UNIVERSIDADE FEDERAL DO RIO DE JANEIRO - UFRJ

LUIZ FELIPE DE ALMEIDA BENITES

DIVERSIDADE VIRAL ASSOCIADA AO ENDOSSIMBIONTE DE CORAIS *Symbiodinium* spp. (DINOPHYTA)

RIO DE JANEIRO

2016

LUIZ FELIPE DE ALMEIDA BENITES

DIVERSIDADE VIRAL ASSOCIADA AO ENDOSSIMBIONTE DE CORAIS *Symbiodinium* spp. (DINOPHYTA)

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biodiversidade e Biologia Evolutiva, Instituto de Biologia, Universidade Federal do Rio de Janeiro, como requisito parcial à obtenção do título de Mestre em Biodiversidade e Biologia Evolutiva.

Orientador: Profº Drº Paulo Sergio Salomon

RIO DE JANEIRO

2016

LUIZ FELIPE DE ALMEIDA BENITES

DIVERSIDADE VIRAL ASSOCIADA AO ENDOSSIMBIONTE DE CORAIS *Symbiodinium* spp. (DINOPHYTA)

Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biodiversidade e Biologia Evolutiva, Instituto de Biologia, Universidade Federal do Rio de Janeiro, como requisito parcial à obtenção do título de Mestre em Biodiversidade e Biologia Evolutiva.

Aprovado em

_____

(Nome do orientador, sua titulação e Instituição a que pertence)

_____

(nome, titulação e instituição a que pertence)

_____

(nome, titulação e instituição a que pertence)

AGRADECIMENTOS

Agradeço minha família, que me entende e apoia em perseguir essa vontade de tentar compreender o mundo e as coisas vivas. Principalmente minha mãe, obrigado.

Queria agradecer a minha amada Lílian, que esteve comigo nos dias mais díficeis e mais bonitos, que me apoia nas decisões mais loucas com um tanto de amor, e que estava sempre com um sorriso convidativo no rosto depois de meses separados. Também agradeço a sua família por todo o carinho, especialmente Imi e Ademar, obrigado.

Obrigado meu velho Victor Valiati, grande professor que me deu chances de começar toda essa história de pesquisa em simbiose, e me incentivou a buscar novos trabalhos.

Agradeço a minha querida Betíssima (Elisabeth Renck) por ter acreditado em mim. Obrigado mesmo.

Aos meus amigos do Sul e alguns que se encontram em outros lugares (Manu, Carol, Renan), espero retomar nossas parcerias, tenho um bocado de coisas para compartilhar com vocês e muita vontade de escutar sobre as suas vidas. Obrigado pelo apoio de longe e de perto.

Aos amigos do Rio dos laboratórios de Microbiologia, Fitoplâncton e Protistas que receberam um cara estranho e me ajudaram a construir uma vida nesta cidade enorme. Principalmente Tati, Rafa, Michele, Denise, Bruno (quantas conversas meu querido), Glau, Gi (a loira), Gi (a morena), Felipe, Pedro, Paulo, Juline, Artur, Felipe Coutinho, Carol, Carol (IC), Cris, Inácio, Marcelo, Anderson, Dona Angélica e Patricia, espero estar com vocês novamente um dia. Obrigado.

Agradeço aos professores e funcionários do PPGBBE por toda atenção e auxílio ao longo do programa e por construirem uma pós tão especial quanto esta que tive o prazer e sorte de fazer parte. Em especial aos professores Michele, Dani, Claudia e Heber, muito obrigado.

Por último, agradeço aos órgãos de fomento (CAPES e CNPq), ao Prof. Fabiano Thompson pela oportunidade de realizar parte do trabalho em seu laboratório, e meu orientador Paulo Salomon, por ter me confiado a condução do trabalho e das ideias, e ter proporcionado uma liberdade fundamental para perseguir as questões aqui apresentadas em forma de pesquisa. Obrigado pela orientação.

Faça, ou não faça. Tentativa não há.
~ Yoda

Se vai tentar, vá até o fim.
Caso contrário, nem comece.
Se vai tentar, vá até o fim. (...)
E você fará a despeito da rejeição e dos piores azares e será melhor do que qualquer coisa que possa imaginar.
Se vai tentar, vá até o fim.
Não há outra emoção como essa.
Você estará sozinho com os deuses e as noites queimarão como fogo.
Você cavalgará a vida diretamente para o riso perfeito.
Essa é a única boa luta que existe.
~ Bukowski

RESUMO

Dinoflagelados do gênero *Symbiodinium* são endossimbiontes associados com diversos hospedeiros protistas e metazoários, como os corais. Corais são holobiontes formados por simbiose entre cnidários, dinoflagelados, fungos, procariontes e vírus. Recifes de corais, estruturas construídas por estes holobiontes, constituem um complexo ecossistema que se encontra em risco de uma rápida extinção. Na região do Banco dos Abrolhos - BA, Brasil, entre alguns dos principais construtores recifais, encontra-se a espécie endêmica de coral, *Mussismilia braziliensis*, muito sensível a anomalias climáticas. Doenças emergentes como o branqueamento de corais ainda não são bem compreendidas. O aumento da temperatura e intensidade solar é apontado como possíveis causas. Recentemente, o componente viral do holobionte foi apontado como possível agente de doenças. Corais doentes demonstraram diversas sequências virais. Vírus são as entidades genéticas mais abundantes nos oceanos, desempenhando importantes papeis nestes ecossistemas. Observou-se que *Symbiodinium* possui uma infecção viral latente, produzindo partículas semelhante a vírus (PSVs) quando em alta temperatura e sob irradiação ultravioleta (UV). Foi sugerida a existência de um provírus latente integrada no genoma ou episssoma de *Symbiodinium*, entrando em ciclo lítico após estresse. Tais experimentos indicariam a possibilidade de que em recifes de corais, tais estresses induziriam a produção e virulência de PSVs. Embora tais vírus sejam assinalados a família *Phycodnaviridae*, desconhece-se quais gêneros ou tipo de interação vírus-hospedeiro. O objetivo geral deste trabalho foi estudar a ocorrência e caracterizar morfologicamente e geneticamente vírus associados com *Symbiodinium* spp., simbionte de corais. Objetivos específicos foram verificar a indução da produção de PSVs em cultivos de *Symbiodinium* do coral *M. braziliensis*, em estresse por UV e alta temperatura; caracterizar morfologicamente PSVs produzidas; verificar, através de análises computacionais, a presença de sequências virais no genoma de *S. minutum*. Para tanto, foram feitas análises por citometria de fluxo, observação por microscopia eletrônica e análises *in-silico*. A indução da produção de PSVs decorrente da manipulação das condições de cultivo foi positiva. Citogramas dos dois cultivos, em ambos os tratamentos possibilitaram distinguir duas populações de putativas PSVs. Concentrações destas PSVs foram de $3,5 \times 10^4$ a $2,0 \times 10^5$, e $0,8 \times 10^4$ a $1,8 \times 10^5$ partículas por ml^{-1} (para estresse com ultravioleta e alta temperatura, respectivamente). Imagens de microscopia eletrônica revelaram PSVs de tamanho "gigante" (~450-500nm), morfologicamente semelhante à Megaviridae. O resultado difere do esperado. Trabalhos anteriores sugeriram uma infecção por Phycodnaviridae. Ainda, nestas imagens, verificaram-

se PSVs assimétricas e disformes, associadas com PSVs de menor tamanho (~100 nm). Sugeriu-se que estas estruturas seriam efeitos de uma infecção por um virófago, hiperparasitas de vírus gigantes. Análises genômicas identificaram genes de origem viral em *Symbiodinium*, adquiridos via transferência horizontal, o que sugere integração viral no genoma deste simbionte. Também, observou-se nestas transferências, funções gênicas de interação vírus-hospedeiro. Em conjunto, este trabalho demonstrou a produção viral em decorrência de estressores ambientais em *Symbiodinium* sp. de *M. braziliensis.* Sugere-se uma infecção por Megaviridade e uma possível hiperinfecção por virófagos. No contexto de mudanças climáticas, é crucial compreender a múltipla simbiose e viralidade dos holobiontes corais.

Palavras-chave: *Symbiodinium.* Vírus gigantes. Tranferência horizontal. Virófagos.

ABSTRACT

Dinoflagellates from the genus *Symbiodinium* are endosymbionts associated with various protist and metazoan hosts, as e.g. corals. Corals are holobionts formed by symbiosis between cnidarian, dinoflagellates, fungi, procarionts and viroses. Coral reefs, structures built by these holobionts are in risk of becoming extinct. In the Abrolhos Bank region, BA, Brazil, among the major reef builders is the endemic coral species, *Mussismillia braziliensis*, higly sensitive to climate anomalies. Emergent diseases as coral bleaching, are not yet well understood. Temperature and irradiance increase are reported as triggers of coral disease. Metagenomes of diseased corals normaly show high abundance of viral sequences. Viruses are the most abundant genetic entities of the oceans, playing important roles in these ecosystems. It was observed that *Symbiodinium* has a latente viral infection, producing viral-like particles (VLPs) when subjected to high temperature and UV irradiation. It was suggested the existence of a latent provirus integrated in the *Symbiodinium* genome, or episome, that enter a lytic cycle upon stress. Such experiments indicated the possibility of coral reefs producing virulent VLPs, when in stressed conditions. Altough these viruses have been associated to the Phycodnaviridae family, their diversity and the kind of vírus-host interaction remains unknown. The main objective of this work was to characterize morphologicaly and geneticly viruses associated with *Symbiodinium* spp., a coral symbiont. Specific objectives where to verify the induction of VLPs in *Symbiodinium* cultures under UV and thermal stress; to characterize the morphology of produced VLPs; to verify through compuational analyses the presence of viral sequences in *S. minutum* genome. For this, flow cytometry, electron microscopy, and in-silico analyses were performed. Induction of VLP production through UV and thermal stresses was successful. Cytograms of*Symbiodinium* strains tested, in both thermal and UV treatments sshowed PSVs populations with high DNA contente. VLPs concentrations ranged from 3,5 x 10^4 to 2,0 x 10^5, and 0,8 x 10^4 to 1,8 x 10^5 particles per ml^{-1} (for UV and high temperature). Microscopic imagens revealed giant sized (~450-500nm), VLPs with morphological similarities with Megaviridae. Previous works suggested an infection by Phycodnaviridae in *Symbiodinium*, differently than revealed by the electron microscopy images. In this same imagens, it was also observed assymetric and irregular VLPs associated with small sized VLPs (~100 nm). It is suggested that these strucutres could be the effects of an unsuspected infection by virophages, hyperparasites of giant viruses. Genomic analyses identified genes of viral origin acquired trough horizontal transfer, wich suggests viral integration in the *Symbiodinium* genome. It was also observed, in this transfer, gene functions related to viral-host interactions. Together, the results of this study demonstrated the viral production due stressors in *Symbiodinium* of *M braziliensis*. It indicates a Megaviridae infection and also a possible hyperinfection by virophages in this zooxanthelae. In the contexto of climate change, it is crucial to undestand the multiple symbiosis and virality of the coral holobiont.

Keywords: *Symbiodinium.* Giant virus. Horizontal gene transfer. Virophage.

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO

1.1 O GÊNERO DE ENDOSSIMBIONTES MARINHOS *Symbiodinium*

Symbiodinium é um gênero de dinoflagelados marinhos que associam-se simbioticamente com diversos hospedeiros metazoários invertebrados e protistas. Este gênero é internamente divido em 9 clados (A-I) e diversos filotipos (SANTOS et al, 2002; POCHON et al, 2004) endossimbióticos de esponjas (CARLOS et al, 1999), platelmintos (TRENCH; WINSOR, 1987), moluscos (BURGHARD et al, 2008), foraminíferos (POCHON; PAWLOWSKI, 2006), radiolários (GAST; CARON, 2001), ciliados (LOBBAN et al, 2002), anêmonas (PALINCSAR, 1988), medusas (ASTORGA et al, 2012), corais rochosos (escleractíneos) e octocorais (GOULET; COFFROTH, 2004).

1.2 O HOLOBIONTE CORAL: SAÚDE, DOENÇA E QUEBRA DE SIMBIOSE

Corais são considerados holobiontes por serem entidades formadas através da simbiose entre metazoários cnidários, dinoflagelados do já citado gênero *Symbiodinium*, e ainda com organismos distintos como fungos, bactérias, arquéias e vírus (ROHWER et al. 2002; KREDIET et al, 2013). A profunda e antiga associação entre *Symbiodinium* e corais, datada em 200 milhões de anos (HESTER et al, 2015), é responsável primariamente pela formação e manutenção dos recifes de corais nas regiões oligotróficas dos oceanos através da fotossíntese e troca de nutrientes entre os organismos do holobionte (MUSCATINE et al, 1975; KNOWLTON; ROHWER, 2003). A saúde do holobionte coral é essencial para manter a sustentabilidade e produtividade dos recifes coralíneos, crucial para comunidades humanas em todo o planeta (RIEGL et al, 2009). Gatilhos ambientais, como elevação das temperaturas da água do mar (BROWN, 1997) ou alta irradiância solar (LESSER; FARRELL, 2004; ANTHONY; KERSWELL, 2007), podem acarretar na quebra da simbiose que mantém o holobionte estruturado, resultando na perda e/ou expulsão das células de *Symbiodinium* dos tecidos dos corais; fenômeno conhecido como “branqueamento de corais” (VAN OPPEN; LOUGH, 2009). Eventos de branqueamento massivos vêm ocorrendo de forma mais frequente ao longo das últimas décadas, ameaçando o futuro dos corais, principalmente em tempos de mudanças climáticas (GOREAU; HAYES, 1994; HOEGH-GULDBERG et al, 2007). Embora alguns possíveis agentes de branqueamento, entre outras doenças de corais,

tenham sido apontados e investigados (ROSENBERG et al, 2009; KNOWLTON, 2011; KREDIET et al, 2013), ainda permanece pouco explorada a possibilidade de infecções virais relacionadas nestes fenômenos (SOFFER et al, 2014), apesar de estudos antigos e correntes (THURBER; CORREA, 2011; LAWRENCE et al. 2014b; NGUYEN-KIM et al, 2014), bem como uma falta da identificação precisa dos residentes virais no holobionte coral.

1.3 OS VÍRUS MARINHOS E SEUS PAPÉIS ECOLÓGICOS

Os vírus são as entidades biológicas mais abundantes dos oceanos. Concentrações de até 10^{11} partículas virais L^{-1} já foram observadas (BERGH et al, 1989), com abundâncias tipicamente na ordem de 10^{10} partículas por litro na água marinha superficial (FUHRMAN, 1999). Em torno de 5.000 genótipos virais foram observados em apenas 100 L de água marinha (ROHWER; VEGA THURBER, 2009). Estima-se que entre 20% a 40% das células de microrganismos marinhos são mortas por vírus a cada dia (SUTTLE, 2007; NAGASAKI, 2008; MONIER et al, 2009). A lise das células do hospedeiro por vírus é responsável pela recirculação de uma considerável fração do carbono total e da regeneração de nutrientes, através da liberação na água dos componentes celulares dos hospedeiros (MIDDELBOE et al, 1996; NOBLE; FUHRMAN, 1999; NOBLE et al, 1999; NOBLE; FUHRMAN, 2000). De fato, 6-26% do carbono fixado na zona fótica do oceano é reciclado como resultado da lise de células hospedeiras através da chamada alça viral (viral shunt) (WILHELM; SUTTLE, 1999), tornando os vírus componentes dinâmicos das alças microbiana nos ambientes marinhos (SUTTLE, 2005). Na escala planetária, vírus marinhos podem modular os ciclos biogeoquímicos através da mortalidade dos hospedeiros, da transferência horizontal de genes (THG) (MONIER et al, 2009), da reprogramação metabólica e aptidão do hospedeiro (MANN et al. 2003; BREITBAR, 2012; HURWITZ et al, 2013; ROSENWASSER et al, 2014). A ação viral nos marestem impacto direto no clima do mundo, tornando possível o aumento das emissões biológicas de moléculas sulfurosas como dimetilsufetos (DMS), as quais agem como núcleos de formação de nuvens, resultando em chuva ácida (BRATBAK et al, 1995; HILL et al, 1998; MALIN et al, 1998). A maior parte da diversidade viral marinha é composta por bacteriófagos (vírus que infectam bactérias) (BREITBART, 2011), enquanto que 1% a 10% da diversidade total é representada por vírus gigantes infectando eucariotos (BRUSSAARD, 2004) com abundâncias estimadas entre 4000 a 170,000 genomas por mL^{-1} na zona fótica do oceano (HINGAMP et al, 2013, WILSON et al, 2014).

1.4 MODULAÇÃO E SUBVERSÃO VIRAL DO MAQUINÁRIO MOLECULAR DO HOSPEDEIRO

O sucesso da infecção viral requer o sequestro de uma célula hospedeira e a subversão das funções metabólicas desta célula, modulando vias celulares de forma a produzir novas partículas e estruturas virais. Uma das vias eucarióticas e virais mais utilizadas é a do Sistema Ubiquitina-Proteossoma (SUP) (MAHON et al, 2014). Essa via media o reparo de DNA, transdução de sinal e desligamento de proteínas (ULTICH; WALDEN, 2010). A via de Ubiquitina é um alvo para os vírus, quando ocorre a construção de um ambiente intracelular favorável para sua replicação (GAO; LUO, 2006) através de modulações epigenéticas dos seus hospedeiros. Genes relacionados a esta via foram observados em eventos de THG entre vírus gigantes e seus hospedeiros (RELLY; GUARINO, 1996; HUGHES; FRIEDMAN, 2003; MA et al, 2015).

1.5 OS VÍRUS GIGANTES E SEUS VIRÓFAGOS PARASITAS: HIPERSIMBIOSE

Claverie et al, (2009) sugeriram que corais seriam potenciais hospedeiros de vírus da família *Mimiviridae*, uma vez que Octocorais possuem em seu genoma um gene característico desta família, MutS – *mismatch repair protein*, adquirido possivelmente por um vírus gigante residente de um holobionte coral ainda não caracterizado (BILEWITCH; DEGNAN, 2011; OGATA et al, 2011). Os vírus gigantes pertencem à superfamília Nucleocytoplasmic Large DNA Virus (NCLDV) (FILÉE; CHANDLER, 2008) e seus membros infectam uma ampla diversidade de hospedeiros eucarióticos como protistas, animais e algas. NCLDVs são caracterizados por genomas grandes e heterogêneos (FILÉE; CHANDLER et al, 2010) com replicação exclusiva no citoplasma do hospedeiros ou através de estágios nucleares e citoplásmicos (KOONIN; YUTIN, 2010). Dentre as famílias de vírus gigantes, uma de particular interesse devido sua abundância e diversidade de hospedeiros, é a família *Megaviridae*. Esta família emerge como um clado que representa vírus que infectam protistas aquáticos como Amebozoários e algas marinhas (CLAVERIE, 2013) e foi proposta como uma extensão da família *Mimiviridae* (SANTINI et al, 2013) bem como uma fusão de alguns indíviduos que outrora eram assinalados para a família *Phycodnaviridae* e recentemente sofreraram uma revisão taxonômica (MONIER et al, 2008; WILSON et al, 2014). Características definidoras desta família geralmente incluem uma associação com virófagos (LA SCOLA et al, 2008; FISCHER; SUTTLE, 2011; YAU et al, 2011), uma proteína MutS (mismatch repair protein) (OGATA et al, 2011) e uma proteína amino acyl tRNA synthetase, adquirida horizontalmente de organismos celulares (CLAVERIE; ABERGEL, 2013).

Virófagos são pequenas partículas de natureza viral associadas a vírus gigantes e que são incapazes de replicarem-se na ausência de seu hospedeiro viral, assim caracterizam-se como hiperparasitas (KATZOURAKIS; ASWAD, 2014). Eles estão associados com os gêneros *Mimivirus* (LA SCOLA et al. 2008; DESNUES et al, 2012), *Cafeteria roenbergensis* virus (CroV) (FISCHER; SUTTLE, 2011), Organic Lake Phycodnavirus (OLPV) (YAU et al, 2011) e, em forma de provirófago no genoma de seu hospedeiro viral, *Phaeocystis globosa* virus (PgV*)* (SANTINI et al, 2013). Usualmente virófagos impactam no desenvolvimento de seus hospedeiros vírus gigantes, resultando em partículas defectivas e assimétricas ao explorar o maquinário molecular do vírus (LA SCOLA et al, 2008; FISCHER; SUTTLE, 2011). Até este momento, desconhecemos a descrição de simbiose entre virófagos, corais e/ou *Symbiodinium*.

1.6 HORIZONTALIDADE E CICLOS VIRAIS

A transferência horizontal de genes (THG) é a transmissão não-genealógica de material genético de um organismo para o outro (GOLDENFELD; WOESE, 2007) e constitui um poderoso e importante fator evolutivo que molda a evolução da vida na Terra nos três domínios (BOTO, 2010; SYVANEN, 2012). Uma vez integrados nos cromossomos dos hospedeiros recipientes, genes horizontais são transmitidos verticalmente de forma mendeliana, ou seja, entrando na linhagem de um organismo (KATZOURAKIS; GIFFORD, 2010). Eventos de THG podem ser utilizados para reconstruir cenários de co-evolução entre vírus-hospedeiro (PATEL et al, 2011). Vírus gigantes carregam em seu genoma diversos genes derivados do hospedeiro através de THG, embora exista uma discussão a respeito da polarização de tais transferências, que trata sobre a direção do processo, ou seja, se ocorreu do vírus para o hospedeiro ou de hospedeiro para vírus (MOREIRA; LOPEZ-GARCIA, 2005; MOREIRA; BROCHIER-ARMANET, 2008; DERELLE et al, 2008; MOREAU et al, 2010; FILÉE, 2014). A transferência e aquisição de vírus para hospedeiro, presumivelmente é mais escassa, contudo ocorrente entre *Megaviridae* e diversos stramenopilas, incluindo algas marinhas (BLANC et al, 2010; HINGAMP et al, 2013; COLSON et al, 2014; FILÉE et al, 2014; PAGARETE et al, 2015). Em pelo menos 14 genomas de algas foram encontrados eventos de endogenização viral, sugerindo que a THG entre estes grupos são recorrentes (WANG et al, 2014). Não são conhecidos vírus na forma proviral da família *Megaviridae*, ao contraste de alguns indivíduos da família *Phycodnaviridae* que integram os genomas de seus hospedeiros (DELAROQUE; BOLAND, 2008), enquanto que integrações de virófagos (provirófagos) em seus hospedeiros virais (DESNUES et al. 2012; SANTINI et al, 2013;

KATZOURAKIS; ASWAD, 2014) já foram descritas. Assim, virófagos poderiam atuar como engrenagens de THG durante hiperinfecções em protistas (LA SCOLA et al, 2008). Recentemente foram descobertos numerosos genes provenientes de virófagos no genoma da alga *Bigellowiella natans* integrados no seu genoma e sendo expressos, sugerindo um papel ativo de genes de virófagos adquiridos horizontalmente (BLANC et al, 2015). Tais processos de integração poderiam ser positivamente selecionados, uma vez que a associação indireta com hospedeiros eucarióticos, em alguns casos, também é benéfica, devido ao impacto causado no vírus gigante, seu hospedeiro direto, atenuando a sua infecciosidade e aumentando a sobrevivência do hospedeiro eucarioto (FISCHER; SUTTLE, 2011; KATZOURAKIS; ASWAD, 2014).

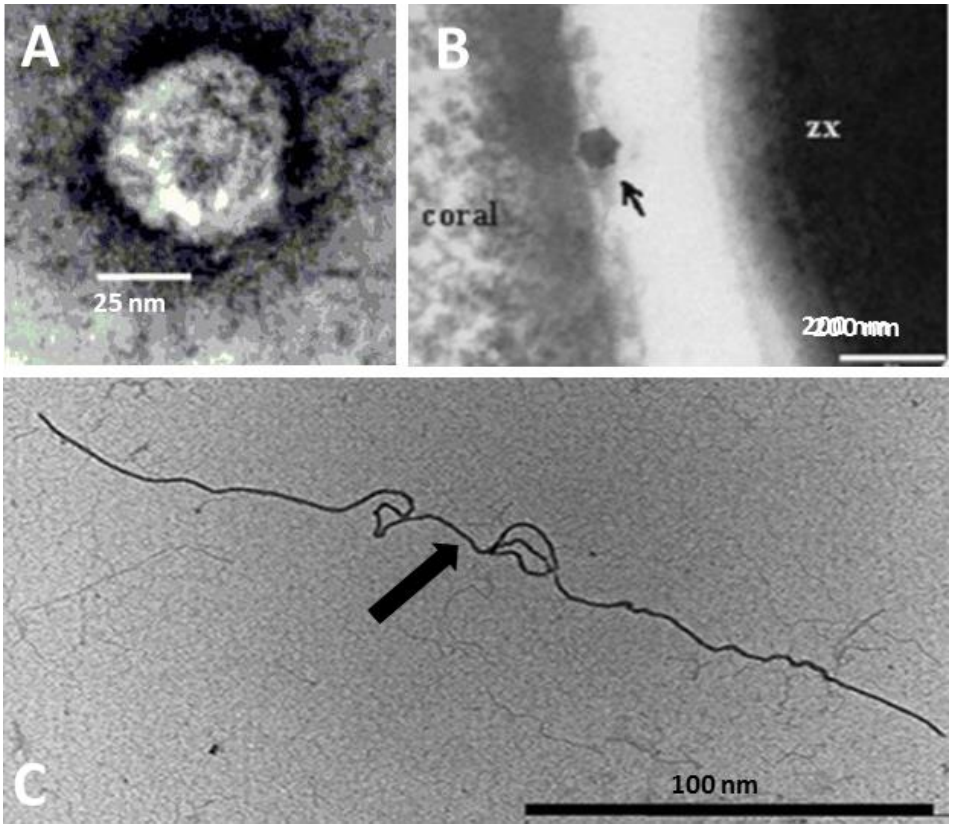
1.7 VÍRUS DE CORAIS E *Symbiodinium*

Oppen et al, (2009) hipotetizaram que vírus eucarióticos e bacteriófagos desempenhariam papéis fundamentais como componentes do holobionte coral, uma vez que tais entidades são as mais abundantes e diversas do holobionte (BLACKALL et al, 2015, HESTER et al, 2015). Em recifes de corais sadios, partículas semelhantes a vírus (PSVs) variam entre 0.3 e 1.25×10^7 partículas por mililitro na água adjacente (SEYMOUR et al, 2005) a $0.8–1\times10^9$ partículas por centímetro cúbico no sedimento (HEWSON et al, 2003). Vírus também foram encontrados em tecidos (BETTAREL et al, 2012), esqueletos (VEGA THURBER et al, 2008) e nas superfícies de corais, onde a abundância alcança a ordem de 10^{10} PSVs por cm^2 (MARHAVER et al, 2008). Embora as identidades taxonômicas precisas das PSVs dos corais não sejam conhecidas (em nível de gênero e espécie), foi possível inferir os prováveis grupos e famílias virais existentes nestes sistemas através de micrografias eletrônicas e análises metagenômicas. O grupo dos bacteriofágos formam provavelmente as entidades mais comuns no holobionte (BARR et al, 2013). Alguns vírus de fita simples e circular de DNA também foram observados em corais doentes, possívelmente infectando tanto a porção metazoária quanto algal do coral (SOFFER et al, 2014). Em corais do gênero *Acropora e Porites*, PSVs observadas remetem a família de vírus de algas *Phycodnaviridae*, *Iridoviridae* (vírus de peixes) e *Hespesviridae* (númerosos hospedeiros animais) (CERVINO et al, 2004; DAVY et al, 2007; WEGLEY et al, 2007; PATTEN et al, 2008; VEGA THURBER et al, 2008). O papel de infecções virais no branqueamento e em outras doenças de corais ainda encontra-se inconclusivo, porém existe um número expressivo de observações. Nas comparações entre *A. millepora* coletadas antes e depois de um evento natural de branqueamento na Grande Barreira de corais (BERKELMANS *et al*, 2004), foi demonstrada

uma proporção elevada de vírus da superfamília NCLDV como *Poxviridae* e *Mimiviridae*, e também bacteriófagos em indivíduos branqueados de corais (LITTMAN et al, 2011). Também foi demonstrado que indivíduos branqueados de *Diploria strigose* possuíam diversas sequencias semelhantes a *Phycodnaviridae* (MARHAVER et al, 2008) e tecidos branqueados de *Montastrea annularis* possuíam uma diversidade alterada e mais abundante de NCLDVs como *Phycodnaviridae, Poxviridae, e Mimiviridae (SOFFER et al, 2014).* Wilson e colaboradores (2001a) demonstraram que algumas linhagens de *Symbiodinium* possuem infecções virais latentes que podem ser ativadas em resposta de estresses ambientais, e juntamente com observações de PSVs associadas em corais, propuseram que o vírus poderia atuar no branqueamento e, possivelmente, em outras doenças de corais (CERVINO et al, 2004; WILSON et al, 2005; DAVY et al, 2006). Os primeiros trabalhos de vírus com *Symbiodinium* encontraram pequenas PSVs (~40–50 nm) em isolados de anêmonas submetidas a estresse térmico com alta temperatura (WILSON et al, 2001b). Foi sugerido que *Symbiodinium* possuiria uma infecção latente por vírus. Em uma infecção latente (também conhecida como lisogênica) os vírus encontram-se incorporados no genoma do hospedeiro como um provírus ou permanecem no citoplasma na forma de um episssoma intracelular (IVEY et al, 1996; MINAROVITS, 2006). Nestes casos, um evento de indução, como um distúrbio ambiental gerador de estresse, pode afetar o hospedeiro desencadeando o ciclo viral latente para o lítico (FUHRMAN, 1999). Lohr e colaboradores (2007) demonstraram em linhagens de *Symbiodinium* uma infecção latente e a produção de PSVs filamentosas quando expostas a radiação ultravioleta (UV). Estes autores sugeriram que um provírus estaria existindo simbioticamente integrado a zooxantela, reforçando as hipóteses de Wilson et al, (2001a) sobre este sistema. Uma infecção ativa de NCLDVs e um vírus de RNA foram descobertos associados com o coral *Montastraea cavernosa*, revelando mais detalhes sobre a identidade e diversidade viral nestes organismos (CORREA et al, 2013). Foi apontado que sequencias de NCLDVs dominavam os viromas (porção genética viral de um organismo ou ambiente) deste coral, com alta identidade com a família *Phycodnaviridae*, embora com identidades significativas com *Mimiviridae*. Comparações entre as sequências de NCLDVs geradas em *M. cavernosa*, com dados transcriptômicos de *Symbiodinium* de clados e hospedeiros diferentes, revelaram 79% de similaridades (BAYER et al, 2012). Neste sentido, seria possível que as interações com vírus e *Symbiodinium* sejam ancestrais, e poderia até mesmo ter ocorrido co-divergência (CORREA et al, 2013). De fato, em consequência de uma provável infecção ancestral, o grupo dos dinoflagelados adquiriu um importante grupo de proteínas de ligação com DNA nuclear, chamadas de DVNPs *(dinoflagellate/viral*

nucleoprotein), através de um evento de THG entre este grupo e um provável ancestral de *Phycodnaviridae*. Tal evento horizontal resultou na expansão massiva do genoma dos dinoflagelados e em outras características que compõe o estado "dinocariótico" (GORNIK et al, 2012). No genoma do *Symbiodinium* (SHOGUCHI et al, 2013) ao menos 19 genes aparentam homologia com DVNPs e provavelmente estão relacionados com a manutenção da estrutura cromossômica (SHINZATO et al, 2014). Estas sequências poderiam representar cópias parálogas de provírus integrado e ainda ativo em *Symbiodinium*, ou somente uma relíquia molecular fossilizada de uma infecção ancestral ou mais recente, como em outros eventos de THG entre algas e vírus (DELAROQUE; BOLAND, 2008; MEINTS et al, 2008; DERELLE et al, 2008; MONIER et al, 2009). Recentemente, PSVs pequenos, grandes (com semelhança de *Phycodnaviridae*), gigantes (similares a *Mimivirus*) e partículas filamentosas parecidas com as observadas em Lohr et al. (2007), foram observadas em *Symbiodinium* de diversos hospedeiros após a ativação por estresse através de tratamentos com UV (LAWRENCE et al, 2014b).

Figura 1. PSVs observadas em corais e *Symbiodinium* spp.

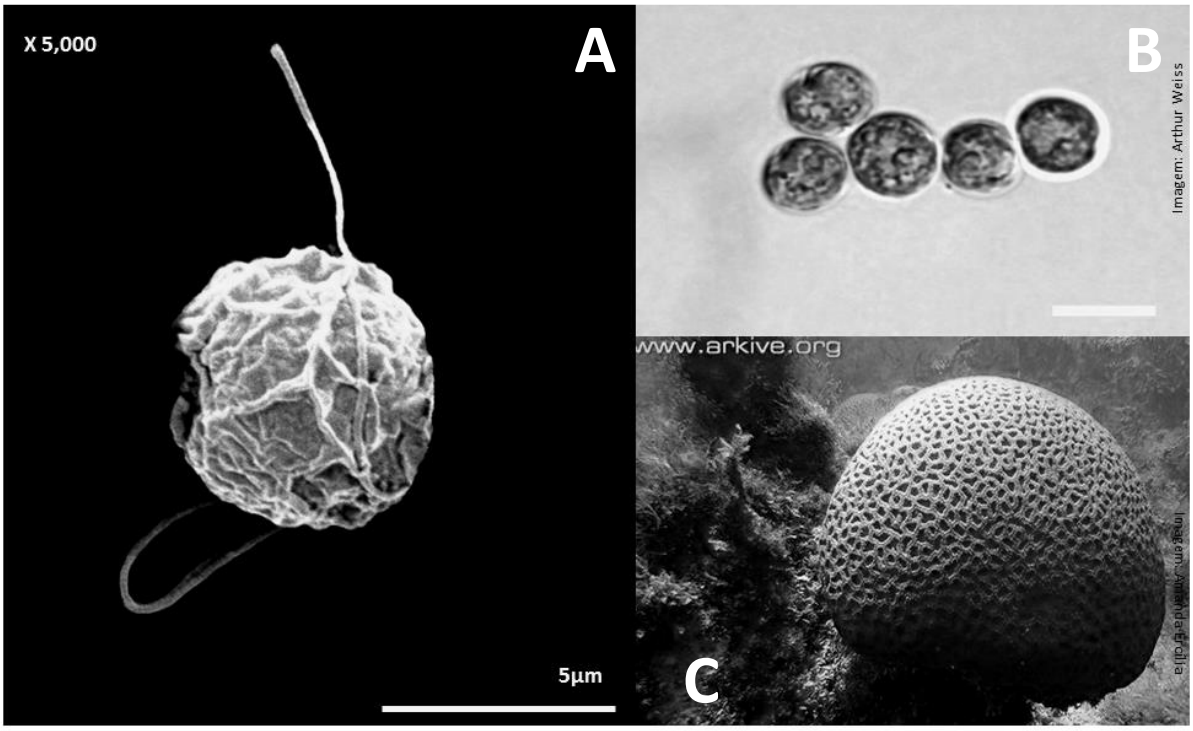


Nota: (A) PSV de *Symbiodinium* isolada de *Anemonia viridis* retirada de Wilson et al. (2001a). (B) PSV observada no coral *Pavona danai* retirada de Wilson et al. (2005). PSV filamentosa observada em cultivos de *Symbiodinium* retirado de Lohr et al. (2007).

1.8 *Symbiodinium* ENDOSSIMBIONTE DO CORAL *Mussismilia braziliensis*

Recentemente foi obtida a primeira caracterização da diversidade genética de *Symbiodinium* do coral *M. braziliensis* do sistema recifal do Banco dos Abrolhos (SILVA-LIMA et al, 2015). *Symbiodinium* dos clados A4 e C3 foram detectados nesta espécie de coral, com mais de um clado habitando o mesmo espécime. Cultivos de *Symbiodinium* destes clados foram estabelecidos a partir do isolamento de células em suspensões de tecidos do coral (SILVA-LIMA et al, 2015). Esta coleção de cepas de *Symbiodinium* é mantida no Instituto de Biologia da UFRJ, sob a responsabilidade e curadoria do Prof. Paulo S. Salomon. *M. braziliensis* é endêmica e constitui 70% dos recifes de corais na região de Abrolhos (Bahia, Brasil) (LEÃO; KIKUCHI, 2005). Altas taxas de doenças de corais ameaçam os ambientes dos corais deste gênero (CASTRO; PIRES, 1999; FRANCINI-FILHO et al, 2008). Este holobionte mantém uma assembleia microbiana espécie-específica (CASTRO et al, 2010). Garcia et al, (2013), identificaram grande abundância de sequências virais nos metagenomas deste coral.

Figura 2: *Symbiodinium* sp. isolado do coral *M. braziliensis*.



Nota: (A) Imagem de microscopia de varredura eletrônica de isolado de *Symbiodinium* do clado A obtida neste estudo; (B) Detalhe do cultivo de *Symbiodinium* do clado A; (C) O holobionte coral *M. braziliensis*.

1.9 OBJETIVO GERAL

Estudar a ocorrência e caracterizar morfologicamente e geneticamente a associação entre vírus e *Symbiodinium* spp., um simbionte de corais.

1.9.1 Objetivos específicos

- Verificar a indução da produção de partículas virais em cultivos de *Symbiodinium* isolados do coral *Mussismilia braziliensis* do Banco dos Abrolhos, quando submetidos a estresse por radiação ultravioleta e alta temperatura;

- Caracterizar morfologicamente as PSVs produzidas nos cultivos de *Symbiodinium* sp. associados a *Mussismilia braziliensis* do Banco dos Abrolhos;

- Verificar, através de análises de bioinformática e reconstruções filogenéticas, a presença de sequências virais no genoma de *Symbiodinium minutum* disponível em bancos de dados públicos.

CAPÍTULO 2

2 GIANT VIRAL-LIKE AND VIROPHAGE-LIKE PARTICLES ASSOCIATED WITH *Symbiodinium* spp. FROM THE CORAL *Mussismilia braziliensis*.

Luiz Felipe Benites[1], Inácio Domingos da Silva Neto[2], Arthur Weiss Lima[1], Fabiano Lopes Thompson[1], Paulo Sergio Salomon[1*].

[1] Department of Marine Biology, Biology Institute, Federal University of Rio de Janeiro

[2] Department of Zoology, Biology Institute, Federal University of Rio de Janeiro

Artigo formatado para submissão à revista: **Coral Reefs**

Giant viral-like and virophage-like particles associated with *Symbiodinium* spp. from the coral *Mussismilia braziliensis*.

Luiz Felipe Benites[1], Inácio Domingos da Silva Neto[2], Arthur Weiss Lima[1], Fabiano Lopes Thompson[1], Paulo Sergio Salomon[1*].

[1] Department of Marine Biology, Biology Institute, Federal University of Rio de Janeiro

[2] Department of Zoology, Biology Institute, Federal University of Rio de Janeiro

Email addresses:

LFB: l.felipebenites@gmail.com

IDSN: idsnet@biologia.ufrj.br

AWL: arthurwlima@gmail.com

FLT: fabiano.thompson@biologia.ufrj.br

***Corresponding author**

PSS: paulosalomon@biologia.ufrj.br

Keywords: *Symbiodinium*, giant virus, Megaviridae, virophage, Abrolhos, coral reefs

Abstract

Coral reefs are one of the most dynamic and productive marine ecosystems. The coral holobiont consists of the coral animal and a variety of associated microorganisms that includes the dinoflagellate *Symbiodinium* as well as bacteria, archaea, fungi, and viruses. The interactions among these components are crucial for coral health and resilience during environmental disturbance. In the present study, by exposures to UV radiation and thermal stresses, we induced the production of virus-like-particles (VLPs) in two strains of *Symbiodinium* spp. (one from clade A and one from clade C) isolated from the coral *Mussismilia braziliensis*, endemic of the Abrolhos reef system in the South Atlantic ocean. VLPs were stained with SYBR green I nucleic acid dye and detected by flow cytometry in the medium of *Symbiodinium* cultures 96 h after exposure to UV and thermal stresses. Control cultures kept in optimal growth conditions did not have these VLPs. Scanning (SEM) and transmission (TEM) electron microscopy images of the stressed cultures confirmed the production of VLPs and revealed the presence of giant viral-like particles (ca. 500 um) with morphological features linking them to the family *Megaviridae*, including hyperinfections by virophage-like particles that are characteristic of this giant-virus family in contrary to previous studies that propose a *Phycodnaviridae* infection for *Symbiodinium* spp. We hypotesize that, in coral holobionts, virophages act in controlling the giant virus infectivity in *Symbiodinium*, and regulating virus-host interactions, as reported in other studies. *Symbiodinium* megaviridae and its virophage may represent important forces driving carbon flows in coral reefs microbial loop, as well as key players in the maintenance of coral holobiont health in times of climatic change.

Introduction

Dinoflagellates of the genus *Symbiodinium* are photosynthetic protists that forge symbioses with a wide range of metazoan and protistan hosts. The genus is divided in 9 clades (A–I) and numerous phylotypes (Pochon et al. 2004) hosted by sponges, flatworms, molluscs, foraminifera, radiolarians, ciliates, and cnidarians as stony (Scleractinian) and octocorals (Goulet and Coffroth 2004).

Corals are considered ‘holobionts’, an entity defined as a symbiotic assemblage formed by polyp animals and endosymbiotic dinoflagellates as *Symbiodinium* (termed zooxanthelae), and a varitey of fungal, bacterial and viral associates (Rohwer et al. 2002).

Coral-*Symbiodinium* interactions are primarily responsible for reef formation in oligotrophic marine environments, through photosynthates fixation and nutritional exchange (Knowlton and Rohwer 2003). Coral holobiont health is essential to the sustainability and productivity of reefs, crucial for human communities across the globe (Riegl et al. 2009).

Environmental triggers, such as elevated seawater temperatures (Brown 1997) or high irradiance (Anthony and Kerswell 2007) can lead to the breakdown of the symbiosis, resulting in the loss and/or expulsion of *Symbiodinium* cells from the coral host, a phenomenon known as "coral bleaching" (Oppen and Lough 2009). Mass bleaching events have become more frequent over the decades, threatening the future of coral reefs in times of climate change (Hoegh-Guldberg et al. 2007).

Although some causative agents of bleaching, along with other coral diseases have been investigated (Rosenberg et al. 2009), the possibility that viral infections are implicated in these phenomena remains largely understudied (Soffer et al. 2014). Despite several efforts (Thurber and Correa 2011; Lawrence et al. 2014a; Nguyen-Kim et al. 2014) there is a lack of precise identification of putative viral residents of coral holobionts. Oppen et al. (2009) hypothesize that viruses could play fundamental roles as components of the coral holobiont, once that they have been found in reef substratum (Dinsdale et al. 2008) in coral tissues, skeleton (Vega Thurber et al. 2008) and mucus (Nguyen-Kim et al., 2014).

Symbiodinium was shown to harbor latent viral infections that can be activated in response to environmental stress (Wilson et al 2001). This finding, together with observations of viral-like particles (VLPs) associated with corals, led to the conclusion that, to some extent, viruses could be implicated in bleaching and possibly other coral reef diseases (Wilson et al. 2005). Recently, a variety of VLPs, including small, large (with Phycodnaviruses resemblance), giant icosahedral (similar to Mimiviruses), and filamentous forms resembling those reported in Lohr et al. (2007), were observed in *Symbiodinum* from various hosts after exposure to UV irradiance (Lawrence et al. 2014b). In the coral *Monstastraea cavernosa,* an active viral infection was discovered (Correa et al. 2013), shedding some light on the identities of holobiont viral residents, where heat stressed samples was dominated by double stranded DNA viruses from the superfamily NCLDV (Nucleocytoplasmic Large DNA Virus) with best identity with Phycodnaviruses (a family of algal viruses), although it was also found significant *Mimiviridae* similarities, that infects algae and other protists (Moniruzzaman et al. 2014). Correa et al. (2013) also suggested that viral symbiotic relationships with zooxanthella

could be from an ancient origin. In fact, as a consequence of a probable ancient viral infection, dinoflagellates gained the gene DVNP (*dinoflagellate/viral nucleoprotein)*, from a *Phycodnaviridae*-like virus in a Horizontal gene transfer event (HGT) (Gornik et al. 2012). In *Symbiodinium* genome (Shoguchi et al. 2013), 19 genes appears homologous to DVNPs (Shinzato et al. 2014) and could represent paralogous copies of an integrated provirus (Delaroque et al. 1999; Meints 2008) or molecular relics of past and/or present viral infections as in other HGT within algae-virus symbiotic systems (Derelle et al. 2008; Monier et al. 2009).

In this study we successfully induced the production of VLPs in cultures of *Symbiodinium* spp. isolated from *Mussismilia braziliensis*, an endemic scleractinian coral from the Abrolhos reef system (South Atlantic ocean). A characterization of these VLPs using scanning and transmission electron microscopy images is presented.

Materials and methods

Symbiodinium cultures

In order to test if *Symbiodinium* from *M. braziliensis* corals harbor a latent viral infection inducible to enter a lytic cycle by influence of environmental stressors, two clonal strains of *Symbiodinium* isolated from this coral species (Silva-Lima et al. 2015), strain 043D10 from clade "A" (phylotype A4) and strain 103C3 from clade "C" (phylotype C3) were usedin controlled experiments where cells were exposed to thermal and UV radiation stress. Batch cultures of both strains were grown in 250 mL Erlenmeyers flasks in seawater-based F/2 medium at 24°C with an irradiance of 80 μmol photons m^{-2} s^{-1} (cool daylight fluorescent tubes) and a 14h light : 10h dark photoperiod. This culture was used to expose the cells to temperature and UV stresses as described below.

Thermal and UV stress experiments

An experiment was set up to follow VLP production in cultures of *Symbiodinium*, after stress-induced conditions, and compare them with non-stressed, control cultures. We followed a modified version of the method described by Wilson et al. (2001) and Correa et al. (2013) on heat shock induction of VLPs in *Symbiodinium*. Briefly, 50 mL aliquots of cultures of both *Symbiodinium* strains grown as explained above were harvested at late exponential growth phase (containing ca. 10^5 cells ml^{-1}) and were subjected to thermal stress (31,5ºC) for a period

of 24 h and then incubate for 96 h (4 days) at control conditions i.e. 80 μE m^{-2} s^{-1}; a 14 light : 10 dark photoperiod and 24°C. For the UV stress assay, we followed the protocol by Lohr et al. (2007) and Lawrence et al. (2014b), with minor modifications. Aliquots of 50 mL of cultures of both *Symbiodinium* strains harvested as describe above were transferred to large Petri dishes without lids, exposed for 2 minutes to direct UV radiation in a transilluminator (MBS-1500A model - 254 nm), and then incubated for 96 h. at control conditions as describe above. Samples (5 ml) were collected at the end of the incubation period, fixed with glutaraldehyde (1% final concentration), incubated at room temperature in the dark for 15 to 30 min, snap frozen in liquid nitrogen and stored at -80 ºC. Control cultures of both strains were maintained at 24 °C. Both *Symbiodinium* strains were characterized by SEM microscopy. Strain 043D10 was used for further TEM characterization.

Analysis of virus-like particles (VLP) by flow cytometry

Cultures of strains 043D10 and 103C3 (control, UV, and heat-shocked treatments) were screened for the presence of extracellular VLPs using a Flow Cytometer BD Accuri C5, following the methods of Brussaard (2004) and Lohr et al. (2007). Briefly, samples were collected and fixed with glutaraldehyde 1%, snap-frozen in liquid nitrogen (N_2; −196°C) and storage at −80 °C. Samples were diluted in 1:10, 1:100 and 1:1000 with TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0) and stained with the nuclei acid dye SYBR Green I (Thermofisher), final concentration $1x10^{-5}$ of stock solution. Stained samples were incubated at 80 ^{o} C for 10 min in the dark, cooled down to room temperature and analyzed in the flow cytometer. Samples were analyzes for 2 min at a flow rate of 14 ul min^{-1}. SYBR Green I fluorescence was collected as FL1-H signal through a 530/30 nm filter mounted in front of the photomultiplier. Both FL1-H and side scatter (SSC-H) signals were collected in logarithmic mode. VLPs were visualized inSSC vs FL1 scatter plots. Blanks (TE buffer), reference (sea water from Abrolhos reef system) and control (non-stressed *Symbiodinium* cultures) were analyzed in the same way as the stressed *Symbiodinium* cultures.

Transmission electron microscopy (TEM)

Aliquots of 2 mL of *Symbiodinium* cultures were fixed with glutaraldehyde (final 2% v/v) buffered in 0,5 M sodium cacodylate (pH 7,0) for 30 min and post-fixated with 1 mL of osmium tetroxide 2% (2 M Sodium cacodylate) for 30 min. Cells were then rinsed 3x with 0,5 M sodium *cacodylate* buffer. Cells were harvested by centrifugation at 1500 x g for 5 min and dehydrated in an acetone gradient (30, 50, 70, 85, 95%) during 10 min and with acetone 100% for 3X, during 15 min. Pelleted cells were included in Epon resin 50% diluted in acetone for

12 h, embedded in Epon resin 100% for 4 h, and let dry at 60ºC for 48 h to polymerize. Ultrathin sections were obtained in an ultramicrotome model LKB and collected on copper grids and samples were observed in a transmission electron microscope FEI Morgagni, with accelerating voltage of 8900 kV. Control and heat stressed samples could not be processed because of the low number of cells that remains after the chemical washes and preparations.

Scanning electron microscopy (SEM)

Aliquots of 2 m L of *Symbiodinium* cultures were fixed with glutaraldehyde (final conc. 1%) and 1 volume of sodium *cacodylate* (0,2 M at 2,5%) and then post fixed with 1 volume of osmium tetroxide (2%). Cells were then rinsed 3 times with sodium *cacodylate* buffer and twice with distilled water. Samples were applied to coverslips with poly-l-lysine, placed inside 1,5 ml microcentrifuge tubes and dehydrated in an ethanol series (30, 50, 70, 85, 95 and 99, 8%) with critical-point-dried in liquid CO_2 (Ladd Research Industries critical). The cover slips were attached to aluminum stubs with double-sided adhesive tape and coated with a thin layer of gold in a sputter coater (Structure Probes Inc., West Chester, PA, USA). Visualization was done in a scanning electron microscope JEOL JSM – 6510.

Results

Detection of VLPs by flow cytometry

Two distinct putative VLPs populations (VLP1 and VLP2) were detected by flow cytometry in UV and thermally stressed cultures of both *Symbiodinium* strains used in this study (Figure 1). Control cultures did not present these populations. VLP concentrations in UV and thermally stressed cultures ranged from $3,5 \times 10^4$ to $2,0 \times 10^5$, and $0,8 \times 10^4$ to $1,8 \times 10^5$ particles ml^{-1} for populations VLP1 and VLP2, respectively.

Scanning Electron Microscopy (SEM)

Cells of both *Symbiodinium* strains showed a healthy morphological state in control, non-stressed cultures (Figure 2a and 2b). Cells from UV-stressed cultures, on the other hand, showed several signs of deformation such as shrinkage and holes in the cell surface (Figure 2c and 2d). Images of thermally-stressed cultures could not be produced because we could not distinguish organic debris from *Symbiodinium* cells in these samples since the material clumped together, probably due to the temperature effect.

In UV-stressed cultures of both *Symbiodinium* strains we observed a large number of VLPs with polyhedral or asymmetric morphology (Figures 3 and 4). Giant VLPs of 450-500 nm dominated these samples (Figure 3a and b). Icosahedral VLPs observed in the culture of *Symbiodinium* strain 043D10 (clade A) were accompanied by small VLPs, some of them asymmetric (Figure 3c). In *Symbiodinium* strain 103C3 (clade C) cultures, we observed polyhedral (Figure 4a), diamond-like (Figure 4b), asymmetric and few quadratic structures with resemblance to defective particles (fig. 4 c-d). Associated with giant VLPS there were small polyhedral virophage-like particles that were much more abundant than giant VLPs (Figures 3a and 4d). Control samples showed only a few giant VLP (data not show).

Transmission Electron Microscopy (TEM)

TEM images of UV-stressed cultures revealed the presence of giant VLPs outside *Symbiodinium* cells with multi-layered, capsid-like structures covered with fibrils (Figure 5a). Icosahedral VLP of 450-500 nm were also observed near *Symbiodinium* cells (Figure 5b). Coiled, ribbon-like structures were observed near this VLPs. Inside *Symbiodinium* cells, we detected icosahedral VLPs (Figure 5c) of similar size as VLPs found outside the cells by SEM (compare to Figure 3a). In the same samples we observed defective VLPs with several accumulated layers (Figure 6a). A great number of small polyhedral hexagonal particles of ca. 40 nm were observed associated with abnormal or defective VLPs (Figure 6b), and curling long filamentous structures with small particles inside (Figure 6c).

Discussion

We demonstrate that *Symbiodinium* from clade A (phylotype A4) and C (phylotype C3) originated from the coral *M. braziliensis* from Abrolhos reef system has a latent viral infection inducible to produce VLPs when subjected to thermal and UV radiation stresses. In a latent infection the virus is hidden, integrated in the host genome as a provirus or as an episome in the cytoplasm, as in herpesvirus-host systems (Minarovits 2006). Under non-stress environmental conditions, the dynamics of latent viruses in *Symbiodinium* probably does not affect its ecological role in the holobiont and could prevent exogenous viral infections (Bettarel et al. 2015) by superinfection immunity conferred to the host as observed in a large virus-algae infecting *Chlorella* sp. (Greiner et al. 2009).

Corals are normally in their upper limit of tolerance to the effects of natural UV radiation (Lesser 1996), and even small increases in temperature, combined with intense solar radiation, could lead to coral bleaching (Hoegh-Guldberg 2007). In the context of global warming is crucial to understand how holobiont coral residents react and responds to stress triggers and the putative viral link between stress and coral health disruption (Wilson et al, 2005; Oppen et al. 2009; Correa et al. 2013).

Lohr et al. (2007) and Lawrence et al. (2014b) reported filamentous VLPs in *Symbiodinium* cultures subjected to UV stress, similar to what we observed in our samples (Supplementary Figure 1). However, in the absence of genomic data we can only speculate on the nature of these filamentous VLPs. Some of them could be indeed viral but it is also possible that such particles are degraded host cytoplasmic structures (Correa et al. 2013) or even defective viral capsid lipid membranes, that curls off the cell host, like open membrane sheets precursors for inner giant viral membranes (Mutsafi et al. 2013).

Our data strongly indicate that, contrary to previous studies that proposed a *Phycodnaviridae* infection for *Symbiodinium* (Thurber and Correa 2011; Correa et al. 2013), this symbiotic dinoflagellate serve as host for giant viruses belonging to the Megaviridae (Arslan et al. 2011). Our TEM images show the presence of giant VLPs outside *Symbiodinium* cells with multi-layered, capsid-like structures covered with fibrils, which are one of the hallmarks and main feature of *Mimiviridae*/*Megaviridae* (Suzan-Monti et al. 2007; Arslan et al. 2011). Claverie et al. (2009) had suggested that marine Mimivirus relatives could infect corals. In line with this results, Correa et al. (2013) found significant gene hits to *Mimiviridae* in the virome of the coral *Montastraea cavernosa*. Moreover, phylogeny of DNA polymerase family B (DNA pol B) generated in their study was shown to cluster with representatives of *Megaviridae*, like Moumouvirus and Terravirus.

Previous studies have indicated a phylogenetic proximity between Mimivirus and giant algae viruses such as those found in the photosythetic protists of the genera *Pyramimonas*, *Phaeocystis* and *Chrysochromulina* (Monier et al. 2008). These algal viruses have been allocated with other protist viruses in the proposed family *Megaviridae* (Santini et al. 2013). One of the defining features of *Megaviridae* is presence of an associated virophage (La Scola et al. 2008; Fischer and Suttle 2011; Yau et al. 2011; Santini et al. 2013). Virophage-like particles observed in our study reinforces our classification of *Symbiodinium* giant VLPs as Megaviruses.

We hypotesize that, in coral holobionts, virophages act in controlling the giant virus infectivity as seen in amoeba populations (Slimani et al. 2013) and regulate virus-host interactions. Lotka-Volterra simulations with OLV (Organic Lake Virophage) and OLPV (Organic Lake Phycodnavirus) show an increase in the flux of secondary production through the microbial loop by reducing overall mortality of the eukaryotic host through inhibition of the infectivity of giant virus hosts (Yau et al. 2011). In Mamavirus-sputnik system, the infectivity of Mimivirus decreases 70% when infected with the virophage sputnik and had a threefold decrease in cell lysis and increases the production of abnormal particles (La Scola et al. 2008). Mavirus virophage increases the survival of the bacterivorous stramenopile *Cafeteria roenbergensis*, when interfering with propagation of the giant virus that infects the protist (Fischer and Suttle 2011). Infection of Samba virus by its virophage rio negro (RNV) results in a reduction of 80% of viral titer, allowing the recovery of the host amoebae population (Campos et al. 2014). On the other hand, Zamilon, a virophage associated with Mont1 virus, does not seem to have a significant impact on the giant virus host (Gaia et al. 2014).

Through gene transfer and incorporation of acquired virophage genes on giant virus or in *Symbiodinium*, as a side effect of its own hyperparasitism, virophages could improve latency of its giant virus that can lead to viral immunization by superinfection immunity of the host against exogenous virus, either in an episomatic/plasmidial or provirophage state. Virophage integration is observed in Sputnik 2 and its Mimivirus host (Desnues et al. 2012), in the virophage PgVV on the genome of *Phaeocystis globosa* virus (Santini et al. 2013), and in the marine algae *Bigelowiella natans*, that has numerous genes with virophage origins in its nuclear genome (Blanc et al. 2015). Viriophages could also, coupled with their giant virus, act as a stabilizing force in the *Symbiodinium*-coral symbiosis (Villarreal, 2007; Marhaver et al. 2008).

The complexity and entanglement of coral, *Symbiodinium*, giant virus and virophages interactions cast new prospects for further studies. We propose that coral Megaviridae giant virus and its virophage may represent important forces driving carbon flows in coral reefs microbial loop, as well as key players in the maintenance of coral holobiont health in times of climatic change.

Acknowledgements

We acknowledge Paulo Iiboshi for assistance in flow cytometer, Glaucia Ank for *Symbiodinium* cultures assistance, and Marcelo Sales for SEM preparations. We greatly appreciate the helpful discussion, corrections and insightful comments provided by Lílian Caesar. This work was made possible by CAPES/CNPQ funding support.

Reference List

Anthony KRN, Kerswell AP (2007) Coral mortality following extreme low tides and high solar radiation. Marine Biology 151:1623-1631

Arslan D, Legendre M, Seltzer V, Abergel C, Claverie JM (2011) Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. Proceedings of the National Academy of Sciences 108:17486-17491

Bettarel Y, Bouvier T, Nguyen HK, Thu PT (2015) The versatile nature of coral-associated viruses. Environmental Microbiology 17: 3433-9

Blanc G, Gallot-Lavallée L, Maumus F (2015) Provirophages in the *Bigelowiella* genome bear testimony to past encounters with giant viruses. Proceedings of the National Academy of Sciences 112: E5318-26

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16: S129–S138

Brussaard CPD (2004) Viral control of phytoplankton populations - A review. Journal of Eukaryotic Microbiology 51: 125-138

Campos RK, Boratto PV, Assis FL, Aguiar ER, Silva LC, Albarnaz JD, Dornas FP, Trindade GS, Ferreira PP, Marques JT, Robert C, Raoult D, Kroon EG, La Scola B, Abrahão JS (2014) Samba virus: a novel mimivirus from a giant rain forest, the Brazilian Amazon. Virology Journal 11: 95

Claverie JM, Grzela R, Lartigue A, Bernadac A, Nitsche S, Vacelet J, Ogata H, Abergel C (2009) Mimivirus and Mimiviridae: Giant viruses with an increasing number of potential hosts, including corals and sponges. Journal of Invertebrate Pathology, 101:172-180

Correa AMS, Welsh RM, Vega Thurber RL (2013) Unique nucleocytoplasmic dsDNA and +ssRNA viruses are associated with the dinoflagellate endosymbionts of corals. The ISME Journal. 7:13–27

Delaroque N, Maier L, Knippers R, Müller DG (1999) Persistent virus integration into the genome of its algal host, *Ectocarpus siliculosus* (Phaeophyceae). Journal of General Virology 80:1367-1370

Derelle E, Ferraz C, Escande ML, Eychenié S, Cooke R, Piganeau G, Desdevises Y, Bellec L, Moreau H, Grimsley N (2008) Life-cycle and genome of OtV5, a Large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*. PLoS ONE 3: e2250

Desnues C, La Scola B, Yutin N, Fournous G, Robert C, Azza S, Jardot P, Monteil S, Campocasso A, Koonin EV, Raoult D (2012). Provirophages and transpovirons as the diverse mobilome of giant viruses. Proc Natl Acad Sci USA 109:18078-18083

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis BL, Azam F, Knowlton N, Rohwer F (2008) Microbial ecology of four coral atolls in the Northern Line Islands. PLoS ONE 3: e1584

Fischer MG, Suttle CA (2011) A virophage at the origin of large DNA transposons. Science (New York, N.Y.) 332: 231-234

Gaia M, Benamar S, Boughalmi M, Pagnier I, Croce O, Colson P, Raoult D, La Scola B (2014) Zamilon, a novel virophage with Mimiviridae host specificity. PloS ONE 9:e94923

Greiner T, Frohns F, Kang M, Van Etten JL, Käsmann A, Moroni A, Hertel B, Thiel G (2009) Chlorella viruses prevent multiple infections by depolarizing the host membrane. The Journal of general virology 90: 2033-9

Gornik SG, Ford KL, Mulhern TD, Bacic A, McFadden GI, Waller RF (2012) Loss of nucleosomal DNA condensation coincides with appearance of a novel nuclear protein in dinoflagellates. Current Biology 22: 2303-2312

Goulet TL, Coffroth MA (2004) The genetic identity of dinoflagellate symbionts in Caribbean octocorals. Coral Reefs 23: 465-472.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007). Coral reefs under rapid climate change and ocean acidification. Science (New York, N.Y.) 318: 1737-1742

Knowlton N, Rohwer F (2003) Microbial mutualisms on coral reefs: the host as a habitat. American Naturalist. 162: S51-S62

La Scola B, Desnues C, Pagnier I, Robert C, Barrassi L, Fournous G, Merchat M, Suzan-Monti M, Forterre P, Koonin E (2008) **The virophage as a unique parasite of the giant mimivirus.** *Nature* **455:**100-104

Lawrence SA, Davy JE, **Wilson WH**, Hoegh-Guldberg O, Davy SK (2014a) Porites white patch syndrome: associated viruses and disease physiology. *Coral Reefs* 34: 249-257

Lawrence SA, **Wilson WH,** Davy JE, Davy SK (2014b) Latent virus-like infections are present in a diverse range of *Symbiodinium* spp. (Dinophyta). *Journal of Phycology* **50:** 984-997

Lesser MP (1996). Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnology and Oceanography 41:271-283

Lohr J, Munn CB, Wilson WH (2007) Characterization of a latent virus-like infection of symbiotic zooxanthellae. Appl Environ Microbiol 73: 2976-2981

Marhaver KL, Edwards RA, Rohwer F (2008) Viral communities associated with healthy and bleaching corals. Environmental Microbiology 10: 2277-2286

Meints RH, Ivey RG, Lee AM, Choi TJ (2008) Identification of two virus integration sites in the brown alga *Feldmannia* chromosome. Journal of virology 82: 1407-1413

Minarovits J (2006) Epigenotypes of latent herpesvirus genomes. Current Topics in Microbiology and Immunology 310: 61-80

Monier A, Larsen JB, Sandaa RA, Bratbak G, Claverie JM, Ogata H (2008) Marine mimivirus relatives are probably large algal viruses. Virology journal 5:12

Monier A, Pagarete A, De Vargas C, Allen MJ, Read B, Claverie JM, Ogata H (2009) Horizontal gene transfer of an entire metabolic pathway between a eukaryotic alga and its DNA virus. Genome Research 19: 1441-1449

Moniruzzaman M, LeCleir, GR, Brown CM, Gobler CJ, Bidle KD, Wilson WH, Wilhelm SW (2014) Genome of brown tide virus (AaV), the little giant of the Megaviridae, elucidates NCLDV genome expansion and host-virus coevolution. Virology 466-467: 60-70

Mutsafi, Y., Shimoni, E., Shimon, A., and Minsky, A. (2013). Membrane assembly during the infection cycle of the giant Mimivirus. PLoS pathogens *9*, e1003367

Nguyen-Kim H, Bouvier T, Bouvier C, Doan-Nhu H, Nguyen-Ngoc L, Rochelle-Newall E, Baudoux AC, Desnues C, Reynaud S, Ferrier-Pages C, Bettarel Y (2014). High occurrence of viruses in the mucus layer of scleractinian corals. Environmental Microbiology Reports 66: 675–682

Oppen MJH, Leong, JA, Gates RD (2009) Coral-virus interactions: A double-edged sword? Symbiosis 47:1-8

Oppen MJH, Lough JM (2009) Introduction: Coral bleaching — patterns, processes, causes and consequences. Coral Bleaching 175-176

Pochon X, LaJeunesse TC, Pawlowski J (2004) Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta). Marine Biology 146: 17-27

Riegl B, Bruckner A, Coles SL, Renaud P, Dodge RE (2009) Coral reefs: Threats and conservation in an era of global change. Annals of the New York Academy of Sciences 1162:136–186

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Marine Ecology Progress Series, 243:1-10

Rosenberg E, Kushmaro A, Kramarsky-Winter E, Banin E, Yossi L (2009) The role of microorganisms in coral bleaching. The ISME journal 3:139-146

Santini S, Jeudy S, Bartoli J, Poirot O, Lescot M, Abergel C, Barbe V, Wommack KE, Noordeloos AAM, Brussaard CPD, Claverie JM (2013) Genome of *Phaeocystis globosa* virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. Proceedings of the National Academy of Sciences of the United States of America, 110: 10800-5.

Shinzato C, Mungpakdee S, Satoh N, Shoguchi E (2014) A genomic approach to coral-dinoflagellate symbiosis: Studies of *Acropora digitifera* and *Symbiodinium minutum*. Frontiers in Microbiology 5: 1-17

Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujiwara M, Hamada M, Seidi A, Fujie M, Usami T, Goto H, Yamasaki S, Arakaki N, Suzuki Y, Sugano S, Toyoda A, Kuroki Y, Fujiyama A, Medina M, Coffroth MA, Bhattacharya D, Satoh N (2013) Draft assembly of the *Symbiodinium minutum* nuclear genome reveals dinoflagellate gene structure. Current Biology 23:1399-1408

Silva-Lima AW, Walter JM, Garcia GD, Ramires N, Ank G, Meirelles PM, Nobrega AF, Siva-Neto ID, Moura RL, Salomon PS, Thompson CC, Thompson FL (2015) Multiple *Symbiodinium* Strains Are Hosted by the Brazilian Endemic Corals *Mussismilia* spp. Microbial Ecolology

Slimani M, Pagnier I, Raoult D, La Scola B (2013) Amoebae as battlefields for bacteria, giant viruses, and virophages. Journal of virology 87: 4783-5

Soffer N, Brandt ME, Correa AMS, Smith TB, Thurber RV (2014) Potential role of viruses in white plague coral disease. The ISME journal 8: 271-83.

Suzan-Monti M, La Scola B, Barrassi L, Espinosa L, Raoult D (2007) Ultrastructural characterization of the giant volcano-like virus factory of *Acanthamoeba polyphaga* Mimivirus. PLoS One 2:e328

Thurber RLV, Correa AMS (2011) Viruses of reef-building scleractinian corals. Journal of Experimental Marine Biology and Ecology 408: 102–113

Vega Thurber RL, Barott KL, Hall D, Liu H, Rodriguez-Mueller B, Desnues C, Edwards RA, Haynes M, Angly FE, Wegley L, Rohwer FL (2008) Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa*. Proceedings of the National Academy of Sciences of the United States of America, 105: 18413-18418

Villarreal LP (2007) Virus-host symbiosis mediated by persistence. Symbiosis, 44:1–9

Wilson WH, Francis I, Ryan K, Davy SK (2001) Temperature induction of viruses in symbiotic dinoflagellates. Aquatic Microbial Ecology 25: 99-102

Wilson WH, Dale AL, Davy JE, Davy SK (2005) An enemy within? Observations of virus-like particles in reef corals. Coral Reefs 24:145–148

Yau S, Lauro FM, DeMaere MZ, Brown MV, Thomas T, Raftery M J, Andrews-Pfannkoch, C, Lewis M, Hoffman JM, Gibson J a, Cavicchioli R (2011) Virophage control of antarctic algal host-virus dynamics. Proceedings of the National Academy of Sciences of the United States of America, 108: 6163-6168

Figure Legends

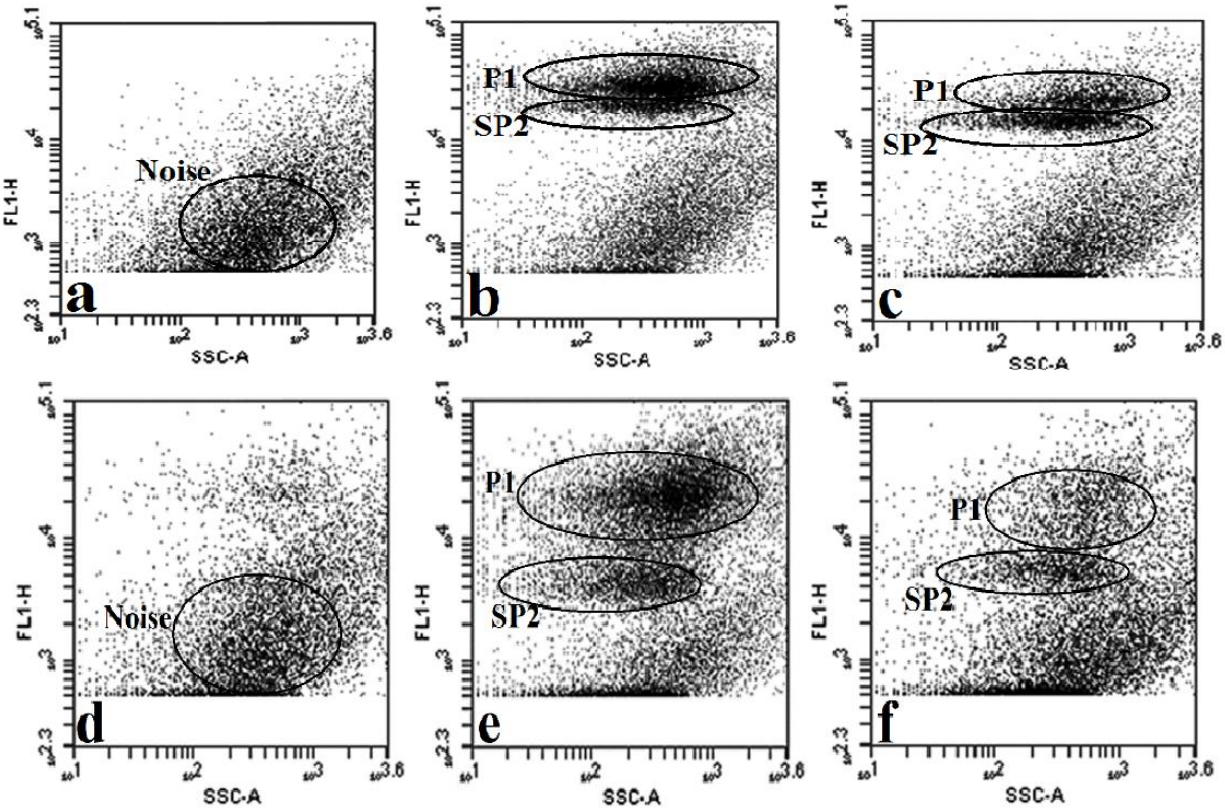


Fig. 1: Detection of VLPs by flow cytometry. Cytograms of side scatter (SSC-H) versus SYBR Green-I fluorescence (FL1-H) of VLPs from *Symbiodinium* spp. cultures. (a and d) Control, non-stressed cultures showing only noise, background fluorescence. (b and c) Cultures of strain 043D10. (e and f) Cultures of strain 103C3. (b and e) Cultures subjected to thermal stress. (c and f) Cultures subjected to UV radiation stress. Two putative VLP populations with high green fluorescence (VLP1 and VLP2) were observed in stressed cultures 96h after UV or thermal stresses.

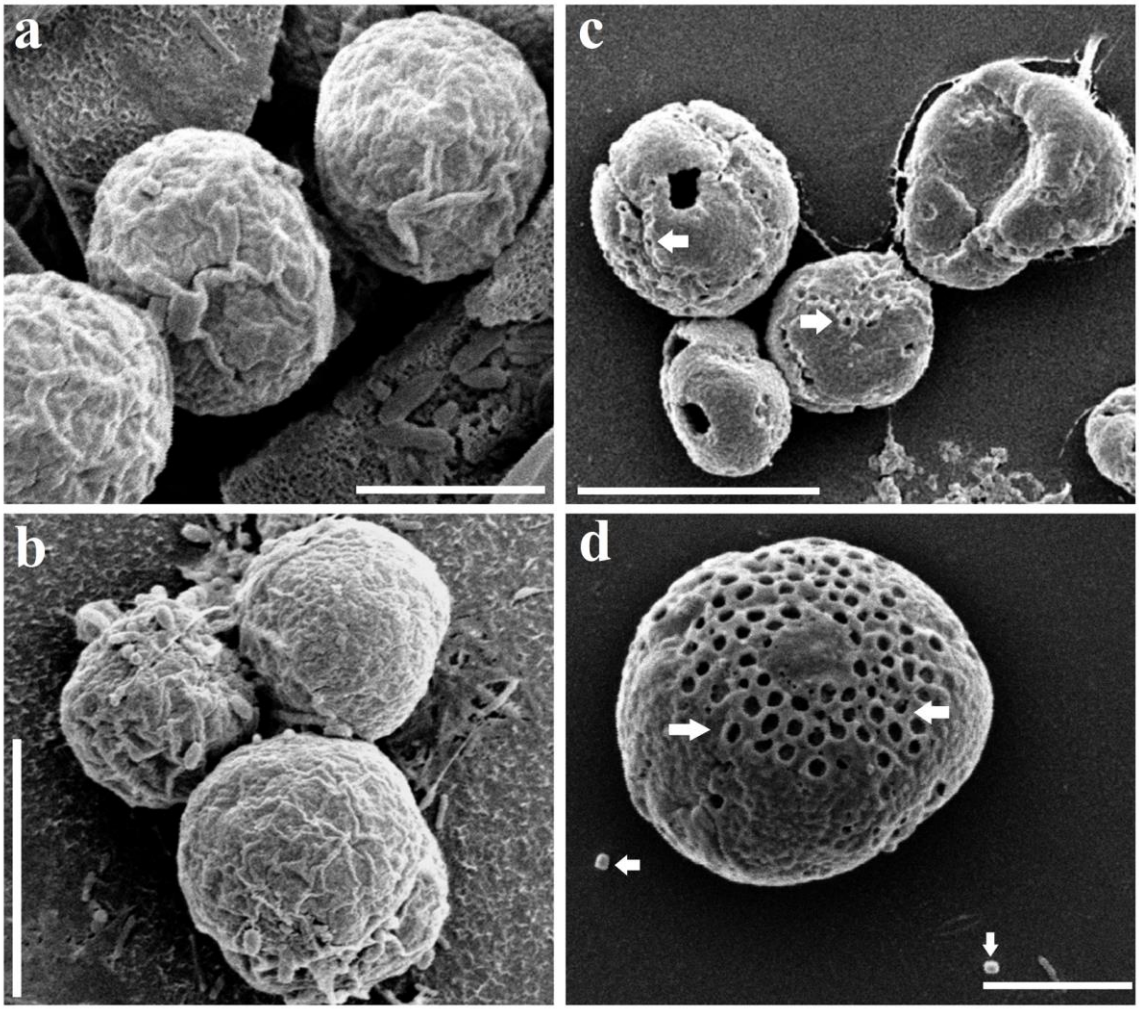


Fig. 2: Scanning electron micrographs of Symbiodinium strain 043D10 (clade A) and 103C3 (clade B). (a and b) Cells from control cultures. (c and d) cells from cultures subjected to UV stress. (c and d) Holes in Symbiodinium cells surface (arrows) likely due to the UV treatment. (d) Putative defective or asymmetric VLPs near *Symbiodinum* (arrows). Scale bars: A: 2 µm, B and C: 5 µm, D: 2 nm.

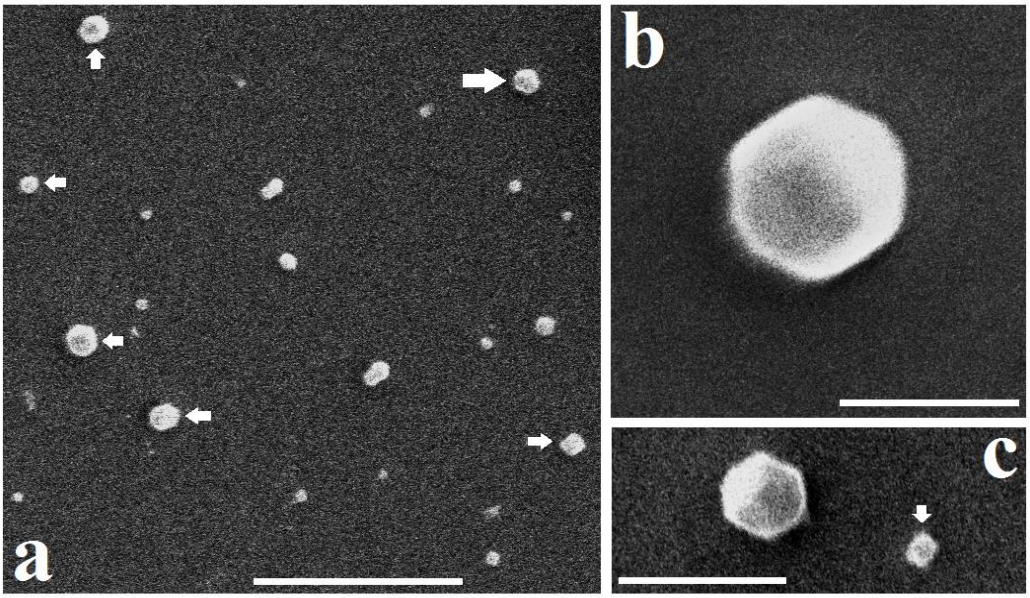


Fig. 3: Scanning electron micrographs of VLPs from UV treated *Symbiodinium* strain 043D10 cultures (clade A). Several giant icosahedral VLPs and small virophage-like particles are observed in cells from the UV treatment (arrows in a). Detail of a giant VLP (b). Giant VLP (~450-500 nm) accompanied by a small particle or a defective VLP (c). Scale bars: A: 2 µm, B: 0,5 µm, C: 1 µm.

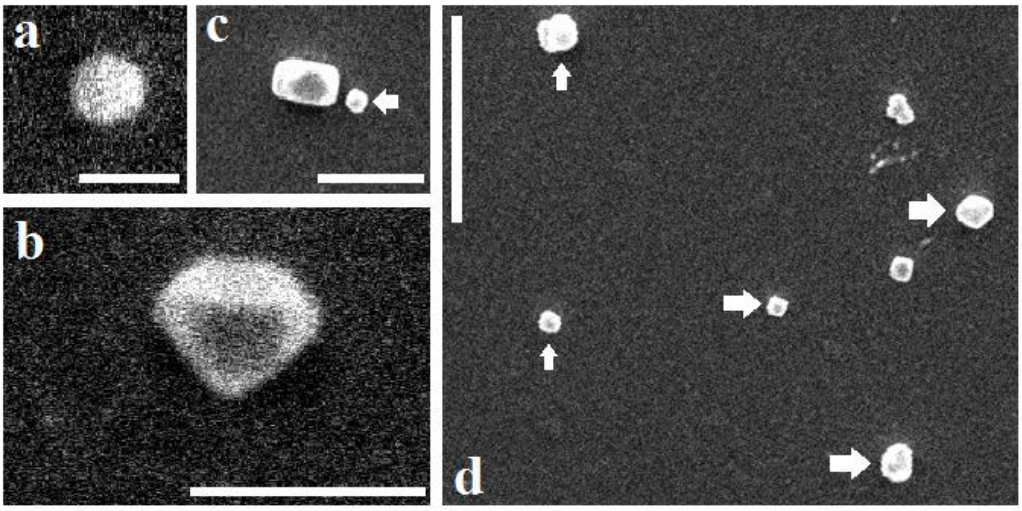


Fig. 4: Scanning electron micrographs of VLPs from UV treated *Symbiodinium* cultures from strain 103C3 of clade C. (a) Icosahedric VLP. (b) A putative defective, diamond-like VLP. (c) Giant VLP (~450-500 nm) accompanied by a small particle or a defective VLP. (d) Several giant icosahedric or asymmetric VLPs with small phage-like particles (arrows). Scale bars: A, B and C: 0,5 µm, D: 2 µm.

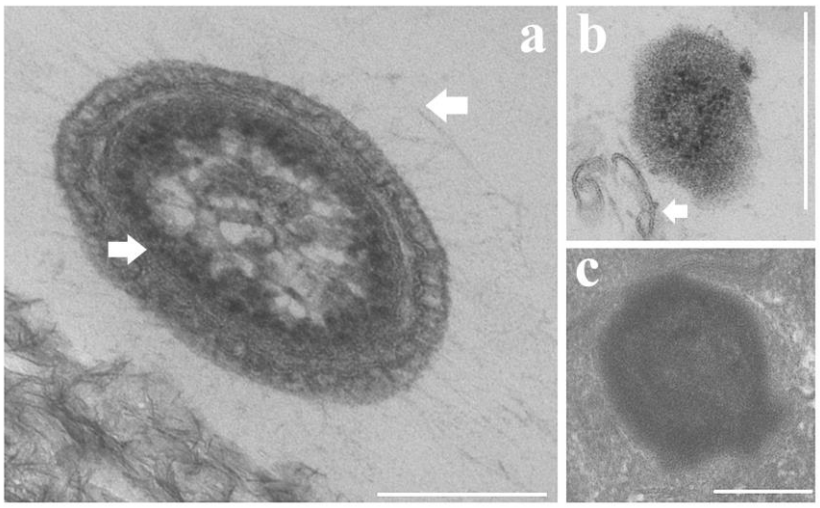


Fig. 5: Transmission electron micrographs of *Symbiodinium* VLPs from strain 043D10 of clade A after the UV treatment. Giant VLP outside *Symbiodinium* cell with characteristics of *Megaviridade* family, as fibril-like structures and multiple layers (a). Icosaedrich VLP near *Symbiodinium* cell (b); arrows show a putative capsid layer reminiscent of *Megaviridae*. Polyhedral VLP inside *Symbiodinium*. Scale bars represents: a –0,2µm, b- 0,5 µm, c – 0,2 µm.

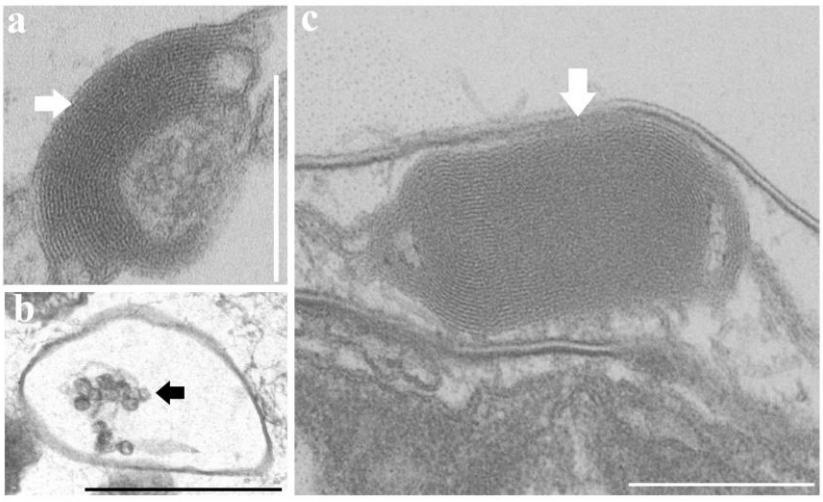


Fig. 6: Transmission electron micrographs of *Symbiodinium* virophage-like particles and asymmetrical structures from UV treated cultures of strain 043D10 of clade A. (a) Multiple layered structure that resembles the parasitic effects of virophages on giant viruses of the *Megaviridae*. (b) Ribbon-like filamentous structure filled with small virophage-like particles outside a *Symbiodinium* cell. (c) Asymmetrical multilayered VLP structure inside *Symbiodinium* cell wall. Scale bars: a: 0,5 µm, b: 0,2 µm, c: 0,2 µm.

CAPÍTULO 3

3 ACQUIRED VIRALITY: INTEGRATION AND GENE TRANSFER BETWEEN GIANT VIRUSES, VIROPHAGES AND *Symbiodinium* FROM THE CORAL HOLOBIONT

Luiz Felipe Benites[1], Paulo Sergio Salomon[1*].

[1] Department of Marine Biology, Biology Institute, Federal University of Rio de Janeiro

Artigo formatado para submissão à revista: **BMC Evolutionary Biology**

Acquired virality: integration and gene transfer between giant viruses, virophages and *Symbiodinium* from the coral holobiont

Luiz Felipe Benites[1], Paulo Sergio Salomon[1*].

[1] Department of Marine Biology, Biology Institute, Federal University of Rio de Janeiro

***Corresponding author**

Email addresses:

LFB:l.felipebenites@gmail.com

PSS: pssalomon@gmail.com

Abstract

Background

Symbiosis between eukaryotes and viruses can lead to viral genes become permanently integrated in their host genome, through horizontal gene transfer. Eukaryotic gene movements could also occur in host-to-virus gene transfer direction. On Earth oceans, viruses are the most abundant and dynamic biological entities. Within marine viral families, one of particular interest encompasses giant viruses that infect both aquatic protists and marine algae, *Megaviridae*, which is associated with hyperparasites, called virophages. Corals could be potential hosts for marine *Megaviridae*-like viruses, since octocorals have horizontally acquired genes from giant viral sources. Dinoflagellates of the genus *Symbiodinium* are also hosted by corals and probably interact with viruses.

Results

Using the draft genome of *Symbiodinium minutum* hosted by corals, we found that *Symbiodinium* has acquired and transferred numerous proteins from and to viral associates through their evolution within coral holobionts.

Conclusions

We suggest that observed horizontally acquired protein coding-genes were transferred through host molecular machinery epigenetic modulation by marine viruses. Our findings provide more evidence of the major influence of symbiosis in enabling the horizontal acquisition of new genes that shape and integrate host-parasite evolutionary trajectories.

Keywords: *Symbiodinium*, giant virus, virophage, horizontal gene transfer, coral reefs

Background

Symbiosis between eukaryotic organisms and viruses can lead to viral genes or whole genomes becoming inherent part of their host through horizontal gene transfer (HGT) [1]. Genes from eukaryotic sources could also be integrated in viral genomes. This non-genealogical transmission of genetic material from one organism to another [2] is an important evolutionary force shaping the evolution in all three domains of life [3; 4]. Once integrated into host chromosomes, acquired genes are vertically inherited, entering the lineage

of recipient organism [5]. HGT events can also be used to reconstruct scenarios of host–virus co-evolution [6]. On earth oceans, viruses are the most abundant biological entities and it is estimated that 20%–40% of oceanic microorganisms are killed by viruses each day [7; 8; 9], making viruses dynamic components of the marine microbial loop [10].

The vast majority of marine viruses are bacteriophages [11], whereas 1 to 10% of this diversity is represented by eukaryotic giant virus [12]. Giant viruses belong to the superfamily of Nucleocytoplasmic Large DNA viruses (NCLDVs) with large double-stranded DNA genomes, whose members infect unicellular eukaryotes and metazoans [13, 14]. Within giant viral families, one of particular interest is *Megaviridae*, that infects both aquatic protists and marine algae [15] and has been proposed as an extension of the *Mimiviridae* family [16]. *Megaviridae* viruses are generally associated with virophages [17]. Virophages are small viral particles which are incapable of replication in the absence of its giant virus host [18].

Giant viruses genomes carry diverse cellular host-originated genes, although there is discussion on the polarization (direction of transfer) [19; 20; 21; 22; 23]. Virus to host HGT (horizontal gene transfer) were presumably more scarce, however it has occured between *Megaviridae* and marine Stramenopiles [24; 25], in the symbiotic Prasinophyceaen algae *Tetraselmis* sp. [26], and within the cryptophyte *Guillardia theta* [23]. Also, endogenized *Phycodnaviridae* genes have been identified in *Chlorella variabilis* [27]. Viral integration were also detected in other 14 algal genomes and transcriptomes, suggesting that viral integrated sequences are scattered and ubiquitous in algae host genomes [28].

Megaviridae are not known to generate integrative proviral forms in host genomes [23]. Integraded *Phycodnaviridae* viruses *Feldmmania* sp. virus and *Ectocarpus silicosus* virus 1 produce viable virion particles [29], whereas virophage integration have been found in mimivirus [18; 30] and in *Phaeocystis globosa* virus [16], which suggests that virophages could act as gene transfer engines during hyperinfections of protist hosts [31]. Recently, it was discovered that the algae *Bigellowiela natans* has numerous endogenous virophages incrusted in its nuclear genome that are being expressed, probably with benefits to the host against exogenous giant viral infections [32]. Virophage endogenization in a provirophage form could be positively selected, since host indirect association, in some cases, is also beneficial, because of its impact on its giant virus host [18; 33].

Claverie et al. [34] propose that corals could be potential hosts for marine *Megaviridae*-like (Mimivirus) viruses, since Octocorals acquired the gene MutS (DNA

mismatch repair protein) in a HGT event between giant viruses and marine bacteria [35]. A possible origin could be from an unknown coral holobiont Megaviridae resident, since large and giant viral sequences have been found associated with corals [36; 37]. Corals are hosted by distinct organisms such as dinoflagellates, bacteria, archaea, fungi and also viruses [38]. In fact, viruses are the most abundant and diverse organisms within the holobiont [39; 40], and possibly play fundamental roles as components of the holobiont community [41]. Dinoflagellates of the genus *Symbiodinium spp.*, in a tighly and profound symbiosis dating from 200 million years [40], allows the holobiont to thrive in tropical oligotrophic waters through absorption of sunlight and delicately processing and transferring of photosynthates within coral tissues [42]. In hospite, coral animals provide conditions that are limited outside of the host [43], however, symbiosis itself could break down, disrupting the animal-algae interaction as a result of stress, manifesting phenotypes as coral bleaching [44]. Today, coral reefs are facing unprecedented levels of degradation especially in times of global climate change, and are at risk to be globally extinct within this century [45; 46].

Environmental triggers as UV radiation and thermal stress promote viral propagation [47; 48; 49], triggers that are also known to be involved in coral diseases [50; 51]. Observations of virus-like particles (VLPs) within *Symbiodinium* and coral led to the hypothesis that latent viruses, likely in a proviral form, could be involved in bleaching and some coral diseases [37; 49; 52; 53]. Currently, there is no *Symbiodinium* viruses isolated or taxonomically identified at genus level, although it was suggested that they are infected by *Phycodnaviridae*-like, +ssRNA and ssDNA [37; 54; 55; 56; 57]. Coral viruses could potentially stabilize coral-algae symbiosis once *Symbiodinium* infected with latent viruses could develope viral resistance [55; 58]. *Phycodnaviridae* and *Mimiviridae* sequences were found in *Monstastraea cavernosa* coral, and in two *Symbiodinium* transcriptomes from different hosts, so in this sense, is possible that these viral symbiotic relationships are ancient ones [57]. In *Symbiodinium* genome [59], 19 genes appear homologous to DVNPs, (*dinoflagellate/viral nucleoprotein*) that was acquired through HGT in dinoflagellates lineage from an ancient viral infection [60; 61], that could represent copies of an integrated and active provirus [29; 62; 63] or “ghosts” of a past giant viruses infections.

In this work we searched for HGT signatures in the draft genome of *S. minutum* from *Orbicella faveolata* (former *Montastraea*) coral [59] and found evidence of integration and multiple transfers of protein-coding genes between giant viruses, virophages and *Symbiodinium*. We suggest that HGT events that lead to movement and horizontal acquisition

of these proteins could be related to host-virus interaction and *Symbiodinium* molecular machinery modulation by marine viruses.

Results and Discussion

We found 210 proteins putatively involved in HGT events on *Symbiodinium* genome (examples in table 1 and complete information in additional file 1), whereas 104 correspond to virus-to-host transfer and 91 correspond host-to-virus transfers. Virophage-to-host transfers corresponds to 15 retrieved putative proteins. On taxonomy level, best hits have occurred with *Megaviridae* (n=77), *Phycodnaviridae* (n=75), Other NCLDVs (n=32) and bacteriophages (n=11). Virophage best hits were with algal *Megaviridae* virophages. It is possible that we overlooked events that have no clear signal of HGT, especially when the timing of the events has occurred too long ago in a lineage. Several lines of evidence support the notion that corals and *Symbiodinium* harbour marine giant viral residents [34; 57] and our results confirm this suggestion, especially because we have found significant hits with virophage-like genes, that are hitherto know to be strictly associated with giant viruses.

Phylogeny and evolution of giant and large viral HGT events

To better understand the evolutionary history of *Symbiodinium* HGTs, and confirm taxonomic identities of viral-like sequences we aligned retrieved and validated sequences along with sequences retrieved from BLAST searches in Genbak, to reconstruct their phylogenies.

Maximum-Likelihood trees were generated with FNIP repeat, hypothetical protein, ABC transporter, E3 ubiquitin-protein ligase and DNA Topoisomerase II representatives of HGT events observed in our searches.

In FNIP repeat phylogeny (Figure 1), *Symbiodinium* clustered together with *Ectocarpus silicosus*, a stramenopile know to harbour the latent virus EsV-1 [29] and with *Cafeteria roebergensis* virus, from *Megaviridae* family that also infects a Stramenopile [64].

Symbiodinium hypothetical protein phylogeny (Figure 2) clustered with Dinophycea and other well knows viral host microalgae, along with *Micromonas pusilla* virus 1 sequence. In a separate clade are marine bacteria. This finding point to a complex scenario of HGT events, and possibly an ancient acquisition of viral genes in Dinophycean lineage from a

Phycodnaviridae-like virus, that evokes probable unknow viral residents of this group of Dinoflagelattes.

In ABC transporter phylogeny (Figure 3), *Symbiodinium* clustered with the marine bacterium *Thalassolituus* sp., one algae, and with the *Megaviridae* representatives Organic Lake phycodnavirus 2 (OLV2) and *Phaeocystis globosa* virus (PgV). Phages, marine bacteria and another giant virus, *Pithovirus sibericum* that is closely related to *Marseilleviridae* [65] are depicted in this tree.

E3 ubiquitin-protein ligase phylogenetic tree (Figure 4) clustered *Symbiodinium* sequences with a *Poxviridae* representative, from the NCLDVs superfamily that infects animals but not algae, nevertheless more related to Mimiviruses [66]. *Feldmannia* sp. virus is show outside this cluster, and more distantly Organic Lake Phycodnavirus 1, from the *Megaviridae* family.

Phylogeny of Topoisomerase II (Figura 5) cluster *Symbiodinium* sequence with representatives of Apicomplexa like *Neospora* sp. and the Chromerid *Vitrella brassicaformis*, organisms described to be associated with corals but unknown to be infected with viruses [67; 68]. Outside this clade there are several viral hosts like *Micromonas* and *Ostreococcus* [69], with *Theobroma* sp. as an exception, since giant viruses HGT was described to have occurred in *Physcomitrella* sp. [70]. More distantly related are Phycodnaviruses representatives at the base of the cluster. This sequence diverged from the previously sequenced NCLDV's *topoisomerase II* observed in *Montastraea cavernosa* virome [57], as it clusters with a separate clade in *Megaviridae*. We interpret this phylogeny as a host-to-virus HGT event because *Symbiodinium* sequence is positioned in a clade of eukariotic organisms rather than viral and is distantly related of the viral topoisomerase II from *M. cavernosa*.

Phylogeny and evolution of virophage-like sequences involved in HGT

Maximum-Likelyhood Tress was generated with DNA-cytosine methylase, GIY-YIG endonuclease and Major Capsid Protein representatives of HGT events observed in our searches.

DNA-cytosine methylase phylogeny of *Symbiodinium* homologues (Figure 6) shows a high divergence from the clade containing a Yellowstone Lake Virophage that infects an unknown giant virus probably associated with algal hosts [71]. Nevertheless, in these clade are *Flavobacterium* sp., a genus that has representatives symbiont of marine algae and corals,

especially diseased ones [72; 73; 74] that clustered with other marine bacteriophages and bacteria.

In GIY-YIG endonuclease phlogeny (Figure 7), *Symbiodinium* sequence cluster with Yellowstone Lake Virophage 6 (YLV6), Mavirus virophage and with the Oomycete *Phytophtora* sp. also knows to have genes with giant viral homology [75]. In this tree, is also depicted a homologue in Cryptophyte algae that have acquired giant virus genes but no virophage genes [23], suggesting a possible virophage integration or association within this cryptophyte.

Phylogeny of the marker gene Major Capsid Protein (Figure 8), *Symbiodinium* sequence had clustered with a representative from *Achromobacter* sp. A a genus that makes associations with corals and marine algae [76; 77], with Mavirus virophage and Sputnik, in a distant and separeted clade, Paenibacilus sp. a genus that has marine relatives with Salpingoeca sp. a marine protist with relatives with gian viral homologous genes are also represented.

Altogether, in these trees, some *Symbiodinium proteins* are represented by long branches, distantly related to known viruses, suggesting they do belong to new viral lineages, yet uncharacterized, in *Symbiodinium*. We have found that *Symbiodinium* genome has numerous homologous proteins, that is know to be involved in epigenetic post-translational modification processes, possible involved in HGT between cellular host and viruses. We further describe selected observed proteins, its related systems in details and hypothesize on their roles in *Symbiodinium*-virus interactions.

Ubiquitin Protease System (UPS)

A successful viral replication requires the hijack and modulation of key cellular pathways as in Ubiquitin-Proteasome System (UPS), by usurping the host protein-degradation machinery [78]. This eukaryotic regulatory system mediates protein turnover, DNA repair, and signal transduction [79]. Ubiquitin pathway is a target for viruses in construction of an intracellular environment that enables their replication [80]. Baculovirus, a giant NCLDV from arthropod hosts, is one of few known viruses to encode Ubiquitin genes [81]. HGT between host and virus of this gene were also detected [82; 83].

Ubiquitin E3 ligases

We have found two homologs of Ubiquitin E3 ligases in *Symbiodinium* genome, possible acquired in a virus-to-host HGT (Table 1 and additional file 1). In host-virus

interactions, a crucial mechanism in hijacking the UPS, is related to these proteins. They modified yet unknow proteins that catalyze ubiquitination [84]. Several ubiquitin-interfering viral proteins interact directly with E3 family proteins [85]. E3 ubiquitin ligases recruits target proteins to the machinery of ubiquitin, and many of viral ubiquitin interferes with cellular pathways that get rewired to serve the viral needs, by host cell functions mimicking. Ubiquitin is also related to selective autophagy in eukaryotes targeting bacteria and viruses [86; 84], also by utilization of E3 ubiquitin ligases [87].

Ubiquitin E3 ligases and viral lytic/latency regulation

UPS is also involved in regulation of viral latency and lytic replication, were a transcription activator protein acts as an E3 ubiquitin ligase, ubiquinating and degrading its own repressors which normally limits lytic replication [88; 89]. In White Spot Syndrome Virus from *Nimaviridae*, viral E3 ubiquitin ligases interacts with host UPS to inhibit virus lytic stage, maintaining latency on its arthropod host, probably through protein phosphorylation [90]. Those cases suggest that host UPS and E3 proteins are crucial in establishing and maintaining viral latency. If *Symbiodinium* indeed harbours a latent viral infection as hypothesized by Wilson et al. [62], the virus could have exploited *Symbiodinium* UPS system utilizing E3 ubiquitin ligases as well.

Kelch Proteins

We found three Kelch Proteins putatively transferred from host to viruses, with best hits with the algae *Crysochromulina* sp. (Table 1 and additional file 1). Kelch proteins are encoded in Poxviruses with ubiquitin ligase activities and involvement in UPS [78]. When these viruses are devoid of Kelch protein, they display altered viral pathogenesis and viral attenuance [91; 92], which suggests that Kelch proteins can manipulate host environment [93].

FNIP repeat protein

We have found six FNIP repeat proteins clearly horizontally acquired from marine giant viruses (Figure 1, Table 1, and additional file 1). FNIP repeats are leucine-rich proteins that mediate protein-protein interactions [94]. These proteins are the most prevalent repetitive elements in *Cafeteria roenbergensis* virus (CroV) genome [64]. They are also present in *Mimivirus*, in the ameobozoan *Dictyostelium discoideum* [95] and in *Ectocarpus silicosus [29]*. *Mimivirus*, FNIP repeats mediates interaction with Ubiquitin pathway probably as a response to host defenses, a general strategy of NCLDVs [13]. The same sequences were

identified in the genome of the brown algae *Saccharina japonica*, suggesting an ancient association of brown algae with viruses leading to a similar HGT event [96].

ANK proteins

We have found 30 proteins possible acquired in a virus-to-host HGT event with best similarities with Phycodnaviruses (Table 1 and additional file 1). ANK repeat is the second largest protein family in *Symbiodinium minutum* genome [61]. ANK proteins play a critical role in reprogramming host signaling cascades [97] and in cell cycle regulation [98]. In *Phycodnaviridae* and Poxviridae ANKs interact with proteins that modulate host cell ubiquitination [85; 99]. ANK proteins also have been described as virulence factors, regulators of viral tropism [100; 101] and in protecting infected cells from virus-induced cell cycle arrest [102]. Symbiotic relationships could promote HGT of ANK proteins to host genomes [103; 104].

Serine/Threonine kinase

Symbiodinium similarities with kinase proteins were found in 8 proteins that have clear signs of virus-to-host HGT and 20 proteins putatively transferred from host to viral associates (Table 1 and additional file 1). Viral infections are associated with apoptosis by modulation of host pathways, used as defense against viruses [105]. In *Iridoviridae*, a serine/threonine kinase is a component of virion particle and is used to induce host apoptosis and protein shutoff that increase its virulence [106]. Protein kinases play important roles in diverse cellular and viral processes, regulating viral morphogenesis and life cycle [107; 108; 109]. They are encoded exclusively by NCLDVs, associated with cytoplasmic viral factories, virion formation [110], and releasing of viral DNA during host entry [111]. Lack of this proteins correlates with accumulation of capsids in the host nucleus [112] and in reduced viral gene expression [113]. High number of similarities with viral proteins strongly suggests HGT events and a virus-*Symbiodinium* interaction trough utilization of kinase proteins as observed here.

DNA J like

Eleven proteins have best similarities with DNAJ-like, and could suggest a giant virus-to-host transfer scenario (Table 1 and additional file 1)**.** DNA J like host proteins interacts with viral capsid proteins [114], and is used by virus to subdue its hosts. These proteins function as cochaperones and regulate heat shock proteins (HSPs) that are related to host resistance and modulation of viral life stages [115]. Chaperones are involved in autophagy and in ubiquitin-dependent proteasomal degradation [116] that suppresses and degrades viral

proteins [117]. DNAJ proteins also assist as cochaperones in stress response and cell cycle progression [118]. It is well known that stresses induce the production of VLPs in *Symbiodinium* and corals [53; 62; 119], so in this sense we could suggest that DNAJ proteins interacting with HSPs that, when expressed in stress situations, could disrupt *Symbiodinium* control of viral latency, triggering viral lytic infection trough signaling cascades similar to the ones described here.

SET domain protein

Only two proteins with matches to SET domain proteins were found, showing a virus-to-host HGT signal (Table 1 and additional file 1). *Phycodnaviridae* members encode this proteins packaged in its virion that methylates histones, and through chromatin degradation and remodeling, repress host transcription, assuming control of the host molecular machinery in order to transcribe viral genes [120]. In latent viruses, transcription is silenced, and host chromatin structure has an active role in controlling the latent genome [121]. Also, chromatin modification of the integrated genomes could be essential for successful latent viral infections [122]. Putative latent viral infection in a proviral state in *Symbiodinium* could follow the same strategy, allowing it to stay integrated in its host genome.

Also other intriguing proteins were found (Table 1 and additional file 1) as MutS (DNA mismatch repair) that is known to have experienced HGT events between octocorals, marine bacteria and giant viruses [35], and one copy of a RNA-dependent RNA polymerase of the dinoflagellate parasite *Heterocapsa circularisquama* virus, suggested to infect *Symbiodinium* [57]. Overall, these hypothetical protein HGT events coupled with their functions predicted to be involved in virus-host interactions reveal a complex and profound insight of *Symbiodinium* biology yet unexplored, and experimentally verifiable in future works.

Conclusion

Our results indicate that *Symbionium* has acquired and transferred multiple gene-coding proteins from and to viral associates trough their evolution. These observations evoke the proviral hypothesis of Wilson et al. [62] who postulates that a virus could be integrated in a latent way in *Symbiodinium* genome entering the lytic cyle when subjected to stress. As we could not find homologs of some structuring proteins as major capsid proteins and DNA viral Polymerases, it remains an open question whether *Symbiodinium* has an active provirus.

Nevertheless, our findings show that HGT events are connected to host-virus interaction, probably trough host molecular machinery epigenetic modulation, that have been occurring in this marine coral symbiotic protist. *Symbiodinium* genome viral and virophage HGT events unveiled here, probably corresponds to a minor fraction of potentially acquired genes, since the draft genome used in this study is ~40% of the predicted whole genome. HGT events might be revealed in its totality in future investigations of whole *Symbiodinium* genomes, as well in other Dinophycean and Alveolate genomic data. If giant viruses and virophages are imperative on *Symbiodinum* and coral holobiont health, then understanding such interactions and its consequences will be of paramount importance. Our findings provide more evidence of the major influence of symbiosis on enabling the horizontal acquisition of new genes or entire organisms, which shapes and integrates their evolutionary trajectories in profound and permanent ways.

Methods

Genomic screening

We use whole predicted proteins of *Symbiodinium minutum* draft genome (available at http://marinegenomics.oist.jp) as a query to perform BLAST searches [123] against all viral proteins downloaded at RefSeq (http://www.ncbi.nlm.nih.gov/refseq/) as an unbiased way to avoid particular types of viruses. For virophage searches, we downloaded data from GenBank [124] and customized a dataset with virophage marker proteins, some of them highly conserved [125]: Major Capsid Protein (MCP), Minor Capsid Protein (mCP), ATPase, Cysteine protease, protein-primed family B DNA polymerase (pPolB), and *GIY*-YIG endonuclease domain, of Mavirus, Organic lake virophage, *Phaeocystis globosa* virophage, Sputnik, Zamilon, and Yellowstone lake virophage.

These two datasets were used separately to conduct BLASTP searches (*E-value* $<10^{-5}$ with 30% minimum identity for viruses and E-value $<10^{-3}$ with 25% minimum identity for virophages) to screen *Symbiodinium* sequences to check for similarity to viral and virophage sequences. Perl scripts were used to collected and organize retrieved BLASTP sequences by taxonomy and amino acid sequence data in order to use in a reciprocal BLASTP search with whole non-redundant protein sequences in National Center for Biotechnology Information (NCBI) database to avoid false positives and to validate that these sequences are indeed viral. We recorded best *E-values* and identities (ID) to viral and virophage best hits

and also non-viral and non-virophage best hits. We also retrieved function and taxonomic assignation for each putative viral protein in *Symbiodinium* genome.

Alignment and Phylogeny

Collected Putative viral protein sequences were aligned with MAFFT [126], manually curated in BioEdit [127] and subjected to PROTTEST [128] to search for the best evolutionary model. Whelan and Goldman (WAG) substitution model was chosen. Phylogenetic trees were constructed with PhyML version 3.0 [129] with 100 replicates for bootstrap support, collapsing branches with bootstrap support of less than 50 %. Trees visualization and edition was done in MEGA5 (Version 3.2.10) [130].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LFB and PSS conceived and designed togheter the study. LFB constructed the databank, conducted data and phylogeny analyses, and drafted the manuscript. PSS advised on data analyses and manuscript preparation. Both authors read and approved the final manuscript.

Acknowledgements

We acknowledge Felipe Coutinho that has provided Perl scripts. We greatly appreciate the helpful discussion, corrections and insightful comments provided by Lílian Caesar. This work was made possible by CAPES/CNPQ funding support.

References

1. Feschotte C, Gilbert C: Endogenous viruses: insights into viral evolution and impact on host biology. *Nat Rev Genet* 2012, 13:283–296.

2. Goldenfeld N, Woese C: Biology's next revolution. *Nature* 2007, 445:369.

3. Boto L: Horizontal gene transfer in evolution: facts and challenges. *Proc Biol Sci* 2010, 277:819–827.

4. Syvanen M: Evolutionary implications of horizontal gene transfer. *Annu Rev Genet* 2012, 46:341–58.

5. Katzourakis A, Gifford RJ: Endogenous viral elements in animal genomes. *PLoS Genet* 2010, 6:e1001191.

6. Patel MR, Emerman M, Malik HS: Paleovirology - Ghosts and gifts of viruses past. *Curr Opin Virol* 2011, 1:304–309.

7. Suttle C a: Marine viruses--major players in the global ecosystem. *Nat Rev Microbiol* 2007, 5:801–812.

8. Nagasaki K: Dinoflagellates, diatoms, and their viruses. *J Microbiol* 2008, 46:235–243.

9. Monier A, Pagarete A, de Vargas C, Allen MJ, Read B, Claverie J-M, Ogata H: Horizontal gene transfer of an entire metabolic pathway between a eukaryotic alga and its DNA virus. *Genome Res* 2009, 19:1441–9.

10. Suttle C a: Viruses in the sea. *Nature* 2005, 437:356–361.

11. Breitbart M: Marine Viruses: Truth or Dare. *Ann Rev Mar Sci* 2012, 4:425–448.

12. Brussaard CPD: Viral control of phytoplankton populations - A review. *J Eukaryot Microbiol* 2004, 51:125–138.

13. Iyer LM, Balaji S, Koonin E V., Aravind L: Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. *Virus Res* 2006, 117:156–184.

14. Yutin N, Koonin E V: Hidden evolutionary complexity of Nucleo-Cytoplasmic Large DNA viruses of eukaryotes. *Virol J* 2012, 9:161.

15. Claverie J-M, Abergel C: Open questions about giant viruses. *Adv Virus Res* 2013, 85:25–56.

16. Santini S, Jeudy S, Bartoli J, Poirot O, Lescot M, Abergel C, Barbe V, Wommack KE, Noordeloos A a M, Brussaard CPD, Claverie J-M: Genome of Phaeocystis globosa virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. *Proc Natl Acad Sci U S A* 2013, 110:10800–5.

17. Yau S, Lauro FM, DeMaere MZ, Brown M V, Thomas T, Raftery MJ, Andrews-Pfannkoch C, Lewis M, Hoffman JM, Gibson J a, Cavicchioli R: Virophage control of antarctic algal host-virus dynamics. *Proc Natl Acad Sci U S A* 2011, 108:6163–6168.

18. Katzourakis A, Aswad A: The origins of giant viruses, virophages and their relatives in host genomes. *BMC Biol* 2014, 12:51.

19. Moreira D, López-García P: Comment on "The 1.2-megabase genome sequence of Mimivirus". *Science* 2005, 308:1114; author reply 1114.

20. Moreira D, Brochier-Armanet C: Giant viruses, giant chimeras: the multiple evolutionary histories of Mimivirus genes. *BMC Evol Biol* 2008, 8:12.

21. Derelle E, Ferraz C, Escande M-L, Eychenié S, Cooke R, Piganeau G, Desdevises Y, Bellec L, Moreau H, Grimsley N: Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga Ostreococcus tauri. *PLoS One* 2008, 3:e2250.

22. Moreau H, Piganeau G, Desdevises Y, Cooke R, Derelle E, Grimsley N: Marine prasinovirus genomes show low evolutionary divergence and acquisition of protein metabolism genes by horizontal gene transfer. *J Virol* 2010, 84:12555–63.

23. Filée J: Multiple occurrences of giant virus core genes acquired by eukaryotic genomes: The visible part of the iceberg? *Virology* 2014, 466-467:53–59.

24. Hingamp P, Grimsley N, Acinas SG, Clerissi C, Subirana L, Poulain J, Ferrera I, Sarmento H, Villar E, Lima-Mendez G, Faust K, Sunagawa S, Claverie J-M, Moreau H, Desdevises Y, Bork P, Raes J, de Vargas C, Karsenti E, Kandels-Lewis S, Jaillon O, Not F, Pesant S, Wincker P, Ogata H: Exploring nucleo-cytoplasmic large DNA viruses in Tara Oceans microbial metagenomes. *ISME J* 2013, 7:1678–95.

25. Colson P, Gimenez G, Boyer M, Fournous G, Raoult D: The giant Cafeteria roenbergensis virus that infects a widespread marine phagocytic protist is a new member of the fourth domain of Life. *PLoS One* 2011a, 6:e18935.

26. Pagarete A, Grébert T, Stepanova O, Sandaa R-A, Bratbak G: Tsv-N1: A Novel DNA Algal Virus that Infects Tetraselmis striata. *Viruses* 2015, 7:3937–3953.

27. Blanc G, Duncan G, Agarkova I, Borodovsky M, Gurnon J, Kuo A, Lindquist E, Lucas S, Pangilinan J, Polle J, Salamov A, Terry A, Yamada T, Dunigan DD, Grigoriev I V, Claverie J-M, Van Etten JL: The Chlorella variabilis NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. *Plant Cell* 2010, 22:2943–55.

28. Wang L, Wu S, Liu T, Sun J, Chi S, Liu C, Li X., Yin J, Wang X, Yu J: Endogenous viral elements in algal genomes. *Acta Oceanologica Sinica* 2014, 33:102-107.

29. Delaroque N, Boland W: The genome of the brown alga Ectocarpus siliculosus contains a series of viral DNA pieces, suggesting an ancient association with large dsDNA viruses. *BMC Evol Biol* 2008, 8:110.

30. Desnues C, La Scola B, Yutin N, Fournous G, Robert C, Azza S, Jardot P, Monteil S, Campocasso A, Koonin E V, Raoult D: Provirophages and transpovirons as the diverse mobilome of giant viruses. *Proc Natl Acad Sci U S A* 2012, 109:18078–18083.

31. La Scola B, Desnues C, Pagnier I, Robert C, Barrassi L, Fournous G, Merchat M, Suzan-Monti M, Forterre P, Koonin E, Raoult D: The virophage as a unique parasite of the giant mimivirus. *Nature* 2008, 455:100–4.

32. Blanc G, Gallot-Lavallée L, Maumus F. Provirophages in the Bigelowiella genome bear testimony to past encounters with giant viruses. *Proc Natl Acad Sci USA* 2015, 112(38):E5318-26.

33. Fischer MG, Suttle CA: A virophage at the origin of large DNA transposons. *Science (80-)* 2011, 332:231–234.

34. Claverie JM, Grzela R, Lartigue A, Bernadac A, Nitsche S, Vacelet J, Ogata H, Abergel C: Mimivirus and Mimiviridae: Giant viruses with an increasing number of potential hosts, including corals and sponges. *J Invertebr Pathol* 2009, 101:172–180.

35. Ogata H, Ray J, Toyoda K, Sandaa R-A, Nagasaki K, Bratbak G, Claverie J-M: Two new subfamilies of DNA mismatch repair proteins (MutS) specifically abundant in the marine environment. *ISME J* 2011, 5:1143–1151.

36. Correa AMS, Welsh RM, Vega Thurber RL: Unique nucleocytoplasmic dsDNA and +ssRNA viruses are associated with the dinoflagellate endosymbionts of corals. *ISME J* 2012, 7:13–27.

37. Soffer N, Brandt ME, Correa AMS, Smith TB, Thurber RV: Potential role of viruses in white plague coral disease. *ISME J* 2014, 8:271–83.

38. Rohwer F, Seguritan V, Azam F, Knowlton N: Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* 2002, 243:1–10.

39. Blackall LL, Wilson B, Van Oppen MJH: Coral—the world's most diverse symbiotic ecosystem. *Mol Ecol* 2015, 24: 5330–5347.

40. Hester ER, Barott KL, Nulton J, Vermeij MJA, Rohwer FL: *Stable and sporadic symbiotic communities of coral and algal holobionts. The ISME Journal* 2015.

41. Oppen MJH, Leong J-A, Gates RD: Coral-virus interactions: A double-edged sword? *Symbiosis* 2009, 47:1–8.

42. Roth MS: The engine of the reef: photobiology of the coral-algal symbiosis. *Front Microbiol* 2014, 5(August):422.

43. Riesgo A, Peterson K, Richardson C, Heist T, Strehlow B, McCauley M, Cotman C, Hill M, Hill A: Transcriptomic analysis of differential host gene expression upon uptake of symbionts: a case study with Symbiodinium and the major bioeroding sponge Cliona varians. *BMC Genomics* 2014, 15:376.

44. Oppen MJH, Lough JM: Introduction: Coral bleaching — patterns, processes, causes and consequences. In *Coral Bleaching*; 2009.

45. Carpenter KE, Abrar M, Aeby G, Aronson RB, Banks S, Bruckner A, Chiriboga A, Cortés J, Delbeek JC, Devantier L, Edgar GJ, Edwards AJ, Fenner D, Guzmán HM, Hoeksema BW, Hodgson G, Johan O, Licuanan WY, Livingstone SR, Lovell ER, Moore J a, Obura DO, Ochavillo D, Polidoro B a, Precht WF, Quibilan MC, Reboton C, Richards ZT, Rogers AD, Sanciangco J, et al.: One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* 2008, 321:560–563.

46. Huang D, Roy K: The future of evolutionary diversity in reef corals. *Philos Trans R Soc Lond B Biol Sci* 2015, 370:20140010.

47. Edgar RS, Lielausis I: Temperature sensitive mutants of phage T4D: their isolation and genetic characterisation. *Genetics* 1964, 49:649 – 662.

48. Jacquet S, Bratbak G: Effects of ultraviolet radiation on marine virus-phytoplankton interactions. *FEMS Microbiol Ecol* 2003, 44:279–289.

49. Davy SK, Burchett SG, Dale AL, Davies P, Davy JE, Muncke C, Hoegh-Guldberg O, Wilson WH: Viruses: agents of coral disease? *Dis Aquat Organ* 2006, 69:101–110.

50. Lesser MP, Farrell JH: Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* 2004, 23:367–377.

51. Bruno JF, Selig ER, Casey KS, Page C a., Willis BL, Harvell CD, Sweatman H, Melendy AM: Thermal stress and coral cover as drivers of coral disease outbreaks. *PLoS Biol* 2007, 5:1220–1227.

52. Wilson WH, Chapman DM: Observation of virus-like particles in thin sections of the plumose anemone, Metridium senile . *J Mar Biol Assoc UK* 2001, 81:879.

53. Lohr J, Munn CB, Wilson WH: Characterization of a latent virus-like infection of symbiotic zooxanthellae. *Appl Env Microbiol* 2007, 73:2976–2981.

54. Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F: Metagenomic analysis of the microbial community associated with the coral Porites astreoides. *Environ Microbiol* 2007, 9:2707–2719.

55. Marhaver KL, Edwards RA, Rohwer F: Viral communities associated with healthy and bleaching corals. *Env Microbiol* 2008, 10:2277–2286.

56. Vega Thurber RL, Barott KL, Hall D, Liu H, Rodriguez-Mueller B, Desnues C, Edwards R a, Haynes M, Angly FE, Wegley L, Rohwer FL: Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. *Proc Natl Acad Sci U S A* 2008, 105:18413–18418.

57. Correa AMS, Welsh RM, Vega Thurber RL: Unique nucleocytoplasmic dsDNA and +ssRNA viruses are associated with the dinoflagellate endosymbionts of corals. *ISME J* 2012, 7:13–27.

58. Villarreal LP: Virus-host symbiosis mediated by persistence. *Symbiosis* 2007, 44:1–9.

59. Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujiwara M, Hamada M, Seidi A, Fujie M, Usami T, Goto H, Yamasaki S, Arakaki N, Suzuki Y, Sugano S, Toyoda A, Kuroki Y, Fujiyama A, Medina M, Coffroth MA, Bhattacharya D, Satoh N: Draft assembly of the symbiodinium minutum nuclear genome reveals dinoflagellate gene structure. *Curr Biol* 2013, 23:1399–1408.

60. Gornik SG, Ford KL, Mulhern TD, Bacic A, McFadden GI, Waller RF: Loss of nucleosomal DNA condensation coincides with appearance of a novel nuclear protein in dinoflagellates. *Curr Biol* 2012, 22:2303–2312.

61. Shinzato C, Mungpakdee S, Satoh N, Shoguchi E: A genomic approach to coral-dinoflagellate symbiosis: Studies of Acropora digitifera and Symbiodinium minutum. *Front Microbiol* 2014, 5(JULY):1–17.

62. Wilson WH, Francis I, Ryan K, Davy SK: Temperature induction of viruses in symbiotic dinoflagellates. *Aquat Microb Ecol* 2001, 25:99–102.

63. Meints RH, Ivey RG, Lee AM, Choi T-J: Identification of two virus integration sites in the brown alga Feldmannia chromosome. *J Virol* 2008, 82:1407–1413.

64. Fischer MG, Allen MJ, Wilson WH, Suttle C a: Giant virus with a remarkable complement of genes infects marine zooplankton. *Proc Natl Acad Sci U S A* 2010, 107:19508–19513.

65. Sharma V, Colson P, Chabrol O, Pontarotti P and Raoult D: *Pithovirus sibericum*, a new *bona fide* member of the "Fourth TRUC" club. *Front. Microbiol* 2015, 6:722.

66. Colson P, Yutin N, Shabalina SA, Robert C, Fournous G, La Scola B, Raoult D, Koonin E V: Viruses with more than 1,000 genes: Mamavirus, a new Acanthamoeba polyphaga mimivirus strain, and reannotation of Mimivirus genes. *Genome Biol Evol* 2011, 3:737–742.

67. Oborník M, Modrý D, Lukeš M, Černotíková-Stříbrná E, Cihlář J, Tesařová M, Kotabová E, Vancová M, Prášil O, Lukeš J: Morphology, Ultrastructure and Life Cycle of Vitrella brassicaformis n. sp., n. gen., a Novel Chromerid from the Great Barrier Reef. *Protist* 2012, 163:306–323.

68. Janouškovec J, Horák A, Barott KL, Rohwer FL, Keeling PJ: Environmental distribution of coral-associated relatives of apicomplexan parasites. *ISME J* 2013, 7:444–7.

69. Bellec L, Clerissi C, Edern R, Foulon E, Simon N, Grimsley N, Desdevises Y: Cophylogenetic interactions between marine viruses and eukaryotic picophytoplankton. *Bmc Evol Biol* 2014, 14:59.

70. Maumus F, Epert A, Nogué F, Blanc G: Plant genomes enclose footprints of past infections by giant virus relatives. *Nat Commun* 2014, 5:4268.

71. Zhou J, Sun D, Childers A, McDermott TR, Wang Y, Liles MR: Three novel virophage genomes discovered from yellowstone lake metagenomes. *J Virol* 2015, 89:1278–85.

72. Miyashita M, Fujimura S, Nakagawa Y, Nishizawa M, Tomizuka N, Nakagawa T, Nakagawa J: Flavobacterium algicola sp. nov., isolated from marine algae. *Int J Syst Evol Microbiol* 2010, 60(Pt 2):344–8.

73. Apprill A, Hughen K, Mincer T: Major similarities in the bacterial communities associated with lesioned and healthy Fungiidae corals. *Environ Microbiol* 2013, 15:2063–72.

74. Garcia GD, Gregoracci GB, Santos EDO, Meirelles PM, Silva GGZ, Edwards R, Sawabe T, Gotoh K, Nakamura S, Iida T, de Moura RL, Thompson FL: Metagenomic analysis of healthy and white plague-affected Mussismilia braziliensis corals. *Microb Ecol* 2013, 65:1076–86.

75. Sharma V, Colson P, Giorgi R, Pontarotti P, Raoult D: DNA-dependent RNA polymerase detects hidden giant viruses in published databanks. *Genome Biol Evol* 2014, 6:1603–1610.

76. Kong MK, Chan K. *A Study on the Bacterial Flora Isolated from Marine Algae*. Botanica Marina 1979, 22:83–98.

77. Bourne D, Iida Y, Uthicke S, Smith-Keune C: Changes in coral-associated microbial communities during a bleaching event. *ISME J* 2008, 2:350–363.

78. Mahon C, Krogan N, Craik C, Pick E: Cullin E3 Ligases and Their Rewiring by Viral Factors. *Biomolecules* 2014, 4:897–930.

79. Ulrich HD, Walden H: Ubiquitin signalling in DNA replication and repair. *Nat Rev Mol Cell Biol* 2010, 11:479–489.

80. Gao G, Luo H: The ubiquitin-proteasome pathway in viral infections. *Can J Physiol Pharmacol* 2006, 84:5–14.

81. Reilly LM, Guarino LA: The viral ubiquitin gene of Autographa californica nuclear polyhedrosis virus is not essential for viral replication. *Virology* 1996, 218:243–7.

82. Hughes AL, Friedman R: Genome-wide survey for genes horizontally transferred from cellular organisms to baculoviruses. *Mol Biol Evol* 2003, 20:979–987.

83. Ma SS, Zhang Z, Xia HC, Chen L, Yang YH, Yao Q, Chen KP2: Evolutionary analysis of the ubiquitin gene of baculovirus and insect hosts. *Ma Genet Mol Res.* 2015, 3:9963-73.

84. Schreiber A, Peter M: Substrate recognition in selective autophagy and the ubiquitin-proteasome system. *Biochim Biophys Acta - Mol Cell Res* 2014, 1843:163–181.

85. Noel E a, Kang M, Adamec J, Van Etten JL, Oyler G a: Chlorovirus skp1-binding ankyrin repeat protein interplay and mimicry of cellular ubiquitin ligase machinery. *J Virol* 2014, 88:13798–810.

86. Thurston TLM, Wandel MP, von Muhlinen N, Foeglein Á, Randow F: Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* 2012, 482:414–418.

87. Orvedahl A, MacPherson S, Sumpter R, Tallóczy Z, Zou Z, Levine B: Autophagy protects against Sindbis virus infection of the central nervous system. *Cell Host Microbe* 2010, 7:115–27.

88. Yang Z, Yan Z, Wood C: Kaposi's sarcoma-associated herpesvirus transactivator RTA promotes degradation of the repressors to regulate viral lytic replication. *J Virol* 2008, 82:3590–603.

89. Gustin JK, Moses A V, Früh K, Douglas JL: Viral takeover of the host ubiquitin system. *Front Microbiol* 2011, 2:161.

90. He F, Kwang J: Identification and characterization of a new E3 ubiquitin ligase in white spot syndrome virus involved in virus latency. *Virol J* 2008, 5:151.

91. Kochneva G, Kolosova I, Maksyutova T, Ryabchikova E, Shchelkunov S: Effects of deletions of kelch-like genes on cowpox virus biological properties. *Arch Virol* 2005, 150:1857–70.

92. Wilton B a, Campbell S, Van Buuren N, Garneau R, Furukawa M, Xiong Y, Barry M: Ectromelia virus BTB/kelch proteins, EVM150 and EVM167, interact with cullin-3-based ubiquitin ligases. *Virology* 2008, 374:82–99.

93. Barry M, van Buuren N, Burles K, Mottet K, Wang Q, Teale A: Poxvirus exploitation of the ubiquitin-proteasome system. *Viruses* 2010, 2:2356–80.

94. Kobe B: The leucine-rich repeat as a protein recognition motif. *Curr Opin Struct Biol* 2001, 11:725–732.

95. O'Day DH, Suhre K, Myre M a., Chatterjee-Chakraborty M, Chavez SE: Isolation, characterization, and bioinformatic analysis of calmodulin-binding protein cmbB reveals a novel tandem IP22 repeat common to many Dictyostelium and Mimivirus proteins. *Biochem Biophys Res Commun* 2006, 346:879–888.

96. Ye N, Zhang X, Miao M, Fan X, Zheng Y, Xu D, Wang J, Zhou L, Wang D, Gao Y, Wang Y, Shi W, Ji P, Li D, Guan Z, Shao C, Zhuang Z, Gao Z, Qi J, Zhao F: Saccharina genomes provide novel insight into kelp biology. *Nat Commun* 2015, 6:6986.

97. Werden SJ, McFadden G: The role of cell signaling in poxvirus tropism: the case of the M-T5 host range protein of myxoma virus. *Biochim Biophys Acta* 2008, 1784:228–37.

98. Siozios S, Ioannidis P, Klasson L, Andersson SGE, Braig HR, Bourtzis K: The Diversity and Evolution of Wolbachia Ankyrin Repeat Domain Genes. *PLoS One* 2013, 8:e55390.

99. Sonnberg S, Seet BT, Pawson T, Fleming SB, Mercer AA: Poxvirus ankyrin repeat proteins are a unique class of F-box proteins that associate with cellular SCF1 ubiquitin ligase complexes. *Proc Natl Acad Sci U S A* 2008, 105:10955–60.

100. Blanié S, Mortier J, Delverdier M, Bertagnoli S, Camus-Bouclainville C: M148R and M149R are two virulence factors for myxoma virus pathogenesis in the European rabbit. *Vet Res* 2009, 40:14.

101. Werden SJ, Lanchbury J, Shattuck D, Neff C, Dufford M, McFadden G: The myxoma virus m-t5 ankyrin repeat host range protein is a novel adaptor that coordinately links the cellular signaling pathways mediated by Akt and Skp1 in virus-infected cells. *J Virol* 2009, 83:12068–83.

102. Johnston JB, Wang G, Barrett JW, Nazarian SH, Colwill K, Moran M, McFadden G: Myxoma virus M-T5 protects infected cells from the stress of cell cycle arrest through its interaction with host cell cullin-1. *J Virol* 2005, 79:10750–63.

103. Werren JH, Richards S, Desjardins CA, Niehuis O, Gadau J, Colbourne JK: Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science (80-)* 2010, 327:343–348.

104. Herbert M, Squire C, Mercer A: Poxviral Ankyrin Proteins. *Viruses* 2015, 7:709–738.

105. Hay S, Kannourakis G: A time to kill: Viral manipulation of the cell death program. *J Gen Virol* 2002, 83:1547–1564.

106. Chitnis NS, Paul ER, Lawrence PK, Henderson CW, Ganapathy S, Taylor P V., Virdi KS, D'Costa SM, May a. R, Bilimoria SL: A Virion-Associated Protein Kinase Induces Apoptosis. *J Virol* 2011, 85:13144–13152.

107. Liu K, Lemon B, Traktman P: The Dual-Specificity Phosphatase Encoded by Vaccinia Virus, VH1, Is Essential for Viral Transcription In Vivo and In Vitro. *J Virol* 1995, 69:7823–7834.

108. Szajner P, Jaffe H, Weisberg AS, Moss B: A complex of seven vaccinia virus proteins conserved in all chordopoxviruses is required for the association of membranes and viroplasm to form immature virions. *Virology* 2004, 330:447–459.

109. Van Vliet K, Mohamed MR, Zhang L, Villa NY, Werden SJ, Liu J, McFadden G: Poxvirus proteomics and virus-host protein interactions. *Microbiol Mol Biol Rev* 2009, 73:730–749.

110. Banham AH, Smith GL: Vaccinia virus gene B1R encodes a 34-kDa serine/threonine protein kinase that localizes in cytoplasmic factories and is packaged into virions. *Virology* 1992, 191(0042-6822 SB - M SB - X):803–812.

111. Wilson ME, Consigli RA: Functions of a protein kinase activity associated with purified capsids of the granulosis virus infecting Plodia interpunctella. *Virology* 1985, 143:526–35.

112. Wolf DG, Courcelle CT, Prichard MN, Mocarski ES: Distinct and separate roles for herpesvirus-conserved UL97 kinase in cytomegalovirus DNA synthesis and encapsidation. *Proc Natl Acad Sci USA* 2001, 98:1895–1900.

113. Krosky PM, Baek M-C, Coen DM: The human cytomegalovirus UL97 protein kinase, an antiviral drug target, is required at the stage of nuclear egress. *J Virol* 2003, 77:905–914.

114. Hofius D, Maier AT, Dietrich C, Jungkunz I, Bornke F, Maiss E, Sonnewald U: Capsid Protein-Mediated Recruitment of Host DnaJ-Like Proteins Is Required for Potato Virus Y Infection in Tobacco Plants. *J Virol* 2007, 81:11870–11880.

115. Attar N: Viral infection: De-chaperoning antivirals. Nat Rev Microbiol 2015, 14(1):2-3.

116. Hayes SA, Dice JF: Roles of molecular chaperones in protein degradation. *J Cell Biol* 1996, 132:255–8.

117. Sohn SY, Kim SB, Kim J, Ahn BY: Negative regulation of hepatitis B virus replication by cellular Hsp40/DnaJ proteins through destabilization of viral core and X proteins. *J Gen Virol* 2006, 87:1883–1891.

118. Susin MF, Baldini RL, Gueiros-Filho F, Gomes SL: GroES/GroEL and DnaK/DnaJ have distinct roles in stress responses and during cell cycle progression in Caulobacter crescentus. *J Bacteriol* 2006, 188:8044–8053.

119. Lawrence SA, Davy, JE, Wilson, WH, Davy SK: Latent virus-like infections are present in a diverse range of *Symbiodinium* spp. (Dinoflagellata). *Journal of Phycology* 2014, 50: 977-997.

120. Manzur KL, Farooq A, Zeng L, Plotnikova O, Koch AW, Sachchidanand, Zhou M-M: A dimeric viral SET domain methyltransferase specific to Lys27 of histone H3. *Nat Struct Biol* 2003, 10:187–196.

121. Lieberman PM: Chromatin organization and virus gene expression. *Journal of Cellular Physiology* 2008:295–302.

122. Williams S a F, Greene WC: Host factors regulating post-integration latency of HIV. *Trends Microbiol* 2005, 13:137–9.

123. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research* 1997:3389–3402.

124. Benson D a., Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW: GenBank. *Nucleic Acids Res* 2013, 41:36–42.

125. Yutin N, Colson P, Raoult D, Koonin E V: Mimiviridae: clusters of orthologous genes, reconstruction of gene repertoire evolution and proposed expansion of the giant virus family. *Virol J* 2013, 10:106.

126. Katoh K, Kuma K, Toh H, Miyata T: MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 2005, 33:511–518.

127. Hall T: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 1999:95–98.

128. Abascal F, Zardoya R, Posada D: ProtTest: Selection of best-fit models of protein evolution. *Bioinformatics* 2005, 21:2104–2105.

129. Guindon S, Lethiec F, Duroux P, Gascuel O: PHYML Online--a web server for fast maximum likelihood-based phylogenetic inference. *Nucleic Acids Res* 2005, 33(Web Server issue):W557–9.

130. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S: MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011, 28:2731–9.

Figure Legends

Table 1. Examples of *Symbiodinium* proteins putatively involved in HGT events.

Virus-to-host, Host-to-virus and Virophage-to-host transfers are indicated.

***Symbiodinium* protein**	**Best viral hit**	**E-value**	**Identity**	**Best Non-viral hit**	**E-value**	**Identity**	**Function**
			Virus-to-Host HGT				
symbB.v1.2.000034.t3|scaffold12.1|size699752|14	Organic Lake phycodnavirus 2	9,00E-17	29%	*Bacillus mannanilyticus*	6,00E-46	44%	ABC transporter
symbB.v1.2.001036.t1|scaffold56.1|size371842|7	*P. bursaria* Chlorella virus	4,00E-26	48%	*Trichomonas vaginalis*	5,00E-29	41%	ankyrin repeat PH and SEC7
symbB.v1.2.014754.t1|scaffold1081.1|size139474|11	*P. bursaria* Chlorella virus	0.0	44%	*Ostreococcus tauri*	0.0	50%	DNA Topoisomerase II
symbB.v1.2.000904.t1|scaffold27.1|size414596|12	Megavirus courdo11	6,00E-13	50%	*Chondrus crispus*	1,00E-32	32%	DnaJ-like protein
symbB.v1.2.001385.t1|scaffold67.1|size356791|11	*Pandoravirus salinus*	3,00E-06	38%	*Perkinsus marinus*	9,00E-20	27%	E3 ubiquitin-protein ligase
symbB.v1.2.000822.t1|scaffold49.1|size384103|6	*Cafeteria roenbergensis* virus	2,00E-60	48%	*Ectocarpus siliculosus*	3,00E-53	42%	FNIP repeat
symbB.v1.2.001648.t1|scaffold86.1|size363240|7	*Micromonas pusilla* virus	3,00E-63	55%	*Symbiodinium* sp. *C3*	7,00E-97	74%	hypothetical protein
symbB.v1.2.000396.t1|scaffold30.1|size407774|12	*Cotesia glomerata* bracovirus	1,00E-22	51%	*Emiliania huxleyi*	5,00E-25	46%	putative histone 4
symbB.v1.2.001365.t1|scaffold71.1|size352893|8	*Heterocapsa circularisquama* virus	2,00E-15	28%	*Toxoplasma gondii*	0,004	29%	RNA-dependent RNA polymerase
symbB.v1.2.001368.t1|scaffold71.1|size352893|11	*P. bursaria* Chlorella virus	4E-15	40%	*Pelodictyon phaeoclathratiforme*	8E-14	34%	SET domain- N-methyltransferase
			Host-to-virus HGT				
symbB.v1.2.000042.t1|scaffold12.1|size699752|22	*P. bursaria* Chlorella virus	2,00E-05	29%	*Karlodinium veneficum*	6,00E-12	49%	ribosomal protein S27
symbB.v1.2.000886.t1|scaffold51.1|size380723|5	*Feldmannia irregularis* virus	6,00E-35	27%	*Vitrella brassicaformis*	4,00E-47	35%	putative serine/threonine protein kinase
symbB.v1.2.000972.t1|scaffold43.1|size391093|1	*Pandoravirus dulcis*	2,00E-38	33%	*Phytophthora sojae*	8,00E-86	44%	tRNA threonylcarbamoyl adenosine
symbB.v1.2.026874.t1|scaffold2720.1|size72291|3	*Chrysochromulina ericina* virus	8E-17	23%	*Vitrella brassicaformis*	6E-73	51%	MutS protein
symbB.v1.2.000034.t1|scaffold12.1|size699752|14	Organic Lake phycodnavirus 2	4,00E-99	46%	*Crypthecodinium cohnii*	2,00E-113	54%	heat shock protein Hsp70 family protein
			Virophage-to-host HGT				
symbB.v1.2.001639.t1|scaffold90.1|size339071|8	Yellowstone Lake virophage 5	6,00E-21	38%	*Bacillus flexus*	2,00E-19	33%	DNA-cytosine methylase
symbB.v1.2.038210.t1|scaffold5874.1|size25606|2	Yellowstone Lake virophage 6	4,00E-05	37%	*Lasius niger*	6,00E-13	30%	GIY-YIG endonuclease
symbB.v1.2.019352.t1|scaffold1564.1|size111405|3	Yellowstone Lake virophage 5	4,00E-05	30%	*Mycobacterium ulcerans*	5.2	29%	Putative packaging ATPase
symbB.v1.2.027015.t1|scaffold2744.1|size71806|1	Yellowstone Lake virophage 5	0,001	31%	*Achromobacter xylosoxidans*	2.5	38%	Major Capsid Protein

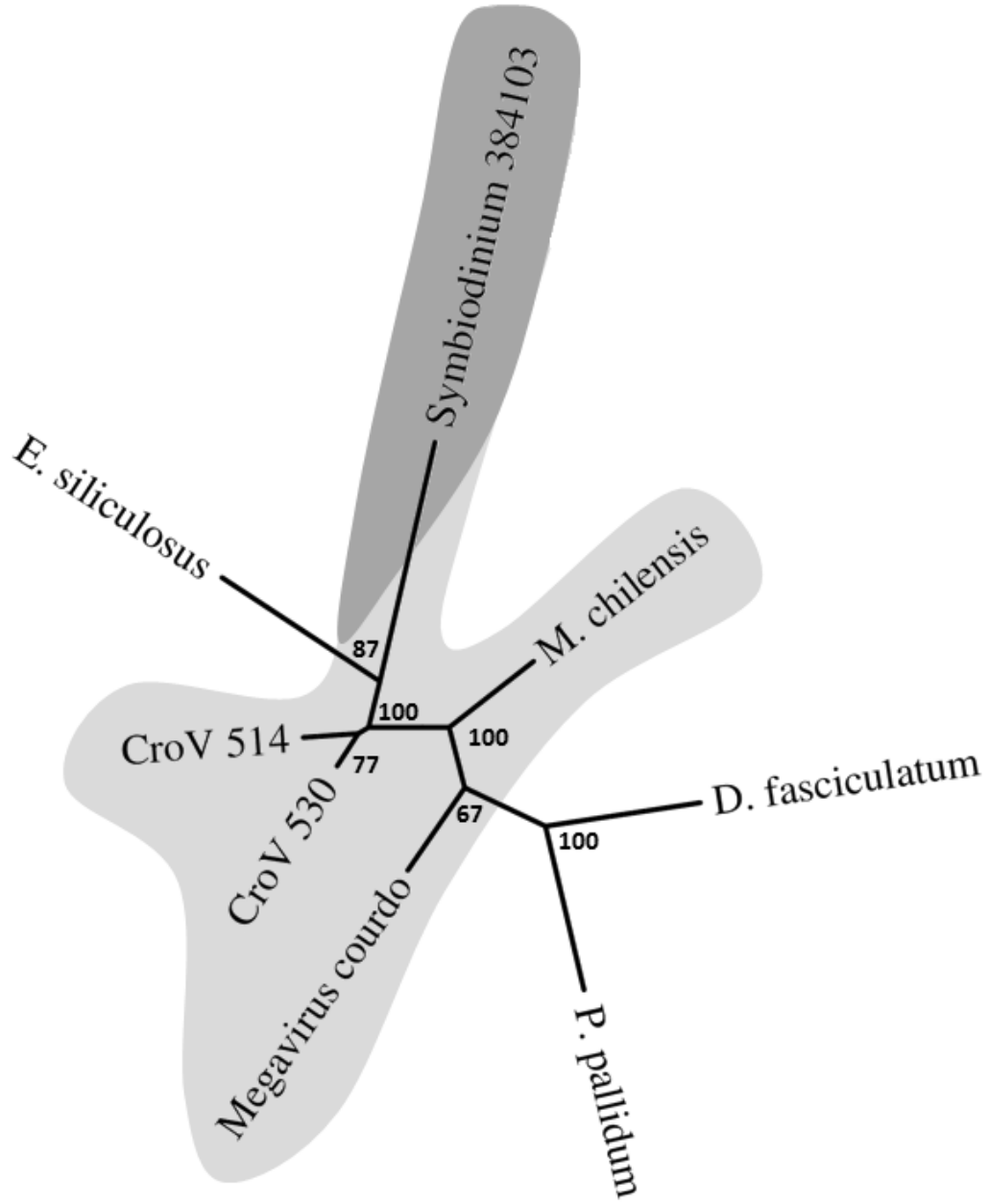


Figure 1. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* FNIP repeat with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: E. siliculosus, *Ectocarpus siliculosus*; CroV, *Cafeteria roenbergensis* vírus; M. chilensis, *Megavirus chilensis*; P. pallidum, *Polysphondylium pallidum; D. fasciculatum, Dictyostelium fasciculatum and* Symbiodinium 384103 corresponds to scaffold49.1|size384103|6.

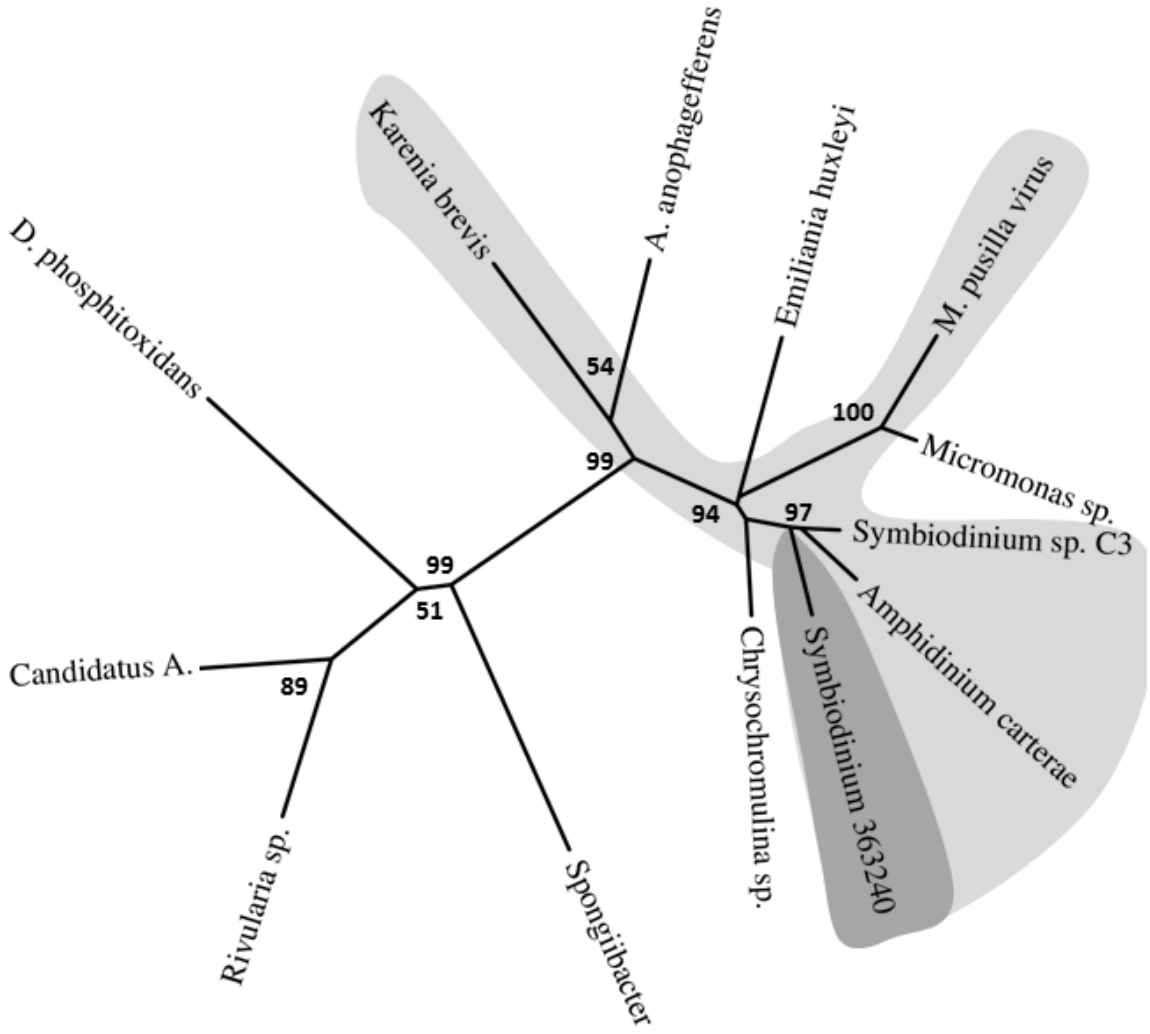


Figure 2. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* Hypothetical protein with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: D. phosphitoxidans, *Desulfotignum phosphitoxidans*; Candidatus A. palustre, Candidatus *Achromatium palustre*; A. anophagefferens, *Aureococcus anophagefferens*; and Symbiodinium 363240 corresponds to scaffold86.1|size363240|7.

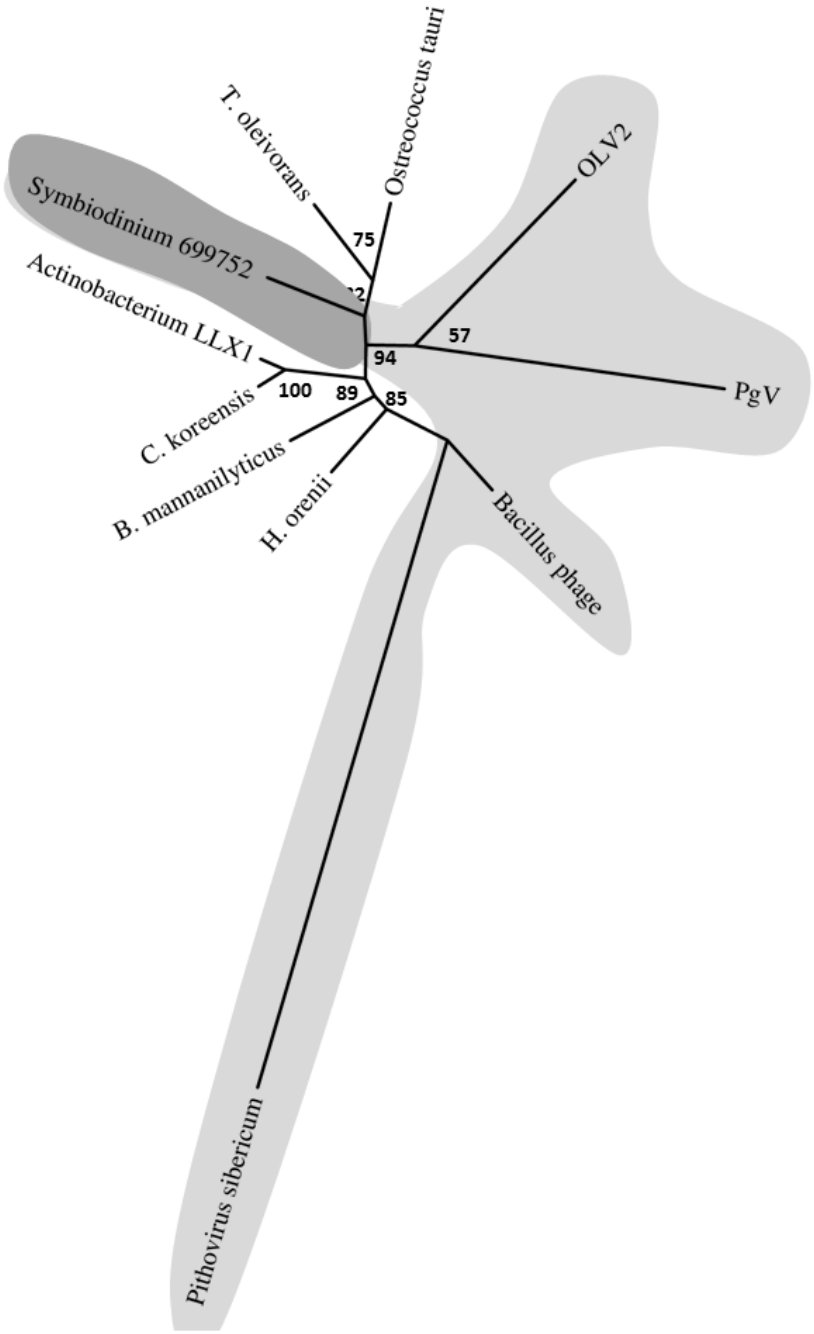


Figure 3. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* ABC transporter with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: H. orenii, *Halothermothrix orenii*; B. mannanilyticus, *Bacillus mannanilyticus*; C. koreensis, *Catelliglobosispora koreensis*; T. oleivorans, *Thalassolituus oleivorans*; OLV2, Organic Lake phycodnavirus 2; PgV, *Phaeocystis globosa* vírus and Symbiodinium 699752 corresponds to scaffold12.1|size699752|14.

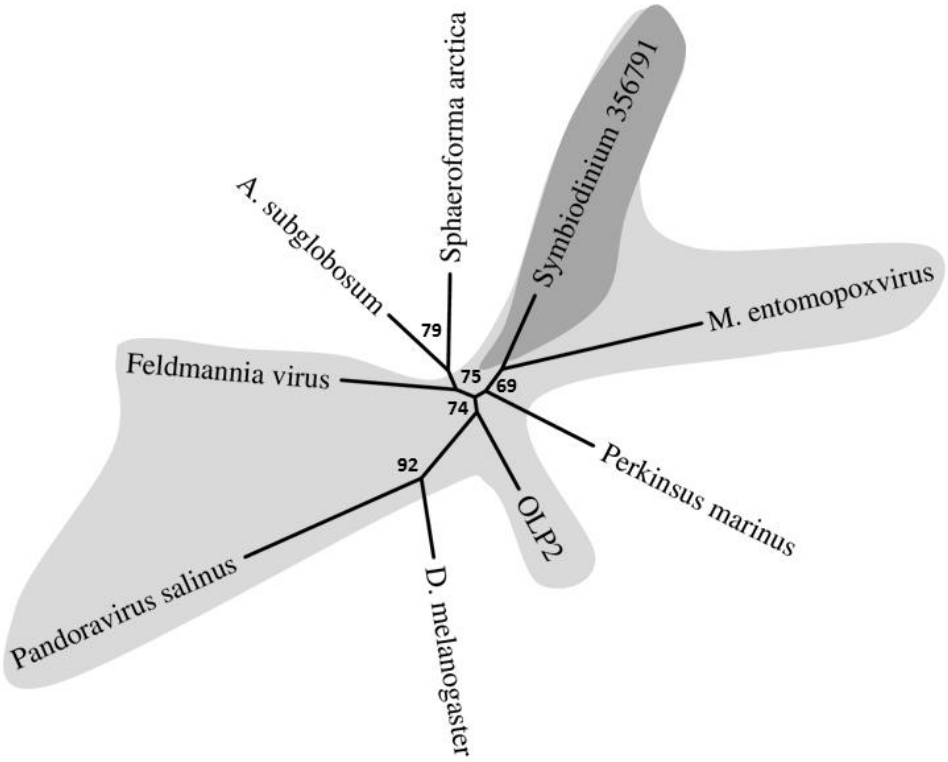


Figure 4. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* E3 ubiquitin-protein ligase with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: OLPV2, Organic Lake phycodnavirus 2; D. melanogaster, *Drosophila melanogaster*; A. subglobosum, *Acytostelium subglobosum*; M. entomopoxvirus, *Melanoplus sanguinipes* entomopoxvirus and Symbiodinium 356791 corresponds to scaffold67.1|size356791|11.

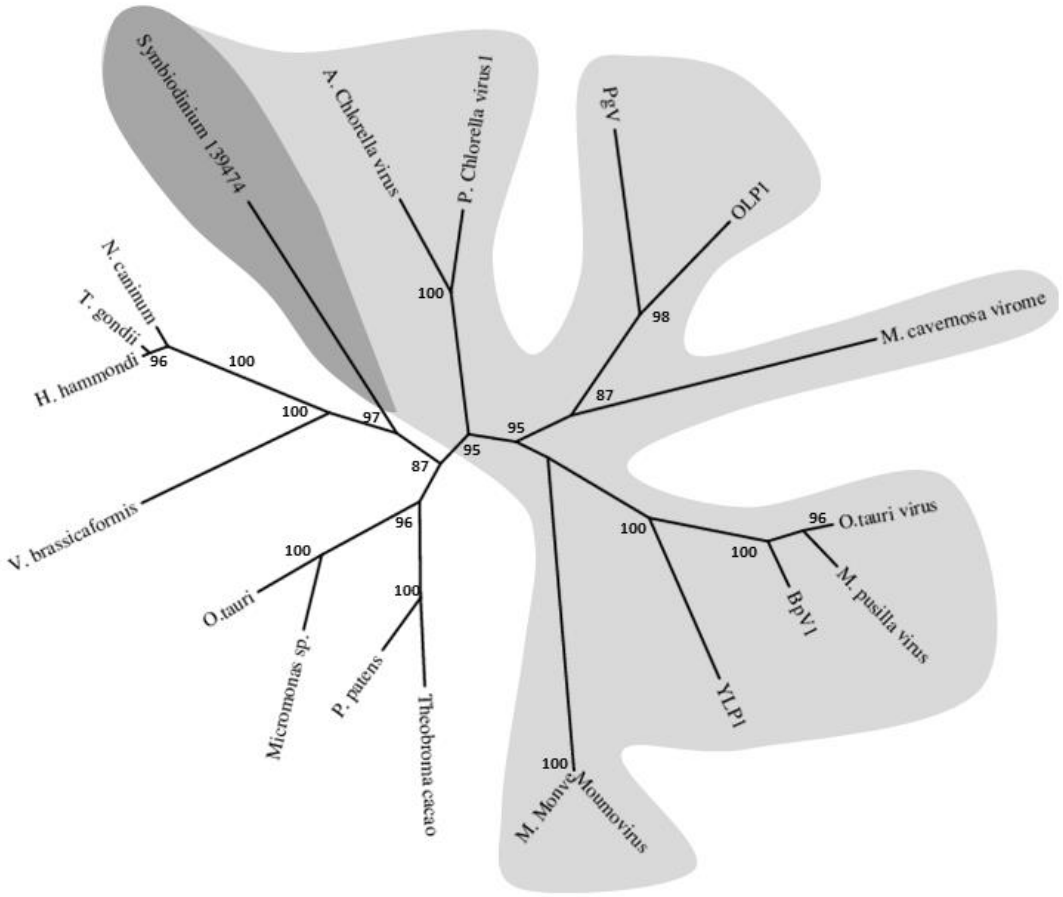


Figure 5. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* Topoisomerase II with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: O. tauri vírus, *Ostreococcus tauri* virus; M. pusilla virus, *Micromonas pusilla* vírus; BpV1, *Bathycoccus* sp. RCC1105 virus; YLP1, Yellowstone lake phycodnavirus 1; M. Monve, Moumovirus monve; Moumovirus, *Acanthamoeba polyphaga* moumouvirus P. patens, *Physcomitrella patens*; O. tauri; *Ostreococcus tauri;* V. brassicaformis, *Vitrella brassicaformis*; H. hammondi, *Hammondia hammondi*; T. gondii, *Toxoplasma gondii*; A. Chlorella vírus, *Acanthocystis turfacea* Chlorella virus; P. Chlorella virus 1, Paramecium bursaria Chlorella virus 1; PgV, *Phaeocystis globosa* virus; OLP1, Organic Lake phycodnavirus 1; M. cavernosa virome, *Montastraea cavernosa* colony-associated virus topoisomerase II-like mRNA, and Symbiodinium 139474 corresponds to scaffold1081.1|size139474|11.

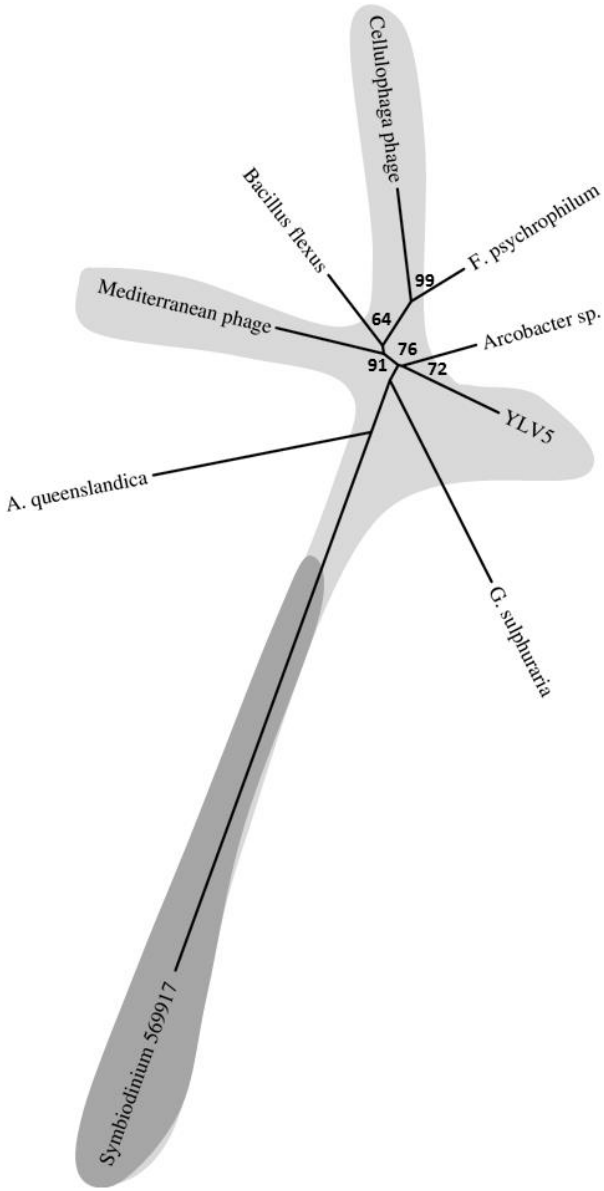


Figure 6. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* DNA-cytosine methylase with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: YLV5, Yellowstone Lake virophage 5; G. sulfuraria, *Galdieria sulfuraria*; A. queenslandica, *Amphimedon queenslandica;* F. psychrophilum, *Flavobacterium psychrophilum*, and Symbiodinium 569917 corresponds to scaffold6.1|size569917|16.

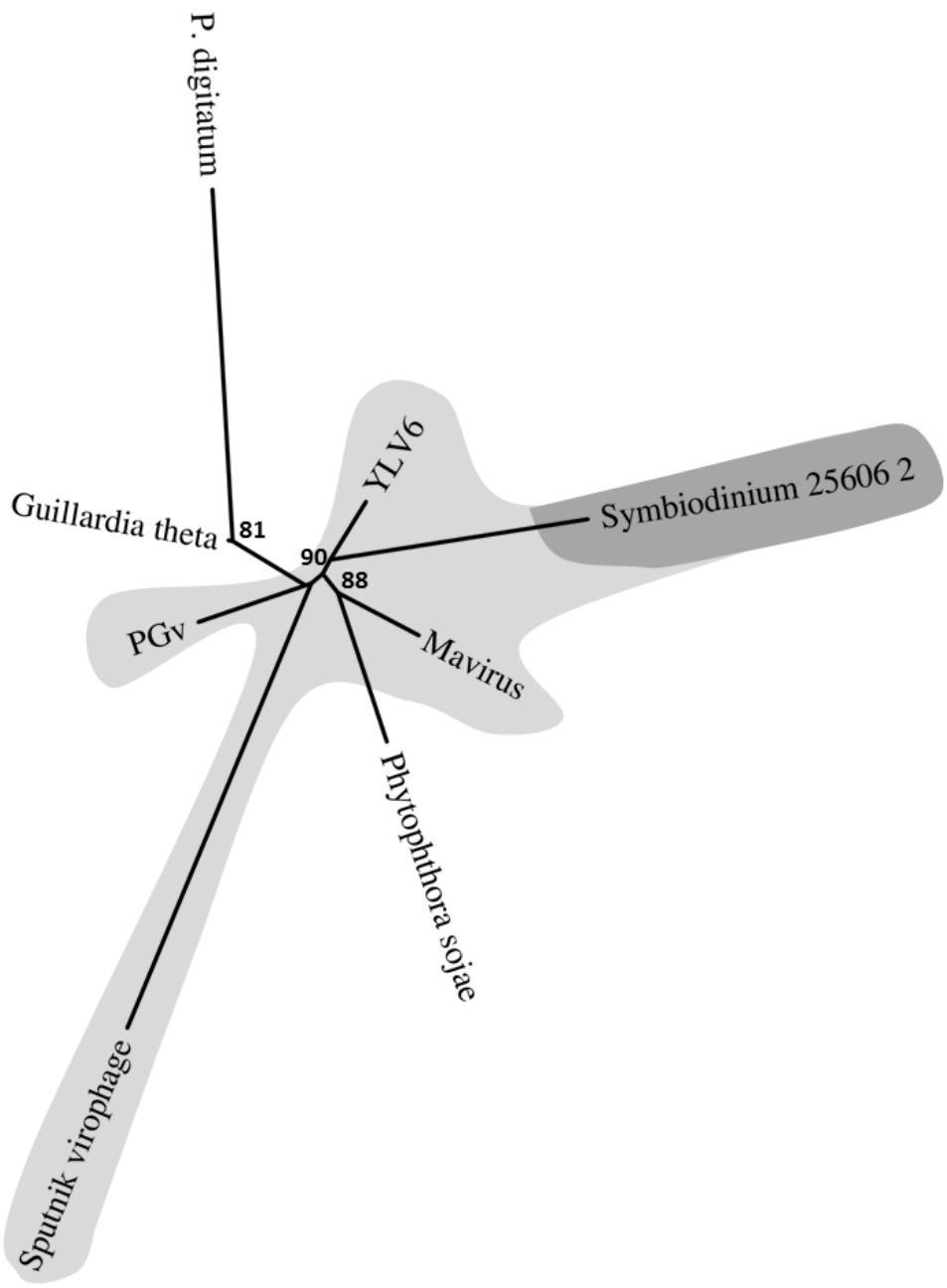


Figure 7. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* GIY-YIG endonuclease with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Species abbreviations: PGv, *Phaeocystis globosa* virophage; *P. digitatum*, *Penicillium digitatum*, YLV6, Yellowstone Lake virophage 6, and Symbiodinium 25606 2 corresponds to scaffold916.1|size152367|3.

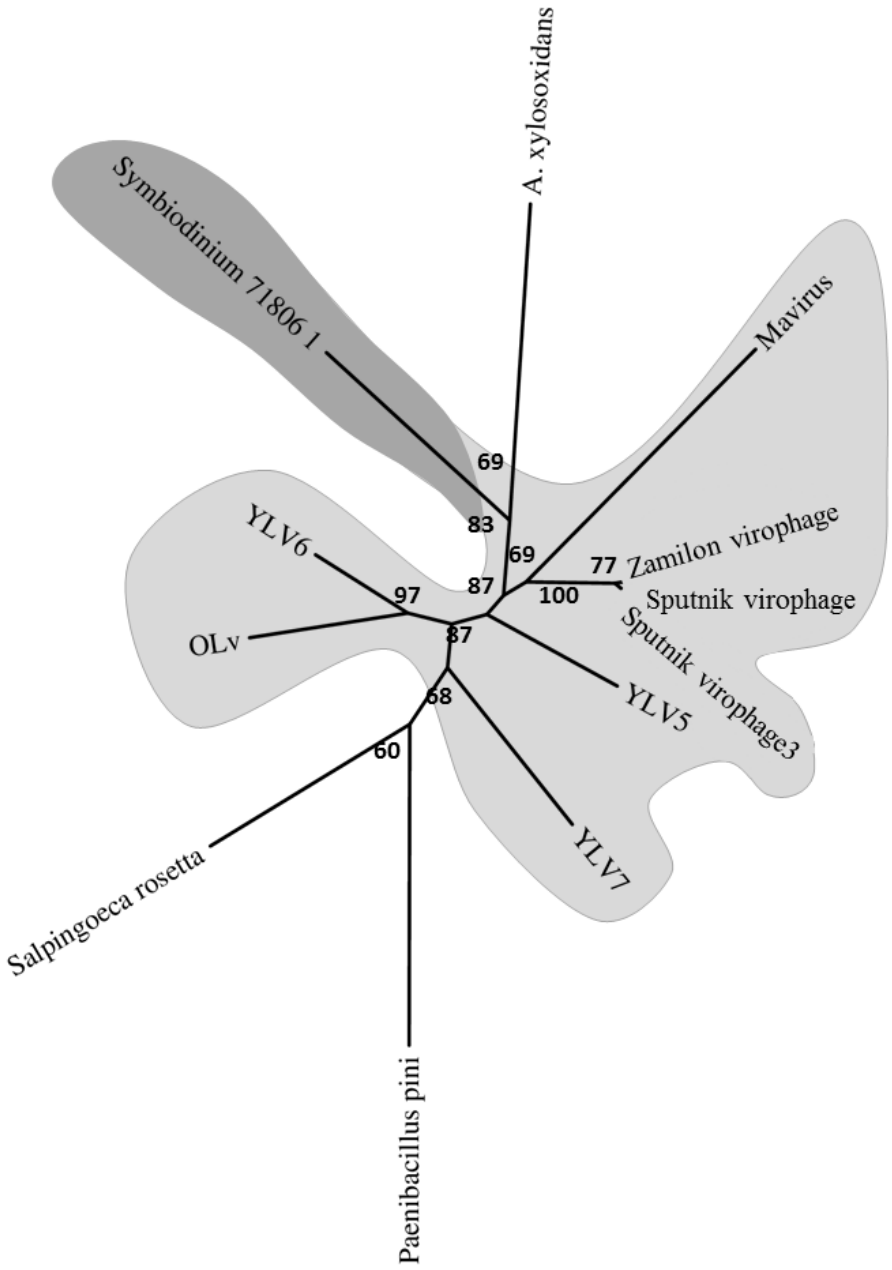


Figure 8. Maximum Likelihood phylogenetic tree generated with *Symbiodinium* major capsid protein (MCP) with 100 bootstrap replicates.

Branches with bootstrap support less than 0.5 were collapsed. Dark gray areas indicate *Symbiodinium* and light gray areas viral genes. Branches with bootstrap support less than 0.5 were collapsed. Gray areas indicate *Symbiodinium* and viral genes. Species abbreviations: YLV5, Yellowstone Lake virophage 5; YLV7, Yellowstone Lake virophage 7, OLv, Organic Lake virophage,; YLV6, Yellowstone Lake virophage 6; A. *xylosoxidans*, Achromobacter *xylosoxidan*, and Symbiodinium 718061 corresponds to scaffold2744.1|size71806|1.

Additional Material 1

Table 1. Complete set of *Symbiodinium* proteins putatively involved in HGT events.

Virus-to-host, Host-to-virus and Virophage-to-host transfers are indicated.

Symbiodinium protein	Best viral hit	E-value	Identity	Best Non-viral hit	E-value	Identity	Function
			Virus to Host HGT				
symbB.v1.2.000034.t3|scaffold12.1|size699752|14	Organic Lake phycodnavirus 2	9,00E-17	29%	*Bacillus mannanilyticus*	6,00E-46	44%	ABC transporter
symbB.v1.2.000450.t1|scaffold32.1|size405148|23	Organic Lake phycodnavirus 1	9,00E-05	25%	*Bacillus* sp.	1,00E-12	23%	ABC transporter related protein
symbB.v1.2.000300.t1|scaffold6.1|size569917|3	*P. bursaria* Chlorella virus	4,00E-14	39%	*Wolbachia endosymbiont wPip*	2,00E-17	45%	Ankirin repeat protein
symbB.v1.2.001212.t1|scaffold66.1|size357995|15	*P. bursaria* Chlorella virus	1,00E-11	30%	*Wolbachia endosymbiont of D. melanogaster*	5,00E-17	40%	Ankirin repeat protein
symbB.v1.2.000627.t2|scaffold26.1|size418576|42	*P. bursaria* Chlorella virus	7,00E-22	38%	*Hydra vulgaris*	1,00E-25	34%	Ankirin repeat protein
symbB.v1.2.001370.t1|scaffold71.1|size352893|13	*Acanthamoeba polyphaga* mimivirus	5,00E-37	32%	*Aedes aegypti*	3,00E-63	36%	Ankirin repeat protein
symbB.v1.2.000946.t1|scaffold45.1|size390604|9	*Megavirus chilensis*	6,00E-08	31%	*Metallosphaera yellowstonensis*	2,00E-10	45%	Ankirin repeat protein
symbB.v1.2.000946.t1|scaffold45.1|size390604|9	*Megavirus chilensis*	6,00E-08	31%	*Metallosphaera yellowstonensis*	2,00E-10	45%	Ankirin repeat protein
symbB.v1.2.000109.t1|scaffold13.1|size649204|20	*P. bursaria* Chlorella virus	4,00E-04	32%	*Burkholderia grimmiae*	9,00E-09	39%	Ankirin repeat protein
symbB.v1.2.000285.t1|scaffold24.1|size427761|1	*P. bursaria* Chlorella virus	3,00E-06	32%	*Sulfolobus islandicus*	9,00E-09	31%	Ankirin repeat protein
symbB.v1.2.001136.t1|scaffold62.1|size503095|2	*P. bursaria* Chlorella virus	1,00E-07	30%	*Vitrella brassicaformis*	1,00E-22	30%	Ankirin repeat protein
symbB.v1.2.001394.t1|scaffold67.1|size356791|20	*Acanthamoeba polyphaga* mimivirus	2,00E-07	31%	endosymbiont of *Acanthamoeba* sp. *UWC36*	4,00E-11	38%	Ankirin repeat protein
symbB.v1.2.000224.t1|scaffold4.1|size633627|6	*Acanthamoeba polyphaga* mimivirus	1,00E-07	25%	*Talaromyces marneffei*	4,00E-15	24%	Ankirin repeat protein
symbB.v1.2.000123.t1|scaffold15.1|size524077|14	*P. bursaria* Chlorella virus	6,00E-28	42%	*Aedes aegypti*	1,00E-158	35%	Ankirin repeat protein
symbB.v1.2.000678.t1|scaffold35.1|size400642|22	*P. bursaria* Chlorella virus	2,00E-27	34%	*Lingula anatina*	7,00E-60	36%	ankyrin repeat PH and SEC7
symbB.v1.2.001201.t1|scaffold66.1|size357995|4	*P. bursaria* Chlorella virus	7,00E-24	43%	*Trichomonas vaginalis*	1,00E-30	51%	ankyrin repeat PH and SEC7
symbB.v1.2.001206.t1|scaffold66.1|size357995|9	*P. bursaria* Chlorella virus	3,00E-14	43%	*Trichomonas vaginalis*	8,00E-20	52%	ankyrin repeat PH and SEC7
symbB.v1.2.001228.t1|scaffold66.1|size357995|31	*P. bursaria* Chlorella virus	1,00E-43	47%	*Trichomonas vaginalis*	6,00E-78	49%	ankyrin repeat PH and SEC7
symbB.v1.2.001300.t1|scaffold57.1|size370615|9	*P. bursaria* Chlorella virus	3,00E-12	38%	*Metallosphaera yellowstonensis*	1,00E-14	47%	ankyrin repeat PH and SEC7
symbB.v1.2.001370.t1|scaffold71.1|size352893|13	*P. bursaria* Chlorella virus	2,00E-35	31%	*Aedes aegypti*	3,00E-63	36%	ankyrin repeat PH and SEC7
>symbB.v1.2.000869.t1|scaffold38.1|size396883|9	*P. bursaria* Chlorella virus	1,00E-21	41%	*Vitrella brassicaformis*	3,00E-33	45%	ankyrin repeat PH and SEC7

symbB.v1.2.000369.t1|scaffold17.1|size449391|6	*P. bursaria* Chlorella virus	6,00E-10	32%	*Halyomorpha halys*	7,00E-12	41%	ankyrin repeat PH and SEC7
symbB.v1.2.000123.t1|scaffold15.1|size524077|14	*P. bursaria* Chlorella virus	2,00E-33	28%	*Aedes aegypti*	1,00E-158	35%	ankyrin repeat PH and SEC7
symbB.v1.2.001201.t1|scaffold66.1|size357995|4	*P. bursaria* Chlorella virus	6,00E-23	43%	*Trichomonas vaginalis*	4,00E-30	51%	ankyrin repeat PH and SEC7
symbB.v1.2.001036.t1|scaffold56.1|size371842|7	*P. bursaria* Chlorella virus	4,00E-26	48%	*Trichomonas vaginalis*	5,00E-29	41%	ankyrin repeat PH and SEC7
symbB.v1.2.000178.t1|scaffold9.1|size550961|34	*P. bursaria* Chlorella virus	2,00E-04	28%	*Cryptosporidium parvum*	3,00E-47	33%	ankyrin repeat PH and SEC7
symbB.v1.2.000678.t1|scaffold35.1|size400642|22	*P. bursaria* Chlorella virus	2,00E-27	34%	*Lingula anatina*	7,00E-60	36%	ankyrin repeat PH and SEC7
symbB.v1.2.000792.t1|scaffold44.1|size390916|5	*P. bursaria* Chlorella virus	2,00E-39	30%	*Amphimedon queenslandica*	6,00E-46	37%	ankyrin repeat PH and SEC7
symbB.v1.2.000678.t2|scaffold35.1|size400642|22	*P. bursaria* Chlorella virus	7,00E-22	38%	*Lingula anatina*	4,00E-35	40%	ankyrin repeat PH and SEC7
symbB.v1.2.000746.t1|scaffold36.1|size400579|18	*P. bursaria* Chlorella virus	3,00E-10	33%	*Pterocles gutturalis*	3,00E-18	39%	ankyrin repeat PH and SEC7
symbB.v1.2.000869.t1|scaffold38.1|size396883|9	*P. bursaria* Chlorella virus	3,00E-25	46%	*Aedes aegypti*	1,00E-33	57%	ankyrin repeat PH and SEC7
symbB.v1.2.001036.t1|scaffold56.1|size371842|7	*P. bursaria* Chlorella virus	4,00E-26	48%	*Trichomonas vaginalis*	5,00E-29	41%	ankyrin repeat PH and SEC7
symbB.v1.2.001580.t1|scaffold88.1|size340390|15	*Bacillus* phage	8,00E-34	23%	*Thermaerobacter marianensis*	1,00E-54	31%	ATP-dependent DNA helicase
symbB.v1.2.001441.t1|scaffold58.1|size370606|10	*Acanthamoeba polyphaga* mimivirus	7,00E-13	29%	*Aspergillus fumigatus*	5,00E-46	48%	ATP-dependent RNA helicase
symbB.v1.2.000165.t1|scaffold9.1|size550961|21	*Moumouvirus goulette*	2,00E-26	27%	*Xanthophyllomyces dendrorhous*	2,00E-52	30%	ATP-dependent RNA helicase
symbB.v1.2.000165.t1|scaffold9.1|size550961|21	*Moumouvirus goulette*	2,00E-26	27%	*Xanthophyllomyces dendrorhous*	2,00E-52	30%	ATP-dependent RNA helicase A
symbB.v1.2.001147.t1|scaffold47.1|size388503|2	*Clostridium* phage	7,00E-06	39%	*Acropora millepora*	6,00E-94	55%	Dimethlysulfoniopropionate lyase
symbB.v1.2.006137.t1|scaffold349.1|size309019|1	*P. bursaria* Chlorella virus	1,00E-160	51%	*Perkinsus marinus*	0.0	61%	DNA Topoisomerase II
symbB.v1.2.006541.t1|scaffold388.1|size215153|2	*P. bursaria* Chlorella virus	0.0	48%	*Ostreococcus lucimarinus*	0.0	53%	DNA Topoisomerase II
symbB.v1.2.011504.t1|scaffold775.1|size163641|1	*P. bursaria* Chlorella virus	0.0	35%	*Albugo laibachii*	0.0	37%	DNA Topoisomerase II
symbB.v1.2.012523.t1|scaffold852.1|size193270|1	*P. bursaria* Chlorella virus	0.0	45%	*Physcomitrella patens*	0.0	52%	DNA Topoisomerase II
symbB.v1.2.014754.t1|scaffold1081.1|size139474|11	*P. bursaria* Chlorella virus	0.0	44%	*Ostreococcus tauri*	0.0	50%	DNA Topoisomerase II
symbB.v1.2.015202.t1|scaffold1129.1|size136302|10	*P. bursaria* Chlorella virus	0.0	48%	*Perkinsus marinus*	0.0	59%	DNA Topoisomerase II
symbB.v1.2.015327.t1|scaffold1138.1|size205980|26	*P. bursaria* Chlorella virus	7,00E-138	42%	*Perkinsus marinus*	3,00E-180	48%	DNA Topoisomerase II
symbB.v1.2.016997.t1|scaffold1313.1|size125722|1	*P. bursaria* Chlorella virus	4,00E-38	36%	*Amanita muscaria*	8,00E-63	39%	DNA Topoisomerase II
symbB.v1.2.020817.t1|scaffold1761.1|size106227|6	*Acanthocystis turfacea* Chlorella virus	2E-09	32%	*Anthracocystis flocculosa*	1,00E-38	48%	DNA Topoisomerase II
symbB.v1.2.033145.t1|scaffold4078.1|size45081|1	*Aureococcus anophagefferens* virus	6,00E-90	47%	*Plasmodium berghei*	3,00E-129	57%	DNA Topoisomerase II
symbB.v1.2.033147.t1|scaffold4078.1|size45081|3	*Moumouvirus monve*	5,00E-20	31%	*Fungal* sp. *11243*	5,00E-37	42%	DNA Topoisomerase II
symbB.v1.2.000588.t1|scaffold26.1|size418576|3	*Phaeocystis globosa* virus	2,00E-07	47%	*Callithrix jacchus*	8,00E-10	50%	dnaj homolog subfamily
symbB.v1.2.001767.t2|scaffold95.1|size600632|15	*Moumouvirus goulette*	3,00E-07	41%	*Homo sapiens*	2,00E-12	57%	dnaJ homolog subfamily B member 2

symbB.v1.2.001622.t1|scaffold80.1|size342472|9	*Cafeteria roenbergensis* virus	6,00E-05	46%	*Beckwithbacteria*	1,00E-08	33%	dnaJ homolog subfamily B member 2
symbB.v1.2.001652.t1|scaffold86.1|size363240|11	*A. polyphaga* moumovirus	2,00E-11	42%	*Charadrius vociferus*	3,00E-16	56%	dnaJ homolog subfamily B member 2
symbB.v1.2.001677.t1|scaffold86.1|size363240|36	*Cafeteria roenbergensis* virus	4,00E-08	52%	*Hymenolepis microstoma*	1,00E-10	61%	dnaJ homolog subfamily B member 2
symbB.v1.2.001767.t1|scaffold95.1|size600632|15	*Moumouvirus goulette*	8,00E-09	41%	*Pan troglodytes*	2,00E-13	57%	dnaJ homolog subfamily B member 2
symbB.v1.2.001073.t1|scaffold33.1|size517934|25	*Moumouvirus goulette*	4,00E-13	44%	*Fukomys damarensis*	4,00E-21	67%	dnaJ homolog subfamily B member 8
symbB.v1.2.000904.t1|scaffold27.1|size414596|12	*Megavirus courdo*11	6,00E-13	50%	*Chondrus crispus*	1,00E-32	32%	DnaJ-like protein
symbB.v1.2.000390.t1|scaffold30.1|size407774|6	*Megavirus courdo*11	3,00E-13	48%	*Opisthorchis viverrini*	3,00E-11	57%	DnaJ-like protein
symbB.v1.2.001077.t2|scaffold33.1|size517934|29	*A. polyphaga* moumovirus	3,00E-10	38%	*Stackebrandtia nassauensis*	4,00E-12	45%	DnaJ-like protein
symbB.v1.2.000154.t1|scaffold9.1|size550961|10	*Feldmannia* species virus	8,00E-06	45%	*Sesamum indicum*	4,00E-11	61%	E3 ubiquitin-protein ligase
symbB.v1.2.001385.t1|scaffold67.1|size356791|11	*Pandoravirus salinus*	3,00E-06	38%	*Perkinsus marinus*	9,00E-20	27%	E3 ubiquitin-protein ligase
symbB.v1.2.000231.t1|scaffold4.1|size633627|13	*Synechococcus* phage	3,00E-30	55%	*Hyalomma asiaticum asiaticum*	3,00E-56	57%	Ferritin
symbB.v1.2.001638.t1|scaffold90.1|size339071|7	*Cafeteria roenbergensis* virus	2,00E-106	41%	*Ectocarpus siliculosus*	5,00E-91	43%	FNIP repeat
symbB.v1.2.000824.t1|scaffold49.1|size384103|8	*Cafeteria roenbergensis* virus	5,00E-42	48%	*Ectocarpus siliculosus*	5,00E-35	41%	FNIP repeat
symbB.v1.2.001043.t2|scaffold56.1|size371842|14	*Cafeteria roenbergensis* virus	4,00E-77	37%	*Ectocarpus siliculosus*	2,00E-63	32%	FNIP repeat
symbB.v1.2.001638.t1|scaffold90.1|size339071|7	*Cafeteria roenbergensis* virus	2,00E-106	41%	*Ectocarpus siliculosus*	5,00E-91	43%	FNIP repeat
symbB.v1.2.001707.t1|scaffold61.1|size362833|6	*Cafeteria roenbergensis* virus	3,00E-67	49%	*Dictyostelium purpureum*	3,00E-50	51%	FNIP repeat
symbB.v1.2.000822.t1|scaffold49.1|size384103|6	*Cafeteria roenbergensis* virus	2,00E-60	48%	*Ectocarpus siliculosus*	3,00E-53	42%	FNIP repeat
symbB.v1.2.000977.t1|scaffold43.1|size391093|6	*Synechococcus* Phage	4,00E-17	36%	*Phaeodactylum tricornutum*	3,00E-33	46%	formylmethionine deformylase
symbB.v1.2.001364.t1|scaffold71.1|size352893|7	*Clostridium* phage	3,00E-07	30%	*Ophiostoma piceae*	3,00E-15	39%	glycoside hydrolase
symbB.v1.2.001702.t1|scaffold61.1|size362833|1	*A. polyphaga* moumovirus	2,00E-08	40%	*Schizosaccharomyces octosporus*	2,00E-12	41%	histone lysine methyltransferase Set5
symbB.v1.2.001669.t1|scaffold86.1|size363240|28	*Cotesia congregata* bracovirus	2,00E-33	49%	*Emiliania huxleyi*	4,00E-34	49%	hypothetical cysteinyl-tRNA synthetase
symbB.v1.2.001670.t2|scaffold86.1|size363240|29	*Cotesia congregata* bracovirus	6,00E-67	52%	*Emiliania huxleyi*	1,00E-90	50%	hypothetical cysteinyl-tRNA synthetase
symbB.v1.2.000858.t1|scaffold37.1|size397765|30	*Klebsiella* phage	2,00E-83	36%	*Thermosipho melanesiensis*	8,00E-108	41%	hypothetical protein
symbB.v1.2.000987.t1|scaffold43.1|size391093|16	*Sphingomonas* phage	2,00E-07	35%	*Drosophila persimilis*	1,00E-38	34%	hypothetical protein
symbB.v1.2.000379.t1|scaffold17.1|size449391|16	*Micromonas pusilla* virus	9,00E-61	60%	*Symbiodinium* sp. *C3*	1,00E-87	80%	hypothetical protein
symbB.v1.2.001648.t1|scaffold86.1|size363240|7	*Micromonas pusilla* virus	3,00E-63	55%	*Symbiodinium* sp. *C3*	7,00E-97	74%	hypothetical protein
symbB.v1.2.001366.t1|scaffold71.1|size352893|9	Organic Lake phycodnavirus 1	2,00E-05	31%	*Clostridium* sp.	3,00E-07	28%	hypothetical protein
symbB.v1.2.001077.t1|scaffold33.1|size517934|29	*Megavirus chilensis*	1,00E-07	38%	*Stemphylium lycopersici*	6,00E-35	28%	hypothetical protein
symbB.v1.2.001579.t1|scaffold88.1|size340390|14	African swine fever virus	2,00E-04	39%	*Emiliania huxleyi*	7,00E-10	27%	hypothetical protein

symbB.v1.2.001198.t1|scaffold66.1|size357995|1	*P. bursaria* Chlorella virus	1,00E-177	100%	*Chrysochromulina* sp.	6,00E-32	36%	prolyl 4-hydrolase-like
symbB.v1.2.000212.t1|scaffold21.1|size436794|31	*Feldmannia* species virus	7,00E-34	33%	*Vitrella brassicaformis*	7,00E-128	47%	protein kinase
symbB.v1.2.000184.t1|scaffold21.1|size436794|3	*Mollivirus sibericum*	3,00E-06	28%	*Aphanomyces invadans*	5,00E-19	27%	protein kinase
symbB.v1.2.000657.t1|scaffold35.1|size400642|1	*Cafeteria roenbergensis* virus	2,00E-60	48%	*Neolamprologus brichardi*	2,00E-35	34%	protein phosphatase 1A-like
symbB.v1.2.001529.t1|scaffold83.1|size345278|13	*Acanthamoeba polyphaga* mimivirus	2,00E-16	27%	*Crassostrea gigas*	4,00E-40	35%	putative ATP-dependent RNA helicase
symbB.v1.2.001082.t1|scaffold33.1|size517934|34	*Megavirus chilensis*	6,00E-24	36%	*Opisthocomus hoazin*	3,00E-105	72%	putative ATP-dependent RNA helicase
symbB.v1.2.001734.t1|scaffold61.1|size362833|33	*Ectocarpus siliculosus* virus 1	4,00E-07	44%	*Arthrobacter* sp. *H41*	3,00E-11	38%	putative chaperone protein
symbB.v1.2.001060.t1|scaffold33.1|size517934|12	*Cafeteria roenbergensis* virus	3,00E-18	45%	*Chaetura pelagica*	3,00E-22	63%	putative DnaJ/Hsp40
symbB.v1.2.000685.t1|scaffold41.1|size391900|2	*Ostreococcus tauri* virus	4,00E-15	52%	*Colletotrichum sublineola*	1,00E-19	51%	putative FMN-dependent dehydrogenase
symbB.v1.2.000396.t1|scaffold30.1|size407774|12	*Cotesia glomerata* bracovirus	1,00E-22	51%	*Emiliania huxleyi*	5,00E-25	46%	putative histone 4
symbB.v1.2.000382.t1|scaffold17.1|size449391|19	*Aureococcus anophagefferens* virus	2,00E-11	33%	*Dicentrarchus labrax*	2,00E-12	33%	putative protein disulfide isomerase
symbB.v1.2.000524.t1|scaffold31.1|size418471|20	*Mollivirus sibericum*	5,00E-12	26%	*Paramecium tetraurelia*	2,00E-24	26%	putative serine/threonine protein kinase
symbB.v1.2.001779.t1|scaffold95.1|size600632|27	*Megavirus courdo*7	4,00E-21	29%	*Crassostrea gigas*	1,00E-31	30%	putative serine/threonine protein kinase
symbB.v1.2.001321.t1|scaffold57.1|size370615|30	*Cafeteria roenbergensis* virus	8,00E-39	28%	*Rhizoctonia solani*	5,00E-40	28%	putative serine/threonine protein kinase
symbB.v1.2.001223.t1|scaffold66.1|size357995|26	*P. bursaria* Chlorella virus	4,00E-11	27%	*Vitrella brassicaformis*	1,00E-31	24%	putative serine/threonine protein kinase
symbB.v1.2.000143.t1|scaffold16.1|size461936|17	*Cotesia congregata* bracovirus	6,00E-13	35%	*Tetrahymena thermophila*	6,00E-30	32%	putative serine/threonine protein kinase
symbB.v1.2.001223.t1|scaffold66.1|size357995|26	*P. bursaria* Chlorella virus	4,00E-11	27%	*Vitrella brassicaformis*	1,00E-31	24%	putative serine/threonine protein kinase
symbB.v1.2.000524.t1|scaffold31.1|size418471|20	*Mollivirus sibericum*	5,00E-12	26%	*Paramecium tetraurelia*	2,00E-24	26%	putative serine/threonine protein kinase
symbB.v1.2.000627.t2|scaffold26.1|size418576|42	*P. bursaria* Chlorella virus	7,00E-22	38%	*Hydra vulgaris*	1,00E-25	34%	putative serine/threonine protein kinase
symbB.v1.2.000792.t1|scaffold44.1|size390916|5	*P. bursaria* Chlorella virus	6,00E-36	30%	*Amphimedon queenslandica*	6,00E-46	37%	putative serine/threonine protein kinase – Ank
symbB.v1.2.001365.t1|scaffold71.1|size352893|8	*Heterocapsa circularisquama* virus	2,00E-15	28%	*Toxoplasma gondii*	4,00E-03	29%	RNA-dependent RNA polymerase
symbB.v1.2.001368.t1|scaffold71.1|size352893|11	P. bursaria Chlorella virus	4,00E-15	40%	*Pelodictyon phaeoclathratiforme*	8,00E-14	34%	SET domain- N-methyltransferase
symbB.v1.2.001702.t1|scaffold61.1|size362833|1	*A. polyphaga* moumovirus	3,00E-09	28%	*Phanerochaete carnosa*	9,00E-20	32%	SET domain protein
symbB.v1.2.000381.t1|scaffold17.1|size449391|18	*Chrysochromulina ericina* virus	1,00E-09	35%	*Aedes aegypti*	5,00E-12	34%	thioredoxin/protein disulfide isomerase
symbB.v1.2.000381.t1|scaffold17.1|size449391|18	*Chrysochromulina ericina* virus	3,00E-11	35%	*Aedes aegypti*	5,00E-12	34%	thioredoxin/protein disulfide isomerase
symbB.v1.2.000257.t1|scaffold20.1|size571870|2	*Phaeocystis globosa* virus	8,00E-37	29%	*Anolis carolinensis*	7,00E-62	39%	ubiquitin specific peptidase C19
			Host to virus HGT				
symbB.v1.2.000094.t1|scaffold13.1|size649204|5	*Pandoravirus dulcis*	1,00E-12	28%	*Coccomyxa subellipsoidea*	4,00E-51	38%	2OG-Fe(II) oxygenase
symbB.v1.2.000036.t1|scaffold12.1|size699752|16	Organic Lake phycodnavirus 2	3,00E-27	27%	*Columba livia*	0.0	34%	ABC transporter permease

symbB.v1.2.001217.t1|scaffold66.1|size357995|20	*Ectocarpus siliculosus virus* 1	6,00E-24	24%	*Perkinsus marinus*	3,00E-56	37%	ATP-dependent RNA helicase A-like
symbB.v1.2.000312.t1|scaffold6.1|size569917|15	*Ectocarpus siliculosus virus* 1	4,00E-12	25%	*Perkinsus marinus*	5,00E-34	35%	calmodulin-domain protein kinase
symbB.v1.2.000750.t1|scaffold36.1|size400579|22	*Moumouvirus goulette*	8,00E-24	24%	*Paramecium tetraurelia*	4,00E-58	39%	CMGC/CDK/CDC2 protein kinase
symbB.v1.2.001015.t1|scaffold52.1|size380577|5	*Marseillevirus marseillevirus*	2,00E-13	26%	*Oxyrrhis marina*	3,00E-109	54%	cysteine proteinase
symbB.v1.2.001277.t1|scaffold64.1|size360880|9	*Ostreococcus tauri* virus	9,00E-09	32%	*Nannochloropsis gaditana*	8,00E-79	41%	cytosine-specific methyltransferase
symbB.v1.2.000979.t1|scaffold43.1|size391093|8	*Acanthocystis turfacea* Chlorella virus	7,00E-26	33%	*Veillonella ratti*	5,00E-46	42%	D-lactate dehydrogenase
symbB.v1.2.001543.t1|scaffold85.1|size341090|3	*Ectocarpus siliculosus* virus 1	2,00E-25	27%	*Aureococcus anophagefferens*	4,00E-70	46%	DnaJ-like protein
symbB.v1.2.000308.t1|scaffold6.1|size569917|11	*Pandoravirus inopinatum*	1,00E-14	38%	*Vitrella brassicaformis*	6,00E-65	40%	dual specificity protein phosphatase
symbB.v1.2.000784.t1|scaffold42.1|size391715|13	*P. bursaria* Chlorella virus	3,00E-52	33%	*Colwellia psychrerythraea*	9,00E-74	51%	elongation factor 3
symbB.v1.2.000934.t1|scaffold54.1|size375170|7	*P. bursaria* Chlorella virus	5,00E-24	25%	*Thalassiosira pseudonana*	4,00E-91	42%	elongation factor 3
symbB.v1.2.001546.t7|scaffold85.1|size341090|6	*P. bursaria* Chlorella virus	4,00E-21	30%	*Aureococcus anophagefferens*	2,00E-47	44%	elongation factor 3
symbB.v1.2.000934.t1|scaffold54.1|size375170|7	*P. bursaria* Chlorella virus	8,00E-26	25%	*Thalassiosira pseudonana*	5,00E-99	42%	elongation factor 3
symbB.v1.2.001090.t1|scaffold33.1|size517934|42	*Acanthocystis turfacea* Chlorella virus	1,00E-23	31%	*Plasmodiophora brassicae*	4,00E-60	45%	GDP-D-mannose 4,6-dehydratase
symbB.v1.2.000034.t1|scaffold12.1|size699752|14	Organic Lake phycodnavirus 2	4,00E-99	46%	*Crypthecodinium cohnii*	2,00E-113	54%	heat shock protein Hsp70 family protein
symbB.v1.2.000695.t1|scaffold41.1|size391900|12	Invertebrate iridescent virus	3,00E-15	36%	*Populus trichocarpa*	1,00E-23	40%	hypothetical protein
symbB.v1.2.000877.t1|scaffold38.1|size396883|17	*Cotesia congregata* bracovirus	9,00E-66	40%	*Plasmodiophora brassicae*	7,00E-151	38%	hypothetical protein
symbB.v1.2.000200.t1|scaffold21.1|size436794|19	*Acanthamoeba polyphaga* mimivirus	3,00E-08	42%	*Strongylocentrotus purpuratus*	2,00E-08	51%	hypothetical protein
symbB.v1.2.000573.t1|scaffold3.1|size669525|13	*P. bursaria* Chlorella virus	1,00E-11	25%	*Emiliania huxleyi*	2,00E-31	35%	hypothetical protein
symbB.v1.2.000756.t1|scaffold25.1|size427608|1	Megavirus lba	2,00E-24	28%	*Mycobacterium gilvum*	1,00E-37	38%	hypothetical protein
symbB.v1.2.000928.t1|scaffold54.1|size375170|1	wVitA of *Nasonia vitripennis* phage	4,00E-07	38%	*Brassica napus*	4,00E-30	44%	hypothetical protein
symbB.v1.2.000974.t1|scaffold43.1|size391093|3	*Megavirus chilensis*	2,00E-14	24%	*Naumovozyma castellii*	5,00E-46	35%	hypothetical protein
symbB.v1.2.000997.t1|scaffold43.1|size391093|26	*Moumouvirus monve*	1,00E-18	34%	*Emiliania huxleyi*	2,00E-58	37%	hypothetical protein
>symbB.v1.2.001529.t1|scaffold83.1|size345278|13	*Acanthamoeba polyphaga* mimivirus	3,00E-15	27%	*Limulus polyphemus*	4,00E-69	32%	hypothetical protein
symbB.v1.2.000002.t1|scaffold5.1|size591573|2	*Feldmannia* species virus	2,00E-11	34%	*Acaryochloris* sp.	1,00E-39	44%	hypothetical protein
symbB.v1.2.000679.t1|scaffold35.1|size400642|23	*Ostreococcus tauri* virus	8,00E-12	38%	*Schizopora paradoxa*	6,00E-26	40%	hypothetical protein
symbB.v1.2.000329.t1|scaffold6.1|size569917|32	*Ostreococcus tauri* virus	9,00E-06	34%	*Ectocarpus siliculosus*	6,00E-57	31%	hypothetical protein
symbB.v1.2.000679.t1|scaffold35.1|size400642|23	*Ostreococcus tauri* virus	8,00E-12	38%	*Schizopora paradoxa*	6,00E-26	40%	hypothetical protein
>symbB.v1.2.000773.t1|scaffold42.1|size391715|2	*Pandoravirus inopinatum*	2,00E-06	39%	*Physcomitrella patens*	3,00E-61	43%	hypothetical protein
symbB.v1.2.001453.t1|scaffold58.1|size370606|22	*Acanthamoeba polyphaga* mimivirus	7,00E-15	26%	*Exophiala spinifera*	1,00E-35	37%	hypothetical protein

symbB.v1.2.000524.t1|scaffold31.1|size418471|20	*Moumouvirus goulette*	2,00E-13	27%	*Paramecium tetraurelia*	1,00E-23	36%	hypothetical protein
symbB.v1.2.001579.t1|scaffold88.1|size340390|14	African swine fever virus	2,00E-04	39%	*Emiliania huxleyi*	7,00E-10	27%	hypothetical protein
symbB.v1.2.001071.t1|scaffold33.1|size517934|23	*Vibrio* phage	9,00E-07	29%	*Emiliania huxleyi*	2,00E-19	38%	hypothetical protein
symbB.v1.2.000634.t1|scaffold7.1|size571927|2	Horsepox vírus	3,00E-25	26%	*Zixibacteria bacterium*	1,00E-45	33%	hypothetical protein
symbB.v1.2.000891.t1|scaffold51.1|size380723|10	*Cafeteria roenbergensis* virus	2,00E-47	31%	*Euphyllia ancora*	1,00E-102	41%	II helicase/eIF-4AIII
symbB.v1.2.001007.t1|scaffold43.1|size391093|36	*Brevibacillus* phage	5,00E-17	29%	*Chrysochromulina* sp.	6,00E-39	36%	kelch repeat protein
symbB.v1.2.000101.t1|scaffold13.1|size649204|12	Swinepox vírus	1,00E-17	26%	*Chrysochromulina* sp.	2,00E-41	36%	kelch repeat protein
symbB.v1.2.000237.t1|scaffold4.1|size633627|19	Swinepox vírus	2,00E-21	25%	*Chrysochromulina* sp.	4,00E-48	38%	kelch repeat protein
symbB.v1.2.000711.t2|scaffold40.1|size395337|15	*Leptospira* phage	6,00E-18	29%	*Leptospira borgpetersenii*	5,00E-23	33%	Leucine rich repeat protein
symbB.v1.2.000509.t1|scaffold31.1|size418471|5	*Pandoravirus inopinatum*	6,00E-29	39%	*Sediminimonas qiaohouensis*	2,00E-35	46%	Morn repeat protein
symbB.v1.2.002929.t1|scaffold162.1|size290285|2	*Pyramimonas orientalis* virus	4,00E-11	28%	*Vitrella brassicaformis*	1,00E-177	40%	MutS protein
symbB.v1.2.013503.t1|scaffold959.1|size148843|12	*Cafeteria roenbergensis* virus	2,00E-16	31%	*Danio rerio*	2,00E-53	38%	MutS protein
symbB.v1.2.026874.t1|scaffold2720.1|size72291|3	*Chrysochromulina ericina* virus	8,00E-17	23%	*Vitrella brassicaformis*	6,00E-73	51%	MutS protein
symbB.v1.2.001008.t1|scaffold43.1|size391093|37	*Moumouvirus goulette*	1,00E-23	30%	*Naegleria gruberi*	8,00E-34	29%	P4Hc domain-containing protein
symbB.v1.2.000661.t1|scaffold35.1|size400642|5	*Marseillevirus marseillevirus*	7,00E-15	25%	*Noctiluca scintillans*	4,00E-123	56%	papain-like cysteine peptidase
symbB.v1.2.001558.t1|scaffold70.1|size352959|8	*Lausannevirus*	1,00E-06	23%	*Tetrahymena thermophila*	2,00E-84	41%	papain-like cysteine peptidase
symbB.v1.2.000661.t1|scaffold35.1|size400642|5	*Marseillevirus marseillevirus*	7,00E-15	25%	*Noctiluca scintillans*	4,00E-123	56%	papain-like cysteine peptidase
symbB.v1.2.000514.t3|scaffold31.1|size418471|10	*Pithovirus sibericum*	8,00E-15	25%	*Eimeria tenella*	1,00E-133	47%	PDZ serine protease
symbB.v1.2.000118.t1|scaffold15.1|size524077|9	*Pithovirus sibericum*	4,00E-09	26%	*Emiliania huxleyi*	2,00E-70	36%	PDZ serine protease
symbB.v1.2.001101.t1|scaffold59.1|size365495|5	*Pithovirus sibericum*	5,00E-07	27%	*Citrus clementina*	3,00E-41	48%	PDZ serine protease
symbB.v1.2.001200.t1|scaffold66.1|size357995|3	*Ostreococcus tauri* virus	3,00E-07	27%	*Chrysochromulina* sp.	7,00E-47	46%	peptide-aspartate beta-dioxygenase
symbB.v1.2.000014.t1|scaffold5.1|size591573|14	*Megavirus courdo*11	9,00E-09	27%	*Oikopleura dioica*	2,00E-12	39%	phosphatidylinositol 3 kinase-like protein age-1
symbB.v1.2.000573.t1|scaffold3.1|size669525|13	*P. bursaria* Chlorella virus	1,00E-11	25%	*Emiliania huxleyi*	2,00E-31	35%	prolyl 4-hydroxylase
symbB.v1.2.001344.t1|scaffold73.1|size366217|8	*Cafeteria roenbergensis* virus	3,00E-35	36%	*Chrysochromulina* sp.	2,00E-157	54%	putative DnaJ/Hsp40
symbB.v1.2.000461.t1|scaffold11.1|size528188|10	*Cafeteria roenbergensis* virus	6,00E-11	44%	*Trypanosoma congolense*	2,00E-12	50%	putative DnaJ/Hsp40
symbB.v1.2.001217.t2|scaffold66.1|size357995|20	*Ectocarpus siliculosus* virus 1	8,00E-24	24%	*Perkinsus marinus*	4,00E-56	37%	putative DnaJ/Hsp40
symbB.v1.2.000658.t1|scaffold35.1|size400642|2	Organic Lake phycodnavirus	2,00E-10	25%	*Thalassiosira pseudonana*	8,00E-59	36%	putative exostosin-like protein
symbB.v1.2.000177.t1|scaffold9.1|size550961|33	*Aureococcus anophagefferens* virus	3,00E-10	30%	*Thalassiosira pseudonana*	4,00E-65	55%	putative protein disulfide isomerase
symbB.v1.2.000377.t1|scaffold17.1|size449391|14	*Aureococcus anophagefferens* virus	8,00E-06	29%	*Vigna angularis*	3,00E-35	27%	putative protein disulfide isomerase

symbB.v1.2.000523.t1|scaffold31.1|size418471|19	*Marseillevirus*	7,00E-09	33%	*Reticulomyxa filosa*	7,00E-18	41%	putative protein disulfide isomerase
symbB.v1.2.000140.t1|scaffold16.1|size461936|14	*Moumouvirus monve*	6,00E-14	32%	*Salmo salar*	7,00E-24	28%	putative serine/threonine protein kinase
symbB.v1.2.000403.t1|scaffold30.1|size407774|19	*Cotesia congregata* bracovirus	1,00E-25	30%	*Perkinsus marinus*	6,00E-46	42%	putative serine/threonine protein kinase
symbB.v1.2.000449.t1|scaffold32.1|size405148|22	*Moumouvirus goulette*	6,00E-28	30%	*Guillardia theta*	2,00E-47	34%	putative serine/threonine protein kinase
symbB.v1.2.000886.t1|scaffold51.1|size380723|5	*Feldmannia species* virus	6,00E-35	27%	*Vitrella brassicaformis*	4,00E-47	35%	putative serine/threonine protein kinase
symbB.v1.2.000921.t1|scaffold27.1|size414596|29	*Megavirus courdo*11	3,00E-30	32%	*Plasmodiophora brassicae*	2,00E-41	36%	putative serine/threonine protein kinase
symbB.v1.2.001249.t1|scaffold63.1|size477159|20	*Pithovirus sibericum*	5,00E-33	36%	*Vitrella brassicaformis*	5,00E-48	41%	putative serine/threonine protein kinase
symbB.v1.2.001336.t3|scaffold57.1|size370615|45	*Acanthamoeba polyphaga* mimivirus	3,00E-13	32%	*Cyanobacterium aponinum*	2,00E-16	41%	putative serine/threonine protein kinase
symbB.v1.2.001421.t1|scaffold34.1|size402451|2	*Moumouvirus goulette*	1,00E-25	34%	*Stylonychia lemnae*	5,00E-36	38%	putative serine/threonine protein kinase
symbB.v1.2.000944.t1|scaffold45.1|size390604|7	*P. bursaria* Chlorella virus	2,00E-24	26%	*Tetrahymena thermophila*	3,00E-33	31%	putative serine/threonine protein kinase
symbB.v1.2.001421.t1|scaffold34.1|size402451|2	*A. polyphaga* moumovirus	5,00E-30	29%	*Toxocara canis*	6,00E-49	35%	putative serine/threonine protein kinase
symbB.v1.2.001591.t1|scaffold88.1|size340390|26	*Feldmannia species* virus	2,00E-18	25%	*Paramecium tetraurelia*	2,00E-30	35%	putative serine/threonine protein kinase
symbB.v1.2.001779.t1|scaffold95.1|size600632|27	Megavirus lba	3,00E-21	29%	*Sorghum bicolor*	5,00E-31	32%	putative serine/threonine protein kinase
symbB.v1.2.000744.t1|scaffold36.1|size400579|16	*Cafeteria roenbergensis* virus	3,00E-13	23%	*Esox lucius*	1,00E-27	30%	putative serine/threonine protein kinase
symbB.v1.2.001421.t1|scaffold34.1|size402451|2	*Acanthamoeba polyphaga* mimivirus	9,00E-29	29%	*Toxoplasma gondii*	2,00E-67	32%	putative serine/threonine protein kinase
symbB.v1.2.001591.t3|scaffold88.1|size340390|26	*Feldmannia species* virus	5,00E-23	26%	*Paramecium tetraurelia*	1,00E-36	37%	putative serine/threonine protein kinase
symbB.v1.2.000253.t1|scaffold18.1|size444001|10	*Aureococcus anophagefferens* virus	1,00E-36	33%	*Allomyces macrogynus*	4,00E-86	46%	putative serine/threonine protein kinase
symbB.v1.2.000431.t1|scaffold32.1|size405148|4	*Cafeteria roenbergensis* virus	1,00E-16	28%	*Vitrella brassicaformis*	3,00E-83	43%	putative serine/threonine protein kinase
symbB.v1.2.000733.t1|scaffold36.1|size400579|5	*Cafeteria roenbergensis* virus	2,00E-18	32%	*Perkinsus marinus*	7,00E-61	38%	putative serine/threonine protein kinase
symbB.v1.2.000886.t1|scaffold51.1|size380723|5	*Feldmannia irregularis* virus	6,00E-35	27%	*Vitrella brassicaformis*	4,00E-47	35%	putative serine/threonine protein kinase
symbB.v1.2.001591.t2|scaffold88.1|size340390|26	*Feldmannia irregularis* virus	7,00E-20	25%	*Blastocystis* sp.	2,00E-54	34%	putative serine/threonine protein kinase
symbB.v1.2.000146.t1|scaffold9.1|size550961|2	*Emiliania huxleyi* virus	0.0	62%	*Perkinsus marinus*	0.0	68%	ribonucleoside-diphosphate reductase
symbB.v1.2.000159.t1|scaffold9.1|size550961|15	*Feldmannia irregularis* virus	2,00E-56	66%	*Plasmodium vivax*	2,00E-59	69%	ribonucleoside-diphosphate reductase
symbB.v1.2.000042.t1|scaffold12.1|size699752|22	*P. bursaria* Chlorella virus	2,00E-05	29%	*Karlodinium veneficum*	6,00E-12	49%	ribosomal protein S27
symbB.v1.2.001396.t1|scaffold75.1|size348941|2	*Acanthocystis turfacea* Chlorella virus	7,00E-14	41%	*Karlodinium veneficum*	5,00E-59	77%	ribosomal protein S27
symbB.v1.2.001396.t1|scaffold75.1|size348941|2	*Acanthocystis turfacea* Chlorella virus	8,00E-14	43%	*Karlodinium veneficum*	5,00E-59	77%	ribosomal protein S27a
symbB.v1.2.001397.t1|scaffold75.1|size348941|3	*Micromonas pusilla* virus	2,00E-15	34%	*Karlodinium veneficum*	2,00E-58	75%	ribosomal protein S27a
symbB.v1.2.001398.t1|scaffold75.1|size348941|4	*Micromonas pusilla* virus	1,00E-09	33%	*Karlodinium veneficum*	5,00E-56	74%	ribosomal protein S27a
symbB.v1.2.000972.t1|scaffold43.1|size391093|1	*Pandoravirus dulcis*	2,00E-38	33%	*Phytophthora sojae*	8,00E-86	44%	tRNA threonylcarbamoyl adenosine

symbB.v1.2.000449.t1|scaffold32.1|size405148|22	*Moumouvirus goulette*	6,00E-28	30%	*Guillardia theta*	2,00E-47	34%	tyrosine kinase
symbB.v1.2.000601.t1|scaffold26.1|size418576|16	*Moumouvirus goulette*	4,00E-16	33%	*Malassezia pachydermatis*	4,00E-37	44%	ubc-like protein
symbB.v1.2.000237.t1|scaffold4.1|size633627|19	Swinepox vírus	2,00E-21	25%	*Chrysochromulina* sp.	4,00E-48	38%	kelch repeat protein
Virophage to host HGT							
symbB.v1.2.001639.t1|scaffold90.1|size339071|8	Yellowstone Lake virophage 5	6,00E-21	38%	*Bacillus flexus*	2,00E-19	33%	DNA-cytosine methylase
symbB.v1.2.000313.t1|scaffold6.1|size569917|16	Yellowstone Lake virophage 5	2,00E-08	31%	*Bacillus flexus*	3,00E-09	27%	DNA-cytosine methylase
symbB.v1.2.038210.t1|scaffold5874.1|size25606|2	Yellowstone Lake virophage 6	4,00E-05	37%	*Lasius niger*	6,00E-13	30%	GIY-YIG endonuclease
symbB.v1.2.013127.t1|scaffold916.1|size152367|3	Yellowstone Lake virophage 6	4,00E-03	31%	*Penicillium digitatum*	5,00E-07	37%	GIY-YIG endonuclease
symbB.v1.2.013516.t1|scaffold795.1|size259473|8	Yellowstone Lake virophage 6	0,004	27%	*Rhizobium selenitireducens*	8.2	33%	GIY-YIG endonuclease
symbB.v1.2.032000.t1|scaffold3753.1|size50886|1	*Phaeocystis globosa* virophage	0,0002	20%	*Dinoponera quadriceps*	0.38	27%	Hypothetical protein
symbB.v1.2.013934.t1|scaffold996.1|size145880|15	*Phaeocystis globosa* virophage	9,00E-05	18%	*Botrytis cinerea*	1,00E-21	99%	Hypothetical protein
symbB.v1.2.027015.t1|scaffold2744.1|size71806|1	Yellowstone Lake virophage 5	1,00E-03	31%	*Achromobacter xylosoxidans*	2.5	38%	Major Capsid Protein
symbB.v1.2.017015.t2|scaffold1316.1|size125546|5	Mavirus virophage	0.0003	21%	*Salpingoeca rosetta*	0.0036	41%	Major Capsid Protein
symbB.v1.2.013818.t1|scaffold984.1|size146690|9	Yellowstone Lake virophage 7	2,00E-03	27%	*Paenibacillus pini*	0.18	27%	Major Capsid Protein
symbB.v1.2.038636.t1|scaffold6091.1|size20991|2	Yellowstone Lake virophage 6	2,00E-06	38%	*Ectocarpus silicosus*	3,00E-33	27%	Putative cysteine protease
symbB.v1.2.017038.t1|scaffold1317.1|size125501|5	Yellowstone Lake virophage 5	0.0002	25%	*Anolis carolinensis*	4,60E-02	29%	Putative packaging ATPase
symbB.v1.2.019352.t1|scaffold1564.1|size111405|3	Yellowstone Lake virophage 5	2,00E-03	30%	*Mycobacterium ulcerans*	5.2	29%	Putative packaging ATPase
symbB.v1.2.019352.t1|scaffold1564.1|size111405|3	Yellowstone Lake virophage 5	4,00E-05	30%	*Mycobacterium ulcerans*	5.2	29%	Putative packaging ATPase
symbB.v1.2.022011.t1|scaffold1935.1|size95652|6	Mavirus virophage	4,00E-04	26%	*Pedobacter* sp.	1.2	36%	Putative protein-primed B-family polymerase

CAPÍTULO 4

4 DISCUSSÕES

Estresses como altas temperaturas oceânicas (FITT et al, 2001) e radiação UV são implicados na indução do branqueamento em corais (GLEASON; WELLINGTON, 1993). Holobiontes corais já encontram-se no limite máximo de tolerância aos efeitos da radiação UV (LESSER, 1996) e pequenos aumentos de temperatura, combinados com alta irradiância solar, podem levar ao branqueamento dos corais (HOEGH-GULDBERG, 1999; FERRIER-PAGÈS et al, 2007). Sabe-se que estes mesmos fatores também promovem a propagação e virulência de partículas virais (EDGAR; LIELAUSIS, 1964; JACQUET; BRATBAK, 2003; DAVY et al. 2006). Em conjunto a observações de PSVs e sequencias virais em *Symbiodinium* de corais doentes e/ou submetidos a estes estresses (THURBER; CORREA, 2011), também observados no presente estudo, sugere-se uma conexão entre estresse, atividade viral e respostas fisiológicas de holobiontes corais e seus associados. De acordo com projeções das respostas dos sistemas recifais ao aquecimento global, é estimado que, até o ano de 2100, estes sistemas poderão estar extintos do planeta se os níveis de impacto antropogênico continuarem na mesma intensidade (HOEGH-GULDBERG et al, 2007). Assim, se a atividade viral em *Symbiodinium* e no holobionte possui de fato relação com estresses ambientais, tais cenários de mudanças climáticas no oceano poderiam intensificar a virulência, impactando e ameaçando a saúde e existência dos recifes de corais.

A hipótese "proviral" de Wilson et al, (2001a) propõe a existência de uma infecção viral latente em *Symbiodinium*, onde a transição para o ciclo ativo, ou seja, lítico, seria estimuladas por estresses como os já mencionados.. Em uma infecção latente o vírus se encontra inativo, integrado ao genoma de seu hospedeiro como um provírus ou como um episssoma no citoplasma (MINAROVITS, 2006). Em condições ambientais não estressantes, o vírus latente provavelmente não afetaria os papéis ecológicos desempenhados por *Symbiodinium* na dinâmica do holobionte e ainda poderia prevenir o sistema contra infecção de vírus exógenos (BETTAREL et al, 2015), processo resultante de uma imunidade adquirida por superinfecção conferida ao hospedeiro (GREINER et al, 2009, LABRIE et al, 2010). Observações realizadas em nosso estudo reforçam a hipótese "proviral", uma vez que cultivos de *Symbiodinium* submetidos a estresse demonstraram a produção de PSVs e, em seu genoma, há diversos genes possívelmente de origem em virus gigantes, embora genes estruturais

(como proteínas de capsídeo e polimerases), que confirmariam um provirus ativo, estejam aparentemente ausentes. Observou-se também diversos genes de virófagos, incluindo genes estruturais e, através desta incorporação gênica, virófagos poderiam, como efeito adverso de seu próprio hiperparasitismo, controlar a latência dos vírus gigantes no sistema, o que também levaria a imunização por superinfecção do hospedeiro contra outros vírus, provavelmente na forma episomática/plasmidial ou em forma de um provirófago ativo (DESNUES et al, 2012; SANTINI et al, 2013; BLANC; GALLOT-LAVALLÉE; MAUMUS, 2015). O presente trabalho constitui a primeira observação de entidades semelhantes a virófagos associados a corais e seus endossimbiontes, incluindo genes e particular semelhantes. Virófagos acoplados com vírus gigantes podem estar atuando como forças estabilizadoras na simbiose entre *Symbiodinium* e corais (VILLARREAL, 2007; MARHAVER; EDWARDS; ROHWER, 2008). Em termos de dinâmicas de alças microbianas em recifes de corais, a interação entre vírus gigantes e virófagos poderia, através do hiperparasitsmo por virófagos, exercer controle e atenuação da infectividade e virulência sobre as populações virais (DESNUES et al, 2012; SLIMANI et al, 2013), logo, aumentando a sobrevivência do hospedeiro eucarioto e impactando diretamente no aumento do fluxo de produção secundária em ecossistemas recifais (LA SCOLA et al, 2008; YAU et al, 2011).

É possível elaborar um cenário de dinâmica hipersimbiótica entre *Symbiodinium*, vírus gigantes e virófagos latentes, em conjunto com a disrupção do sistema por estresse, baseado em nossas observações e em dados da literatura (LA SCOLA et al, 2008; YAU et al, 2011; SANTINI et al, 2013). Estresses ambientais atuariam como gatilhos de produção viral, e promoveriam a transição do ciclo latente para o ciclo lítico, em um indivíduo ou uma pequena população de *Symbiodinium*. A intensidade da infecção (número de partículas virais em dado tempo) seria proporcional a intesidade do estresse. Após o estresse e produção viral inicial, ocorreria o espalhamento da infecção e, em um tipo de reação em cadeia, partículas poderiam infectar outros *Symbiodinium* residentes do holobionte e, através do parasitismo de vírus gigantes, uma população inteira de *Symbiodinium* poderia ser eliminada, impactando o holobionte e posteriormente alcançando um recife inteiro. Se os corais não conseguissem repovoar sua população de *Symbiodinium*, ou estas não conseguirem suportar a infecção, poderia ocorrer a morte do coral. Em condições ambientais estáveis, virófagos acoplados atuariam exercendo controle dessas populacões de vírus gigantes, garantindo o equilíbrio da hipersimbiose. Contudo, este controle seria ineficaz em um cenário de intesidade alta de

estresse pois tal equilibrio seria dependente da intensidade da infecção, tamanho da população viral e rapidez de espalhamento.

Em termos de evolução genômica do *Symbiodinium*, as identidades das proteínas observadas e hipotetizadas em possuirem evidências de THG (transferência horizontal de genes) verificadas no nosso trabalho, indicam que existe uma relação antiga e muito provavelmente ainda atuante de vírus gigantes, virófagos e outros tipos de virus, com *Symbiodinium*. Além disso, estas identidades estão relacionadas a processos de interação e subversão viral do maquinário do hospedeiro, bem como manutenção de latência viral, respostas à estresse, modificações epigenéticas (pós-traducionais) e construção de partículas virais.

5 CONCLUSÃO

Neste estudo foi demonstrado que linhagens de *Symbiodinium* pertencentes ao clado A (filotipo A4) e ao clado C (filotipo C3), hospedados pelo coral *M. braziliensis*, possuem uma infecção viral latente induzida a produzir partículas semelhantes a vírus (PSVs) quando submetidas a estresse termal e radiação ultravioleta (UV). Através da caracterização morfológica das PSVs observadas nos cultivos de *Symbiodinium* isolados de *M. braziliensis*, apontou-se também que, contrariamente aos estudos prévios que sugeriram infecção viral pela família *Phycodnaviridae* (THURBER; CORREA, 2011; CORREA; WELSH; VEGA THURBER, 2013), PSVs observadas nos cultivos indicam uma infecção por um representante ainda não caracterizado da família *Megaviridae* (ARSLAN et al, 2011), assim como uma hiperinfecção por vírofagos. Estas observações estão alinhadas com a Claverie et al, (2009) que sugeriram uma infecção por Mimivírus marinhos (família *Megaviridae*) em corais, e ainda com Correa et al. (2013) que encontraram semelhanças significativas nos viromas de *M. cavernosa* também com Mimivirus e outros vírus gigantes. Ao utilizar o genoma de *S. minutum* isolado do coral *Orbicella faveolata* (conhecido anteriormente como *M. faveolata*), também foram descritos diversos genes codantes de proteínas envolvidos em eventos putativos de THG (transferência horizontal de genes) com vírus gigantes e virófagos, o que poderia indicar a existência de um provírus e um provirófago em forma latente neste genoma.

Por fim, este trabalho adiciona observações e fenômenos ao montante de evidências que demonstram a grande influência que mutiplos níveis de simbiose possuem na construção, moldura e integração imanente das linhas de vida e trajetórias evolutivas dos simbiontes. No contexto de mudanças climáticas (LESSER, 2007) torna-se crucial compreender de que forma esta multiplicidade que compõe os holobiontes corais interagem e respondem, e a relação entre a viralidade, e futura existência dos corais e seus endossimbiontes em um planeta que se encontra em profunda mudança, ainda em tempo.

6 REFERÊNCIAS BIBLIOGRÁFICAS

ANTHONY, KRN; KERSWELL, AP. Coral mortality following extreme low tides and high solar radiation. **Mar Biol**, v.151, p.1623–1631, 2007.

ARSLAN, D; LEGENDRE, M; SELTZER, V; ABERGEL, C; CLAVERIE, JM. Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. **Proceedings of the National Academy of Sciences**, v.108, p.17486-17491, 2011.

ASTORGA, D; RUIZ, J; PRIETO, L. Ecological aspects of early life stages of Cotylorhiza tuberculata (Scyphozoa: Rhizostomae) affecting its pelagic population success. **Hydrobiologia**, v.690, p.141-155, 2012.

BARR, JJ; AURO, R; FURLAN, M; WHITESON, KL; ERB, ML; POGLIANO, J; STOTLAND, A; et al. Bacteriophage adhering to mucus provide a non–host-derived immunity. **Proceedings of the National Academy of Sciences**, v.110, p.10771-10776, 2013.

BAYER, T; ARANDA, M; SUNAGAWA, S; YUM, LK; DESALVO, MK; LINDQUIST, E; COFFROTH, MA; VOOLSTRA, CR; MEDINA, M. Symbiodinium transcriptomes: Genome insights into the dinoflagellate symbionts of reef-building corals. **PLoS ONE**, v.7, p.e35269, 2012.

BERGH, O; BORSHEIM, KY; BRATBAK, G; HELDAL, M. High abundance of viruses found in aquatic environments. **Nature**, v.340, p.467-468, 1989.

BERKELMANS, R; DE'ATH, G; KININMONTH, S; SKIRVING, WJ. A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns and predictions. **Coral Reefs**, v.23, p.74–83, 2004.

BETTAREL. Y; BOUVIER, T; NGUYEN, HK; THU, PT. The versatile nature of coral-associated viruses. **Environmental Microbiology**. v.17, p.3433-9, 2015.

BILEWITCH, JP; DEGNAN, SM. A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. **BMC Evol. Biol**. 11:228, 2011.

BLACKALL, LL; WILSON, B; OPPEN, MJH. Coral—the world's most diverse symbiotic ecosystem. **Mol Ecol**, v.24, p.5330–5347, 2015.

BLANC, G; DUNCAN, G; AGARKOVA, I; BORODOVSKY, M; GURNON, J; KUO, A; LINDQUIST, E; LUCAS, S; PANGILINAN, J; POLLE, J; SALAMOV, A; TERRY, A; YAMADA, T; DUNIGAN, DD; GRIGORIEV, IV; CLAVERIE, JM; VAN ETTEN, JL. The Chlorella variabilis NC64A genome reveals adaptation to photosymbiosis, coevolution with viruses, and cryptic sex. **Plant Cell**, v.22, p.2943–55, 2010.

BLANC, G; GALLOT-LAVALLÉE, L; MAUMUS, F. Provirophages in the Bigelowiella genome bear testimony to past encounters with giant viruses. **Proceedings of the National Academy of Sciences**, v.112, p.(38):E5318-26, 2015.

BOTO, L. Horizontal gene transfer in evolution: facts and challenges. **Proc Biol Sci**, v.277, p.819–827, 2010.

BRATBAK, G; LEVASSEUR, M; MICHAUD, S; CANTIN, G; FERNANDEZ, E; et al. Viral activity in relation to Emiliania huxleyi blooms: a mechanism of DMSP release? **Mar Ecol Prog Ser**, v.128, p.133–142, 1995.

BREITBART, M. Marine viruses: Truth or dare. **Annu Rev Mar Sci**, v.4, p.425–448, 2012.

BROWN, BE. Coral bleaching: causes and consequences. **Coral Reefs**, v.16, p.S129–S138, .

BRUSSAARD, CPD. Viral control of phytoplankton populations - A review. **Journal of Eukaryotic Microbiology**, v.l, p.125-138, 2004.

BURGHARDT, I; STEMMER, K; WÄGELE, H. Symbiosis between Symbiodinium (Dinophyceae) and various taxa of Nudibranchia (Mollusca: Gastropoda), with analyses of long-term retention. **Organisms Diversity and Evolution**, v.8, p.66-76, 2008.

CARLOS, AA; BAILLIE, BK; KAWACHI, M; MARUYAMA, T. Phylogenetic position of Symbiodinium (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. **Journal of Phycology**, v.35, p.1054-1062, 1999.

CASTRO, AP; DE ARAÚJO, SD; REIS, AMM; MOURA, RL; FRANCINI-FILHO, RB; PAPPAS, G; RODRIGUES, TB; et al. Bacterial community associated with healthy and diseased reef coral Mussismilia hispida from Eastern Brazil. **Microbial Ecology**, v.59, p.658-667, 2010.

CASTRO, CB; PIRES, DO. A bleaching event on a Brazilian coral reef. **Brazilian Journal of Oceanography**, v.47, p.87–90, 1999.

CERVINO, JM; HAYES, R; GOREAU, TJ; SMITH, GW. Zooxanthellae regulation in yellow blotch/band and other coral diseases contrasted with temperature related bleaching: In situ destruction vs expulsion. **Symbiosis**, v.37, p.63–85, 2004.

CLAVERIE, JM; ABERGEL, C. Open Questions About Giant Viruses. **Advances in Virus Research**, v.85, p.25-58, 2013.

CLAVERIE, JM; GRZELA, R; LARTIGUE, A; BERNADAC, A; NITSCHE, S; VACELET, J; OGATA, H; ABERGEL, C. Mimivirus and Mimiviridae: Giant viruses with an increasing number of potential hosts, including corals and sponges. **Journal of Invertebrate Pathology**, v. 101, p.172-180, 2009.

CORREA, AMS; WELSH, RM; VEGA THURBER, RL. Unique nucleocytoplasmic dsDNA and +ssRNA viruses are associated with the dinoflagellate endosymbionts of corals. **The ISME Journal**, v.**7**, p.13–27, 2012.

DAVY, J. E; PATTEN, NL. Morphological diversity of virus-like particles within the surface microlayer of scleractinian corals. **Aquatic Microbial Ecology**, v.47, p.37-44, 2007.

DAVY, SK; BURCHETT, SG; DALE, AL; DAVIES, P; DAVY, JE; MUNCKE, C; HOEGH-GULDBERG, O; WILSON, WH. Viruses: agents of coral disease? **Dis Aquat Organ**, v.69, p.101–110, 2006.

DELAROQUE, N; BOLAND, W. The genome of the brown alga Ectocarpus siliculosus contains a series of viral DNA pieces, suggesting an ancient association with large dsDNA viruses. **BMC Evol Biol**, v.8, p.110, 2008.

DERELLE, E; FERRAZ, C; ESCANDE, ML; EYCHENIÉ, S; COOKE, R; PIGANEAU, G; DESDEVISES, Y; BELLEC, L; MOREAU, H; GRIMSLEY, N. Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga Ostreococcus tauri. **PLoS One**, v.3, p.e2250, 2008.

DESNUES, C; LA SCOLA, B; YUTIN, N; FOURNOUS, G; ROBERT, C; AZZA, S; JARDOT, P; MONTEIL, S; CAMPOCASSO, A; KOONIN, EV; RAOULT, D. Provirophages and transpovirons as the diverse mobilome of giant viruses. **Proc Natl Acad Sci USA**, v.109, p.18078–18083, 2012.

EDGAR, RS; LIELAUSIS, I. Temperature sensitive mutants of phage T4D: their isolation and genetic characterisation. **Genetics**, v.49, p.649 – 662, 1964.

FERRIER-PAGÈS, C; RICHARD, C; FORCIOLI, D; ALLEMAND, D; PICHON, M; SHICK, JM. Effects of temperature and UV radiation increases on the photosynthetic efficiency in four scleractinian coral species. **Biological Bulletin**, v.213, p.76-87, 2007.

FILÉE, J. Multiple occurrences of giant virus core genes acquired by eukaryotic genomes: The visible part of the iceberg? **Virology**, v.466-467, p.53–59, 2014.

FILÉE, J; CHANDLER, M. Convergent mechanisms of genome evolution of large and giant DNA viruses. **Research in Microbiology**, v.159, p.325-331, 2008.

FILÉE, J; CHANDLER, M. Gene exchange and the origin of giant viruses. **Intervirology**, v.53, p.354–361, 2010.

FISCHER, MG; SUTTLE, CA. A virophage at the origin of large DNA transposons. **Science**, v.332, p.231–234, 2011.

FITT, WK; BARBARA, EB; MARK EW; RICHARD, PD. Coral Bleaching: Interpretation of Thermal Tolerance Limits and Thermal Thresholds in Tropical Corals. **Coral Reefs**, v.20, p.51-65, 2001.

FRANCINI-FILHO, RB; MOURA, RL; THOMPSON, FL; REIS, RM; KAUFMAN, L; KIKUCHI, RKP; LEÃO, ZMAN. Diseases leading to accelerated decline of reef corals in the largest South Atlantic reef complex (Abrolhos Bank, eastern Brazil). **Marine Pollution Bulletin**, v.56, p.1008-1014, 2008.

FUHRMAN, JA. Marine viruses and their biogeochemical and ecological effects. **Nature**, v.399, p.541-548, 1999.

GAO, G; LUO, H. The ubiquitin-proteasome pathway in viral infections. **Can J Physiol Pharmacol**, v.84, p.5–14, 2006.

GARCIA, GD; GREGORACCI, GB; SANTOS, EO; MEIRELLES, PM; SILVA, GGZ; EDWARDS, R; SAWABE, T; et al. Metagenomic Analysis of Healthy and White Plague-Affected Mussismilia braziliensis Corals. **Microbial Ecology**, v.65, p.1076-1086, 2013.

GAST, RJ; CARON, DA. Photosymbiotic associations in planktonic foraminifera and radiolaria. **Hydrobiologia**, v.461, p.1-7, 2001.

GLEASON, DF; WELLINGTON, GM. Ultraviolet radiation and coral bleaching. **Nature**, v.365, p.836-838, 1993.

GOLDENFELD, N; WOESE, C. Biology's next revolution. **Nature**, v.445, p.369, 2007.

GORNIK, SG; FORD, KL; MULHERN, T D; BACIC, A; MCFADDEN, GI; WALLER, RF. Loss of nucleosomal DNA condensation coincides with appearance of a novel nuclear protein in dinoflagellates. **Current Biology**, v.22, p.2303-2312, 2012.

GOULET, TL; COFFROTH, MA. The genetic identity of dinoflagellate symbionts in Caribbean octocorals. **Coral Reefs**, v.23, p.465-472, 2004.

GREINER, T; FROHNS, F; KANG, M; VAN ETTEN, JL; KÄSMANN, A; MORONI, A; HERTEL, B; THIEL, G. Chlorella viruses prevent multiple infections by depolarizing the host membrane. **The Journal of general virology**, v.90, p.2033-9, 2009.

HESTER, ER; BAROTT, KL; NULTON, J; VERMEIJ, MJA; ROHWER, FL. Stable and sporadic symbiotic communities of coral and algal holobionts. **The ISME Journal**, 2015.

HEWSON, I; VARGO, GA; FUHRMAN, JA. Bacterial Diversity in Shallow Oligotrophic Marine Benthos and Overlying Waters: Effects of Virus Infection, Containment, and Nutrient Enrichment. **Microbial Ecology**, v.46, p.322-336, 2003.

HILL, RW; WHITE, BA; COTTRELL, MT; DACEY, JWH. Virus-mediated total release of dimethylsulfoniopropionate from marine phytoplankton: A potential climate process. Aquatic **Microbial Ecology**, v.14, p.1-6, 1998.

HINGAMP, P; GRIMSLEY, N; ACINAS, SG; CLERISSI, C; SUBIRANA, L; POULAIN, J; FERRERA, I; SARMENTO, H; VILLAR, E; LIMA-MENDEZ, G; FAUST, K; SUNAGAWA, S; CLAVERIE, JM; MOREAU, H; DESDEVISES, Y; BORK, P; RAES, J; DE VARGAS, C; KARSENTI, E; KANDELS-LEWIS, S; JAILLON, O; NOT, F; PESANT, S; WINCKER, P; OGATA, H. Exploring nucleo-cytoplasmic large DNA viruses in Tara Oceans microbial metagenomes. **ISME J**, v.7, p.1678–95, 2013.

HOEGH-GULDBERG, O. Climate change, coral bleaching and the future of the world's coral reefs. **Mar. Freshwater Res**, v.50, p.839-866, 1999.

HOEGH-GULDBERG, O; MUMBY, PJ; HOOTEN, AJ; STENECK, RS; GREENFIELD, P; GOMEZ, E; HARVELL, CD; SALE, PF; EDWARDS, AJ; CALDEIRA, K; KNOWLTON, N; EAKIN, CM; IGLESIAS-PRIETO, R; MUTHIGA, N; BRADBURY, RH; DUBI, A;

HATZIOLOS, ME. Coral reefs under rapid climate change and ocean acidification. **Science**, v.318, p.1737–1742, 2007.

HUGHES, AL; FRIEDMAN, R. Genome-wide survey for genes horizontally transferred from cellular organisms to baculoviruses. **Mol. Biol. Evol**, v.20, p.979–987, 2003.

HURWITZ, BL; HALLAM, SJ; SULLIVAN, MB. Metabolic reprogramming by viruses in the sunlit and dark ocean. **Genome biology**, v.14, p.R123, 2013.

IVEY, RG; HENRY, EC; LEE, AM; KLEPPER, L; KRUEGER, SK; MEINTS, RH. A Feldmannia algal virus has two genome size-classes. **Virology**, v.220, p.267-273, 1996.

JACQUET, S; BRATBAK G: Effects of ultraviolet radiation on marine virus-phytoplankton interactions. **FEMS Microbiol Ecol**, v.44, p.279–289, 2003.

KATZOURAKIS, A; ASWAD, A. The origins of giant viruses, virophages and their relatives in host genomes. **BMC Biol**, v.12, p.51, 2014.
KATZOURAKIS, A; GIFFORD, RJ. Endogenous viral elements in animal genomes. **PLoS Genet**, v.6, p.e1001191, 2010.

KNOWLTON, N. The future of coral reefs. **Proceedings of the National Academy of Sciences of the United States of America**, v.98, p.5419-5425, 2001.

KNOWLTON, N; ROHWER, F. Microbial mutualisms on coral reefs: the host as a habitat. **American Naturalist**, v.162, p.S51-S62, 2003.

KOONIN, EV; YUTIN, N. Origin and evolution of eukaryotic large nucleo-cytoplasmic DNA viruses. **Intervirology**. v.53, p.284-92, 2010.

KREDIET, CJ; RITCHIE, KB; PAUL, VJ; TEPLITSKI, M. Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proceedings. **Biological sciences / The Royal Society**, v.280, p.20122328, 2013.

LA SCOLA, B; DESNUES, C; PAGNIER, I; ROBERT, C; BARRASSI, L; FOURNOUS, G; MERCHAT, M; SUZAN-ONTI, M; FORTERRE, P; KOONIN, E; RAOULT, D. The virophage as a unique parasite of the giant mimivirus. **Nature**, v.455, p.100–4, 2008.

LABRIE, SJ; SAMSON, JE; MOINEAU, S. Bacteriophage resistance mechanisms. Nature reviews. **Microbiology**, v.8, p.317-327, 2010.

LAWRENCE, SA; DAVY, JE; **WILSON, WH;** HOEGH-GULDBERG, O; DAVY, SK. Porites white patch syndrome: associated viruses and disease physiology. **Coral Reefs**, p.1-9, 2014a.

LAWRENCE, SA; **WILSON, WH;** DAVY, JE; DAVY, SK. Latent virus-like infections are present in a diverse range of Symbiodinium spp. (Dinophyta). **Journal of Phycology 50:**984-997, 2014b.

LEÃO, ZMAN; KIKUCHI, RKP. A relic coral fauna threatened by global changes and human activities, Eastern Brazil. **Marine Pollution Bulletin**, v.51, p.599-611, 2005.

LERUSTE, A; BOUVIER, T; BETTAREL, Y. Enumerating viruses in coral mucus. Appl **Environ Microbiol**, v.78, p.6377-6379, 2012.

LESSER, M. P. Coral reef bleaching and global climate change: can corals survive the next century? **Proceedings of the National Academy of Sciences of the United States of America**, v.104, p.5259-5260, 2007.

LESSER, MP. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. **Limnology and Oceanography**, v.41, p.271-283, 1996.

LESSER, MP; FARRELL, JH. Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. **Coral Reefs**, v.23, p.367–377, 2004.

LITTMAN, R; WILLIS, BL; BOURNE, DG. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. **Environmental Microbiology Reports**, v.3, p.651-660, 2011.

.

LOHR, J; MUNN, CB; WILSON, WH. Characterization of a latent virus-like infection of symbiotic zooxanthellae. **Appl Env Microbiol**, v.73, p.2976–2981, 2007.

MA, SS; ZHANG, Z; XIA, HC; CHEN, L; YANG, YH; YAO, Q; CHEN, KP. Evolutionary analysis of the ubiquitin gene of baculovirus and insect hosts. **Ma Genet Mol Res**, v.3, p.9963-73, 2015.

MAHON, C; KROGAN, N; CRAIK, C; PICK, E. Cullin E3 Ligases and Their Rewiring by Viral Factors. **Biomolecules**, v.4, p.897–930, 2014.

MALIN, G; WILSON, WH; BRATBAK, G; LISS, PS; MANN, NH. Elevated production of dimethylsulfide resulting from viral infection of cultures of Phaeocystis pouchetii. **Limnol. Oceanogr**, v.43, p.1389–1393, 1998.

MARHAVER, KL; EDWARDS, RA; ROHWER, F. Viral communities associated with healthy and bleaching corals. **Env Microbiol**, v.10, p.2277–2286, 2008.

MEINTS, RH; IVEY, RG; LEE, AM; CHOI, TJ. Identification of two virus integration sites in the brown alga Feldmannia chromosome. **J Virol**, v.82, p.1407–1413, 2008.

MEINTS, RH; IVEY, RG; LEE, AM; CHOI, TJ. Identification of two virus integration sites in the brown alga Feldmannia chromosome.**Journal of virology**, v.82, p.1407-1413, 2008.

MIDDELBOE, M; JORGENSEN, NOG; KROER, N. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. **Applied and Environmental Microbiology**, v.62, p.1991-1997, 1996.

MINAROVITS, J. Epigenotypes of latent herpesvirus genomes. **Current Topics in Microbiology and Immunology**, v.310, p.61-80, 2006.

MONIER, A; LARSEN, JB; SANDAA, RA; BRATBAK, G; CLAVERIE, JM; OGATA, H. Marine mimivirus relatives are probably large algal viruses. **Virology journal**, v.5, p.12, 2008.

MONIER, A; PAGARETE, A; DE VARGAS, C; ALLEN, MJ; READ, B; CLAVERIE, JM; OGATA, H. Horizontal gene transfer of an entire metabolic pathway between a eukaryotic alga and its DNA virus. **Genome Research**, v.19, p.1441-1449, 2009

MOREAU, H; PIGANEAU, G; DESDEVISES, Y; COOKE, R; DERELLE, E; GRIMSLEY, N. Marine prasinovirus genomes show low evolutionary divergence and acquisition of protein metabolism genes by horizontal gene transfer. **J Virol**, v.84, p.12555–63, 2010.

MOREIRA, D; BROCHIER-ARMANET, C. Giant viruses, giant chimeras: the multiple evolutionary histories of Mimivirus genes. **BMC Evol Biol**, v.8, p.12, 2008.

MOREIRA, D; LÓPEZ-GARCÍA, P. Comment on "The 1.2-megabase genome sequence of Mimivirus". **Science**, v.308, p.1114, 2005.

MUSCATINE, L; POOL, RR; TRENCH, RK. Symbiosis of algae and invertebrates: aspects of the symbiont surface and the host-symbiont interface. **Transactions of the American Microscopical Society**, v.94, p.450-469, 1975.

NAGASAKI, K. Dinoflagellates, diatoms, and their viruses. **Journal of Microbiology**. v.46, p.235–43, 2008.

NGUYEN-KIM, H; BOUVIER, T; BOUVIER, C; DOAN-NHU, H; NGUYEN-NGOC, L; ROCHELLE-NEWALL, E; BAUDOUX, AC; et al. High occurrence of viruses in the mucus layer of scleractinian corals. **Environmental Microbiology Reports**, v.6, p.675–682, 2014.

NOBLE, RT; FUHRMAN, JA. Breakdown and microbial uptake of marine viruses and other lysis products. **Aquatic Microbial Ecology**, v.20, p.1-11, 1999.

NOBLE, RT; FUHRMAN, JA. Rapid virus production and removal as measured with fluorescently labeled viruses as tracers. **Applied and Environmental Microbiology**, v.66, p.3790-3797, 2000.

NOBLE, RT; MIDDELBOE, M; FUHRMAN, JA. Effects of viral enrichment on the mortality and growth of heterotrophic bacterioplankton. **Aquatic Microbial Ecology**, v.18, p.1-13, 1999.

.

OPPEN, MJH; LOUGH, JM. Introduction: Coral bleaching — patterns, processes, causes and consequences. In **Coral Bleaching**, 2009.

PAGARETE, A; GRÉBERT, T; STEPANOVA, O; SANDAA, RA; BRATBAK, G. Tsv-N1: A Novel DNA Algal Virus that Infects Tetraselmis striata. **Viruses**, v.7, p.3937–3953, 2015.

PALINCSAR, JS. Effects of Isolation of the Endosymbiont Symbiodinium microadriaticum (Dinophyceae) from its host Aiptasia pallida (Anthozoa) on cell wall ultrastructure and mitotic rate. **Transactions of the American Microscopical Society**, v.107, p.53-66, 1988.

PATEL, MR; EMERMAN, M; MALIK, HS. Paleovirology - Ghosts and gifts of viruses past. **Curr Opin Virol**, v.1, p.304–309, 2011.

PATTEN, NL; HARRISON, PL; MITCHELL, JG. Prevalence of virus-like particles within a staghorn scleractinian coral (Acropora muricata) from the Great Barrier Reef. **Coral Reefs**, v.27, p.569-580, 2008.

POCHON, X; PAWLOWSKI, J. Evolution of the soritids-Symbiodinium symbiosis. **Symbiosis**, v.42, p.77-88, 2006.

POCHON, X; LAJEUNESSE, TC; PAWLOWSKI, J. Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (Symbiodinium; Dinophyta).**Marine Biology**, v.146, p.17-27, 2004.

REILLY, LM; GUARINO, LA. The viral ubiquitin gene of Autographa californica nuclear polyhedrosis virus is not essential for viral replication. **Virology**, v.218, p.243-7, 1996.
RIEGL, B; BRUCKNER, A; COLES, S L; RENAUD, P; DODGE, RE. Coral reefs: Threats and conservation in an era of global change. **Annals of the New York Academy of Sciences**, v.1162, p.136–86, 2009.

ROHWER, F; THURBER, RV. Viruses manipulate the marine environment. **Nature**, v.459, p.207-212, 2009.

ROHWER, F; SEGURITAN, V; AZAM, F; KNOWLTON, N. Diversity and distribution of coral-associated bacteria. **Mar Ecol Prog Ser**, v.243, p.1–10, 2002.

ROSENBERG, E; KUSHMARO, A; KRAMARSKY-WINTER, E; BANIN, E; YOSSI, L. The role of microorganisms in coral bleaching. **The ISME journal**, v.3, p,139-146, 2009.

ROSENWASSER, S; MAUSZ, MA; SCHATZ, D; SHEYN, U; MALITSKY, S; AHARONI, A; WEINSTOCK, E; et al. Rewiring Host Lipid Metabolism by Large Viruses Determines the Fate of Emiliania huxleyi, a Bloom-Forming Alga in the Ocean. **The Plant cell**, v.26, p.2689-2707, 2014.

SANTINI, S; JEUDY, S; BARTOLI, J; POIROT, O; LESCOT, M; ABERGEL, C; BARBE, V; et al. Genome of Phaeocystis globosa virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. **Proceedings of the National Academy of Sciences of the United States of America**, v.110, p.10800-5, 2013.

SANTOS, SR; TAYLOR, DJ; KINZIE, RA; HIDAKA, M; SAKAI, K; COFFROTH, MA. Molecular phylogeny of symbiotic dinoflagellates inferred from partial chloroplast large subunit (23S)-rDNA sequences. **Molecular Phylogenetics and Evolution**, v.23, p.97-111, 2002.

SEYMOUR, JR; PATTEN, N; BOURNE, DG; MITCHELL, JG. Spatial dynamics of virus-like particles and heterotrophic bacteria within a shallow coral reef system. **Marine Ecology Progress Series**, v.288, p.1-8, 2005.

SHINZATO, C; MUNGPAKDEE, S; SATOH, N; SHOGUCHI, E. A genomic approach to coral-dinoflagellate symbiosis: Studies of Acropora digitifera and Symbiodinium minutum. **Front Microbiol**, v.5, p.1–17, 2014.

SHOGUCHI, E; SHINZATO, C; KAWASHIMA, T; GYOJA, F; MUNGPAKDEE, S; KOYANAGI, R; TAKEUCHI, T; et al. Draft assembly of the Symbiodinium minutum nuclear genome reveals dinoflagellate gene structure. **Current Biology**, v.23, p.1399-1408, 2013.

SILVA-LIMA, AW; WALTER, JM; GARCIA, GD; RAMIRES, N; ANK, G; MEIRELLES, PM; NOBREGA, AF; SIVA-NETO, ID;, MOURA, RL; SALOMON, PS; THOMPSON, CC; THOMPSON, FL. Multiple Symbiodinium Strains Are Hosted by the Brazilian Endemic Corals Mussismilia spp. **Microb. Ecol**, 2015.

SLIMANI, M; PAGNIER, I; RAOULT, D; LA SCOLA, B. Amoebae as battlefields for bacteria, giant viruses, and virophages. **Journal of virology**, v.87, p.4783-5, 2013.
SOFFER, N; BRANDT, ME; CORREA, MAS; SMITH, TB; THURBER, RV. Potential role of viruses in white plague coral disease. **ISME J**, v.8, p.271–83, 2014.

SUTTLE, CA. Viruses in the sea. **Nature**, v.437, p.356-361, 2005.

SUTTLE, C. A. Marine viruses--major players in the global ecosystem. **Nature reviews. Microbiology**, v.5, p.801-812, 2007.

SYVANEN, M. Evolutionary implications of horizontal gene transfer. **Annual review of genetics**, v.46, p.341-58, 2012.

THURBER, RLV; CORREA, AMS. Viruses of reef-building scleractinian corals. **Journal of Experimental Marine Biology and Ecology**, v.408, p.102–13, 2011.

TRENCH, RK; WINSOR, H. Symbiosis with dinoflagellates in two pelagic flatworms, Amphiscolops sp. and Haplodiscus sp. **Symbiosis**, v.3, p.1-21, 1987.

ULRICH, HD; WALDEN, H. Ubiquitin signalling in DNA replication and repair. Nature reviews. **Molecular cell biology**, v.11, p.479-489, 2010.

VEGA THURBER, RL; BAROTT, KL; HALL, D; LIU, H; RODRIGUEZ-MUELLER, B; DESNUES, C; EDWARDS, RA; HAYNES, M; ANGLY, FE; WEGLEY, L; ROHWER, FL. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. **Proc Natl Acad Sci USA**, v.105, p.18413–18418, 2008.

VILLARREAL, LP. Virus-host symbiosis mediated by persistence. **Symbiosis**, v.44, p.1–9, 2007.

WANG, L; WU, S; LIU, T; SUN, J; CHI, S; LIU, C; LI, X; YIN, J; WANG, X; YU, J. Endogenous viral elements in algal genomes. **Acta Oceanologica Sinica**, v.33, p.102-107, 2014.

WEGLEY, L; EDWARDS, R; RODRIGUEZ-BRITO, B; LIU, H; ROHWER, F. Metagenomic analysis of the microbial community associated with the coral Porites astreoides. **Environ Microbiol**, v.9, p.2707–2719, 2007.

WILHELM, SW; SUTTLE, CA. Viruses and Nutrient Cycles in the Sea aquatic food webs. **BioScience**, v.49, p.781-788, 1999.

WILSON, WH; GILG, IC; DUARTE, A; OGATA, H. Development of DNA mismatch repair gene, MutS, as a diagnostic marker for detection and phylogenetic analysis of algal Megaviruses. **Virology**, v.466–67, p.123–28, 2014.

WILSON, WH; CHAPMAN, DM. Observation of virus-like particles in thin sections of the plumose anemone, Metridium senile. **J Mar Biol Assoc UK**, v.81, p.879, 2001b.

WILSON, WH; DALE, AL; DAVY, JE; DAVY, SK. An enemy within? Observations of virus-like particles in reef corals. **Coral Reefs**, v.24, p.145–148, 2005.

WILSON, WH; FRANCIS, I; RYAN, K; DAVY, SK. Temperature induction of viruses in symbiotic dinoflagellates. **Aquatic Microbial Ecology**, v.25, p.99-102, 2001a.

YAU, S; LAURO, FM; DEMAERE, MZ; BROWN, MV; THOMAS, T; RAFTERY, MJ; ANDREWS-PFANNKOCH, C; et al. Virophage control of antarctic algal host-virus dynamics. **Proceedings of the National Academy of Sciences of the United States of America**, v.108, p.6163-6168, 2011.