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**VIRIOPLÂNCTON NA AMAZÔNIA: ESTUDO DA  
DIVERSIDADE DOS VÍRUS DAS FAMÍLIAS  
*PHYCODNAVIRIDAE* E *MYOVIRIDAE* NOS RIOS NEGRO,  
SOLIMÕES E CUIEIRAS**

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## RESUMO

Gimenes MV. Virioplâncton na Amazônia: estudo da diversidade dos vírus das famílias *Phycodnaviridae* e *Myoviridae* nos rios Negro, Solimões e Cuieiras [tese (Doutorado em Microbiologia)]. São Paulo: Instituto de Ciências Biomédicas da Universidade de São Paulo; 2010.

Os vírus são os agentes biológicos mais abundantes em ambientes aquáticos, podendo ser encontrados em concentrações de até  $3 \times 10^9$  partículas por litro. Infectam, provavelmente, todos os organismos e micro-organismos aquáticos e estão presentes nos mais diversos sistemas, como, oceanos, rios, lagos, estromatólitos, recifes de corais, fumarolas submarinas, lagos hipersalinos, lagos alcalinos, fontes termais e gelo polar. Hoje se sabe que a comunidade viral - virioplâncton - é parte ativa e indispensável a ambientes aquáticos, tendo grande importância ecológica, uma vez que está envolvida em processos como ciclagem de nutrientes, diversificação e distribuição de algas e bactérias, controle de florações fitoplanctônicas, formação de nuvens e transferência gênica horizontal. Os objetivos deste projeto foram analisar as populações de ficodnavírus (*Phycodnaviridae*) e miofagos (*Myoviridae*) nos rios Cuieiras, Negro e Solimões, localizados na bacia Amazônica, próximo à região de Manaus, e determinar um possível impacto do lançamento de efluentes domésticos sobre essas populações. Para tanto, foram utilizadas as técnicas moleculares de *PCR* (*Polimerase Chain Reaction*), *DGGE* (*Denaturing Gradient Gel Electrophoresis*), clonagem e sequenciamento, e microscopia eletrônica de transmissão (MET), além de análises filogenéticas e de filodinâmica, e levantamento da flora fitoplanctônica. A presença de ficodnavírus só foi constatada nas amostras dos rios Solimões e Cuieiras, *PCR*, mas não por MET, enquanto que miofagos foram detectados em todas as amostras dos rios Negro, Cuieiras e Solimões, por ambos os métodos. O perfil eletroforético da população de miofagos, resultante da *DGGE*, foi característico para cada rio, e mostrou-se constante nas amostras de um mesmo rio; a aplicação da técnica para análise dos ficodnavírus não foi possível. A partir das amostras de água coletadas, foram obtidos 104 clones de ficodnavírus e 180 de miofagos, cujas sequências foram utilizadas em estudos filogenéticos e de filodinâmica. Estes revelaram que os miofagos não se agrupam em função das características do ambiente nem da localidade, havendo uma relação mais próxima entre os clones ambientais do que com os fagos isolados. Com relação aos ficodnavírus, os resultados mostraram que os clones provenientes de ambientes de água doce são mais proximamente relacionados com os vírus marinhos *MpV*, *BpV* e *OsV*, do que com o vírus *PBCV-1*, também de água doce, e que os clones ambientais agrupam-se em clados distintos dos de vírus isolados; os ficodnavírus não são panmíticos em relação ao ambiente que ocupam, havendo fluxo gênico entre os ambientes lótico e lêntico, e do marinho para os de água doce, mas não o contrário; houve variação no número de populações de ficodnavírus no último 1,5 milhão de anos, com um mínimo alcançado no período de 500 a 300 mil anos, seguido de recuperação até o presente. Contrariamente ao esperado, a interferência do lançamento de efluentes domésticos sobre as características da água e sobre as populações locais virais das famílias *Phycodnaviridae* e *Myoviridae* e fitoplanctônicas não foi observada, apesar de ter sido sugerida pela diminuição do número de táxons de fitoplâncton.

**Palavras-chave:** Amazônia. DGGE. Filodinâmica. *Myoviridae*. *Phycodnaviridae*.

## ABSTRACT

Gimenes MV. Virioplankton in the Amazon: diversity study of viruses from *Phycodnaviridae* and *Myoviridae* families in the Negro, Solimões and Cuieiras rivers [Ph.D. Thesis (Microbiology)]. São Paulo: Instituto de Ciências Biomédicas da Universidade de São Paulo; 2010.

Viruses are the most abundant agents in aquatic environments, found in concentrations up to  $3 \times 10^9$  particles per liter. They infect a wide spectrum of organisms and microorganisms, and are present in a variety of aquatic systems such as, oceans, rivers, lakes, stromatolites, coral reefs, submarine fumaroles, hypersaline and alkaline lakes, thermal vents and polar ice. It is now known that the viral community (virioplankton) is an active and essential part of aquatic environments, being of great ecological importance since it is involved in processes such as nutrient cycling, diversification and distribution of algae and bacteria, phytoplankton bloom termination, cloud formation and horizontal gene transfer. The aims of this work were to analyse the populations of phycodnaviruses (*Phycodnaviridae*) and myophages (*Myoviridae*) in the Cuieiras, Negro and Solimões rivers, located in the Amazon Basin, close to the city of Manaus, and to determine a possible impact of sewage discharge in the Negro river on these populations. In order to accomplish that, PCR (Polimerase Chain Reaction), DGGE (Denaturing Gradient Gel Electrophoresis), cloning and sequencing, and Transmission Electron Microscopy (TEM) were used. Phylogenetics and phylodynamics analyses and a phytoplankton survey were also conducted. Phycodnaviruses were only detected in the samples from the Solimões and Cuieiras rivers, by PCR, but not by TEM, whereas myophages were detected in all samples from the Negro, Cuieiras and Solimões rivers by both PCR and MET. Myophages' DGGE fingerprints were unique for each river and constant in all samples from the same river; the use of DGGE fingerprinting for phycodnaviruses was not possible. A total of 104 and 180 clones of phycodnavirus and myophages were obtained, respectively, and these sequences were used in phylogenetic and phylodynamic analyses. Results showed that myophages do not cluster accordingly neither to the environment nor to the geographic location, and that environmental clones tend to cluster among themselves more than with isolated phages. Phycodnaviruses' phylogeny showed that freshwater clones are more closely related to the marine viruses *MpV*, *BpV* and *OsV* than to the freshwater virus *PBCV-1*, and that environmental clones cluster separately from isolated viruses. Results also showed that phycodnaviruses are not panmitic and that there is gene flux between riverine and lacustrine environments and also from marine to freshwater ones, but not the contrary. A fluctuation on the number of phycodnavirus' populations was observed in the last 1.5 million years, reaching the lowest values around 500 to 300 thousand years ago, followed by a recovery towards the present. Adversally to what was expected, the interference of sewage discharge on the water characteristics and the local viral (phycodnavirus and myophage) and phytoplanktonic populations was not detected, even though indicated by the decrease of phytoplankton taxa.

**Keywords:** Amazon. DGGE. Phylodynamics. *Myoviridae*. *Phycodnaviridae*.

## 1 INTRODUÇÃO

*“What is a scientist after all?  
It is a curious man looking through a keyhole,  
the keyhole of nature, trying to know what's going on.”*  
(Jacques Cousteau)

A história e a atividade da vida na Terra têm sido guiadas pela presença e ação de micro-organismos, uma vez que eles foram e continuam sendo agentes indispensáveis na evolução climática, geológica, geoquímica e biológica do planeta. Por muitos anos, estudos ecológicos sobre a comunidade microbiológica aquática estiveram restritos à pesquisa de bactérias. A descoberta da existência de grande número de partículas virais (virioplâncton) em ambientes pelágicos marinhos, em 1979 (Torrella e Morita, 1979), entretanto, marcou o início de um novo campo de pesquisa, que vem se expandindo rapidamente nos últimos 15 anos, principalmente após o reconhecimento da importância dos vírus aquáticos, em 1983, por Azam et al.

Não há dúvidas de que os vírus sejam parte ativa e de grande importância em ecossistemas aquáticos. Em ambientes marinhos, essa comunidade tem sido estudada nas diversas latitudes, do Ártico (Steward et al., 1996) à Antártica (Steward et al., 1992), passando pelos Oceanos Atlântico e Pacífico, em ambas as porções temperada e tropical (Bergh et al., 1989; Cochlan et al., 1993; Wilhelm et al., 2003). Tais estudos têm focalizado não somente a zona fótica da coluna d'água, mas também o sedimento (Hewson et al., 2001; Danovaro et al., 2008), fumarolas submarinas (Ortmann e Suttle, 2005), *wetlands* (Jackson e Jackson, 2008), recifes de corais (Patten et al., 2006; Davy e Patten, 2007; Patten et al., 2008) e estromatólitos (Desnues et al., 2008).

Apesar de o mesmo não ocorrer para ambientes de água doce, onde um número significativamente menor de estudos tem sido conduzido somente em ambientes lacustres do hemisfério norte (Colombet et al., 2006; Lymer et al., 2008a; Lymer et al., 2008b; Tijdens et al., 2008a; Clasen e Suttle, 2009), na Antártica (Madan et al., 2005; Vanucci et al., 2005; Sävström et al., 2007), e em alguns rios europeus e norte-americanos (Leroy et al., 2008; Peduzzi e Luef, 2008; Short e Short, 2008), sabe-se, hoje, que o conhecimento referente a ambientes marinhos estende-se aos de água doce. Além de a importância dos vírus na cadeia alimentar microbiana já ter sido verificada em ambos os ambientes, a abundância do virioplâncton, o tamanho da progênie viral, assim como taxa de produção e porcentagem de células infectadas, também parecem ser independentes da salinidade, mas relacionadas ao estado trófico do ambiente e às variações sazonais. Apesar de o papel dos vírus nos ambientes aquáticos ser bastante complexo e ainda permanecerem muitas questões em aberto, a literatura disponível indica que, apesar de haverem algumas diferenças, muitos dos controles sobre a atividade e a diversidade do virioplâncton são similares em ambientes marinhos e de água doce (Wilhelm e Matteson, 2008), o que nos permitiu caracterizar o grupo com base em dados provenientes de ambos os ambientes.

Em âmbito nacional, a totalidade dos estudos realizados na área da virologia “ambiental”, até o presente momento, enfoca apenas aspectos de Saúde Pública e agentes causadores de doenças (Santos et al., 2004; De Paula et al., 2007; Miagostovich et al., 2008; Ferreira et al., 2009). Em adição à ausência de estudos brasileiros sobre a ecologia de vírus aquáticos verdadeiramente ambientais (autóctones), a importância da região amazônica, principalmente em termos de biodiversidade, a riqueza de ambientes da região e a importância ecológica do virioplâncton justificaram a realização do presente estudo, juntamente com a necessidade de se conhecer melhor esses vírus e seus papéis, na busca por uma compreensão mais abrangente da ecologia dos ambientes aquáticos e das famílias virais envolvidas.

## 6 CONCLUSÕES

- Os dados físicos e químicos das amostras amazônicas confirmaram sua classificação como “águas-pretas” para os rios Negro e Cuieiras e “águas-brancas” para o Solimões.
- A presença de ficodnavírus nas amostras dos rios Solimões e Cuieiras foi constatada por *PCR*, mas não por *MET*. Nas amostras do rio Negro a presença desse vírus não foi constatada por nenhum dos métodos.
- A presença de miofagos em todas as amostras dos rios Negro, Cuieiras e Solimões foi constatada tanto por *PCR* quanto por *MET*.
- O perfil eletroforético da população de miofagos foi característico para cada rio e mostrou-se constante nas amostras de um mesmo rio. Tal caracterização, porém, não foi possível para ficodnavírus.
- Foi gerado um banco de dados com 104 sequências de ficodnavírus e 180 sequências de miofagos, originárias dos rios amostrados.
- O estudo filogenético de ficodnavírus revelou que os clones provenientes de ambientes de água doce são mais proximamente relacionados com os vírus marinhos *MpV*, *BpV* e *OsV* do que com o vírus *PBCV-1*, também de água doce, e que os clones ambientais agrupam-se em clados distintos dos de vírus isolados.
- O estudo filogenético de miofagos revelou que estes não se agrupam em função das características do ambiente nem da localidade, havendo uma relação mais próxima entre os clones ambientais do que com os fagos isolados.
- Os ficodnavírus não são panmíticos em relação ao ambiente que ocupam. Verificou-se fluxo gênico entre os ambientes lótico e lêntico, e do marinho para os de água doce, mas não o contrário.
- O estudo filodinâmico revelou que o número de populações de ficodnavírus variou no último 1,5 milhão de anos, alcançando seu mínimo no período de 500 a 300 mil anos atrás, seguido de recuperação até o presente.
- Os grupos de fitoplâncton encontrados nas amostras analisadas referendam os dados da literatura.
- A interferência do lançamento de efluentes domésticos sobre as características da água e sobre as populações locais virais das famílias *Phycodnaviridae* e *Myoviridae* e fitoplanctônicas não foi observada, apesar de ter sido sugerida pela diminuição do número de táxons de fitoplâncton.

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