column, nutrient availability represents one of the main determinants of bacterial growth and viability. However, the influence of trophic environment on bacterial epibionts of corals remains unclear. Although the presence of high concentrations of inorganic nutrients has been shown to promote coral diseases (Fabricius 2005, Voss & Richardson 2006) the underlying mechanisms have not yet been elucidated. Also, to date, the abundance of mucosal cells has not been evaluated and compared in *in situ* biomes of contrasting trophic regime.

In this study, a total of 14 different coral species were sampled, but only 2 (i.e. *P. decussata* and *L. flabelliformis*) were common at both sites; being also capable of producing a sufficient amount of mucus for the various analyses, these species allowed us to make the inter-site comparison of coral-associated viral and bacterial traits. Thus, this comparison should be taken with caution and clearly needs further investigation. However, although bacterial communities are species-specific (Rohwer et al. 2002, Ceh et al. 2011), such a low-resolution comparison still remains of interest, providing a global snapshot of bacterial and viral ecological traits in scleractinians.

Surprisingly in our study, despite important discrepancies in the concentrations of nutrients (nitrate and phosphate), dissolved organic carbon and chl *a* between the 2 different sampling stations (see also Faxneld et al. 2011), no significant differences could be detected for either bacterial or viral abundances measured in *P. decussata* and *L. flabelliformis* (see Table 2). Interestingly, like for their abundance, epibiotic bacteria did not show any significant difference in their community composition between

these 2 coral species (PERMANOVA test). Again, given the low replication of coral samples, the lack of significant differences should be interpreted with prudence. Coral–microbe relationships are susceptible to sudden rises in organic matter inputs (Voss & Richardson 2006, Vega Thurber et al. 2009). For example, an experimental increase in dissolved organic carbon concentrations stimulated the growth rate of microbes living on corals' superficial layer by an order of magnitude (Kline et al. 2006). Numerically, the absence of significant difference in these 2 species could be explained by the recently documented ability of corals to shed bacteria (Garren & Azam 2012). By using high-speed laser scanning confocal microscopy on live corals, these authors observed that scleractinians can get rid of excess of bacterial cells during times of organic matter stress. In other words, this mechanism may counteract bacterial growth stimulated by organic inputs and may potentially help explain the equivalent levels of abundance of epibiotic viruses and prokaryotes in both Cat Ba and Long Chau. However, we have no direct evidence for this to occur in the present study. Another recent study on cold water corals reported that an experimental enrichment of viral and bacterial abundance in surrounding water did increase the abundances in the coelenteron but not in the mucus of corals, indicating some sort of ecological stability of epibiotic microbes (Weinbauer et al. 2012). Alternatively, coral-associated bacterial communities have also been recognized for their ecological adaptation, being capable of strong physiological and genetic adjustments to cope with environmental disturbances and to ultimately ensure coral viability (Reshef et al. 2006, Rosenberg et al. 2009, Bourne et al. 2011). Finally, the maintenance of relatively stable abundances and phylogenetic composition of epibiotic bacteria and viruses may be crucial for corals to avoid the excessive accumulation of these particles in mucus beyond a threshold that would otherwise threaten the balance between corals and their associated microbiota. Further investigations are now necessary to gain a deeper insight into the molecular and ecological processes allowing corals to regulate the abundance of their symbionts and how such symbionts can also auto-adjust their abundance in the mucus. Overall, our results provide support for the hypothesis that coral mucus represents a confined environment for an adapted consortium of bacterial cells (and their viral parasites) whose development seems preserved from some variability of the trophic characteristics of the water column.

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