Deep sequencing of amplified Prasinovirus and host green algal genes from an Indian Ocean transect reveals interacting trophic dependencies and new genotypes

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Running title: Marine algae and their viruses in the Indian Ocean

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Summary

High-throughput sequencing of *Prasinovirus* DNA polymerase and host green algal (Mamiellophyceae) ribosomal RNA genes was used to analyse the diversity and distribution of these taxa over a ~10,000 km latitudinal section of the Indian Ocean. New viral and host groups were identified among the different trophic conditions observed, and highlighted that although unknown prasinoviruses are diverse, the cosmopolitan algal genera *Bathycoccus*, *Micromonas* and *Ostreococcus* represent a large proportion of the host diversity. While *Prasinovirus* communities were correlated to both the geography and the environment, similar links were not found for host communities. Nevertheless, analysis of single environmental variables showed that eutrophic conditions strongly influence the distributions of both hosts and viruses. Moreover, these communities were not correlated, in their composition or specific richness. These observations could result from antagonistic dynamics, such as that illustrated in a prey-predator model, and/or because hosts might be under a complex set of selective pressures. Both reasons must be considered to interpret environmental surveys of viruses and hosts, since covariation does not always imply interaction.

Introduction

Microbes are the most abundant organisms in the sea, where they shape the structure and function of ecosystems (Azam et al., 1983), but they are still one order of magnitude less abundant than microbe-infecting viruses (Suttle, 2005). Viruses are thus important players in microbial mortality and strongly influence biogeochemical cycles and the structure of host communities (Proctor and Fuhrman, 1990; Gustavsen et al., 2014). Marine microbes and their associated viruses are thought to have high dispersal capacities because of their abundance, (Finlay, 2002; Angly et al., 2006), although community composition might differ according to environmental conditions (Angly et al., 2006; Martiny et al., 2006).

However, little is known concerning the environmental factors that best explain their distribution and whether or not host and virus communities are correlated. To answer these questions, this study focus on the genus *Prasinovirus*, a member of the *Phycodnaviridae* family (Wilson et al., 2009) that infect an abundant and widespread picoeukaryotic algal class referred to as the Mamiellophyceae (Marin and Melkonian, 2010). Known *Prasinovirus* host species include the three dominant genera: *Bathycoccus*, *Micromonas* and *Ostreococcus*, infected respectively by *Bathycoccus* viruses (BpV), *Micromonas* viruses (MpV) and *Ostreococcus* viruses (OV). Several species have been described for each host genus (Marin and Melkonian, 2010; Piganeau et al., 2011b) that might be adapted to different environments. For example, *Ostreococcus* species might contain different ecotypes adapted to different light intensities (Rodriguez et al., 2005). Prasinoviruses are large, double-stranded DNA viruses and form a monophyletic group within the *Phycodnaviridae* family (Bellec et al., 2009). They are also abundant and widespread (Short and Short, 2008; Bellec, Grimsley, and Desdevises, 2010; Park et al., 2011; Hingamp et al., 2013; Zhong and Jacquet, 2014). Previous studies suggested that both *Prasinovirus* and Mamiellophyceae have high dispersal

capacities (Slapeta et al., 2006; Bellec, Grimsley, and Desdevises, 2010) and that occurrence of genotypes is related to environmental conditions (Lepère et al., 2009; Bellec, Grimsley, Derelle, et al., 2010). However, culture-dependent methods were mainly used to study these groups so far, with no overview at the scale of communities.

The occidental part of the Indian Ocean was chosen for this analysis. This large region is affected disproportionally by global warming, since modeling and recent observations revealed a substantial temperature increase in the upper 700 m of the Indian Ocean (Lee et al., 2015), driving El Niño/Southern Oscillation cycles and climate change. Warm waters arriving on the Equatorial Currents from around Malaysia and Western Australia drive the warm Aghulas current southwards along the East African coast, that in turn meets colder water from the South Atlantic and Benguela currents in an upwelling area. Thus this region provided contrasting conditions well-suited for our objectives: (i) to describe the diversity of prasinoviruses and Mamiellophyceae at a community scale using a culture-independent sequencing approach; (ii) to disentangle the influence of the geographical and the environmental variables; (iii) to determine whether or not host and viral communities are correlated. We hypothesized that dispersal capacities of these communities are not limited within this oceanic region, but that compositions are highly constrained by the environment. Furthermore, *Prasinovirus* distribution might be strongly correlated to host communities, because their own replication depends on the cellular machinery.

Results and discussion

From oligotrophic to eutrophic samples. The 11 samples came from 8 stations in the occidental part of the Indian Ocean (Figure 1). Most samples were taken from the surface, but three came from the deep chlorophyll maximum (hereafter named DCM); stations 58, 65 and 66. The sampling sites and the environmental variables are described in detail as supplementary information for methods. The first component (C1) of principal component analysis (PCA) for the 11 samples (Figure 2) divides them mainly according to potential temperature, oxygen and density (Table S1). Beam attenuation and backscattering coefficient of light by particles (bbp) are both proxies of the particle load of seawater (e.g. Neukermans et al., 2012) and contributed to build the second component (C2) such as heterotrophic bacteria, which divide stations 36, 38, 39, 46, 66 from 57, 58, 65. This ordination highlighted high variability of environmental conditions, from oligotrophic (57, 58), to mesotrophic (36, 38, 39, 46, 65) and eutrophic (66). Stations 57 and 58 were located in the Mozambique channel, an oligtrophic area (Lévy et al., 2007; Leal et al., 2009), and contained low concentrations of particles and heterotrophic bacteria, which are more abundant in higher nutrient situations (e.g. Thingstad et al., 2008). In contrast, station 66 was particularly different from the other samples, probably because it was sampled within an area of high primary production (Villar et al., 2015) due to upwelling from the Benguela, South Atlantic and Agulhas currents (Figure 1; Summerhayes et al., 1995; Boebel et al., 1998; Lutjeharms et al., 2000). Station 66 was characterized by motion of dense, cooler and nutrient-rich water towards the surface that increased the concentration of oxygen through enhanced photosynthetic activity. Notably, this station contained among the highest concentrations of chlorophyll *a* and photosynthetic picoeukaryotes (Table S2).

Uncultured prasinoviruses were very diverse. Although the *Prasinovirus* sequences are available for the 11 samples, the data for Mamiellophyceae concern 6 samples from 4 stations (Figure 1). The sampling strategy is described in details as supplementary information for methods, including the number of sequences, genotypes and OTUs (Tables S3 and S4). In order to describe virus and host diversity of this oceanic region, phylogenetic reconstructions (Figures 3 and 4) and sequence annotations of viral DNA polymerase (*polB*) and host green algal RNA ribosomal (18S) genes were performed (see supplementary information for methods, Figures S1-S3, Table S5). Known host species of prasinoviruses are all species within dominant genera of the order Mamiellales (Bellec et al., 2009; Marin and Melkonian, 2010; reviewed in Grimsley et al., 2012). However, the culture-independent approach used here highlighted that although BpV and MpV were the richest groups, OV was only the seventh richest, and that unknown *Prasinovirus* contributed a high proportion of the diversity (OTU7, OTU11, OTU15 and OTU39; Figure 3 and Figure S2).

In contrast, the diversity of the Mamiellophyceae was consistent with previous studies; *Bathycoccus*, *Micromonas* and *Ostreococcus* were the most abundant (Figure S3) (Not et al., 2004; Viprey et al., 2008). Notably, *Bathycoccus* and *Ostreococcus* were found in higher proportions in this region, whereas *Micromonas* dominated the eukaryotic picoplankton in the English Channel (Not et al., 2004) and at a Mediterranean Sea coastal site (Zhu et al., 2005). This composition was nevertheless realistic, since the genus *Ostreococcus* can dominate picoeukaryote communities: it is known to form blooms (O'Kelly et al., 2003; Treusch et al., 2012) and can contribute to up to 70 % of the phototrophic picoeukaryotes (Countway and Caron, 2006). Moreover, phylogenetic reconstruction of Mamiellophyceae sequences also highlighted a new environmental clade related to *Crustomastix* and *Dolichomastix* (Figure 4 box with dashed lines (OTUs were defined for a nucleotide identity of 95 % instead of 97 % to produce a clearer tree); and Table S6). Remarkably, a few related sequences were found in

144 samples from a deep-sea methane cold seep (Takishita et al., 2007), the sediment of a
145 hydrothermal vent (Edgcomb et al., 2002), and in gut content of a bivalve (Duplessis et al.,
146 2004).
147 **Most unknown prasinoviruses might infect Dolichomastigales.** Only representatives of
148 BpV, MpV and OV are so far available in culture (Cottrell and Suttle, 1995; Derelle et al.,
149 2008, 2015; Bellec et al., 2009; Weynberg et al., 2009, 2011). This lack of virus cultures for
150 other genera might be biased, because mostly coastal areas were sampled using cultures of
151 coastal algal strains, whereas *Mamiella*, *Crustomastix* and *Dolichomastix* were more
152 commonly represented in oligotrophic waters (Viprey et al., 2008). Since unknown
153 *Prasinovirus* genotypes were very rich in our dataset (particularly OTU7, OTU11, OTU15
154 and OTU39; Figure S2), the prediction of host identities was carried out.
155 First, a CCA highlighted that 2 Mamiellophyceae OTUs were correlated to the distribution of
156 *Prasinovirus*: OTU28 and OTU126 (p-value = 0.005). These 2 OTUs belong to the robust
157 clade described above using the phylogenetic analysis (Figure 4). A BLASTn search against
158 the NCBI nucleotide collection suggested that they are most similar to *Crustomastix*
159 *stigmatica* (Table S7), and these sequences came mostly from stations 36 and 38 where they
160 represent ~14 % of genotypes compared to an average of 2 % in other samples.
161 Secondly, since *Prasinovirus* are mainly genus specific (Clerissi et al., 2012; Bellec et al.,
162 2014), a co-distribution analysis was computed using genus annotation for Mamiellophyceae
163 and the *Prasinovirus* annotation (Figure 5, Figure S2, Table S5). While *Ostreococcus* and
164 *Bathycoccus* displayed a homogeneous distribution within the 6 samples, the correspondence
165 analysis (CA) shows similar distributions for (i) *Micromonas* and OV in the station 66, (ii)
166 OTU7, OTU26, Mamiellaceae_unknown and *Dolichomastix* in station 65, (iii) OTU11,
167 OTU14, OTU15, OTU58, *Crustomastix*, *Mantoniella*_unknown and
168 Dolichomastigales_unknown in stations 36 and 38. However, only the link between

Dolichomastigales_unknown and OTU11 was significant (r = 0.99; p-value = 0.01). Thus both analyses suggested that uncultured *Prasinovirus* groups possibly infected Mamiellophyceae strains from the Dolichomastigales order.

The distribution of communities is influenced mainly by trophic conditions. Given the results of previous studies (Slapeta et al., 2006; Lepère et al., 2009; Bellec, Grimsley, and Desdevises, 2010; Bellec, Grimsley, Derelle, et al., 2010; Clerissi, Grimsley, Subirana, et al., 2014), links with environmental conditions were expected, but not with geographical distances (locations) for both communities in this oceanic region.

First, *Prasinovirus* were correlated to both locations (Mantel test, r = 0.722, p-value = 0.001) and environment (Mantel test, r = 0.626, p-value = 0.001) (see supplementary information for methods, with details about the statistical and multivariate procedures). This spatial structure was surprising, since no links were observed between genetic distances of *Ostreococcus lucimarinus* viruses and sampling locations at a global scale (Bellec, Grimsley, and Desdevises, 2010; Derelle et al., 2015). However, locations and environment were also correlated in our dataset (Mantel test, r = 0.521, p-value = 0.001), and no differences were found between the genotypic structures of *Prasinovirus* communities in the 11 samples (P-test, p-value = 1). These observations might indicate a key role of the environment, and that *Prasinovirus* were actually dispersed in the occidental part of the Indian Ocean.

Secondly, significant links for the Mamiellophyceae communities were not found using Mantel tests (location: r = 0.275, p-value = 0.141, environment: r = 0.342, p-value = 0.092). This lack of correlations could be the result of a low statistical power, because the dataset contains 6 samples, but such correlations were still significant for *Prasinovirus* communities when using the same reduced dataset (location: r = 0.852, p-value = 0.003; environment: r = 0.771, p-value = 0.004). Hence, Mamiellophyceae might be highly dispersed and homogeneously distributed in this region.

However, to further decipher the influence of environmental variables on both communities, canonical correspondence analyses (CCA) were computed with a forward-selection procedure. This analysis highlighted that (i) potential temperature, density and beam attenuation constrained *Prasinovirus* distribution in the 11 samples (p-value = 0.005) (Figure 6; a similar trend was observed for the reduced dataset of 6 samples, Figure S4), and (ii) that potential temperature influenced Mamiellophyceae in the 6 samples (p-value = 0.015). Because potential temperature and density tend to separate station 66 from the other samples for both analyses, the eutrophic conditions of the station 66 seem to highly constrain communities of this host-virus system.

Few links between *Prasinovirus* and Mamiellophyceae communities. Since *Prasinovirus* entirely depend on hosts for their replication, a strong correlation between both communities was expected, but links were significant neither for community compositions (r = 0.397, p-value = 0.172) (Table 1), nor for specific richness (Spearman correlation, $\rho = 0.6$, p-value = 0.242). This lack of correlation can be explained by at least three hypotheses: (i) a poor resolution of membership content of both viral and host communities according to different unknown biases (DNA extraction, PCR, sequencing), (ii) a non-corresponding taxonomic threshold between viruses and hosts, and (iii) antagonistic oscillations between hosts and viruses.

A non-corresponding taxonomic threshold might result from an overestimation of *Prasinovirus* diversity and/or an underestimation of host diversity. On one hand, since the environmental diversity of prasinoviruses was not known, their phylogenetic limit was defined arbitrarily by the *Chlorovirus* sister clade (see supplementary information for methods). In addition, it is possible that the thresholds used to define virus and host OTUs did not correspond to the taxonomic interaction and that not all were able to infect Mamiellophyceae. On the other hand, some evidence suggests that host diversity is

219 underestimated when using the 18S as genetic marker (Piganeau et al., 2011a), especially
220 since strains with identical sequences display different susceptibilities to prasinoviruses
221 (Clerissi et al., 2012).
222 Antagonistic oscillations between hosts and viruses are also a plausible source of noise for
223 correlation analyses. Indeed, viruses might shape the structure of host communities via the
224 top-down elimination of different members (Thingstad and Lignell, 1997; Winter et al.,
225 2010). They can terminate blooms of hosts and be present when hosts are not (Bratbak et al.,
226 1993; Schroeder et al., 2003). As a consequence, an increasing abundance of viral genotypes
227 is expected to be associated with a decrease of their specific hosts. However links are not
228 necessarily linear and can be complex because host ranges vary widely for example (Winter et
229 al., 2010). Since free viral particles were sampled independently of host cells (fraction below
230 0.2 µm for viruses), it is tempting to speculate that the antagonistic dynamics observed is a
231 likely hypothesis to explain the lack of correlations between *Prasinovirus* and
232 Mamiellophyceae communities in this study. In particular, OV were mainly found in
233 station 66 with *Micromonas* (Figure 5). Their occurrence suggests a bloom of the genus
234 *Ostreococcus* before an algal succession dominated by *Micromonas*.
235 Lastly, while viruses mainly depend on the presence of hosts and on factors involved in their
236 decay, hosts must face not only bottom-up (nutrients) and top-down factors (viruses and
237 grazers such as ciliates and flagellates), but also sideways controls such as competition for
238 nutrients against other algae and heterotrophic bacteria (e.g. Thingstad et al., 2008). Thus,
239 host occurrence depends on a complex set of selective pressures, and this might explain
240 absence of correlations for Mamiellophyceae communities with viruses and environments in
241 this study.
242

To conclude, *Prasinovirus* and Mamiellophyceae communities were compared in the West part of the Indian Ocean, and the results suggest that trophic conditions influenced their distribution. Until now, known *Prasinovirus* were characterized mainly in samples from eutrophic waters, but here we showed that related communities also occur in nutrient-limited waters and that unknown genotypes possibly infect Dolichomastigales.

In addition, geographic barriers seemed inexistent for viruses and hosts in this region, and taxa represented in each sample probably arose from growth of adapted genotypes before further dispersal. Our analysis also highlighted that host-virus interactions in natural environments can be difficult to study because these partners may follow complex antagonistic dynamics. Hence, future projects should focus on temporal analyses of specific sites or using a unique sampling strategy that describes both viruses and hosts (e.g. cell sorting using flow cytometry or sampling through 0.8 µm filters).

Finally, the link between *Prasinovirus* communities and the environment suggested the presence of different propagation strategies, such as described for OtV2, a virus that infects the low-light adapted *Ostreococcus tauri* strain and that contains specific genes certainly acquired laterally (Weynberg et al., 2011). This observation leads to exciting new questions from an evolutionary point of view: do *Prasinovirus* genomes contain adaptive genes to promote infections of their hosts in different trophic conditions? If so, are they acquired by lateral transfers from hosts or other viruses during coinfection events?

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Data deposition footnote

The sequence datasets have been submitted to the Sequence Read Archive of the European Nucleotide Archive under the following accession numbers: 36SUR (ERR632179; ERR562665), 38SUR (ERR632184; ERR562391), 39SUR (ERR632191), 46SUR (ERR632186), 57SUR (ERR632175), 58DCM (ERR632185), 58SUR (ERR632181), 65DCM

(ERR632174; ERR562488), 65SUR (ERR632195; ERR562667), 66DCM (ERR632194; ERR562660), 66SUR (ERR632169; ERR562457).

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Figure legends

Figure 1. Locations of sampling sites. Numbers in station names are in chronological order. Seawater samples were collected on the schooner *Tara* at 2 depths: surface (SUR) and Deep Chlorophyll Maximum (DCM). Free *Prasinovirus* particles and Mamiellophyceae were sampled using 0.1 and 0.8 µm filters, respectively. ●: *Prasinovirus*; ○: Mamiellophyceae. Arrows indicate known water currents (adapted from Boebel et al., 1998).

Figure 2. Principal component analysis of the 11 samples according to the environmental variables. A. Distances between samples. B. Correlations between variables. Numbers in station names are in chronological order. SUR: Surface; DCM; Deep Chlorophyll Maximum. The following environmental variables were measured by the CTD: salinity ($g.L^{-1}$), potential temperature (°C; i.e. pressure-corrected temperature), density (kg/m^{-3}), oxygen ($\mu mol.kg^{-1}$), chlorophyll *a* (Chla; mg $Chl.m^{-3}$), backscattering coefficient of light by particles (bbp; 470 nm; m^{-1}), beam attenuation (m^{-1}). Moreover, flow cytometry was used to estimate concentrations of *Prochlorococcus*, *Synechococcus*, heterotrophic bacteria (Het_Bact), picoeukaryotes (Peuk; mL^{-1}), the proportion of high-nucleic acid bacteria (HNA), and of small picoeukaryotes (Peuk1; putative Mamiellophyceae).

Figure 3. Phylogenetic tree of environmental OTUs and 23 reference sequences of *Prasinovirus* and *Chlorovirus*, reconstructed using Bayesian inference. PCR amplifications, sequencing and sequence cleaning were performed such as described in Clerissi, Grimsley, Ogata et al., (2014). OTUs are defined for a nucleotide identity of 90 %. Phylogenetic reconstructions were based on DNA sequences that were partitioned according to codon position, and the estimation of model parameters was unlinked across partitions. Bayesian analysis was carried out with MrBayes 3.2 (Ronquist *et al.*, 2012), with 4 chains of 2,000,000 generations, trees sampled every 1000 generations, and burnin value set to 20 % of the

475 sampled trees. The tree was rooted using the chloroviruses. Numbers are posterior
476 probabilities (%) reflecting clade support. Twenty-three reference sequences representing 475
477 *Prasinovirus* and *Chlorovirus* isolates for an OTU cutoff of 90 % are indicated by an asterisk
478 (*). Four abundant but unknown OTUs are indicated by a lozenge, The cultured *Prasinovirus*-
479 containing clade is indicated by an arrow.

480 Figure 4. Phylogenetic tree of environmental OTUs and 16 reference sequences of
481 Mamiellophyceae, reconstructed using Bayesian inference. PCR amplifications of of V9
482 region of the 18S were conducted using the PCR primers 1389f (5'-TTG TAC ACA CCG
483 CCC-3') and 1510r (5'-CCT TCY GCA GGT TCA CCT AC-3'). Amplicons were sequenced
484 using Illumina, sequences were cleaned and chimeras were removed using usearch (Edgar
485 2010). Phylogenetic reconstructions were based on DNA sequences, with an evolutionary
486 model selected via Akaike Information Criterion and jModelTest v2 (Darriba *et al.*, 2012).
487 Bayesian analysis was carried out with MrBayes similarly to *Prasinovirus*. The tree was
488 rooted using *Monomastix* strains. Numbers are posterior probabilities (%) reflecting clade
489 support. Sixteen reference sequences representing Mamiellophyceae diversiy (Marin and
490 Melkonian, 2010) for an OTU cutoff of 97 % are indicated by an asterisk (*). The known
491 *Prasinovirus* host-containing clade is indicated by an arrow and a new environmental clade is
492 outlined in a box with dashed lines.

493 Figure 5. Correspondence analysis of the relative abundance matrix for *Prasinovirus* and
494 Mamiellophyceae. Clustering analyses with reference sequences were computed to annotate
495 *Prasinovirus* OTUs and Mamiellophyceae genotypes at the genus level.

496 Figure 6. Canonical correspondence analysis of the 11 samples on *Prasinovirus* assemblages
497 constrained by environmental data. Numbers in station names are in chronological order.
498 SUR: Surface; DCM; Deep Chlorophyll Maximum. OTUs are defined for a nucleotide

identity of 90 %. Only the significant variables are shown.(i.e. variables that significantly explained changes in the distribution of OTU). They were selected using a forward-selection procedure associated to the canonical correspondence analysis.

Tables

Table 1. Mantel test correlations. *Prasinovirus* and Mamiellophyceae OTUs are defined for a nucleotide identity of 90 and 97 %, respectively. N.A. Not Available. Numbers indicate correlation coefficients and significant correlations (p-value < 0.05) are in bold. The distance matrices were computed using the Bray-Curtis dissimilarity for virus and host communities, and the Euclidean metric for the environmental variables and the geographic coordinates after a standardization step.

	11 samples		**6 samples**		
	Environment	**Location**	**Mamiellophyceae**	**Environment**	**Location**
Prasinovirus	**0.626**	**0.722**	0.397	**0.771**	**0.852**
Mamiellophyceae	N.A.	N.A.	-	0.342	0.275
Environment	-	**0.521**	0.342	-	**0.775**
Location	**0.521**	-	0.275	**0.775**	-