

carbyne equivalents, or through the development of alternative ones. ■

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## MARINE VIROLOGY

# A non-tailed twist in the viral tale

Microscopy studies indicate that the most common viruses in the sea lack a tail structure. However, most cultured marine viruses have tails. A family of these elusive non-tailed marine viruses has now been identified. [SEE LETTER P.118](#)

**JULIO CESAR IGNACIO-ESPINOZA & JED A. FUHRMAN**

**B**acteriophages, the viruses that infect bacteria, are thought to be the most abundant biological entities on Earth<sup>1</sup>. It has been estimated that, if lined up end to end, bacteriophages from the oceans alone would cover a distance of 3 million parsecs, past many distant galaxies<sup>2</sup>. Most bacteriophages cultured in the laboratory or represented in DNA-sequence databases have a ‘tail’ structure, which might take the form of a tube with spider-leg-like protrusions (similar in shape to the base of the Apollo Moon lander). However, electron-microscopy analysis of ocean samples indicates that oceanic viruses

are predominantly non-tailed<sup>3–5</sup>. Therefore, identifying the ‘missing’ non-tailed marine viruses might improve our understanding of how viruses regulate the microbial systems that control a large fraction of global carbon and nitrogen cycling<sup>2,6</sup>. On page 118, Kauffman *et al.*<sup>7</sup> report the discovery of a non-tailed viral family that they suggest might be an important component of the missing viruses, and they explain why this group might have eluded detection until now.

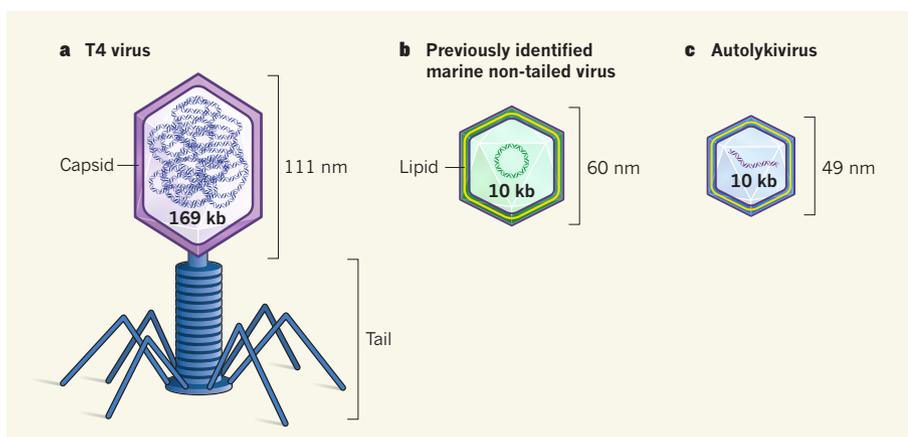
Viruses can be key drivers of the evolution, community composition and mortality of microorganisms, although their major role in the oceans was not recognized until the 1990s<sup>2,6</sup>. The number of species a virus can infect is a crucial factor that can influence

gene transfer between species and the spread of viral infection. Bacteriophage action is usually studied using models in which a virus infects only one or a small group of closely related host strains or species, because this is the pattern observed for most of the bacteriophages cultured so far. And yet such cultures represent just a small proportion<sup>8</sup> of the hundreds of known bacterial phyla<sup>9</sup>.

Kauffman and colleagues sought to expand the breadth of marine viruses known. They analysed water samples collected off the coast of Massachusetts on three separate days, and attempted to identify bacteriophages that could infect any of the 1,334 strains of Vibrionaceae bacteria that they had isolated. The Vibrionaceae are an easily cultured bacterial group that contains the cholera-causing pathogen *Vibrio cholerae*, as well as more-benign relatives, such as bioluminescent bacteria that form symbiotic relationships with fishes and squid.

Of all the bacteria that the authors tested, 239 strains became infected with viruses, and Kauffman *et al.* isolated 241 previously unknown viruses, of which 18 were non-tailed. They named this non-tailed family (Fig. 1) the *Autolykiviridae*, after Autolykos, an elusive thief from Greek mythology who could not be caught. DNA-sequence analysis revealed that autolykiviruses have small genomes (approximately 10 kilobases in length), which diverge enough from those of known viruses to form their own distinct lineage. They differ from other bacteriophages in sequences encoding a specific structural fold in a capsid protein, which forms the outer viral shell. The putative capsid-encoding sequences identified by Kauffman and colleagues were most like those that encode a fold known as a double jelly roll. This fold was previously associated with non-tailed viruses, in contrast to the HK97 fold found in tailed bacteriophages<sup>10</sup>.

To investigate the ecological role of autolykiviruses, the authors performed a monumental host-range analysis, testing the ability of their 241 marine-isolated viruses to infect and kill 318 of their marine bacterial strains. They found that the 18 non-tailed autolykiviruses were responsible for a disproportionately high number of bacterial cell deaths. This was mainly because the autolykiviruses had a substantially wider host range than the tailed group, and could infect multiple Vibrionaceae genera, whereas the tailed viruses studied could not. The authors therefore propose that the impact of autolykiviruses on the marine environment might be fundamentally



**Figure 1 | Different viral forms.** Viruses that contain a tail structure are the most common type of bacterium-infecting virus (bacteriophage) cultured in the laboratory or represented in DNA databases. However, in samples taken from marine environments, non-tailed viruses are more common<sup>3–5</sup>. Kauffman *et al.*<sup>7</sup> report a previously unknown family of non-tailed marine viruses. **a**, T4, an example of a tailed virus. Its 169-kilobase genome is enclosed in a capsid structure, made of protein (dark purple), that is 111 nanometres long<sup>15</sup>. The average capsid length for tailed marine viruses<sup>5</sup> is 65 nm. The tail structures in certain other types of tailed virus have a different shape from that of T4. **b**, The cortovirus PM2, one of the few non-tailed marine bacteriophages identified so far. PM2 has lipid (yellow) associated with its capsid. Non-tailed marine viruses have an average capsid size<sup>5</sup> of 54 nm. **c**, An autolykivirus, a member of a family of non-tailed marine viruses identified by Kauffman *et al.*<sup>7</sup>. The properties of these bacteriophages are consistent with the presence of lipid.

different from that of tailed bacteriophages. Although this is possible, it is perhaps premature to generalize. Kauffman *et al.* examined only Vibrionaceae. Moreover, some tailed marine bacteriophages have wide host ranges<sup>11</sup>.

How did these viruses evade previous detection in highly studied systems? The authors note that this could be because some standard viral isolation approaches for lab culture and DNA analysis have sampling biases, which might arise if a virus particle contains lipid, as seems to be the case for the autolykiviruses.

Chloroform treatment is commonly used to disrupt cell membranes as a way of limiting bacterial contamination during bacteriophage isolation; however, Kauffman *et al.* report that chloroform can inactivate autolykiviruses. And when a density-gradient centrifugation approach is used to isolate bacteriophages for DNA-sequence analysis, autolykiviruses are found in a low-density fraction separate from the heavier fraction that contains most bacteriophages (and which is thus usually analysed). An additional snag is the presence of protein covalently bound to autolykiviral DNA that necessitates treatment with a protease enzyme for DNA extraction, an uncommon step in standard large-scale virus-sequencing studies. These technical obstacles should be considered both in future surveys and in the interpretation of existing ones.

Are these autolykiviruses the missing non-tailed marine viruses? Although this discovery certainly illuminates the value of analysing host–virus systems found in marine environments<sup>11–13</sup>, the *Autolykiviridae* constitutes just a fraction of the missing non-tailed viruses — the authors' quantitative isolation technique demonstrated that only around 7% of the viruses (18 out of 241) cultured on Vibrionaceae were autolykiviruses. Autolykivirus-related sequences are found in DNA sequences of many phyla of bacteria and archaea (another single-celled group that lacks a nucleus). This suggests that other *Autolykiviridae*-like viruses exist, although the authors were unable to estimate the frequency of autolykivirus-like sequences. They observed that the autolykiviral infection cycle was slower than that of tailed bacteriophages in laboratory experiments, which might imply a disproportionately lower effect of autolykiviruses when viruses compete in nature.

Because the autolykiviruses were found by studying hosts representing only a tiny portion of the overall diversity of marine bacteria, the use of hosts from multiple phyla might reveal other viral groups previously missed. Modified experimental protocols might capture those excluded for the same types of technical reason that prevented autolykiviral identification. Finding previously unknown viral groups is crucial, because large-scale DNA surveys across organisms require reference sequences, and this study points us in the right direction. Luckily, a method is available for extracting

viral DNA that avoids the problematic step of density gradients and includes a protease-treatment step<sup>14</sup>. This puts the search for further non-tailed viral relatives, and the quantitative study of their effect on marine microbial systems, within reach. ■

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## PALAEOCLIMATE

# Pollen weighs in on a climate conundrum

**Simulations by climate models show that Earth warmed during the Holocene epoch, whereas ocean sedimentary cores suggest that global cooling occurred. An analysis of fossil pollen samples now sides with the models. [SEE LETTER P.92](#)**

JEREMY D. SHAKUN

The iconic 'hockey stick' graph shows that global warming of 1°C during the past century has reversed a 2,000-year-long, modest cooling trend<sup>1</sup>. But longer trends are also of interest: how did temperatures change during the rest of the Holocene epoch, which began about 11,700 years ago at the end of the last ice age, and during which human civilization arose? It was previously thought that the Holocene coincided with long-term global cooling<sup>2</sup>, but on page 92, Marsicek *et al.*<sup>3</sup> present a reconstruction of Holocene temperatures from North America and Europe that indicate a long-term warming trend. The findings cast light on how well climate models and proxies agree with each other and reproduce the ancient climate, the drivers of climate change during interglacial periods, and the geological context for the current state of the climate.

Global temperature might seem a simple quantity, but its changes during the Holocene are not easy to deduce theoretically. The factors that had the largest effect on climate during this period were gradual variations in the tilt and wobble of Earth's axis. These orbital changes altered the insolation (the amount of sunlight received) for different regions of the world and the seasonal patterns of this

insolation. However, such orbital variations do not change the global mean annual insolation, and thus would not affect global temperature if the climate system responded in a linear way.

But the climate system's response might have been nonlinear — for instance, if responses occurred more strongly in some areas or at some times of the year than others<sup>4</sup>. Indeed, it is well established that orbital forcing gave rise to the glacial–interglacial cycles of the past few million years, with most of the change in global temperature typically being attributed to feedbacks associated with ice sheets and greenhouse gases. Climate models suggest that these two factors would also have dominated global temperature during the Holocene: retreating ice sheets early in the epoch, and rising levels of greenhouse gases later on, both nudged the planet towards warmer temperatures<sup>5</sup>.

The first reconstruction<sup>2</sup> of Holocene global temperatures to be derived from the geological record was based mainly on sea surface temperatures obtained from the analysis of marine sediment cores. In contrast to the models, this showed that the early Holocene was the warmest part of the epoch, and that global temperature has dropped by about 0.7°C during the past 5,000 years. The disparity between models and the reconstruction has been dubbed the 'Holocene temperature conundrum'<sup>5</sup>, and two possible explanations