*Journal of the Marine Biological Association of the United Kingdom*, 2013, 93(4), 909–912. 
doi:10.1017/S0025315411002062

# Observation of virus-like particles in thin sections of the bleaching scleractinian coral *Acropora cytherea*

YVAN BETTAREL[1], NGUYEN THANH THUY[2], TRAN QUANG HUY[2], PHAN KIM HOANG[3] AND THIERRY BOUVIER[1]

[1]IRD, UMR 5119 ECOSYM, Université Montpellier 2, France, [2]National Institute of Hygiene and Epidemiology (NIHE), Laboratory of Electron Microscopy, Hanoi, Vietnam, [3]Vietnamese National Institute of Oceanography (VNIO), Nha Trang, Vietnam

*Virus-like particles were observed in thin sections of partially bleaching colonies of the scleractinian* Acropora cytherea*, collected in the Hon Mun Marine Protected Area (Vietnam). The most common particles were found in the cytoplasm of epidermal cells, exhibiting icosahedral symmetry and measuring 90–140 nm in diameter. Some had a tail-like structure resembling that of certain Hepadnaviridae such as the hepatitis B virus. Some others were tailless with a virion size. Overall, less than 10% of the cells showed obvious signs of infection. Images of virally-parasitized tissues of* A. cytherea *provide further evidence that corals are a target for viral infection. However, more research is required into their pathogenicity and involvement in bleaching events.*

**Keywords:** virus-like particles, hepatitis B virus, thin sections, partially bleaching scleractinian colonies, *Acropora cytherea*

Submitted 10 November 2011; accepted 13 November 2011; first published online 5 July 2012

## INTRODUCTION

Coral reefs are amongst the most productive biological zones on Earth and are home to an extraordinary biodiversity which provides food for nearly 500 million people (Wilkinson, 2008). These ecosystems are certainly the most vulnerable marine environments and have declined dramatically over the last 30 years owing to the combined effect of global warming and increasing anthropogenic pressure (Hoegh-Guldberg, 2010). The most visible and widespread manifestation of this decline is coral bleaching and to a lesser extent the emergence of other microbial pathologies (yellow blotch, black band disease, white band disease, white plague, etc) which now affect coral reefs throughout the world (Normile, 2010). However, despite efforts made by the scientific community to protect this biological heritage, the ecological and physiological processes that lead to these coral diseases are still unproven and often challenged.

The most common explanation for bleaching is related to the expulsion of endosymbiotic zooxanthellae (*Symbiodinium* spp.) by the coral polyps as a result of prolonged increase in temperature or other environmental changes (Rosenberg *et al.*, 2007). An alternative theory, put forward during the past ten years by an increasing number of microbiologists, is that bleaching could be the result of a sudden structural change in the bacterial and archaeal communities in the mucus in response to environmental disturbance (increase in temperature, in particular, but also changes in nutrients, acidification, etc) (Bourne *et al.*, 2008; Rosenberg *et al.*, 2009).

Viruses are surely the least studied of the biological entities that form the coral holobiont. Their role remains uncertain and little is known about the possible pathogenic or mutualistic relationships that they may have with the host animal (Davy *et al.*, 2006; van Oppen *et al.*, 2009). A few recent studies have shown that the surface microlayer of mucus contains a large number of viruses that are morphologically and genetically very diverse and differ from the viral communities in the surrounding water (Davy *et al.*, 2007; Marhaver *et al.*, 2008), suggesting specific interactions between these viruses and the other members of the holobiont. The high levels of viral abundance found in healthy corals also suggest that their activity may stabilize or even improve the coral biological balance rather than disturbing it. For example, Rohwer *et al.* (2002) and van Oppen *et al.* (2009) suggested that viruses could be determinant in preventing the emergence of pathogens. Viruses were also thought to improve coral viability by encoding for genes that provide benefits to the coral host (Patten *et al.*, 2008).

Viruses have also been found in thin sections of zooxanthellae from thermally-stressed corals (Wilson *et al.*, 2001, 2005; Lohr *et al.*, 2007), implying that a fraction of these dinoflagellate symbionts could harbour latent viruses. Unfortunately, such observations were only made in corals that had been cultured under artificial conditions (aquarium) and not in a natural environment. Therefore, the hypothesis that viral infection of zooxanthellae is a mechanism involved in temperature-induced coral bleaching clearly lacks supporting evidence. Furthermore, observations of virally-infected coral tissues are rare and no scleractinian diseases have yet been firmly attributed to viral origin. Viruses were first

**Corresponding author:**
Y. Bettarel
Email: yvan.bettarel@ird.fr

reported in Cnidaria cells of the plumose anemone *Metridium senile* (Wilson & Chapman, 2001) and the scleractinian *Pavona danai* (Wilson *et al.*, 2005). Although these two species were also grown artificially in aquaria, a more recent study conducted in the Great Barrier Reef (Australia) revealed the presence of intracellular viruses in epidermal and gastrodermal cells of the coral *Acropora muricata* (Patten *et al.*, 2008). Viruses were found in both healthy and diseased colonies (white syndrome), suggesting that they might not be directly involved in coral morbidity or mortality. Different sizes and morphologies of viruses were found in the tissues of *P. danai*, which led the authors (Patten *et al.*, 2008) to conclude that 'viruses are infecting different organisms within the holobiont, or that one host is susceptible to infection from more than one type of virus'. Although there is conclusive evidence that viruses are a component of the coral holobiont, little is known about whether or not they contribute to the worldwide decline of coral reef environments. This study showed that bleaching colonies of the scleractinian *Acropora cytherea* harboured intracellular viruses in their epidermal cells.

## MATERIALS AND METHODS

Our study used a single sample of those taken in the Hon Mun Marine Protected Area (MPA) in Vietnam (12°09′53″N 109°18′38″E) on 11 November 2010. Coral colonies were sampled at depths between 2 and 4 m in a zone where coral tissues were subject to partial bleaching. The superficial microlayer of *A. cytherea* was removed gently from the coral surface using 10 ml syringe (Davy & Patten, 2007). It was then fixed in a solution of 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4 and stored overnight at 4°C. Samples were then washed three times in 0.1M cacodylate buffer for 15 minutes and post-fixed in 1% osmium tetroxide for 1 hour, at room temperature. Samples were then rinsed twice in 0.1M cacodylate buffer (10 minutes), dehydrated in an ethanol series (50%, 70%, 90% and 100%) and propylene oxide (10 minutes/each), embedded in Epon 812. Thin 60–80 nm sections were obtained by ultra-microtome (Leica, UC6), using collodion coated copper grids (300 meshes). The sections were double-stained for 5 minutes with 5%

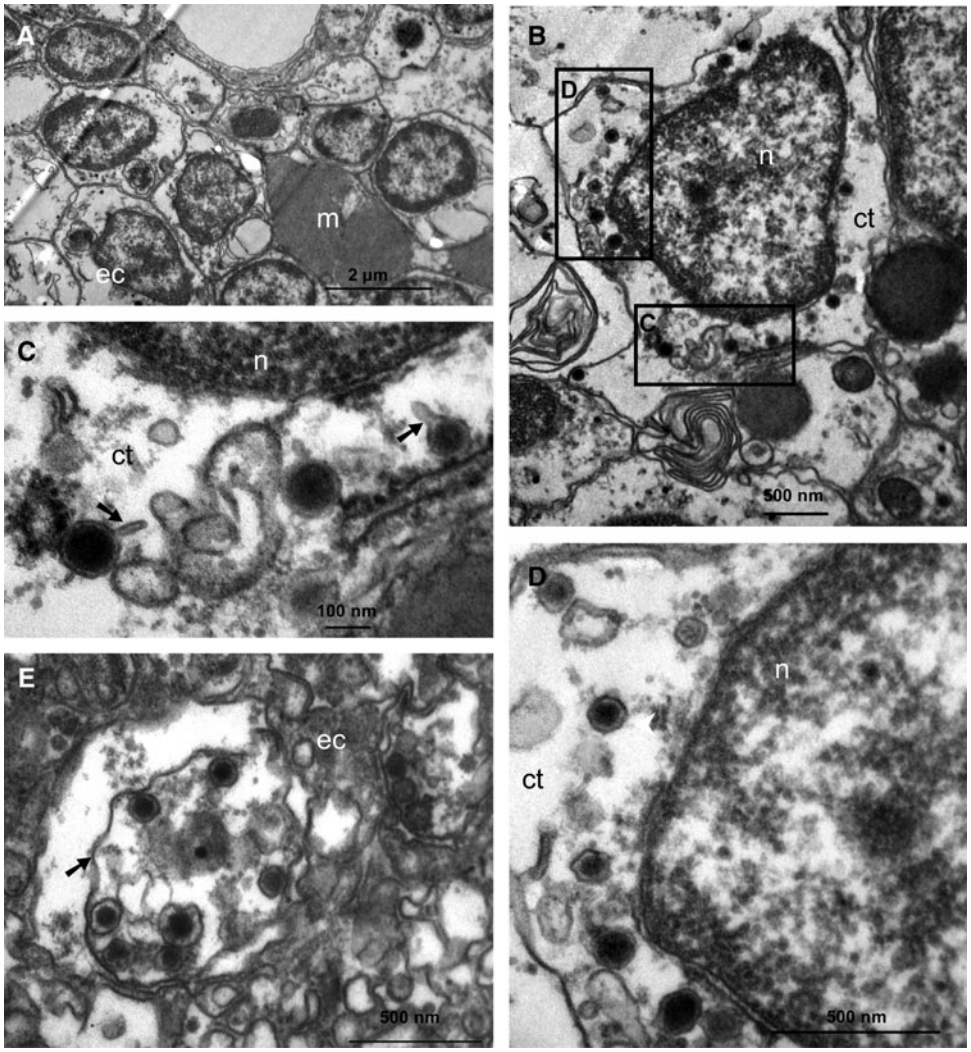


**Fig. 1.** Virus-like particles (VLPs) within epidermis cells of *Acropora cytherea* coral colonies from the Hon Mun Marine Protected Area (Vietnam). (A) Uninfected polymorphic epidermal cells (ec) and mesoglea (m); (B) overall view of epidermal cells with numerous VLPs within the cytoplasm (ct); nucleus (n); (C) detail of (B). Spherical VLPs with a capsid diameter of 90–140 nm, showing an electron-dense core surrounded by an electron-lucent layer. Note the presence of a tail-like structure (arrow) in two of the three VLPs; (D) second detail of (B). Here, the VLPs lack tails and are hexagonal and spherical in shape with capsid diameter of 80–130 nm; (E) an inclusion of untailed VLPs, 130–140 nm in diameter within a membrane bound vacuole (arrowed) in epidermal tissue.

https://doi.org/10.1017/S0025315411002062 Published online by Cambridge University Press

uranyl acetate and 10 minutes with lead citrate, and examined at 80 kV by transmission electron microscopy (TEM:JEOL 1010).

## RESULTS AND DISCUSSION

In the single sample that was analysed, only two out of more than 20 epidermal cells showed obvious signs of viral infection. The virions observed by TEM display different stages of maturation, suggesting that the viruses effectively replicate in the coral host. Virus-like particles (VLPs) were all found in the cytoplasm, were pentagonal or hexagonal in section, indicating icosahedral symmetry (Figure 1B). These VLPs had an electron-dense core surrounded by a thin double layer. The diameter of these VLPs ranged from 90 to 140 nm. The VLPs did not have an outer envelope. Although it was difficult to assign them to a viral family from their morphology, their characteristics resembled those previously published for another subspecies of *Acropora* (i.e. *muricata*) in the Great Barrier Reef (Patten *et al.*, 2008), which were thought to belong to the Phycodnaviridae and Iridoviridae viral families. Recent metagenomic analyses also revealed the presence of abundant viruses of plant, algae, prokaryotes and also, more surprisingly, of viruses with herpes-like sequences within the tissues of *Diploria strigosa* (Marhaver *et al.*, 2008) and *Porites compressa* (Thurber *et al.*, 2009). However, unlike herpes viruses, those observed in *A. cytherea* had no envelope. Some even had tail-like structure similar to that of phages of the order Caudovirales. Although eukaryotic viruses are typically tailless, the protein of the virion coat is sometimes produced in excess and extends as a tubular tail on one side of the virus particle that becomes partially filamentous. For example, some viruses of the Hepadnaviridae family such as hepatitis B virions are known to vary between tubular and spherical forms (Kaito *et al.*, 2006).

Herein, *A. cytherea* was likely infected by more than one type of virus. Indeed, the epidermal cells were also infected by tailless viruses whose size of the virions varied from 80–130 nm and 130–140 nm (see insets B, E Figure 1). The disparity between the virions could not simply be explained by the fact they were sectioned in different planes. In their recent study, Patten *et al.* (2008) also showed that coral tissues can harbour viruses differing in size and/or morphology.

However, no clear causal link between their presence and the current bleaching event can be established. Pathogenicity is in question as viruses have also been found in healthy (and diseased) *Acropora muricata* (Patten *et al.*, 2008) and *Diploria strigosa* (Marhaver *et al.*, 2008). This raises several questions: (i) is there a threshold beyond which the abundance of intracellular viruses becomes lethal to the host? This is not unrealistic as previous experiments showed that herpes-like viral sequences were abundant in most ultraviolet/nutrient/temperature-stressed *Porites compressa* but were rarely detected in healthy individuals (Thurber *et al.*, 2009). It was, therefore, suspected that stress could trigger the activation of latent viruses in coral tissues. However, further investigation is needed to determine the potential pathogenicity of these viruses; (ii) are corals simply subjected to chronic or asymptomatic infection? In the first case, viruses are still able to persist and viral replication continues despite the coral immune response (Reed *et al.*, 2010), the balance of the immune response thus being in favour of the virus. In the case of asymptomatic infection, infected corals still produce numerous viruses without developing disease; and finally (iii) can viruses manipulate corals? In other words, are viruses a source of functions that are beneficial for the corals by providing additional genes that may optimize their performance in nutrition, reproduction, resistance to pathogens, etc? (Rohwer & Thurber, 2009). It is also possible that, as suggested for zooxanthellae (Wilson & Chapman, 2001), the presumed latent infection of coral tissues may confer immunity against other viral infections and thus ensure the stability of the polyps. These are all valid hypotheses and they are not mutually exclusive. Clearly, the successful isolation and characterization of these holobiont viruses is imperative if we are to understand their ecological role.

## ACKNOWLDEGEMENTS

The research reported here was financially supported by the EC2CO project 'CORINE' and the Total Foundation. We gratefully acknowledge advice, discussion, encouragement and assistance provided by Drs Vo Si Tuan, Bui Hong Long, Nguyen Van Long, Chu Van Thuoc, Jean-Pascal Torréton and Aurore Trottet. We also sincerely thank the staff of the Electron Microscopy Laboratory at the National Institute of Hygiene and Epidemiology of Hanoï for their help with the sample preparation for TEM analyses.

## REFERENCES

**Bourne D., Lida Y., Uthicke S. and Smith-Keune C.** (2008) Changes in coral-associated microbial communities during a bleaching event. *The ISME Journal* 2, 350–363.

**Davy S.K., Burchett H.E., Dale A.L., Davies P., Davy J.E., Muncke C., Hoegh-Guldberg O. and Wilson W.H.** (2006) Viruses: agents of coral disease? *Diseases of Aquatic Organisms* 69, 101–110.

**Davy J.E. and Patten N.L.** (2007) Morphological diversity of virus-like particles within the surface microlayer of scleractinian corals. *Aquatic Microbial Ecology* 47, 37–44.

**Kaito M., Ohba H., Chiba J., Kohara M., Tanaka H., Fujita N., Gabazza E.C., Watanabe S., Konishi M. and Adachi Y.** (2006) The ultrastructural morphology of native hepatitis B virus. *Medical Molecular Morphology* 39, 136–145.

**Hoegh-Guldberg O.** (2010) Dangerous shifts in ocean ecosystem function? *The ISME Journal* 4, 1090–1092.

**Lohr J., Munn C.B. and Wilson W.H.** (2007) Characterization of a latent virus-like infection of symbiotic zooxanthellae. *Applied and Environmental Microbiology* 73, 2976–2981.

**Marhaver K.L., Edwards R.A. and Rohwer F.** (2008) Viral communities associated with healthy and bleaching corals. *Environmental Microbiology* 10, 2277–2286.

**Normile D.** (2010) Hard summer for corals kindles fears for survival of reefs. *Science* 329, 1001.

**Patten N.L., Harrison M.L. and Mitchell G.J.** (2008) Prevalence of virus-like particles within a staghorn scleractinian coral (*Acropora muricata*) from the Great Barrier Reef. *Coral Reefs* 27, 569–580.

**Reed K.C., Muller E.M. and van Woesik R.** (2010) Coral immunology and resistance to disease. *Diseases of Aquatic Organisms* 90, 85–92.

**Rosenberg E., Koren O., Reshe L., Efrony R. and Zilber-Rosenberg I.** (2007) The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology* 5, 355–362.

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

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

**Rohwer F. and Thurber R.V.** (2009) Viruses manipulate the marine environment. *Nature* 459, 207–212.

**Thurber R.V., Willner-Hall D., Rodriguez-Mueller B., Desnues C., Edwards R.A., Angly F., Dinsdale E., Kelly L. and Rohwer F.** (2009) Metagenomic analysis of stressed coral holobionts. *Environmental Microbiology* 11, 2148–2163.

**van Oppen M.J.H., Leong J.O., Gates D.R.** (2009) Coral–virus interactions: a double-edged sword? *Symbiosis* 47, 1–8.

**Wilkinson C.** (2008) *Status of coral reefs of the world: 2008*. Townsville, QL: Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, 296 pp.

**Wilson W.H. and Chapman D.M.** (2001) Observation of virus-like particles in thin sections of the plumose anemone, *Metridium senile*. *Journal of the Marine Biological Association of the United Kingdom* 81, 879–880.

**Wilson W.H., Francis I., Ryan K. and Davy S.K.** (2001) Temperature induction of viruses in symbiotic dinoflagellates. *Aquatic Microbial Ecology* 25, 99–102.

and

**Wilson W.H., Dale A.L., Davy J.E. and Davy S.K.** (2005) An enemy within? Observations of virus-like particles in reef corals. *Coral Reefs* 24, 145–148.

**Correspondence should be addressed to:**
Y. Bettarel
IRD, UMR 5119 ECOSYM
Université Montpellier 2, France
email: yvan.bettarel@ird.fr

https://doi.org/10.1017/S0025315411002062 Published online by Cambridge University Press