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Genetic data generated from virus–host complexes obtained by membrane co-immobilization are equivalent to data obtained from tangential filtrate virus concentrates and virus cultures

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Abstract The sieving and immobilization of virus–host complexes using impact filtration (aka membrane co-immobilization or MCI) is a novel approach to the study of plankton viruses. One of the most interesting characteristics of the method is the possibility of generating data on potential viral hosts without the need of culturing hosts cells. MCI has demonstrated to be useful for studying viruses of picoalgae, but studies comparing data generated by MCI to data obtained by other techniques are lacking. In this work, *Ostreococcus* virus (OV) and *Ostreococcus* sp. sequences generated from virus–host complexes obtained by MCI were compared to sequences obtained from tangential filtration (TF) concentrates and virus cultures (VC). Statistical parsimony, phylogenetic analyses, pairwise distance comparisons, and analysis of molecular variance showed that the viral and host sequences obtained by the three methods were highly related to each other, indicating that MCI, TF, and VC produce equivalent results. Minor differences were observed among viral sequences obtained from VC and TF concentrates as well as among host sequences generated from VC and MCI. As discussed in the body of the paper, the divergence observed for cultured cells could be due to selective pressures exerted by culture conditions, whereas the correlate observed for the corresponding viral sequences could obey to a hitchhiking effect.

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Introduction

Electron microscope studies performed in the last decades demonstrated that viruses are important components of aquatic ecosystems [1–3]. It is estimated that these agents are responsible for the death of ~1e25 microbes, which is equivalent to about 100 million metric tons, per minute [4]. Viruses also constitute a major source of plankton, large metazoans, and macroalgae mortality, further contributing to the sinking of particulate organic matter. Thus, viral mortality exerts a significant control of carbon and nitrogen fluxes up and down the food chain [4–7].

Picoplanktonic microorganisms (<2 μm) are responsible for a large proportion of the oceanic primary production [8]. The majority of carbon fixation is tough to be achieved by *cyanobacteria* from the genera *Prochlorococcus* and *Synechococcus* [9]. However, recent works indicate that photosynthetic picoeukaryotes from the genera *Bathycoccus*, *Micromonas*, and *Ostreococcus* (Mamiellophyceae) could be important primary producers in many marine environments as well as in coastal and estuarine waters [10–14]. These microorganisms are commonly associated to icosahedral, dsDNA viruses from the *Prasinovirus* (*Phycodnaviridae*) genus [15, 16]. Despite its abundance, the study of environmental viruses generally depends on sample concentration [17]. To date, the study of prasinoviruses has been grounded on virus isolation and tangential filtration [15, 16, 18–24]. Culture-based methods require specific hosts to be available [25], and the approach is relatively laborious and time consuming. Furthermore,

neither tangential filtration nor in vitro culture provide information on the hosts naturally infected by the studied viruses.

Recently, we devised an approach based on impact filtration to isolate viral genetic material from membrane-immobilized picoeukaryotic cells [26]. The technique, which we called membrane co-immobilization (MCI), immobilize host cells onto filter membranes of different pore sizes, resulting in the sorting and concentration of host cells and the corresponding intracellular viruses. As genetic material can be readily recovered from these membranes [26, 27], it is possible to obtain sequences from both virus and hosts from the samples of interest, thus generating data on potential virus–host interactions in the studied communities without the need of host culturing. In addition, MCI is a relatively simple procedure that does not require expensive equipment. Although MCI has proven useful for the study of picoplankton [26, 27], studies comparing data generated by MCI to data obtained by other techniques are lacking. In the present work, we evaluated how viral and host sequences generated from virus–host complexes obtained by MCI compare to sequences obtained from TF virus concentrates and in vitro virus cultures.

Materials and methods

Sampling

A surface water sample was taken in January 2013 near to the Chubut River mouth (43.34003°S, 65.02472°W) using an acid-cleaned, opaque carboy tank that was washed twice with sea water prior to sampling. The sample was taken during the high tide, which ensures seawater condition at the sampling coordinates (to date, no Mamiellophyceae cells have been detected in fresh estuarial waters). Besides, this ensures a relatively clear and particle-free condition of the water, which contrast with the comparatively high particle load displayed by fresh river water, which makes filtering difficult due to filter clogging. This sample was immediately transported to the laboratory and submitted to MCI, in vitro culture and tangential filtration.

Membrane co-immobilization

Around 2 L of the sea water sample were filtered using filters with pore sizes of 20, 10, 5, 1.2, and 0.2 μm (47-mm diameter polycarbonate filters, MSI Westboro) and a pressure of 20 mmHg. This procedure combines both virus concentration and sorting. Concentration of picoplankton (and any intracellular virus contained by these cells) is attained onto the 0.22 μm membrane, and sorting is given by previous sieving through membranes with pore sizes of 20, 10, 5, and 1.2 μm. The filters were stored at −80 °C until used.

Virus culture

Two aliquots of 30 mL from the 1.2-μm filtrate were mixed with an equal volume of K medium [28] supplemented with a mix of streptomycin, ampicillin, and kanamycin, and the preparations were incubated at 22 °C with cycles of 12 h of light (100 μmol m^{-2} s^{-1}) and 12 h of darkness. Cell counts were made using a hemocytometer. Once the cells reached a density of 20–30 × 10^6 cells mL^{-1}, the presence of prasinoviruses was confirmed by PCR, and the cultures were maintained by aseptic sub-culturing using a 1:10 (culture:fresh medium) dilution every seventh day [15, 19]. Picoalgal growth was observed in both cultures and the presence of prasinoviruses was corroborated in both of them. One of the cultures was randomly selected and used for subsequent experiments.

Tangential filtration

Two liters of the 0.22 μm filtrate were processed using a VivaFlow 200 (30,000 MWCO PES) device. The sample was allowed to circulate through the system at a pressure of 2.5 bar until the volume was reduced to 200 mL. The obtained concentrate was then diafiltrated against 1 L of deionized water in order to dilute salts and any possible component of the sample that could led to PCR inhibition. Finally, the sample was further concentrated to a final volume of 30 mL, dispensed into 1 mL aliquots and maintained at −80 °C until used.

Nucleic acids extraction

The nucleic acids retained by the 0.22 μm filters were extracted following procedures described elsewhere [26]: first, the filters were incubated for 30 min in 720 μL of pre-heated CTAB buffer (2 % [w/v] CTAB Sigma, 1.4 M NaCl, 0.2 % [v/v] β-mercaptoethanol, 20 mM EDTA, 100 mM Tris–HCl pH 8.0), at 60 °C. After an extraction with chloroform:isoamyl alcohol (1:1), the suspension was digested for 1 h at 37 °C with RNase A (Sigma-Aldrich) at a final concentration of 10 μg mL^{-1}. An organic extraction with one volume of chloroform was performed and then nucleic acids were precipitated with isopropanol. The sample was centrifuged at 20,000×*g* for 30 min at 4 °C, and the pellet was washed with 70 % ethanol. The DNA pellet was air dried and resuspended in ultrapure, DNase-free water (Invitrogen). Nucleic acid extractions from cultured cells were performed using a 1.5 mL aliquot of the culture, which was centrifuged in a micro centrifuge at 4 °C for 10 min at 10,000×*g*. The obtained cell pellet was

resuspended in Proteinase K buffer (50 mM Tris–HCl pH 8, 1.5 mM $CaCl_2$, 1 % sodium lauroyl sarcosinate) containing 50 μg mL^{-1} of Proteinase K and incubated at 37 °C for 60 min. After incubation, the sample was extracted once with one volume of chloroform and the nucleic acids were precipitated with sodium acetate (final concentration 0.3 M; pH 5.2) and ethanol, over night, at −20 °C. Then, the sample was centrifuged at 20,000×*g* for 30 min at 4 °C, and the pellet was washed with 70 % ethanol, dried and resuspended in ultrapure, DNase free water (Invitrogen). As mentioned above, tangential filtrate concentrates were diafiltrated in order to eliminate salts and any potential PCR inhibitor. Thus, these viral concentrates were used directly for PCR amplification. The first step of the PCR reactions (94 °C for 60 s, see below) ensures both heat-disintegration of the viral particles and denaturation of the released viral DNA.

Nucleic acids amplification and cloning

The nucleic acids suspensions obtained as described above were used, separately, as templates for amplifying 18S sequences and/or a portion of the viral DNA polymerase B (polB) gene. The viral sequences were amplified with primers AVS-1/AVS-2 [29], using the PCR conditions described elsewhere [26]. Briefly, the reactions were performed using 1 U of AccuPrime™ *Taq*DNA Polymerase High Fidelity (Invitrogen), 5 μL of Buffer II provided by the manufacturer, the AVS primer set [15, 29] with optimized annealing temperature (45.3 °C) and 35 amplification cycles. Previous to molecular cloning of the obtained amplicons, specificity was corroborated by a second round amplification performed with primers AVS-1/POL [29, 30] using gel purified amplicons as template. Host sequences were obtained using primers UNI7F (5′-ACCTGGTT GATCCTGCCAG-3′) and UNIR1534 (5′-TGATCCTT CYGCAGGTTCAC-3′) complementary to sequences of the 18S rRNA gene [31–33]. These reactions were also performed using 1 U of AccuPrime™ *Taq*DNA Polymerase High Fidelity (Invitrogen), with optimized annealing temperature (62.2 °C) and 35 amplification cycles [26]. PCR amplifications were performed using a BioRad My Cycler thermal cycler (Bio-Rad Laboratory, Inc.). All the reactions were performed in triplicate and the obtained products were pooled to avoid potential PCR biases. Then, these products were purified by the QIAquick PCR Purification Kit (QIAGEN) and quantitated using a Nanovue Plus spectrophotometer (GE Health care).

PCR amplification products were cloned with the pGEM®-T Easy System II kit (Promega), using *Escherichia coli* strain TOP 10 (Invitrogen). Recombinant plasmids were purified using the turboprep method [34] and the corresponding amplicons were obtained as described elsewhere [35], using pGEM®-T-specific primers T7 and SP6, 1 U of AccuPrime™ *Taq*DNA Polymerase High Fidelity (Invitrogen), 5 μL of 10× AccuPrime™ Buffer I (Invitrogen), 5 μL of Buffer I provided by the manufacturer, 2.5 μL of each primer (20 μM) and ultrapure, DNase free water (Invitrogen) to a final volume of 50 μL. The amplification conditions consisted of an initial denaturation step at 94 °C for 60 s followed by 30 cycles of amplification (94 °C for 30 s, 53 °C for 30 s, and 68 °C for 1 min kb^{-1} extension). PCR primers were used for direct and reverse sequencing of viral polymerase. The 18S gene was sequenced using primer *UNIR1534*. Sequence positions with *phred* quality scores below 20 that could not be confirmed by direct and reverse sequencing were marked as missing data, which ensures a 99 % sequence accuracy [36, 37]. The datasets were cleaned of chimerical sequences using the de novo mode of the program UCHIME [38]. The obtained sequences were deposited in GenBank (accession numbers KC210884-KC211009 and KF577953-KF577978).

Data analyses

Sequence alignments were obtained with the program *MAFFT* using iterative refinement and default *op* and *ep* [39]. Haplotype analyses were performed with *Mothur* [40] and *Ape* [41]. Inter-library sequence similarities were obtained as described elsewhere [26]. The obtained data were analyzed by the boxplot tool [42–44] provided in the *R* statistical package [45]. Hierarchical analysis of molecular variance was performed by AMOVA [46], which tests the differences among population and/or groups of populations in a way similar to traditional analysis of variance (ANOVA), as implemented in *pegas* [47]. For this last analysis, evolutionary distances were obtained using the *Ape* package, under evolutionary models inferred by MrAIC [48]. Hypothesis tests were based on 1,000 permutations (default value in *pegas*).

Statistical parsimony analyses were performed with the *TCS* program using a connection probability of 95 %. Distance-based phylogenetic analyses were performed by the balanced minimum evolution algorithm [49] implemented in the *Ape* package, with trees optimized by subtree pruning and regrafting and tree bisection reconnection permutations. Parsimony analyses were performed with the program TNT [50]. Tree searches were performed by hitting the shortest-length trees many times until the consensus tree did not change on addition of RAS + TBR cycles [51–53]. The maximum likelihood trees were obtained by PhyML 3.0 [54], under evolutionary models inferred with MrAIC and model parameters estimated during the searches, which consisted of ten starting trees obtained by BioNJ that were optimized by SPR and TBR.

Table 1 Sequence lengths, variable and informative positions, number of haplotypes and sequence divergence[a] among the studied sequences

Gene	Length	Haplotypes	Variable	Informative	Divergence
DNA *pol*	433–442	28	201	142	4.0e−3 (5.1e−3)
18S	932–958	36	102	24	3.3e−3 (3.0e−3)

[a] Mean and standard deviation (in parenthesis) of uncorrected, pairwise distances. Gaps were penalized with the exception of terminal ones

The Bayesian phylogenetic analyses were performed with MrBayes 3.2.1 [55, 56]. The program was set to perform four Monte-Carlo chains (MCMC) that were run for 10E6 generations, sampling parameters every 500 generations. Fifty percent majority rule consensus trees were used to summarize the post-burnin posterior samples of trees.

Results

The presence of picoeukaryotic cells and OVs in the 0.22-µm filters, the obtained cultures and the TF concentrates was corroborated by PCRs directed against the viral polymerase and the 18S ribosomal gene (not shown). The corresponding amplicons were used to construct five gene libraries from which virus and host sequences were obtained. The DNA polymerase and 18S datasets exhibited 28 and 36 haplotypes, respectively. The sequences from each gene, however, were highly similar to each other (Table 1). As expected from a previous study [26], BLAST analyses shown that all the obtained viral sequences were highly similar to genotype 1 OV sequences. Likewise, the host sequences obtained from both immobilized and cultured cells was highly similar to previously described *Ostreococcus* sp. sequences. The viral sequences were combined with reference sequences from the seven OV genotypes described before (GQ412094, EU889370, FJ267501, FJ267502, NC_013288, GQ412088, JQ691969, JQ691949, JQ691960, JQ691951, JQ692032, JQ691952, and JQ691996) and the host sequences were combined with reference sequences from the four *Ostreococcus* sp. clades described previously (GenBank accession numbers AB058376, AY329636, GQ426343, AY425307, AY425310, AY425311, and AY425313) [26, 57, 58]. Once aligned, the viral dataset presented 201 variable positions, of which 142 were parsimony informative. The host dataset presented 24 informative positions out of 102 variable ones (Table 1).

Statistical parsimony analyses of the viral sequences resulted in 8 networks. Six of them corresponded to OV genotypes 2 to 7. One sequence obtained from cultured viruses (KC210923) could not be connected to any haplotype. The rest of sequences obtained from cultured viruses, as well as the ones obtained by MCI and TF, clustered into a single network together with previously described genotype 1 sequences (Fig. 1). The majority of viral haplotypes were connected to the most likely outgroup haplotype by a single mutational step, with the exception of six sequences from cultured viruses and three haplotypes originated from the TF concentrate. Furthermore, four of the haplotypes from cultured viruses were more related to each other than to the rest of the haplotypes but to a single sequence corresponding to the TF concentrate. In addition, three such sequences were connected to other sequences by missing haplotypes. Host haplotypes from the MCI and culture libraries were clustered into a single network. This network also included the *Ostreococcus* sp. clades A to D reference sequences (Fig. 1). The majority of sequences were identical to previously described clade A (aka *O. lucimarinus*) sequences. The rest of sequences described here were separated from clade A sequences by one to twelve mutational steps, though in no case they were connected directly to clades B, C, or D (Fig. 1). Thus, both culture and MCI-based data were congruent with previous results indicating that the *Ostreococcus* sp. from the Chubut river estuary belongs to clade A [26]. As observed for the cultured viruses, some sequences obtained from cultured *Ostreococcus* sp. cells were relatively divergent in comparison to the MCI ones. In addition, these sequences were connected to their closest haplotypes by up to eight missing haplotypes (Fig. 1).

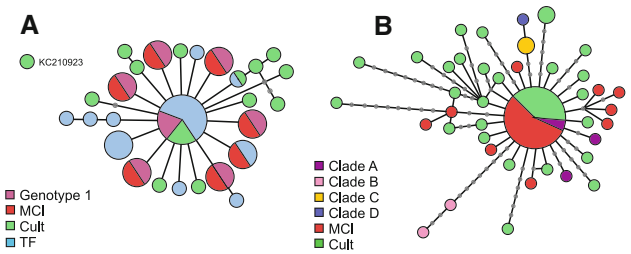

Fig. 1 Haplotype network analyses of DNA polymerase (**a**) and 18S (**b**) sequences obtained from tangential filtration concentrates (*TF*) and immobilized (*MCI*) and cultured (*Cult*) cells. *Circles* correspond to haplotype groupings, with the circles' radiuses proportional to the number of accrued sequences. Reference sequences from the seven *Ostreococcus* virus genotypes and the four previously described *Ostreococcus* sp. clades were included in the analyses. Missing haplotypes are represented by *gray dots*. *Ostreococcus* virus genotypes 2 to 7 were accreted into six unconnected clusters (not shown) (Color figure online)

Pairwise sequence comparisons were highly congruent with statistical parsimony ones, showing that sequences

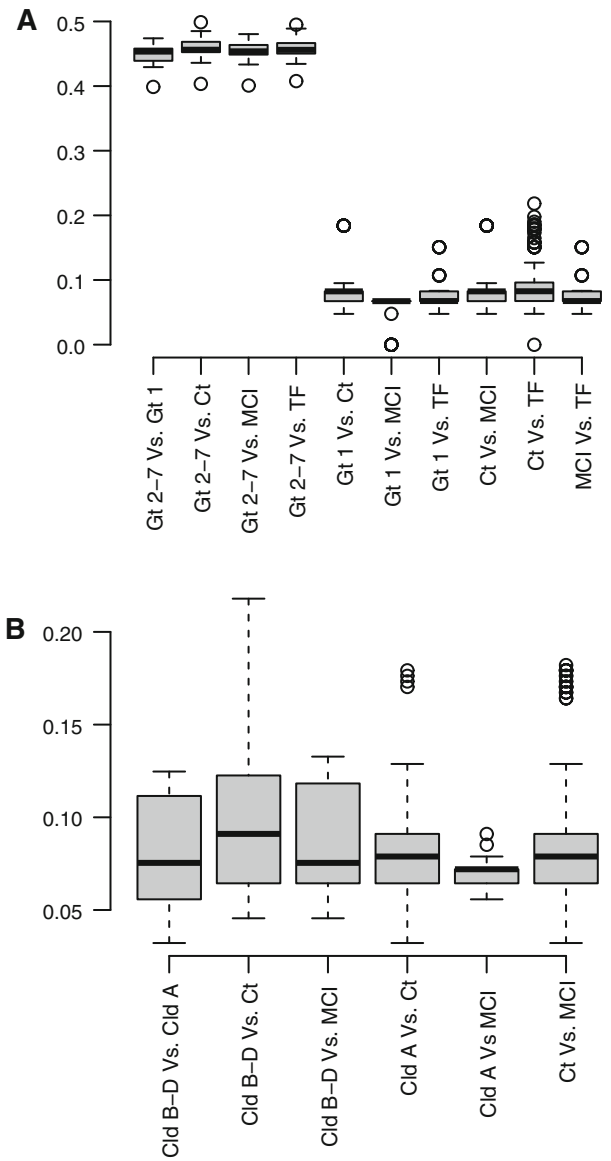

Fig. 2 Divergence among viral (**a**) and host (**b**) sequences obtained in this work and reference sequences from the seven *Ostreococcus* virus genotypes and the four *Ostreococcus* sp. clades described before. Boxplots were obtained from the square roots of substitutions per aligned position in pairwise sequence comparisons. *TF* tangential filtration; *Ct* in vitro culture; *MCI* membrane co-immobilization; *Gt 2–7* genotypes 2–7; *Gt 1* genotype 1; *Cld B–D* Clades B–D; *Cld A* Clade A

obtained by MCI, culture, and TF were highly similar to each other (Fig. 2). The presence of some extreme values, however, was evident from the boxplots, and the AMOVA analyses reveled a slightly significant level of structuring among the viral TF and culture libraries (Table 2). Some sequences from cultured cells also were associated with outlying pairwise distances, although AMOVA analysis indicated no structuring among host sequences (Table 2).

Phylogenetic analyses displayed a high degree of concordance with the statistical parsimony ones and the pair

Table 2 Genetic differentiation[a] among the gene libraries obtained by membrane co-immobilization (MCI), tangential filtration (TF), and culture (Cult)

Gene	MCI-TF-Cult	MCI-Cult	TF-Cult	TF-MCI
DNA *pol*	2.7e−06 (0.11)	2.7e−6 (0.11)	5.0e−6 (0.02)	2.7e−6 (0.13)
18S	–	−1.4e−6 (0.99)	–	–

[a] Sigma squared (*p* value)

wise sequence comparisons. The viral sequences obtained by MCI, TF, and culture were intermingled along single clades of the obtained phylogenetic trees (Fig. 3), indicating that the corresponding libraries harbored sequences that were highly related to each other and that the three techniques (i.e., MCI, TF, and culture) resulted in equivalent inferences about the taxonomic composition of the studied populations. In agreement with the statistical parsimony and pairwise sequence comparisons, some tree terminals corresponding to cultured viruses displayed relatively large branches in comparison to the branches corresponding to MCI and TF viruses (Fig. 3). Some of the viral sequences obtained by TF displayed a tendency to cluster to each other, though neither bootstrap nor Bayesian analyses provided support for these groupings (not shown). As observed for the viral sequences, host sequences were interspersed along the obtained phylogenetic trees (Fig. 4). The four *Ostreococcus* sp. clades were not recovered in the analyses, as could be expected from the scarce number of informative alignment positions present in the dataset studied here (Table 1). In agreement with the statistical parsimony analysis and pairwise sequence comparisons, some tree branches corresponding to sequences obtained from cultured cells were larger than other tree branches (Fig. 4).

Discussion

The results described here show that clone libraries from virus–host complexes obtained by MCI are equivalent to gene libraries from TF virus concentrates and virus cultures. Furthermore, all the obtained data are congruent with previous, independent studies from our group [26].

The majority of viral sequences obtained from MCI virus–host complexes were identical to genotype 1 OV reference sequences. Likewise, most host sequences were identical to Clade A *Ostreococcus* sp. sequences. The small differences detected among some of the libraries studied here (Figs. 3, 4; Table 2) could be attributed to minor biases related to the fraction of the viral population that is targeted by each

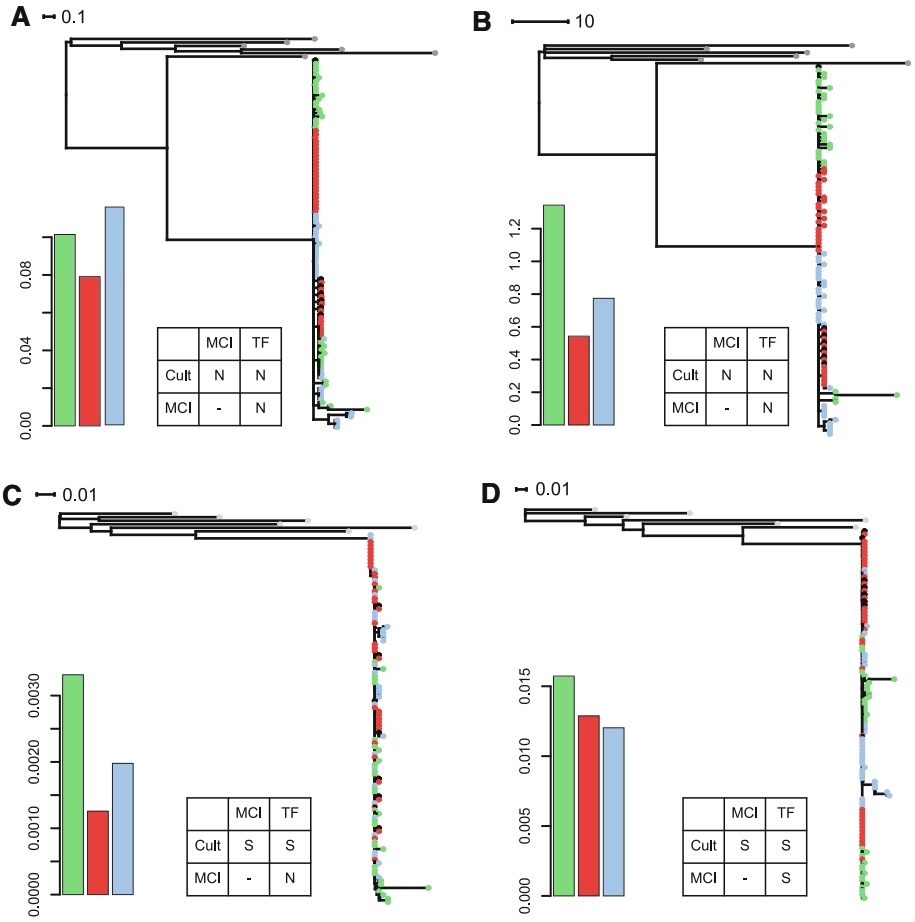

Fig. 3 Phylogenetic trees of viral sequences obtained by tangential filtration (*blue circles*), membrane co-immobilization (*red circles*) and in vitro culture (*green circles*). The trees were inferred by Bayesian (**a**), Parsimony (**b**), Distance (**c**), and Maximum Likelihood (**d**) techniques. Reference sequences from genotype 1 *Ostreococcus* virus were included (*closed, black circles*). Outgroup sequences (genotypes 2–7) are indicated with *gray circles*. The scale bar units are substitutions per aligned position (**a, c, d**) or substitutions (**b**). The barplots correspond to mean patristic distances from tree terminals to genotype 1 base node. *Inset* tables indicate whether the observed differences were significant ($p < 0.05$; Kolmogorov–Smirnov test; *S* significant, *N* non significant) (Color figure online)

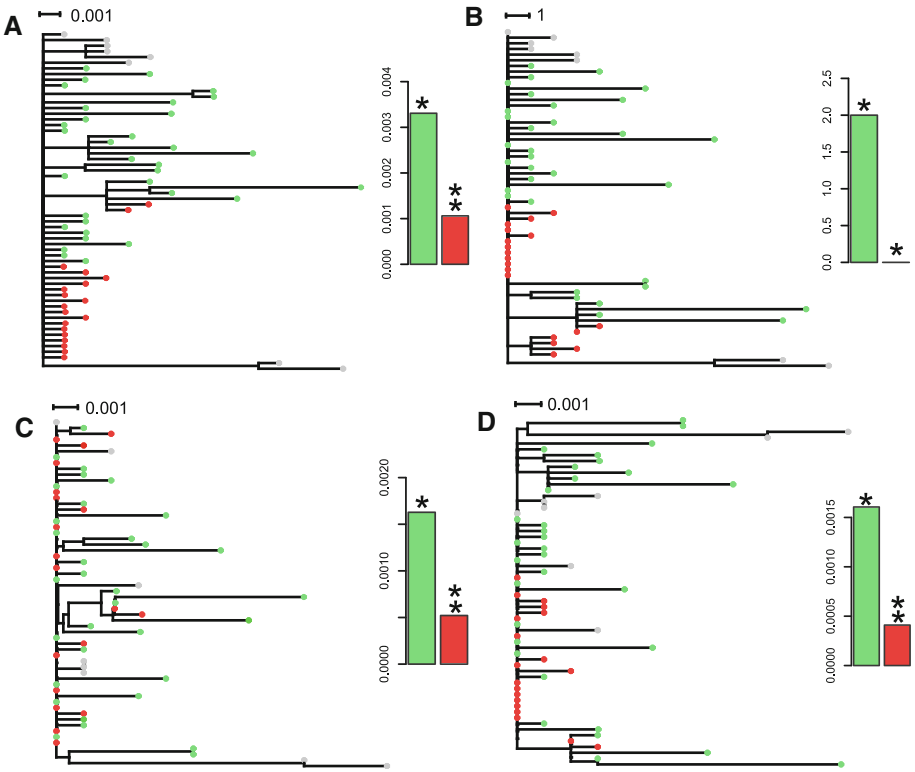

Fig. 4 Phylogenetic trees of *Ostreococcus* sp. sequences obtained by membrane co-immobilization (*red circles*) and in vitro culture (*green circles*). The trees were inferred by Bayesian (**a**), Parsimony (**b**), Distance (**c**),and Maximum Likelihood (**d**) techniques. Reference sequences from the previously described *Ostreococcus* sp. clades were included (*gray*). The scale bar units are substitutions per aligned position (**a, c, d**) or substitutions (**b**). *TF* tangential filtration; *Ct* in vitro culture; *CI* membrane co-immobilization. The barplots correspond to mean patristic distances from tree terminals to tree root. *Asterisks* above bars indicate if samples are significantly different ($p < 0.05$; Kolmogorov–Smirnov test) (Color figure online)

method. For example, some of the cells that develop in cultures may represent minority population fractions that have enhanced fitnesses under the culture conditions. Likewise, MCI is likely to be biased towards replicating viruses, whereas TF targets free viral particles. These data may seem to disagree with previous works such as Marin and Melkonian [59] and Subirana et al. [60], which describe relatively little variation among 18S sequences of *Ostreococcus* spp. However, this apparent disagreement is due to the fact that these authors used direct PCR sequencing, which results in the sequence corresponding to the major molecular variant present in the sample. In contrast, we could encompass and dissect all the diversity present in our samples by intercalating cloning steps. It can be appreciated from Figs. 1b and 4 that the major haplotypes obtained from MCI-immobilized and cultured cells were identical to each other and to the Clade A reference sequence. It is to say that if we would performed bulk PCR sequencing, we probably would have obtained identical sequences from both MCI-immobilized and cultured cells [35]. As in all PCR-based studies, we cannot be sure that all the observed polymorphisms are not due to polymerase errors. However, given that polymerase errors occur at random, these errors could not have biased our analyses because they would have randomly distributed among the libraries studied.

As mentioned above, we think that the differences observed between some individual host sequences could obey to the selective effect exerted by the culture conditions, which could have driven the emergence of minority fractions of the natural host population. Although the studied gene hardly could be involved in the adaptation to culture conditions, it is possible that the selective pressure imposed by culturing conditions, combined with the short generation time of these microorganisms, could have produced a genetic hitchhiking effect that resulted in the emergence of rare allelic variants. Some of the viral sequences obtained from cultured cells also were divergent in comparison to the rest of viral sequences (Figs. 1, 2, 3), strongly suggesting the occurrence of a hitchhiking effect affecting also the viruses harbored by cultured cells. In addition, a fraction of the viral sequences obtained from the TF concentrate also were comparatively divergent regarding the rest of viral sequences (Figs. 1, 2, 3; Table 2). Tangential filtration results in the concentration of free viral particles, whereas MCI and culture are focused on viral particles that are capable of replicate. We think that the presence of divergent sequences in the TF library could obey to this fact. Furthermore, the fact that significant differences were observed among the culture and TF viral samples (Fig. 3; Table 2) supports the idea that these two libraries might have harbored some outlier representatives of the original virus population.

An interesting property of MCI is the possibility of concentrating and sorting both viruses and hosts, which has the potential of providing information on virus–host relationships. Beside the *Ostreococcus* sp. sequences present in the MCI library, we found one sequence related to an *Halichondria* sp. (possible from small spermatozoa), one related to an *Amoebophyra* sp., one distantly related (88 % sequence similarity) to *Amphidinium* sp. sequences (attributable to cell fragments that could have passed through the 1.2-μm filter, tinny cysts, or perhaps unknown alveolates), one sequence distantly related (90 % sequence similarity) to a *Kephyrion* sp., one sequence putatively corresponding to a *Cafeteria roenbergensis*, a sequence related to *Islandium minutum* (possible due to cell fragments) and three sequences corresponding to *Micromonas* sp. cells (data not shown). For viruses with unknown host, these kinds of data, combined with the phylogenetic evidence that could be derived from viral sequences, can be used to significantly narrow the possible host range of any virus detected.

Assessing the diversity of marine microorganisms is complicated due to the high diversity displayed by these ecosystem members, a fact that has encouraged the use of cell sorting approaches for dissecting the taxonomic composition of some planktonic fractions [11–14, 61]. Aquatic viruses also display important degrees of variability and, in consequence, techniques have been developed with the aim of reducing the complexity of viral samples [62]. The results described here show that impact filtration using filters with adequate pore sizes can be used to sort, concentrate, and immobilize the viruses hosted by particular host cells, and that the viruses obtained in this way are equivalent to the ones obtained by both TF and in vitro culture. However, cell populations other than picoplanktonic ones, which seem to be relatively homogeneous [14, 26], might encompass difficulties related with the diversity of such cell populations. Thus, this study is preliminary and the adequacy of MCI for the analysis of other viral groups must be evaluated. Without prejudice of the latter, we foresee a promissory future for MCI and other cell sorting-based approaches in the study of micro-, nano-, and picoplankton viruses.

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