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

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

6

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34 diversity.

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

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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

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

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

Results and discussion

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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 oligotrophic 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).

119 **Uncultured prasinoviruses were very diverse.** Although the *Prasinovirus* sequences are
120 available for the 11 samples, the data for Mamiellophyceae concern 6 samples from 4 stations
121 (Figure 1). The sampling strategy is described in details as supplementary information for
122 methods, including the number of sequences, genotypes and OTUs (Tables S3 and S4). In
123 order to describe virus and host diversity of this oceanic region, phylogenetic reconstructions
124 (Figures 3 and 4) and sequence annotations of viral DNA polymerase (*polB*) and host green
125 algal RNA ribosomal (18S) genes were performed (see supplementary information for
126 methods, Figures S1-S3, Table S5). Known host species of prasinoviruses are all species
127 within dominant genera of the order Mamiellales (Bellec et al., 2009; Marin and Melkonian,
128 2010; reviewed in Grimsley et al., 2012). However, the culture-independent approach used
129 here highlighted that although BpV and MpV were the richest groups, OV was only the
130 seventh richest, and that unknown *Prasinovirus* contributed a high proportion of the diversity
131 (OTU7, OTU11, OTU15 and OTU39; Figure 3 and Figure S2).

132 In contrast, the diversity of the Mamiellophyceae was consistent with previous studies;
133 *Bathycoccus*, *Micromonas* and *Ostreococcus* were the most abundant (Figure S3) (Not et al.,
134 2004; Viprey et al., 2008). Notably, *Bathycoccus* and *Ostreococcus* were found in higher
135 proportions in this region, whereas *Micromonas* dominated the eukaryotic picoplankton in the
136 English Channel (Not et al., 2004) and at a Mediterranean Sea coastal site (Zhu et al., 2005).
137 This composition was nevertheless realistic, since the genus *Ostreococcus* can dominate
138 picoeukaryote communities: it is known to form blooms (O'Kelly et al., 2003; Treusch et al.,
139 2012) and can contribute to up to 70 % of the phototrophic picoeukaryotes (Countway and
140 Caron, 2006). Moreover, phylogenetic reconstruction of Mamiellophyceae sequences also
141 highlighted a new environmental clade related to *Crustomastix* and *Dolichomastix* (Figure 4
142 box with dashed lines (OTUs were defined for a nucleotide identity of 95 % instead of 97 %
143 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

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

172 **The distribution of communities is influenced mainly by trophic conditions.** Given the
173 results of previous studies (Slapeta et al., 2006; Lepère et al., 2009; Bellec, Grimsley, and
174 Desdevises, 2010; Bellec, Grimsley, Derelle, et al., 2010; Clerissi, Grimsley, Subirana, et al.,
175 2014), links with environmental conditions were expected, but not with geographical
176 distances (locations) for both communities in this oceanic region.

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

187 Secondly, significant links for the Mamiellophyceae communities were not found using
188 Mantel tests (location: $r = 0.275$, $p\text{-value} = 0.141$, environment: $r = 0.342$, $p\text{-value} = 0.092$).
189 This lack of correlations could be the result of a low statistical power, because the dataset
190 contains 6 samples, but such correlations were still significant for *Prasinovirus* communities
191 when using the same reduced dataset (location: $r = 0.852$, $p\text{-value} = 0.003$; environment: $r =$
192 0.771 , $p\text{-value} = 0.004$). Hence, Mamiellophyceae might be highly dispersed and
193 homogeneously distributed in this region.

194 However, to further decipher the influence of environmental variables on both communities,
195 canonical correspondence analyses (CCA) were computed with a forward-selection
196 procedure. This analysis highlighted that (i) potential temperature, density and beam
197 attenuation constrained *Prasinovirus* distribution in the 11 samples (p-value = 0.005) (Figure
198 6; a similar trend was observed for the reduced dataset of 6 samples, Figure S4), and (ii) that
199 potential temperature influenced Mamiellophyceae in the 6 samples (p-value = 0.015).
200 Because potential temperature and density tend to separate station 66 from the other samples
201 for both analyses, the eutrophic conditions of the station 66 seem to highly constrain
202 communities of this host-virus system.

203 **Few links between *Prasinovirus* and Mamiellophyceae communities.** Since *Prasinovirus*
204 entirely depend on hosts for their replication, a strong correlation between both communities
205 was expected, but links were significant neither for community compositions ($r = 0.397$, p-
206 value = 0.172) (Table 1), nor for specific richness (Spearman correlation, $\rho = 0.6$, p-value =
207 0.242). This lack of correlation can be explained by at least three hypotheses: (i) a poor
208 resolution of membership content of both viral and host communities according to different
209 unknown biases (DNA extraction, PCR, sequencing), (ii) a non-corresponding taxonomic
210 threshold between viruses and hosts, and (iii) antagonistic oscillations between hosts and
211 viruses.

212 A non-corresponding taxonomic threshold might result from an overestimation of
213 *Prasinovirus* diversity and/or an underestimation of host diversity. On one hand, since the
214 environmental diversity of prasinoviruses was not known, their phylogenetic limit was
215 defined arbitrarily by the *Chlorovirus* sister clade (see supplementary information for
216 methods). In addition, it is possible that the thresholds used to define virus and host OTUs did
217 not correspond to the taxonomic interaction and that not all were able to infect
218 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

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

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

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

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273 flat.svg#mediaviewer/File:BlankMap-World-162E-flat.svg](http://commons.wikimedia.org/wiki/File:BlankMap-World-162E-flat.svg#mediaviewer/File:BlankMap-World-162E-flat.svg)). This work was supported by an
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Data deposition footnote

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

293 (ERR632174; ERR562488), 65SUR (ERR632195; ERR562667), 66DCM (ERR632194;
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296

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- 450

451 **Figure legends**

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

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

467 Figure 3. Phylogenetic tree of environmental OTUs and 23 reference sequences of
468 *Prasinovirus* and *Chlorovirus*, reconstructed using Bayesian inference. PCR amplifications,
469 sequencing and sequence cleaning were performed such as described in Clerissi, Grimsley,
470 Ogata et al., (2014). OTUs are defined for a nucleotide identity of 90 %. Phylogenetic
471 reconstructions were based on DNA sequences that were partitioned according to codon
472 position, and the estimation of model parameters was unlinked across partitions. Bayesian
473 analysis was carried out with MrBayes 3.2 (Ronquist et al., 2012), with 4 chains of 2,000,000
474 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
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 diversity (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

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

502 **Tables**

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

	11 samples		Mamiellophyceae	6 samples	
	Environment	Location		Environment	Location
<i>Prasinovirus</i>	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	-

509