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Key Points:
- High prokaryotic and viral abundances were observed in surface sediments from the Pearl River Estuary to the deep western South China Sea
- The transport of psbA-containing phytoplankton and cyanophages from the photic zone may provide an important source of sedimental microbes
- Different vertical export behaviors induce different communities of phytoplankton and cyanophages between the photic zone and seafloor

Supporting Information:
- Supporting Information S1

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Abstract The transport of planktonic microbes from the upper ocean to sediments plays an important role in marine ecology and biogeochemistry (e.g., the downward transport of organic matter). In order to have a better understanding of this important process, we investigated the diversity of allochthonous cyanophages, cyanobacteria, and eukaryotic algae in the sediments from the Pearl River Estuary and South China Sea based on psbA, the gene encoding the D1 protein involved in photosynthesis. The psbA is prevalent and diverse in sediments regardless of water depth, revealing that the vertical export of psbA gene-containing planktonic microbes provide an important source of sedimental microbes. Phylogenetic analysis showed the presence of Synechococcus, Synechococcus myovirus, Synechococcus podovirus, Prochlorococcus, Prochlorococcus myovirus, Prochlorococcus podovirus, and eukaryotic algae groups in the sediments. More than 80% of psbA amplicons belong to cyanophages, suggesting the potential prevalent downward transport of cyanophages from the photic zone to the seafloor. Diatoms Pseudosolenia calcarea-avis and Opephora were dominant in some sediment samples, different from planktonic community in the photic zones, indicating a potentially different vertical export efficiency among phytoplankton.

The principal coordinate analysis indicated that psbA gene-containing microbial community varied in estuarine, continental shelf and deep sea sediments. This study investigated the diversity of psbA gene-containing microorganisms in marine sediments and could facilitate the evaluation of the output of photosynthetic organic carbon.

Plain Language Summary The export of planktonic cells and phages from the upper ocean to seafloor offers essential organic substrates, affecting the sediment microbial community and biogeochemical cycles. Based on molecular technique, we observed eukaryotic algae, cyanobacteria, and cyanophages present widely in sediments of the South China Sea and Pearl River Estuary regardless of water depth. The contrasting community structure of these microorganisms between sediments and water column suggested their export behaviors are spatially different. The diversity of allochthonous microbes obtained in our study could provide a helpful reference for evaluating the efficiency of downwards transport of organic matter.

1. Introduction
The export of particulate organic carbon (POC) from the surface ocean to depth, with a global rate of 4–13 Pg C/y (Lima et al., 2014), is thought to be an important factor controlling the “biological pump” (Boyd & Trull, 2007), thereby impacting the ocean carbon storage and the atmospheric carbon dioxide concentrations (Passow & Carlson, 2012). Two main types of exported POC are composed of fecal pellets and aggregates, which include planktonic cells, phytodetritus and inorganic matter (Riley et al., 2012). With high sinking rate (fecal pellets: 5–2,700 m/d; aggregates: 10–386 m/d) (Riley et al., 2012; Turner, 2002), they not only provide the primary substrates of microbial metabolism (e.g., heterotrophic bacterial production and respiration), but also affect the microbial communities in the deep sea and seafloor (McDonnell et al., 2015).
Phytoplankton cells are generally regarded as the important contributors to the transport of organic matter to the ocean interior, even deep into the sediment, being evidenced by the presence of photosynthetic cells in various deep sea ecosystems (Honjo et al., 2008; Rice et al., 1986; Richardson & Jackson, 2007). For example, the ubiquitous presence of healthy photosynthetic cells, dominated by diatoms, has been reported in the bathypelagic dark ocean (2,000–4,000 m depth layer), confirming the prevalence of fast-sinking mechanisms of fresh organic matter in the oligotrophic ocean (Agusti et al., 2015). Eukaryotic algae, Cryptomonadales were observed to be abundant in the polymetallic nodules of the abyssal Pacific Ocean floor (Shulse et al., 2017). Although cyanobacterial cells are too small to sink individually, they have been recognized to be transported to aphotic water and sediments with sinking particles (e.g., phytodetritus and fecal pellets) (Noffke et al., 2003), and accounted for 4.5% of the bacterial community at a depth of 3,000 m (Tseng et al., 2015). Jiao et al. (2014) have detected the presence of Prochlorococcus in the deep dark region of the western Pacific Ocean. Furthermore, phytodetritus deposited on the abyssal seafloor contained abundant cyanobacteria in the northeast Atlantic Ocean (Lochte & Turley, 1988) and northeast Pacific Ocean (Beaulieu & Smith, 1998). In addition, allochthonous import was also shown to be an important source of sedimental viruses (Hewson & Fuhrman, 2003). Viral particles could absorb to sinking particle or maintain in the infected host to accelerate their downward transport (Mari et al., 2007; Mojica et al., 2014). Several investigations on the viral diversity in marine sediments have confirmed the important contribution of planktonic viruses (e.g., cyanophages, the hosts of which are cyanobacteria) to the sedimental viral community. For instance, cyanophages, which should originate from seawater, have been observed in multiple coastal sediments (Lawrence et al., 2002; Suttle, 2000). The detection of Coccolithovirus and its host Emiliania huxleyi at a depth of 971 m in sediment core of the Black Sea suggested a sinking and long-term preservation of the virus-host system (Coolen, 2011).

Summarily, the downwards transport of planktonic microorganisms (cells and viruses) significantly impacted the microbial communities in sediments. This highlights the need to study this ecologically vital process and the associated microbes. However, although the sedimentation of photosynthetic cells and viruses have been observed, studies on their diversity are scarce (Corinaldesi, 2015). The psbA gene, which encodes a critical protein of photosynthetic system II, is present in all oxygenic photosynthetic organisms, including eukaryotic algae, Synechococcus, and Prochlorococcus, and also found in many cyanophages (Mann et al., 2003; Zeidner et al., 2003). It is an efficient gene marker that facilitates investigations into the diversity of photosynthetic cells and cyanophages in various environments (Chenard & Suttle, 2008; Sullivan et al., 2006; Thureborn et al., 2016; K. Wang & Chen, 2008; G. Wang et al., 2009; Zheng et al., 2013). Hence, in this study, we investigated the diversity of exported photosynthetic cells (i.e., eukaryotes and cyanobacteria) and cyanophages from the upper ocean, based on psbA genes in surface sediment of the South China Sea (SCS) and Pearl River Estuary (PRE).

The SCS, locating in the tropical-subtropical rim of the western Pacific Ocean, is a semi-enclosed basin with extensive continental shelves on the northwestern and southern parts and a 4,700 m deep basin (Shaw & Chao, 1994). The Pearl River is the largest river discharging into the SCS, and it contributes ~80% of the suspended particulates (~80 Mt/y) in the estuary plume (Zhou et al., 2004). Since planktonic microorganisms associated with suspended particles are deposited, sedimental microbial communities in the estuarine transition area are strongly affected by the Pearl River discharge (Fu et al., 2015; J. Liu et al., 2014). Additionally, suspended particulates originating from the island and continent directly influence the sedimentation condition in the surrounding sea. For instance, owing to the low sediment flux (~1 Mt/y) from Hainan Island, the mass accumulation rates of the continental shelf are small (P. Wang, 1999), while 40–100 Mt of sediments are annually supplied from the Annamite Chain and maintain the relatively high sedimentation rate of the western SCS (Schimanski & Stattegger, 2005). The community data of microbial plankton being transported from the upper ocean to the sea floor in the PRE and SCS will provide a helpful reference to investigate the vertical export behaviors of planktonic microorganism and their ecological and biogeochemical impacts.
2. Materials and Methods

2.1. Station Locations and Sampling

Sediments were collected from the PRE and the SCS (Figure 1). The samples from sites A, B, and C, which were located in the PRE and its adjacent coastal regions, were collected in July and August 2013. Further details about the sampling methods have been reported in our previous study (He et al., 2017). Other samples from sites S1∼S8 were collected in September 2015, using a rectangular-core box-corer that sealed the sample in situ with a hinged cutting arm. The sediment samples were placed into sterile Ziploc bags after the surface layer was scraped off. The samples were then stored at −80°C until further analysis.

2.2. Enumeration of Microorganisms

The abundances of virus-like particles and prokaryotes were determined by epifluorescence microscopy, according to the methods of our previous study (He et al., 2017). Briefly, 1.0 g of sediment was diluted with 9 mL SM buffer (50 mM Tris-HCl, 10 mM MgSO₄·7H₂O, 100 mM NaCl, 0.01% gelatin, pH = 7.5) and fixed with glutaraldehyde (0.5% v/v). Sodium pyrophosphate solution (5 mM final concentration) was added to the mixture and the samples were shaken violently for 15 min. Sonication was applied to optimize the extraction. Subsamples were diluted 1,000–5,000 times and then filtered on 0.02 µm pore size Anodisc alumina filters (6809-6002, Whatman). The filters were then stained with SYBR Green-I, and evaluated.
using epifluorescence microscopy (Olympus BX51) under blue light (wavelength of 450–490 nm). Each sample was assessed 3 times.

2.3. DNA Extraction, Polymerase Chain Reaction, Cloning, and Sequencing

Total DNA was extracted from 0.5 g of sediment samples using the FastDNA Spin Kit for Soil (Qiagen Inc.), following the manufacturer’s protocol. The psbA genes of the cyanobacteria, microalgae, and cyanophages were amplified using the degenerate primers, Pro-psbA-1F (5′-AAC ATC ATY TCW GGT GCW GT-3′) and Pro-psbA-1R (5′-TCG TGC ATT ACT TCC ATA CC-3′) (Chenard & Suttle, 2008). The template (1 μL) was added to 25 μL of the polymerase chain reaction (PCR) mixture, containing ExTaq buffer (without Mg²⁺; Takara Bio), 2.5 mM magnesium chloride, 0.2 mM of each deoxyribonucleoside triphosphate polymerase, 10 nM of each of the primers, 0.08% bovine serum albumin (Takara), and 2.5 U ExTaq DNA polymerase (Takara). Negative controls contained all the reagents and sterile water instead of a template.

The PCRs were performed with the following cycle parameters: denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 1 min; annealing at 52°C for 1 min; extension at 72°C for 1.5 min; and a final extension at 72°C for 10 min (Chenard & Suttle, 2008). Amplification products were run on a 1% agarose gel. Bands of the expected size were excised and purified using an agarose gel DNA purification kit (Takara). The PCR products were cloned into the pMD18-T vector (Takara) and then transformed into competent cells of Escherichia coli DH5α. Positive clones were screened using PCR re-amplification with the vector primers M13-F/M13-R, and randomly selected for sequencing using an automated DNA sequence analyzer with BigDye Terminator Cycle Sequencing Kit (ABI model 3730, Applied Bio Systems, Perkin-Elmer).

2.4. Phylogenetic Diversity Analysis

Sequence quality of psbA clones was examined using the Molecular Evolutionary Genetics Analysis software (MEGA 6.0). Low quality sequences were removed and the remaining sequences were aligned based on the ClustW program. Aligned sequences were grouped into operational taxonomic units (OTUs), based on a 3% DNA sequence divergence cut-off using the DOTUR program (Schloss et al., 2009). Rarefaction and phylotype richness estimators (Chao 1, Shannon, and Simpson indices) for each clone library were also calculated using DOTUR.

The most similar amino acid sequences of psbA OTUs were searched against the RefSeq non-redundant protein data using the Basic Local Alignment Search Tool (BLAST) 2.7.1 software. A phylogenetic tree of psbA OTUs was constructed using the FastTree program of the Galaxy software (http://zhoulab5.rccc.ou.edu:8080/root) and edited by the ITOL software (http://itol.embl.de/). The GC contents of psbA groups were calculated using the Bioedit software, and the δi/δt, ETTXXXSδi/δt model of different psbA groups was analyzed using the MEGA software. The DNA sequences of the psbA clones were deposited in GenBank under accession numbers MF564305 to MF565374 (https://www.ncbi.nlm.nih.gov/popset/?term=1391907823). Box plots and histograms were constructed with the Origin 2020 software. The gmodels package of the R software (version 3.4.4) was used for principal coordinate analysis (PCA) of psbA OTU assemblages.

3. Results

3.1. Sampling Environments

A total of 11 sediment samples were collected during this study, including seven stations (A, B, C, and S1–S4) along the area from the PRE to SCS; two stations (S5 and S6) located in the continental shelf of the east outer Hainan Island; and two stations (S7 and S8) of the western SCS. The water depths of sampling stations varied from 14 m to 2,689 m (Table S1). The matrix of sediment samples from the PRE comprised silt gyttja, which usually contains small-diameter particles and >2% organic carbon (Borgendahl & Westman, 2007). The sediments from other stations of the continental shelf, east of Hainan Island and in the western SCS comprised silty sand.
3.2. Microorganism Counts

The abundances of virus-like particles in sediments ranged from $4.55 \times 10^7$ to $1.17 \times 10^9$ particles/g, with maximum and minimum values being obtained from sites A and S6, respectively. Prokaryotic abundances ranged from $1.71 \times 10^7$ (S3) to $9.55 \times 10^8$ (A) cells/g (Figure 2a). The abundances of microorganisms (including virus-like particles and prokaryotes) of S3 and S4 were much lower than those of four other stations (B, C, S1, and S2), which were closer to the PRE. Similarly, the microbial abundances of S6 (prokaryotes: $2.83 \times 10^7$ cells/g; virus-like particles: $4.55 \times 10^7$ particles/g) were lower than those of S5 (prokaryotes: $2.65 \times 10^8$ cells/g; virus-like particles: $3.91 \times 10^8$ particles/g).

In addition, microorganisms in the deep sea stations, S7 and S8 (prokaryotes: $1.14–1.40 \times 10^8$ cells/g; virus-like particles: $1.42–1.85 \times 10^8$ particles/g) were more abundant than those in sediments from S3, S4, and S6. The abundances of virus-like particles and prokaryotes were significantly correlated (t-test, $P < 0.01$) (Figure 2b). The virus-to-prokaryote ratios (VPR) ranged from 0.95 to 4.15. Generally, high VPR values indicate high and ongoing viral dynamics and low values could be attributed to diminished viral activity, the absence of viruses, or high viral decay (Parikka et al., 2016). The range of VPR values is suggestive of the different viral dynamics in sediments of the PRE and SCS.

3.3. Phylogenetic Diversity of psbA Sequence

A total of 1,070 psbA clones were obtained from 11 clone libraries, and clustered into 687 OTUs with a cut-off of 97% nucleotide identity. The number of OTUs in each sample varied from 48 to 94. The rarefaction curves of clone libraries (Figure S1) and diversity indices (Table S2), including the Chao1 index (81–732), Shannon index ($H'$: 3.24–4.51), and Simpson index (1/D: 12.44–631.25), suggested the high diversity of psbA gene-containing microbes in marine sediments, regardless of the water depth.

The sedimentary psbA OTUs showed a range of 90.16%–100% identities, the majority (578 of 687 OTUs) of which were higher than 97%, with sequences in the NCBI RefSeq non-redundant protein database. Two OTUs showed 100% identity with psbA sequences from eukaryotic algae *Pseudosolenia calcar-avis* (AKF18093) and *Opephora* sp. HK296 (AKF18128). Likewise, OTUs showing 100% identity with sequences from *Prochlorococcus* MIT9312 (AAT98416), cyanophage S-RIM49 (ACY42515), S-RIM 24 (ACY42503), P-SSM3 (ABF55984), and P-SSM1 (ABF56004) were obtained. Moreover, two OTUs with 99.6% identity, were similar to the sequence from *Synechococcus* BL3 (AAX94058). Furthermore, 312 OTUs (45.4% of the total number of OTUs), representing 485 clones (45.3%), showed the highest similarity to the sequences of
uncultured organisms, originating from surface water samples of the Mediterranean Sea and the East China Sea (Sharon et al., 2007; Zheng et al., 2014).

Phylogenetic relationships between the psbA OTUs obtained in the present study and sequences from cultured organisms (including cyanobacteria, cyanophages, and eukaryotic algae) and environmental psbA clones (Chenard & Suttle, 2008; Sullivan et al., 2006; Zheng et al., 2014) are shown in Figure 3. Seven groups were clustered in the phylogenetic tree: the Synechococcus; Synechococcus myovirus; Synechococcus podovirus; Prochlorococcus; Prochlorococcus myovirus; Prochlorococcus podovirus; and eukaryotic algae groups. A total of 18 psbA OTUs were clustered within “UC Syn-virus I” (14 OTUs, 16 clones) and “UC Syn-virus II” (four OTUs, 13 clones) groups. Based on the GC content and triplet peptides of sedimental psbA OTUs, these two groups were considered as part of the novel clades of the Synechococcus myovirus group (see below).
The eukaryotic algae group comprised 61 psbA OTUs, and some psbA sequences were associated with cultured algae (such as Coscinodiscus sp. KM009566, Asterionellopsis glacialis KM009478, Auxenochlorella protothecoides EU043045, Eustigmatophyceae sp. HQ710713). The most abundant OTU (OTU-6, with 26 clones) clustered in the eukaryotic algae group was only detected in samples from site A (Figure 3). This suggests that this OTU originated from freshwater algae, even though the most similar sequence (97.95% identity, AGL788741) of OTU-6 was from the East China Sea. Five OTUs were clustered into the Prochlorococcus group, which could be differentiated into “high-light” and “low-light” subgroups. The high-light subgroup included three OTUs, with psbA sequences of Prochlorococcus MIT9302, MIT9515, and MED4. The low-light subgroup included two OTUs and psbA sequences from the Mediterranean Sea (EU728206 and EU728168) and Prochlorococcus NATL1A, NATL2A, and MIT9211. Furthermore, 25 sediment OTUs were affiliated in the Synechococcus group with psbA sequences from SYNBL3, WH8101, WH8109, and SYN9902.

Based on the original environments of reference psbA sequences, the Synechococcus myovirus group was further divided into two subgroups, the freshwater subgroup and the marine subgroup. These results are similar to the phylogenetic results of a previous study (Chenard & Suttle, 2008). The abundant OTU-385, OTU-62, and OTU-82, comprising 18, 12, and 11 clones, respectively, fell within the marine subgroup. The OTU-62 and OTU-82 showed 100% identity with the cyanophages S-RIM24 (ACY42503) and S-RIM49 (ACY42515), respectively, while OTU-385 shared 93.03% identity with an uncultured cyanophage sequence (AKZ31960). In the Synechococcus myovirus freshwater group, the psbA OTUs from sediment samples were clustered with the sequences from East Lake, China (KP775373 and KP775365); Lake Erie, North America (EU404145 and EU404156); and Lake Constance, Germany (EU258992 and EU258993) (Figure 3).

According to the phylogenetic results, the relative abundances of psbA OTUs and clones of different groups were analyzed (Figure 4). In the freshwater site A, 81.6% of the OTUs were clustered within the eukaryotic algae group, and a few were associated with the Synechococcus myovirus group (freshwater subgroup, 12.3%; marine subgroup, 6.2%). In site B, most OTUs were clustered to the Synechococcus myovirus, with 73.0% in the marine subgroup and 23.2% in the freshwater subgroup. Furthermore, six OTUs of S1, and one OTU of S2 were also clustered within the Synechococcus myovirus freshwater subgroup.

Among the remaining nine PRE and SCS sites (C and S1–S8), the Synechococcus myovirus marine subgroup and Prochlorococcus myovirus group occupied a majority of psbA OTUs as well as clones. The OTUs and clones in the Synechococcus myovirus marine subgroup ranged from 8.3% (S6) to 67.1% (C), and those in the Prochlorococcus myovirus group ranged from 12.5% (S1) to 52.6% (S5). The percentages in podovirus groups were much lower than those in myovirus groups. The OTUs of the Prochlorococcus podovirus group were mainly apparent at sites S5 (15.8%), S6 (27.8%), and S7 (26.9%); and those of the Synechococcus podovirus group were relatively abundant at sites S2 (13.8%), S7 (11.5%), and S8 (17.3%) (Figure 4a). The distribution of psbA clones showed a similar pattern to that of OTUs (Figure 4b). Various psbA-containing microbial groups were obtained from sediments in the PRE and SCS. Among these groups, cyanomyoviruses infecting both Synechococcus and Prochlorococcus were abundant.

PCA showed that the psbA-containing microbial community varied in different sediments (Figure 5). The distant point in Figure 5 corresponding to the psbA OTUs from site A indicated considerable differences in the psbA-containing microbial community between freshwater sediment and seawater sediment. The heatmap of the OTUs also revealed that site A harbors a microbial composition that differs from those of other sites (Figure S2). In addition, the samples from estuarine stations were in close proximity to each other, while other samples representing the continental shelf (S5 and S6) and deep sea (S7 and S8) sites were distributed further apart (Figure 5). The lowest values of diversity indices (H': 3.74; 1/D: 49.41; Chao1: 81, Table S2) were observed in S7. The PCA results also indicated different psbA-containing microbial compositions between S7 and other marine sediment stations.

### 3.4. Identification of Two Novel Synechococcus Myovirus Groups Based on GC Content and Variable Triplet Peptides

Previous studies have shown that the GC content of psbA sequences, as well as the variable triplet peptides associated with the D1 protein motif $^{H}/^{K}$ ETTXXS $^{G}/^{H}$ (Figure S3) can facilitate determination of the specific to which the psbA sequences belong (Chenard & Suttle, 2008; Sharon et al., 2007; Zeidner...
The average GC contents were 43.46% in Prochlorococcus; 43.82% in Prochlorococcus phages (Prochlorococcus myovirus: 42.95%; and Prochlorococcus podovirus: 44.69%); 48.71% in Synechococcus phages (Synechococcus myovirus: 49.41%; Synechococcus podovirus: 48.02%); and 57.32% in Synechococcus. These findings confirm those of previous reports (Figure 6, Table 1) (Sullivan et al., 2006). The average GC contents of “UC Syn-virus I” and “UC Syn-virus II” were 48.84% and 49.25%, respectively, falling within the range of the Synechococcus myovirus group. Thus, we propose “UC Syn-virus I” and “UC Syn-virus II” as novel clades of the Synechococcus myovirus group.

In this study, there were 36 variable triplet sequences of psbA OTUs and five triplets containing ETE, ESE, ENE, EQE, and GLD accounted for the majority of those detected (Table 1). The triplets, EQE and ENE, appeared in several viral groups. These findings are similar to those from the Global Ocean Sampling metagenomic data (Sharon et al., 2007). Nine triplet sequences were detected in “UC Syn-virus I” and “UC Syn-virus II” as novel clades of the Synechococcus myovirus group.

In this study, there were 36 variable triplet sequences of psbA OTUs and five triplets containing ETE, ESE, ENE, EQE, and GLD accounted for the majority of those detected (Table 1). The triplets, EQE and ENE, appeared in several viral groups. These findings are similar to those from the Global Ocean Sampling metagenomic data (Sharon et al., 2007). Nine triplet sequences were detected in “UC Syn-virus I” and “UC Syn-virus II.” Among them, five abundant triplets, including ESE, ETE, EKE, ENE, and ETV, were also predominant in the Synechococcus myovirus group.

Two triplets, EDM and ESV, were detected only in “UC Syn-virus I” and “UC Syn-virus II,” respectively. These findings provide further proof that these two branches are novel clades of the Synechococcus myovirus group.
Interestingly, the triplets, EEV and EDV, were detected in both of the novel groups and the Synechococcus podovirus group (Table 1) (K. Wang & Chen, 2008). This suggests the relatively close evolutionary distance between these novel clades and the podovirus group. The GLD, ETE, and ESE triplets have been reported previously in both viral and bacterial groups (Sharon et al., 2007), and in the present results, were more evident in viral groups than in host groups. The ESE triplet was not detected in the Prochlorococcus, or Prochlorococcus myovirus or podovirus groups; however, GLD was only detected among these groups (Table 1). These results show considerable differences from previous distribution analyses of psbA triplets. As far as we know, the reliability of triplet peptides in the determination of specific viruses or bacteria associated with psbA sequences, requires increased accuracy, and should be based on an analysis of a considerably higher number of psbA sequences.

Figure 6. Box plots of the GC content of psbA OTUs of various groups. In each box plot, the central point represents the median, the rectangle indicates the interval between the 25% and 75% percentiles, and the whiskers indicate the range. Syn denotes Synechococcus and Pro denotes Prochlorococcus.

Table 1
The GC Content and D1 Protein Motif[^k ETTXX S^H] of psbA OTUs of Various Groups

| Group                  | GC content (average, %) | ^k ETTXX S^H          |
|------------------------|-------------------------|------------------------|
| Synechococcus          | 57.32                   | ESE(18), ETE(3), EQU(1) |
| Syn myovirus freshwater | 48.82                   | ENE(19), ETE(1), ESE(6), EQE(2) |
| Syn myovirus seawater  | 49.99                   | ENE(28), ESE(29), ETE(140), ENE(6), EEE(1), ETA(1), ETG(2), ETV(11), ENV(1), EQV(3) |
| Syn podovirus          | 48.02                   | ETE(4), EIE(1), EEF(2), EIT(1), ENV(3), ENE(20), EEE(1), ETE(1), EDF(2), ESE(3) |
| Prochlorococcus        | 43.46                   | ETE(3), GLD(1)        |
| Pro myovirus[^a]       | 42.95                   | GLD(75), ETE(65), EVE(4), EQE(35), DNE(4), EEE(33), EEF(2), |
| Pro podovirus          | 44.69                   | DNE(5), ENE(35), DLE(2), DAE(1), EVE(6), ELE(2), EEE(1), EDF(4), GLD(4), EVG(1) |
| Eukaryotic algae       | 42.58                   | EIE(2), ETE(3), EEF(50), DGV(2), DGE(1), EGE(1), EQE(1), EEE(1) |
| UC Syn-virus I         | 48.84                   | ESE(2), ETE(6), EEF(2), EDM(1), ENE(2), EEF(1) |
| UC Syn-virus II        | 49.25                   | EDV(1), ENE(1), ESV(1), ETV(1) |

[^a]Syn, Synechococcus. [^b]Pro, Prochlorococcus.
4. Discussion

The abundance of microbes in surface sediments is usually related to the input of fresh detritus or nutrient conditions in the sediments (Boetius & Damm, 1998; Smith et al., 1997). This could explain the higher microbial and viral counts in sediments from the stations (A, B, C, S1, S2, and S5) closer to the mainland or Hainan Island. As the largest river discharging into the SCS, the Pearl River carries a considerable quantity of suspended particulate matter, organic carbon, nitrogen, and phosphorus, and plays a key role in producing estuary suspended particulates (Zhao, 1990; Zhou et al., 2004). Therefore, the large input of the Pearl River strongly affects the microbial community in the plume area, and the degree of its influence gradually decreases with increasing distance from the river mouth (J. Liu et al., 2014), which leads to the decreasing trend of viral and bacterial abundances in waters and sediments of the PRE area (Figure 2a). Similarly, massive sedimental particles from the Annamite Chain may maintain the relatively high sedimentation rate in the western SCS (Schimanski & Stattegger, 2005) and thereby lead to the high microbial abundances observed at the S7 and S8 stations.

4.1. Prevalence of Viral psbA Genes and Vertical Transmission of Cyanophages

High identities with reference sequences from freshwater or upper ocean communities (90.16%–100%) were observed among the sedimentary psbA OTUs in the present study. Considering the lack of sunlight in dark marine sediments, photosynthetic microorganisms are not likely to be indigenous. Therefore, the presence of psbA genes in PRE and SCS sediment samples could be attributed to the downward export of psbA gene-containing microbes from the upper water column. In addition, the similar distribution trends of psbA clones and OTUs among different groups revealed the potential positive relationship between the abundance and diversity of psbA genes (Figure 4).

In this study covering typical estuarine, coastal, and open ocean sediments, the majority of psbA OTUs originated from cyanophages, including cyanomyoviruses and cyanopodoviruses (Figure 3). Viral particles could adhere to sinking particles or be maintained in the infected host to accelerate their transport to the seafloor, where they may become important components of the sedimental viral community (Mari et al., 2007; Mojica & Brussaard, 2014). A recent study found that with an adhesive value of 0.04–24 viruses µm⁻² (bacterial surface-area), viruses could attach to non-host bacteria, and a large proportion of viruses (7%–48%) could adhere to model abiotic surfaces (polycarbonate, polypropylene, and glass) (Yamada et al., 2019). These phenomena should accelerate viral sedimentation in particle-rich marine environments. It was reported that cyanophages remain stable in sediments for more than thousands of years (L. Cai et al., 2019; Wommack & Colwell, 2000). Similarly, Hurwitz et al. (2015) determined the prevalence of viral genes, including psbA, in a large-scale quantitative viral metagenomic data set of the aphotic zone (Pacific Ocean Virome), and suggested that those genes originated from the vertical transport of photic zone viruses.

In the present study, 28 OTUs were affiliated in the *Synechococcus* myovirus freshwater subgroup. Freshwater psbA-containing cyanophages are likely transported by the input of the Pearl River. Owing to the sinking of many suspended particles and strong mixed hydrologic movements, these cyanophages become important components of the sedimental viral community in the PRE. Among the estuarine stations, B, S1, S2, and S3, more psbA OTUs and clones were clustered within *Synechococcus* and *Synechococcus* viruses than within *Prochlorococcus* and *Prochlorococcus* viruses. However, the opposite pattern was observed at the continental shelf stations, S4, S5, and S6. Previous studies have indicated that in the SCS, *Synechococcus* is more abundant in the nutrient-rich coastal zone, while *Prochlorococcus* is more abundant in the continental slope and open sea (Y.-M. Cai et al., 2007). To a certain extent, the distribution pattern of cyanobacteria in sediment samples is similar to that in the upper water column, which also contributes to the distribution of cyanophages in the sediment.

4.2. Sedimentation of psbA Gene-Containing Microphytoplankton and Picophytoplankton

About 12% of psbA clones were clustered within the eukaryotic algae group, with sequences from the diatoms, *Coscinodiscus* CCMP3377 (KM009566) and CVPan-4 (KM009562) (Figure 3). It has reported that the downward transport of phytoplankton cells has the fast sinking rate of 124–732 m/d (Agusti et al., 2015).
Therefore, the sedimentation of phytoplankton cells is an important means by which the net primary production of the photic zone can be conveyed to the ocean interior, including the deep seafloor. The exported organic matter could provide metabolic substrates and thereby affect the benthic microbial community (Thomas et al., 2000). Previous investigations on phytoplankton diversity have indicated that diatoms can be found predominantly in the photic zone of the northern SCS. Although the Coscinodiscus spp. accounts for only a small percentage (0.08%–1.28%) of the phytoplanktonic community in euphotic waters (H. Liu et al., 2016; Wei et al., 2018), they can be found predominantly in surface sediments of the SCS (Jiang et al., 2004; Wu et al., 2013), which suggests their relatively high deposition rate. Furthermore, some 100% identity sequences of OTUs belong to the diatoms, Pseudosolenia calcar-avis and Opephora spp. HK296. Pseudosolenia calcar-avis is a common small centric diatom and occasionally dominates the phytoplankton community of the temperate zone (Kaiser et al., 2016). Previous investigations have shown that Pseudosolenia calcar-avis was widely distributed along coastal areas of the SCS and East China Sea (Boonyapiwat, 1999). Pseudosolenia calcar-avis and Opephora spp. HK296 were also prevailing species in marine sediments. For example, Pseudosolenia calcar-avis was reportedly prevalent in the Caspian Sea and a sediment core of the Baltic Sea, and even dominated the flora, reaching more than 80% of total assemblages in both environments (Karpinsky, 2010; Sohlenius et al., 1996). Opephora was commonly found in brackish and marine sediments worldwide (Sabbe & Vyverman, 1995). As expected, these psbA OTUs of phytoplankton cells were present in sediment samples of the PRE and SCS. However, although Pseudo-nitzschia delicatissima, Skeletonema costatum, and Chaetoceros curvisetus were reportedly abundant in the North SCS (Ma, 2016; Wei et al., 2018), they were not detected among our clone libraries. This may indicate a slower rate of transport and/or faster consumption of these phytoplankton than Pseudosolenia calcar-avis, Opephora spp., and other species found in the sediments in the present study. Besides, it is worth noting that the lack of some phytoplanktonic psbA sequence in the NCBI database, such as Thalassionema nitzschioides, one of abundant diatoms in the upper zone of SCS, could also cause them to be unidentified in our study.

In conclusion, our findings revealed that different phytoplankton species may show varied vertical export behaviors and the major contributors to the export of organic matter might not be the predominant primary producers near the ocean’s surface.

Picophytoplankton in open waters of the SCS, including Synechococcus and Prochlorococcus, occupy most (up to 64.2%) of the phytoplanktonic communities (Y.-M. Cai et al., 2007). In the present study, 0.7% of clones was associated with Prochlorococcus and 3.6% was associated with Synechococcus groups. These values are lower than those of eukaryotic algae (11.7% of total clones), indicating that the sedimentation of picophytoplankton was less efficient than that of eukaryotic algae, most likely because of their small particle sizes and higher consumption rate (Boyd & Newton, 1999). However, the downwards export of picophytoplankton is still prevalent and facilitated by multiple transport mechanisms. This leads to the presence of picophytoplankton in marine sediments, even along the abyssal sea floor (Lindh et al., 2017). Vilibic and Santic (2008) reported that the combination of winter convection and density currents on the downslope of the South Adriatic Sea leads to the downward movement of Synechococcus from overlying water to the sea floor (1,150 m).

Interestingly, most of the picophytoplankton psbA sequences were from deep sea sites (S7 and S8) in the western SCS (Figure 4b), where a dipole comprising a cyclonic eddy and an anticyclonic eddy occurs each summer (Jing & Liu, 2012). The lowest value of diversity indices and the specific psbA-containing microbial composition of site S7 (Table S2, Figure 6) likely resulted from complicated hydrological movements and varying nutrient supplies. In the same area, Yan et al. (2018) had reported a high Synechococcus abundance and chlorophyll florescence in the cyclonic eddy, owing to the higher nutrient supply generated by upwelling, and the presence of Prochlorococcus at a depth of 500 m in an anticyclonic eddy due to strong vertical mixing and horizontal advection. In site S7, with a high sedimentation rate (Schimanski & Stattegger, 2005) and strong eddy-induced hydrologic movement, abundant picophytoplankton and their viruses could be more quickly transferred from the upper layer to seafloor, which may result in the specific psbA-containing microbial community. In the western SCS, clade HL II was predominant among Prochlorococcus clades and clade II accounted for the majority among seven Synechococcus clades (Jing & Liu, 2012). Another study reported that clade HL II and LL IV were the predominant Prochlorococcus ecotypes in both the euphotic and aphotic zones (up to 2,000 m in depth) of the Luzon Strait (Jiao et al., 2014). In this study, seven psbA clones of the “high-light” and “low-light” subgroups could be affiliated in clades HL I,
HL II, LL I, and LL III, with sequences from Prochlorococcus MIT 9515, MIT 9302, NATL2A, MIT9211, and PSSM2. Furthermore, 39 psbA clones are associated with Synechococcus clades I and VIII, with WH8020 and WH8101 (Scanlan, 2012). The present results suggest a difference in the predominant picophytoplankton, including Synechococcus and Prochlorococcus, between the upper ocean and sediment, possibly due to different vertical export rates of various phylotypes.

5. Conclusions

The present study investigated the vertically exported planktonic microorganisms in the surface sediments of SCS and PRE, based on the photosynthesis-related gene psbA. The majority of sediment psbA clones and OTUs originated from cyanophages, indicating their extensive transport from the photic zone down to the sea floor. Spatially, Synechococcus and their phages were more abundant in the coastal sediments while Prochlorococcus and their phages were prevalent in the sediments of open sea, which was similar to their distribution in the water column. Meanwhile, our findings indicated the potentially different vertical export behavior of eukaryotic algae and cyanobacteria, which lead to the different predominant phylotypes in the sediments and in the overlying waters. These allochthonous microorganisms could affect substrate transport, microbial metabolism, and community composition of the dark ocean and marine sediments.

Data Availability Statement

Data and samples were collected on board the R/V “SHIYAN3” implementing the open research cruise NORC2015-07, supported by the NSFC Shiptime Sharing Project (Project number: 41449907). All sequences obtained in this study were deposited in the National Center for Biotechnology Information (NCBI) GenBank under accession numbers MF564305 to MF565374 (https://www.ncbi.nlm.nih.gov/nuccore?term=1391907823). The other data were listed in the table and supporting information and can be available on Zenodo https://zenodo.org/record/4527850#.YCNv-xa-vIU.

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