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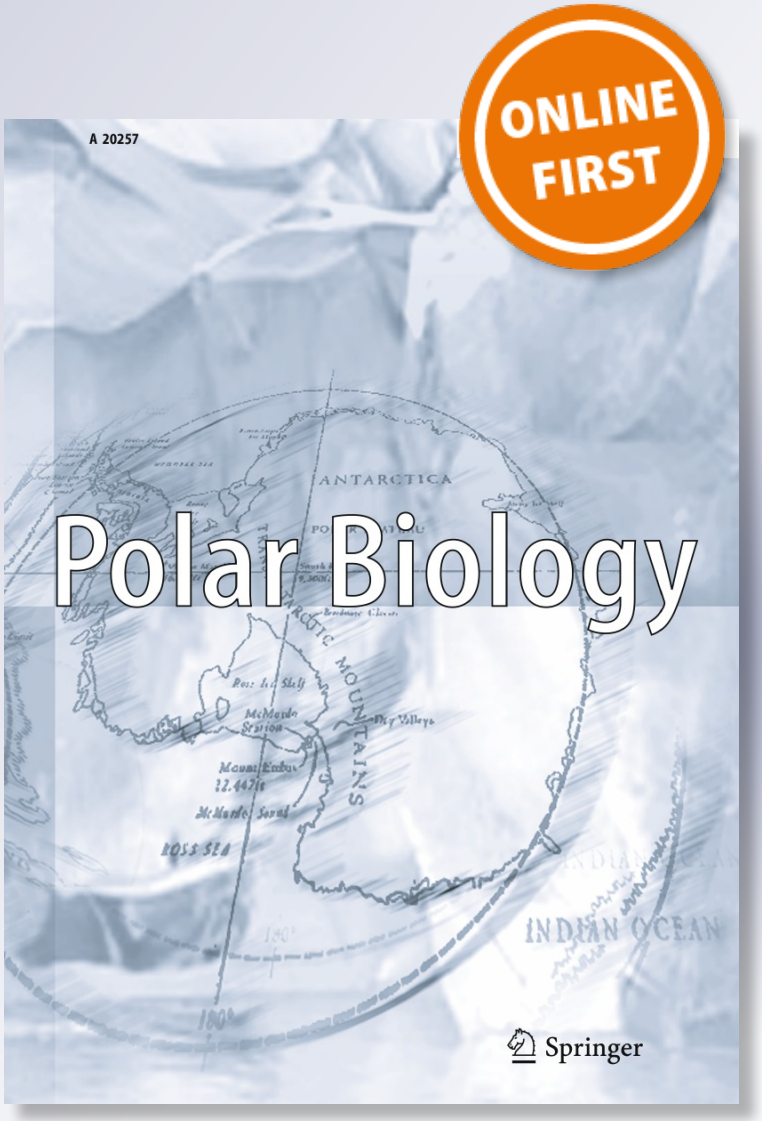
Polar Biology

ISSN 0722-4060

Polar Biol
DOI 10.1007/s00300-018-2288-3



Springer

Polar Biology
https://doi.org/10.1007/s00300-018-2288-3

ORIGINAL PAPER

Abundance and observations of thermophilic microbial and viral communities in submarine and terrestrial hot fluid systems of the French Southern and Antarctic Lands

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Received: 19 May 2017 / Revised: 14 February 2018 / Accepted: 20 February 2018

Abstract

Studies investigating viral ecology have mainly been conducted in temperate marine and freshwater habitats. Fewer reports are available on the often less accessible "extreme environments" such as hot springs. This study investigated prokaryotic- and virus-like particles (VLP) associated to hot springs, themselves situated in cold environments of the Southern Hemisphere (i.e. in the French Southern and Antarctic Lands). This was performed by examining their abundance in hot springs and surrounding temperate seawater using both epifluorescence microscopy (EFM) and flow cytometry (FCM), which was applied for the first time to such ecosystems. On one hand, prokaryotic abundances of 4.0×10^5–2.2×10^6 cell mL^{-1} and 7.0×10^4–2.8×10^6 cell mL^{-1} were measured using EFM and FCM, respectively. The abundances of virus-like particles (VLP), on the other hand, ranged between 9.8×10^5 and 7.5×10^6 particles mL^{-1} when using EFM, and between 1.3×10^5 and 6.2×10^6 particles mL^{-1} when FCM was applied. A positive correlation was found between VLP and prokaryotic abundances, while the virus-to-prokaryote ratio was generally low and ranged between 0.1 and 6. In parallel, samples and culture supernatants were also visualised using transmission electron microscopy. For this, enrichment cultures were prepared using environmental samples. Both raw sample and enrichment culture—supernatants were analysed for the presence of VLPs. Observations revealed the presence of Caudovirales, membrane vesicles and possibly a new type of virion morphology, associated to members of the order Thermotogales, a thermophilic and anaerobic bacterium.

Keywords Abundance · Thermophilic · Virus-like particle · Flow cytometry · Epifluorescence microscopy · Hot spring

Introduction

The discovery of the high abundance of viruses infecting microbes in aquatic ecosystems has led to an increasing interest in their involvement in microbial processes (Wommack and Colwell 2000; Suttle 2005, 2007; Brum and Sullivan 2015). Most studies have focused on temperate aquatic habitats, both marine and freshwater ecosystems. Within the marine field, coastal and offshore waters have been the most investigated habitats, whereas lakes represent unambiguously the most studied environment within the freshwater systems (Parikka et al. 2017). In contrast to temperate aquatic ecosystems, extreme environments have been barely explored. This is probably the result of the locations of these habitats, which are often far, difficult or hard to access for sampling. Among those, hot springs are attractive for microbial studies as temperatures exceeding ca. 60 °C preclude the presence of eukaryotic life (Rothschild and Mancinelli 2001) and harbour very specialised

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00300-018-2288-3) contains supplementary material, which is available to authorized users.

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Published online: 02 March 2018

(i.e. thermophilic to hyperthermophilic) microorganisms from both bacterial and archaeal domains. As prokaryotes are essentially top-down controlled by protozoans and viruses, the absence of eukaryotic communities simplifies the trophic interactions inside these habitats, as viruses are the only known predators (Breitbart et al. 2004). Because hot springs are extreme environments that behave as islands where hot temperatures prevail, several studies have focused on the hyperthermophilic archaea, which are abundant and often dominant in extreme environments (Valentine 2007). The ability of these prokaryotes, not only to withstand but also to thrive in extremely hot ecosystems, has drawn a lot of attention from both structural and physiological aspects, but also for potential biotechnological applications of their enzymes (Canganella and Wiegel 2014; Mehta et al. 2016). Their study has led to the discovery of representatives of new taxonomic divisions of Archaea (Itoh 2003) and also to viruses infecting this group. Indeed, several new and original archeoviruses have been described and characterised during the past years (Prangishvili 2013) and most of these viral families (or groups of family level) are composed of thermophilic viruses (Ackermann and Prangishvili 2012).

In viral ecology, one of the first important steps in the study of these particles (generally associated to a new habitat of interest) is the assessment of both prokaryotic and viral abundances. Since only a small fraction of prokaryotes found in environmental samples can be cultured on conventional media, indirect methods used for (infectious) viral enumeration (i.e. counting plaque-forming units (PFU) [Adams 1959) or using the most probable number method (Kott 1965)] were quickly abandoned when direct (and more global) techniques [i.e. transmission electron microscopy (TEM) (Ewert and Paynter 1980; Børsheim et al. 1990; Ackermann and Heldal 2010; Brum and Steward 2010; Brum et al. 2013), epifluorescence microscopy (EFM) (Hara et al. 1991; Noble and Fuhrman 1998; Chen et al. 2001; Suttle and Fuhrman 2010; Cunningham et al. 2015) and flow cytometry (FCM) (Marie et al. 1999; Brussaard et al. 2000)] became available. Recently, some of these direct techniques have even been revisited to improve accuracy and to reduce the time devoted for enumeration [such as for instance the "wet-mount method" (Cunningham et al. 2015)].

For the study of microbes in high temperature ecosystems, classical direct enumeration techniques such as TEM (Chiura et al. 2002) and EFM (Juniper et al. 1998; Breitbart et al. 2004; Wommack et al. 2004; Ortmann and Suttle 2005; Lee et al. 2007; Manini et al. 2008; Schoenfeld et al. 2008; Williamson et al. 2008; Yoshida-Takashima et al. 2012; Peduzzi et al. 2013) have already been used. Viral abundance has been found to be generally low, with a reported overall average of 5.6×10^6 particles mL^{-1} (Parikka et al. 2017). FCM has been commonly employed to enumerate viral numbers in temperate or tropical ecosystems (Marie et al. 1999; Duhamel and Jacquet 2006; Payet and Suttle 2008; Brussaard et al. 2010), but, to the best of our knowledge, no attempts in thermal environments have been performed yet, although it has been applied on virus–host kinetics of thermophilic viruses in vitro (Lossouarn et al. 2015) and on the detection of their genomes within their host cells (Bize et al. 2009; Okutan et al. 2013). Whereas data have been published on the prokaryotic and viral abundances of different hot springs (both marine and terrestrial), so far, all studies have been conducted on the habitats of the Northern Hemisphere of our planet.

Some hot ecosystems have similar properties to environments in which life could have begun, such as high temperatures, low pH, low levels of oxygen and reducing gasses (e.g. H_2S) (Canganella and Wiegel 2014). Natural hot environments typically include terrestrial hot springs, fumaroles, mud-puddles, geothermal soil and submarine both shallow and deep vents. Temperatures range from mildly hot (50–60 °C) to boiling temperatures (100 °C) in terrestrial habitats, but can reach up to 300 °C and more in hydrothermal vents, due to high pressure associated to depth. In the Southern Hemisphere, several centres of hydrothermal vents are located around Kerguelen and Saint Paul Islands (French Southern and Antarctic Lands—TAAF, i.e. *Terres Australes et Antarctiques Françaises*) (Fig. 1a), i.e. sub-Antarctic islands well isolated from industrial and other anthropogenic activities. The Kerguelen Islands (KI) is located 49°20′South, 69°60′East, in the Southern Ocean, in the oceanic domain of the Antarctic plate. It belongs to the northern part of the Kerguelen Plateau (KP) (Fig. 1b). Over the last 45 Ma, KI moved from a location near the South East Indian Ridge (SEIR) to a present-day intraplate setting together with the emplacement of a huge volume of flood basalts (Giret 1983). The last volcanic activity dated at 26 ± 3 Ka (Gagnevin et al. 2003) took place on the Rallier du Baty (RB) peninsula, south-east part of the KI (Fig. 1c). The present-day volcanic activity, due to the Kerguelen Hot Spot (Charvis et al. 1995), is evidenced by fumaroles, mud pots and hydrothermal discharges located on the RB Peninsula and the Plateau Central [PC, (Delorme et al. 1994)]. A multitude of terrestrial hot springs rise from sea level to 500 m in altitude. They are charged with minerals, and their pH range from acidic to basic (pH 3–10) under hot conditions (55–100 °C). Rainwater at the same place was also sampled and analysed to compare with hot springs composition and be able to estimate contamination via percolation.

The Saint Paul Island (SP) is located 38°43′South, 77°31′East, close to the South East Indian Ridge (Fig. 1a). It consists of the emerged part of a volcano crater and the hydrothermal activity is the consequence of fluid percolations after historical volcanic activity. Its crater (ca. 1 km diameter) is covered by seawater, but with a limited exchange by a narrow passageway (a few metres wide and

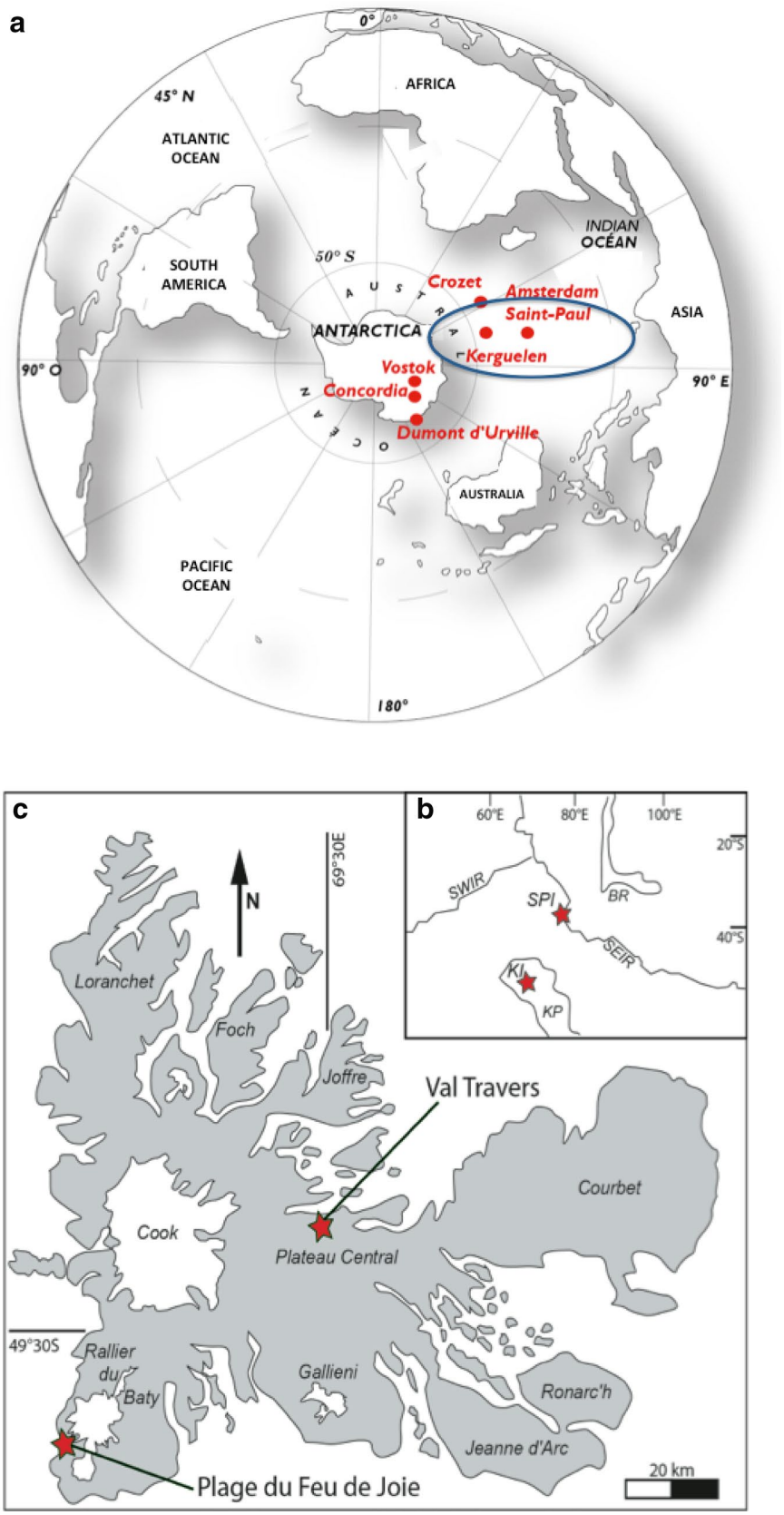

Fig. 1 Locations of the Kerguelen Island (KI; 49°20′S, 69°60′E) and Saint Paul Island (SPI; 38°43′S, 77°31′E) on the globe (circled in blue) (**a**) and their positions (**b**) on the Kerguelen Plateau (KP), in relation to the South West Indian Ridge (SWIR), South East Indian Ridge (SEIR) and Broken Ridge (BR) in the Indian and Southern Oceans. Exact locations of studied vent sites on KI: Plage du Feu de Joie (PFJ) on the Rallier du Baty (RB) Peninsula and Val Travers (VT) on the Plateau Central

deep) with the surrounding Indian Ocean (Online Resource Fig. 1). It harbours both terrestrial and shallow submarine hot springs (45–98 °C), which range from mildly acidic (5.7) to neutral (6.8) pH.

The aim of this study was to explore prokaryotic and viral abundances of both submarine and terrestrial hot springs found in the very remote Saint Paul Island and Kerguelen Islands of the Southern Hemisphere. For this, we used both EFM and FCM. Raw samples, as well as enrichment cultures, were also visualised with transmission electron microscopy to explore the morphology of potentially new virus-like particles (VLP).

Materials and methods

Sampling locations and procedure

Water samples from both terrestrial and submarine hot springs were collected during the HOTVIR sampling campaign of the austral summer of 2011 (02nd of December–19th of January) organised by the French Polar Institute Paul Emile Victor (www.institut-polaire.fr).

Four terrestrial hot springs were sampled at the Kerguelen Archipelago. One of these was located on the RB Peninsula, "Plage du Feu de Joie (PFJ)" (73 °C; pH 7.2) (Online Resource Fig. 2), merging in a rocky slope on the beach slightly above sea level at high tide, into which it discharged. The three others (pH 8–10; 60–61.5 °C) (Online Resource Fig. 3) were sampled at Val Travers (VT), on PC of KI. Two of them (VT1 and VT2) are separated from each other by about 10 m and the third (VT3) is about 200 m apart. They all have a high flow around 8 L s^{-1} (Nougier et al. 1982).

Submarine and coastal hot springs were sampled at the SP. Three submarine vents (6 m below sea level) were sampled by trained scientific French scuba divers. Samples were collected using sterile 50-mL Becton, Dickinson and Company-syringes, which were then subdivided directly into 2- and 5-mL cryotubes (Nalgene™) at the surface. The two main vents (SP1 and SP2) are separated from each other by about 1 km and the third one (SP2 bis) is situated a few metres from SP2. The sampling of SP1 and SP2 was performed by taking samples in small transects, i.e. a sample from the heart of the output of each vent (SP1 I and SP2 I), a sample from 10 cm from the output (SP1 II and SP2 II) and a final sample from the surrounding seawater (SP1 III and SP2 III) at 1 m. Additionally, samples from hot fluid outlets (or discharges) SP3, SP9, SP11, SP11 bis, SP12 and SP13 were also collected. These are small hot fluid emanations that have merged on a rocky shore a few metres from the sea-line, and are only covered by seawater during high tides.

Terrestrial hot spring samples were collected similarly to the submarine hot springs [i.e. using sterile 50-mL Becton, Dickinson and Company-syringes, subdivided afterwards directly into 2- and 5-mL cryotubes (Nalgene™)]. All samples (submarine and terrestrial) were fixed in situ, conserved using 1% glutaraldehyde (final concentration) and "flash-frozen" (Brussaard 2004) in liquid nitrogen within an hour of sampling, and finally stored at − 80 °C. Samples for enrichment cultures were conserved at 4 °C until inoculation. The characteristics of each hot spring are summarised in Table 1.

Method for fluid chemical composition

Fluid chemical composition was obtained following routine protocoles at GET laboratory (Toulouse, France) described in Chavagnac et al. (2013).

The concentrations of major element concentrations (Fe, Mg, Mn, Si, Ca, Na, K) were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, *Horiba Jobin–Yvon Ultima 2*). The instrument was calibrated using synthetic standards and achieved a precision of 2% or better. The full set of standards was run before and after each group of analyses to check the performance of the instrument. In addition, one standard was run as a sample before, during and after each group of analyses, to assess the instrument drift through the course of the analyses. All the concentrations reported in Table 1 are, therefore, drift and blank corrected. Anion concentration (Cl^-, SO_4^{2-}, F^-) were measured by ion chromatography (IC, *Dionex ICS 2000*) which was calibrated using synthetic standards. The analyses achieved a precision of 2% or better. Dissolved inorganic carbon was measured using a *Shimadzu TOC-VCSN* instrument and the B concentration were determined by colorimetry.

Prokaryotic and viral enumeration

Prokaryotic and viral counts were performed using both epifluorescence microscopy and flow cytometry. For enumeration by EFM, the counting technique was based on the protocol of Patel et al. (Patel et al. 2007). Briefly, samples were thawed on ice and filtered onto 0.02-µm filters (Whatman™ Anodisc 25). Filters were then dried and stained using SYBR© Gold (at a final concentration of 5×10^{-4}, Invitrogen™) and subsequently incubated for 15 min in the dark. Anti-fade mounting buffer (composed of phosphate saline buffer and glycerol (v:v), as well as 0.1% *N*,*N*-Dimethyl-1,4-phenylenediamine sulphate) was added, and filters were observed with an Olympus BX60 microscope under blue light excitation. Enumeration was performed by counting manually at least 10 fields and 200 particles on triplicate samples, without the use of any computer software.

For FCM analyses, the protocol is outlined by Jacquet et al. (2013). Briefly, samples were thawed in a 37 °C water bath and then diluted into TE buffer (0.1 mM Tris and 1 mM

Table 1 Locations, characteristics of studied sites and chemical compositions of hydrothermal fluid discharges collected on the Kerguelen Islands (KI) and Saint Paul Island (SP) in comparison with seawater and rainwater at KI

Sample	T	pH	Salinity	Cond[a]	B	F^-	SO_4^{2-}	NO_3^-	Cl^-	Fe	Mg	Mn	Si	Ca	K	Na
Unit	°C		PSU	µS cm^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	µmol L^{-1}	mmol L^{-1}	mmol L^{-1}	mmol L^{-1}	mmol L^{-1}
Kerguelen Island, Val Travers																
VT1	60.8	9.8	0	265	nd	0.01	151.85	0.64	630.89	bdl	bdl	bdl	1.04	0.02	bdl	2.46
VT2	61.5	9.8	0	nd	nd	0.01	150.36	0.59	625.09	bdl	bdl	bdl	1.9	0.02	bdl	2.18
VT3	60.1	8.1	0	nd	nd	0.01	146.49	0.14	613.93	bdl	bdl	bdl	0.76	bdl	bdl	2.04
Kerguelen Island. Rallier du Baty—Plage du Feu de Joie																
PFJ	73.2	7.2	0	1102	52.21	0.58	279.95	103.88	nd	0.17	1.3	bdl	3.79	1.81	0.36	6.01
Seawater	5	8.1	34	29680	1202	181.59	nd	nd	53592	0.29	6411.74	1.20	0.03	10.21	22.27	272.42
Rainwater	8.7	6.6	nd	nd	9.68	157.77	0.1	nd	1086.9	bdl	9.00	bdl	bdl	0.09	bdl	bdl
Saint Paul Island																
SP1 I	70.00	6.80	34	nd	nd	nd	nd	nd	nd	bdl	17679.91	3.25	3.51	7.31	19.63	91.08
SP1 II	31.00	nd	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP1 III	16.00	nd	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP2 I	80.00	6.80	34	nd	nd	nd	nd	nd	nd	bdl	31.74	10.06	0.35	13.01	19.26	88.66
SP2 II	35.00	nd	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP2 III	15.00	nd	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP2 bis	45.00	nd	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP3	98.00	6.80	34	nd	nd	nd	nd	nd	nd	6.83	9.08	bdl	1.60	15.16	20.67	91.66
SP9	98.00	6.40	34	nd	nd	105.82	461.67	25.09	nd	5.52	4961.79	5.66	3.08	nd	nd	nd
SP11	54.00	6.30	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP11 bis	64.00	6.50	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP12	78.50	6.70	34	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SP13	64.30	5.70	34	42.00	735.43	109.59	bdl	bdl	nd	59.90	5700.13	4.13	2.65	13.14	23.47	48.45

BDL below detection limit, *ND* not determined, *PFJ* Plage du Feu de Joie, *SP* Saint Paul, *VT* Val Travers

[a]Conductivity

EDTA, pH 8). A known concentration of commercial 1-µm Invitrogen™ beads was added as a control to each sample. SYBR© Green I was added to the sample (at a final concentration of 5×10^{-5}, Molecular Probes) and incubated in the dark for 5 min at room temperature. A 10 min heat treatment at 75 °C was then performed, followed by a 5 min incubation at ambient temperature before analysis with a FACSCalibur flow cytometer (Becton–Dickinson), equipped with original set-up and blue laser providing 15 mW at 488 nm. Prokaryotic and viral counts were obtained from triplicate samples. The different communities were classically discriminated based on scattering and dye-DNA complex fluorescence levels.

Enrichment cultures

Enrichment cultures were made using samples from all hot springs (except SP1 II, SP1 III, SP2 II and SP2 III, as they were not geothermal) to select and proliferate new thermophilic archaeal and bacterial strains harbouring potentially new types of VLPs. Growth of thermophilic bacteria from the order Thermotogales and hyperthermophilic archaea from the order Thermococcales were promoted using a modified Ravot medium (Postec et al. 2010) containing per litre (L^{-1}): 0.3 g NH_4Cl, 0.5 g $MgCl_2 \times 6H_2O$, 0.1 g $CaCl_2 \times 2H_2O$, 0.5 g KCl, 0.83 g sodium acetate trihydrate, 2 g yeast extract (Difco), 2 g Biotrypcase (Difco), 2 g maltose, 30 g sea salts, 3.3 g PIPES, 1 mL polyvitamin solution (Balch et al. 1979) and 1 mg resazurin (all compounds from Sigma unless otherwise indicated). The medium was prepared aerobically and pH was adjusted to 6.0 before autoclaving. After autoclaving the medium, 5 mL of sterile K_2HPO_4 (7%) and KH_2HPO_4 (7%) were added to the medium. Oxygen was then removed and replaced by a 100% N_2 gas phase; the medium was reduced with 0.5 g $Na_2S \times 9H_2O$ and sterile sulphur was added. Medium inoculation with samples was performed at 1% (v:v or w:v). For the promotion of the growth of Thermotogales, enrichments were incubated for 24–48 h at 70 °C whereas Thermococcales were subjected to 80 °C for the same incubation time. All cultures of 20 mL were supplied with 0.5 g of sterile sulphur.

Microscopy

Raw samples, enrichment cultures and isolated Thermotogales and Thermococcales strain cultures were screened for the presence of positive signals for VLPs and subsequently observed using transmission electron microscopy (TEM). Raw samples (5 mL) were thawed on ice and ultracentrifuged at 100,000 g for 60 min at 4 °C in a Beckman swing-bucket SW41. The ultracentrifuge tubes contained either a Formvar copper-grid mounted on a polyester resin (Sime-Ngando et al. 1996) or a Casco® epoxy resin (Børsheim et al. 1990; Ackermann and Heldal 2010). After ultracentrifugation, the grids were stained with uranyl acetate (2%), incubated 1 min in the dark and rinsed with sterile H_20 before observation with a Jeol 1200 EX or a JEOL 100 CX transmission electron microscope.

Prior to observations by TEM, enrichment cultures were grown for 24–48 h and isolated strains were induced at the exponential growth phase (defined by monitoring culture optical density at $\lambda = 600$ nm) with Mitomycin C (1 µg mL^{-1} final concentration) for the induction of potential prophages. Cells were removed by centrifugation at 6000×*g* for 30 min at 4 °C. Ultracentrifugation of the filtrate was then performed at 100,000×*g* for 60 min at 4 °C in a 70.1 Ti fixed-angle rotor. The pellet was suspended in sterile 50 µl of λ diluent (10 mM Tris–Hcl, pH 7.5; 8 mM$MgSO_4$ and 50 mM NaCl) (Sambrook et al. 1989) and placed on a Formvar copper-grid, which was stained as described above.

Isolates were obtained by three successive streaks on petri dishes containing the same medium (containing Gelzan™ CM Gelrite® gellan gum 1.6%, w:v) as used in enrichment cultures. Both strands of the almost complete 16S rRNA gene of obtained isolates were amplified from a single colony using the universal primers 8F, 1492R (Weisburg et al. 1991) and Eub-int (5′-GCG CCA GCA GCC GCG GTA A-3′), and then sequenced with the BigDye technology (Beckman Coulter Genomics, Essex, UK). Contig assembly was performed from five overlapping sequence fragments. The sequence obtained was a continuous stretch of 1455 bp. A comparison of obtained sequences to those in available databases was performed by use of the BLAST programme (Altschul et al. 1990).

Statistics and software

The prokaryotic and viral abundances obtained using flow cytometry were analysed using the custom-designed freeware CYTOWIN (Vaulot 1989). All prokaryotic and viral abundance data obtained using the two enumeration methods (EFM and FCM) were compared and analysed for significance by calculating non-parametric Spearman's rank correlation coefficients.

Results

The composition of the waters

Chemical compositions of all hydrothermal fluids are presented in Table 1 and compared to seawater and rainwater at KI. The hot springs of KI present variable pH, from neutral (pH 7.2) to basic (pH 9.8). The data are fragmented because of the small amount of fluid available for some samples compared to the volume required for the

analyses. However, the data obtained for hydrothermal fluids compared to rainwater at KI and seawater in the vicinity of KI indicate that the composition of the fluids is mainly influenced by the composition of sea water with moderate (for SPI vents) or significant (for KI vents) contamination by rainwater. Most of the element concentrations also indicate that the composition of the fluids has been modified by volcanic rock dissolution. This is in particular demonstrated by the Cl and B concentrations (Truesdell 1975) in the same way as Icelandic geothermal springs (Armórsson and Andrésdóttir 1995). Hydrothermal fluids are thus derived from a seawater groundwater reservoir, which rises up to the surface into different paths, interacting with rocks at different temperatures and likely different water–rock ratios, and are diluted with percolating meteoric water.

Prokaryotic and viral abundances

Samples from the terrestrial hot springs of Kerguelen Archipelago and coastal marine hot springs from Saint Paul Island were analysed using EFM and FCM, covering for hot springs gradients from moderately (60.1 °C) to hot environments (100 °C) as well as from acidic (pH 3.5), neutral (pH 7.2) to alkaline (pH 9.8) conditions. Table 2 lists all abundances (prokaryotic and VLP) of both terrestrial and marine hot springs obtained in this study. Most of the samples displayed very low numbers of prokaryotic microorganisms (i.e. bacteria and archaea) and virus-like particles. The three terrestrial hot springs of Val Travers (VT1, VT2, VT3) revealed cellular abundances of 5.5×10^5–2.2×10^6 cells mL^{-1} and of 0.7×10^5–1.0×10^6 cells mL^{-1}, when enumerated using EFM and FCM, respectively (Fig. 2a). VLP concentrations, on the other hand, ranged between 1.3×10^6 and 5.4×10^6 particles mL^{-1} when counted with

Table 2 Average prokaryotic and viral abundances as well as their ratios with standard deviations in studied samples

Site	PA (EFM) $\times 10^5$ cells mL^{-1}	PA (FCM) $\times 10^5$ cells mL^{-1}	VLPA (EFM) $\times 10^5$ particles mL^{-1}	VLPA (FCM) $\times 10^5$ particles mL^{-1}	VPR (EFM)	VPR (FCM)
VT 1	6.53 (± 1.35)	0.7	53.96 (± 8.62)	1.33 (± 0.07)	8.28 (± 3.04)	1.89
VT 2	5.48 (± 0.58)	0.97 (± 0.2)	12.77 (± 1.59)	1.52 (± 0.07)	2.33 (± 3.04)	1.56 (± 0.34)
VT 3	21.78 (± 3.73)	10.41 (± 1.25)	19.11 (± 1.63)	27.8 (± 0.99)	0.88 (± 0.07)	2.67 (± 0.36)
SP1 I	8.50 (± 1.75)	3.62 (± 0.19)	9.76 (± 1.62)	2.95 (± 0.32)	1.15 (± 0.22)	0.82 (± 0.09)
SP1 II	4.04 (± 0.66)	8.37 (± 0.53)	8.79 (± 2.6)	11.21 (± 1.7)	2.18 (± 0.84)	1.34 (± 0.2)
SP1 III	11.56 (± 0.93)	16.89 (± 0.65)	75.21 (± 4.03)	46.93 (± 3.34)	6.51 (± 0.9)	2.78 (± 0.09)
SP2 I	17.59 (± 0.85)	15.58 (± 0.74)	70.7 (± 0.85)	61.76 (± 4.92)	4.02 (± 0.21)	3.97 (± 0.25)
SP2 II	6.93 (± 0.46)	8.38 (± 0.43)	31.55 (± 0.46)	24.4 (± 1.41)	4.55 (± 0.1)	2.91 (± 0.06)
SP2 III	12.92 (± 0.97)	18.61 (± 3.03)	56.09 (± 0.97)	34.32 (± 8.4)	4.34 (± 0.13)	1.84 (± 0.18)
SP2 bis	5.56 (± 0.78)	5.48 (± 1.72)	33.11 (± 6.76)	32.96 (± 0.17)	4.41 (± 0.63)	6.01 (± 2.43)
SP9	ND	1.48 (± 1.01)	ND	4.27 (± 7.03)	ND	2.89 (± 0.24)
SP12	ND	1.22 (± 0.16)	ND	1.09 (± 0.13)	ND	0.89 (± 0.23)
SP13	ND	28.2 (± 0.4)	ND	3.43 (± 0.06)	ND	0.12 (± 0)

EFM epifluorescence microscopy, *FCM* flow cytometry, *ND* not determined, *PA* prokaryotic abundance, *SP* Saint Paul, *VLPA* Virus-like particle abundance, *VPR* virus-to-prokaryote ratio, *VT* Val Travers

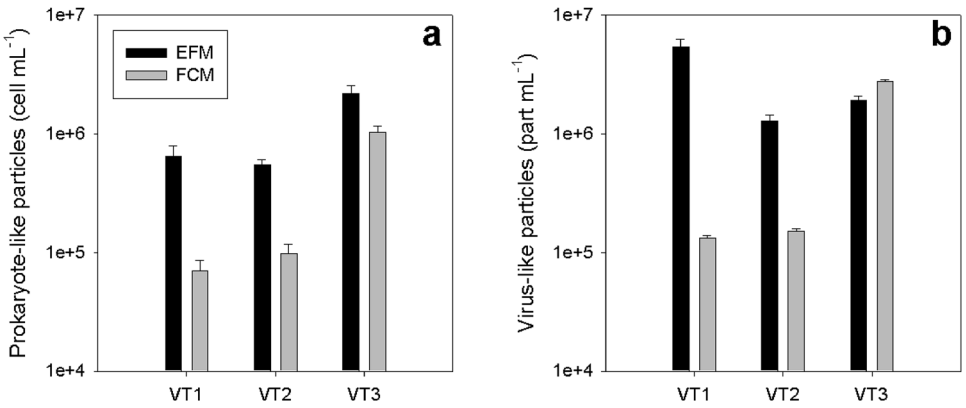

Fig. 2 Average prokaryotic (**a**) and VLP (**b**) abundances with standard deviations from samples collected from the terrestrial hot springs of Val Travers (Kerguelen Island). *EFM* epifluorescence microscopy, *FCM* flow cytometry, *VT* Val Travers

EFM and between 1.3×10^5 and 2.8×10^6 particles mL^{-1}, when using FCM (Fig. 2b). The VPR ranged from 0.9 to 8.3 and from 1.6 to 2.7, when using EFM and FCM, respectively. When all data were plotted together (except for two), a fairly good correlation (with a positive relationship) was found between prokaryotic and VLP abundances ($r = 0.77$, $p < 0.001$, $n = 21$, Online Resource 4).

The shallow marine underwater springs of Saint Paul displayed prokaryotic and VLP abundances roughly of the same order as the terrestrial hot springs of Val Travers. Within the transect of SP1, prokaryotic abundance was higher in the surrounding seawater (SP1 III, 11.6×10^5 cells mL^{-1} with EFM; 16.9×10^5 cells mL^{-1} with FCM) when compared to the output of the hot spring (SP1 I, 8.5×10^5 cells mL^{-1} with EFM; 3.6×10^5 cells mL^{-1} with FCM) (Fig. 3a). This trend was also observed for the transect of SP2 using EFM (SP2 III, 1.3×10^6 cells mL^{-1}; SP2 I, 1.8×10^6 cells mL^{-1}), but was opposite when using FCM (SP2 III, 1.9×10^6 cells mL^{-1}; SP2 I, 1.6×10^6 cells mL^{-1}) (Fig. 3c). VLP abundances were also higher in the surrounding seawater (SP1 III, 7.5×10^6 particles mL^{-1} with EFM; 4.7×10^6 particles mL^{-1} with FCM) than in the output of hot spring SP1 (SP1 I, 9.8×10^5 particles mL^{-1} with EFM; 3.0×10^5 particles mL^{-1} with FCM) (Fig. 3b). However, for SP2, the inverse appeared to be the case, regardless of the method used (i.e. output SP2 I 7.1×10^6 particles mL^{-1} *vs* surrounding seawater SP2 III 5.7×10^6 particles mL^{-1} using EFM and 6.2×10^6 particles mL^{-1} *vs* 3.4×10^6 particles mL^{-1} using FCM) (Fig. 3d). Prokaryotic counts of the third shallow submarine hot spring SP2 bis revealed an abundance of 5.5×10^5 cells mL^{-1} and VLP numbers of 3.3×10^6 particles mL^{-1}, with very close counts obtained with the two enumeration methods (Fig. 4a and b).

For the springs situated on the shore (SP3, SP9, SP11, SP11 bis, SP12 and SP13), mainly FCM was used and prokaryotic abundances ranged from 1.5×10^5 to 2.8×10^6 cells mL^{-1} and VLP abundance between 1.1×10^5 and 3.3×10^6 particles mL^{-1} (Fig. 4a and b). The VPR in coastal marine and the beach hot springs varied between 1.2 and 6.5 and between 0.12 and 6.0 when using EFM and FCM, respectively.

Comparison between EFM and FCM counts

When compared, the two enumeration methods generated relatively similar prokaryotic and VLP counts (Tables 2 and 3, Fig. 5). EFM often produced higher numbers for both prokaryotic and viral populations. This was the case for every hot spring (SP1 I, SP2 I, SP2 bis, VT1, VT2 and VT3) for prokaryotic numbers, and the case for all

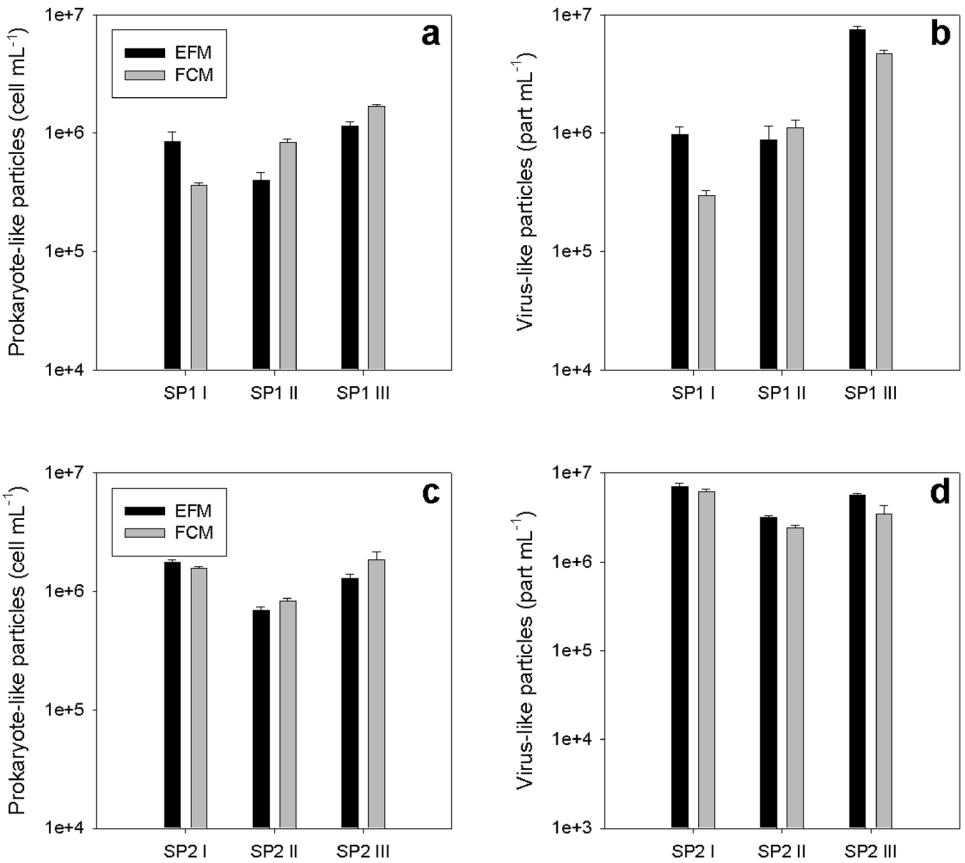

Fig. 3 Average prokaryotic (**a, c**) and VLP (**b, d**) abundances with standard deviations from Saint Paul samples SP1 and SP2 collected from the submarine hot vents of Saint Paul Island. *EFM* epifluorescence microscopy, *FCM* flow cytometry, *SP* Saint Paul

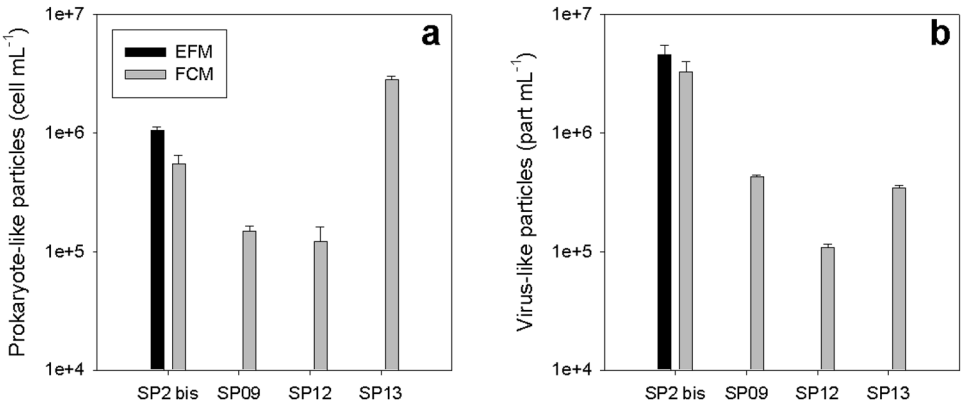

Fig. 4 Average prokaryotic (**a**) and VLP (**b**) abundances with standard deviations from samples collected from the Saint Paul submarine hot spring (SP2 bis) and geothermal emanations of the beach (SP09, SP12, SP13). *EFM* epifluorescence microscopy, *FCM* flow cytometry, *SP* Saint Paul

Table 3 Ratios of microbial and VLP abundances in studied samples obtained by two different enumeration methods: epifluorescence microscopy and flow cytometry

Site	EFM/FCM ratio PA	EFM/FCM ratio VLPA	EFM/FCM ratio VPR
VT 1	9.27	40.64	4.38
VT 2	5.63	8.41	1.49
VT 3	2.09	0.69	0.33
SP1 I	2.35	3.31	1.41
SP1 II	0.48	0.78	1.63
SP1 III	0.68	1.60	2.34
SP2 I	1.13	1.14	1.01
SP2 II	0.83	1.29	1.56
SP2 III	0.69	1.63	2.36
SP2 bis	1.90	1.40	0.73

EFM epifluorescence microscopy, *FCM* flow cytometry, *ND* not determined, *PA* prokaryotic abundance, *VLPA* virus-like particle abundance, *SP* Saint Paul, *VPR* virus-to-prokaryote ratio, *VT* Val Travers

except VT3 for VLP abundances. However, when more or less temperate surrounding seawater samples (SP1 II, SP1 III, SP2 II and SP2 III) were analysed, lower prokaryotic numbers were obtained with EFM as compared to FCM. VLP abundances of temperate samples (except SP1 II), on the other hand, remained higher with EFM. A positive and significant relationship ($r = 0.66$, $p = 0.04$, $n = 10$) was found between the prokaryotic abundances obtained with EFM and FCM (Fig. 5a), but no correlation was observed between VLP numbers enumerated with EFM and FCM ($r = 0.59$, $p = 0.080$, $n = 10$) (Fig. 5b). However, this was only due to the considerable difference between the VLP abundance of VT1 acquired with EFM, which were 40 times higher than those observed with FCM (Table 3). Indeed, when VT1 was not taken into account, a significant correlation (i.e. a positive relationship) was obtained ($r = 0.90$, $p = 0.002$, $n = 9$) (Fig. 5c).

When prokaryotic abundances were compared to the VLP counts, a positive correlation ($r = 0.72$, $p = 0.012$,, $n = 13$) was noted with data obtained using FCM (Table 4), but not with EFM ($r = 0.56$, $p = 0.089$, $n = 10$). However, all prokaryotic and VLP data analysed together, regardless of the method used, also gave a positive correlation ($r = 0.62$, $p = 3.6 \times 10^{-3}$, $n = 23$).

Observations using TEM

The presence of identifiable phages was scarce in all raw samples that were observed under TEM. However, three VLPs were observed in samples from Saint Paul and Rallier du Baty (Kerguelen): an icosahedral particle (Fig. 6a) and two possible members of the Caudovirales order (Fig. 6 b and c). The first particle (Fig. 6a), observed in the SP1 sample, had a size of 65 nm and could either be a member of the *Tectiviridae* family or a detached part of a capsid of a member of the Caudovirales order, consisting of a "head" lacking a tail. The second particle (Fig. 6b) had a capsid of 75 nm and a tail of 130 nm. The third particle (Fig. 6c), found in the sample of the "Plage du Feu de Joie (PFJ)", had a capsid composed of a head of ca. 70 nm attached to a tail of 325 × 15 nm. It appears to be attached to debris, the presence of which was amply visible and impeded performing counts on the FCM charts (data not shown).

Observations of the supernatant of enrichment cultures revealed the presence of membrane vesicles, tailed phages and potentially new viral structures. In the enrichments made using samples originating from SP3 (Saint Paul) in the modified Ravot medium and incubated at 80 °C, only membrane vesicles were observed (Fig. 7a–c). The membrane vesicles varied in size from 100 to 160 nm. They were also present in culture supernatants of several strains of the *Thermococcus* genus (Fig. 7d, e), isolated after enrichment from the sample SP11 bis (Saint Paul) and induced with Mitomycin C. Both vesicular and tubular structures strongly resembled descriptions of the "virus-like vesicles" reported previously (Soler et al. 2008), subsequently characterised and renamed

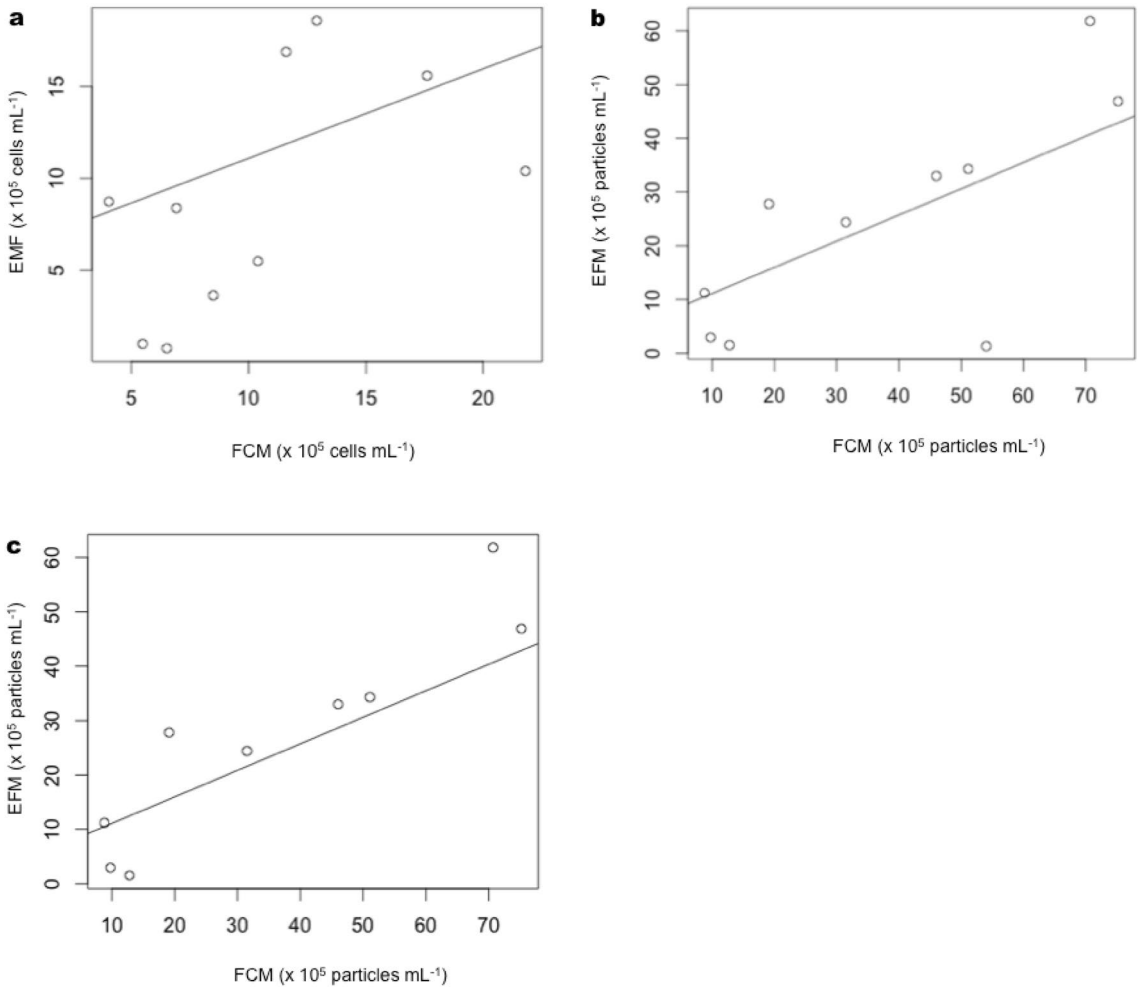

Fig. 5 Comparison of prokaryotic (**a**) and VLP (**b**) abundance data obtained using two enumeration methods: epifluorescence microscopy (EFM) and flow cytometry (FCM). Additional comparison of viral abundance (**c**) excluding data from sample VT1

"membrane vesicles" by Gaudin et al. (2013, 2014). The isolation of Thermococcales from enrichments, the use of specific medium and temperature, observations using light microscopy and the presence of membrane vesicles typical of Thermococcales suggest that these membrane vesicles were indeed produced by members of the Thermococcales.

Table 4 Spearman's rank correlations between prokaryotic abundance (PA) and virus-like particle abundance (VLPA) using two different enumeration methods: epifluorescence microscopy (EFM) and flow cytometry (FCM)

	PA/VLPA (EFM)	PA/VLPA (FCM)	PA/VLPA (EFM & FCM)
P	0.089	**0.012**	**0.0036**
r	0.56	0.72	0.62
n	10	13	23

Significant correlations ($P < 0.05$) are in bold

SP1 enrichment cultures in the same media, but at 70 °C, contained VLPs that resembled *Siphoviridae*—type phages. One VLP was characterised by a capsid of a diameter of 55 nm attached to a tail of 245 nm long (Fig. 8a). Another VLP had a capsid of 65 nm large and a tail of 165 nm long (Fig. 8b). The latter appeared to be attached to a cellular fragment. In another enrichment from SP11, unusual structures were observed (Fig. 9a, b). Similar structures were also observed in the culture supernatant of a *Thermosipho africanus* (named MC6) (Fig. 9c, d), isolated from SP11bis in the vicinity of SP11. All these elements shared a morphological trait: a ring-like structure attached to a filament. Each of the ring-like structures had the same dimensions: a width of 15 nm and a length of 30 nm. These ring-structures were attached to filaments of the same width, 7 nm, but with variable lengths, going up to 850 nm (although the filament of Fig. 9d appears to exceed this length, a part of it being bungled). The other side of the ring-structure differed among all these elements. The two elements observed in

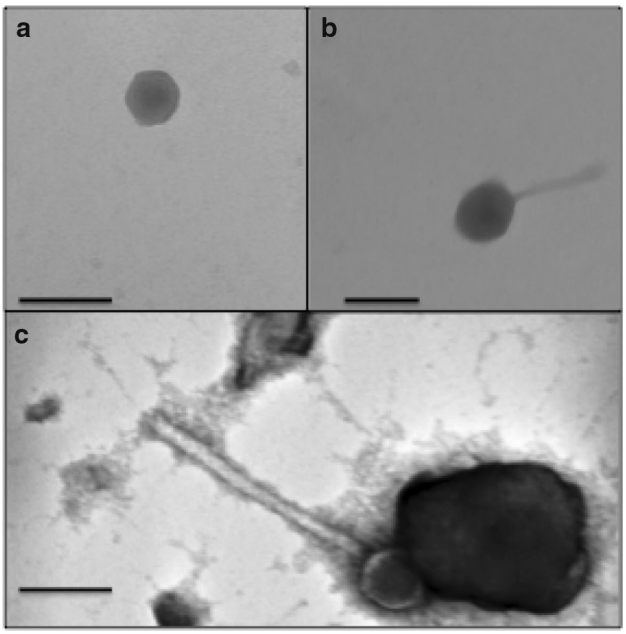

Fig. 6 Transmission electron micrographs of observations of virus-like particles made on raw samples of Saint Paul Island submarine hot vent SP1 (**a, b**) and of terrestrial hot spring Plage du Feu de Joie PFJ (**c**) in Rallier du Baty Peninsula. Kerguelen. Scale bar = 100 nm

enrichment culture supernatants were attached to either a cylinder (40 × 25 nm) and an angular part (50 nm diameter) (Fig. 9a) or a wider cylinder (40 × 55 nm) and a tubular piece (115 × 25 nm) (Fig. 9b). In the other two elements, observed in the culture supernatant of *T. africanus* isolate MC6, the ring-structure was attached to a 65 × 75 nm bulb (Fig. 9c) in one and to a cylinder (20 × 35 nm) and 65 × 55 nm bulb (Fig. 9d) in the other. All together (i) the use of the modified Ravot medium combined with a temperature of 70 °C, (ii) the identification of a cellular sheath, the *toga,* specific to Thermotogales cells, and (iii) the fact that one strain was phylogenetically related to the species *T. africanus* based on 16S rRNA suggest that these structures originate from strains belonging to the Thermotogales order.

Discussion

Methodological aspects

Before the final enumeration provided in this study, exploratory analyses and tests were necessary for both flow cytometry and epifluorescence microscopy. Protocols were adapted based on the nature of the samples, rendering enumeration more efficient and reliable (e.g. getting rid of background fluorescence, using the appropriate buffer dilution, etc.). While on some occasions it was not always possible to detect

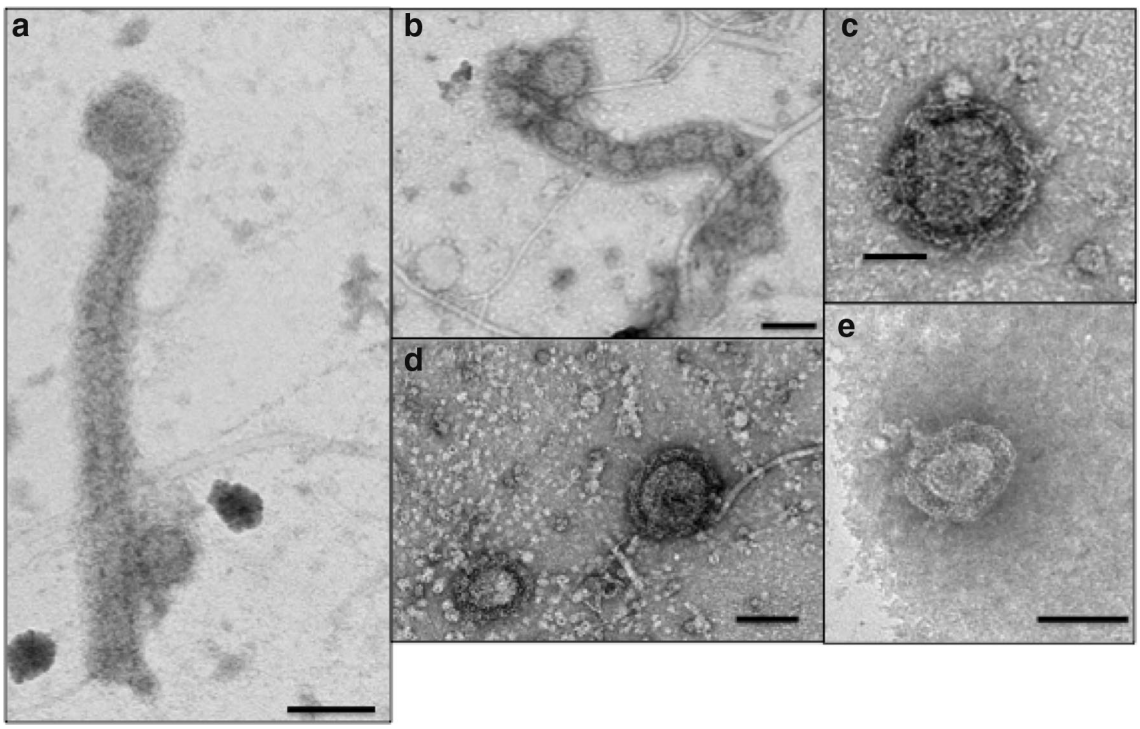

Fig. 7 Transmission electron micrographs of observations of membrane vesicles made in supernatants of an enrichment culture of Saint Paul Island (SP3. incubated at 80 °C) (**a–c**) and of various strains of *Thermococcus* sp. (**d, e**). Scale bar = 100 nm

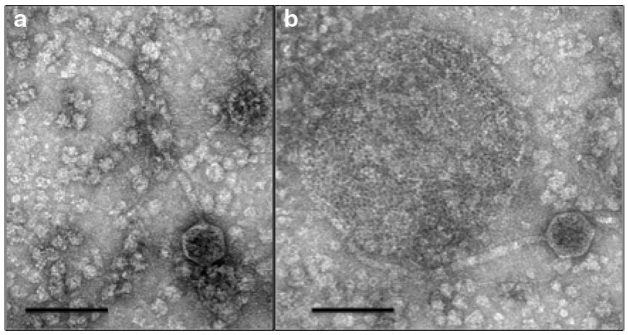

Fig. 8 Transmission electron micrographs of observations of virus-like particles made in supernatant of an enrichment culture of Saint Paul Island (SP1, incubated at 70 °C). Scale bar = 100 nm

and give precise abundances for both VLPs and microorganisms, most of the samples contained enough cells and particles to be counted. Exploratory analyses of samples originating from the Rallier du Baty terrestrial hot springs often revealed undetectable numbers of VLPs and cells, but occasionally prokaryotic of ca. 10^5 mL^{-1} and viral numbers up to of ca. 10^6 mL^{-1}, like in the case of the PFJ-sample.

Considering other samples, the comparison showed that both enumeration methods globally produced very similar prokaryotic and VLP concentrations, with usually higher abundances generated by EFM hot springs. There seemed to be a more important bias towards overestimating VLPs, most of the ratios of EFM to FCM of the VPR being above 1.0 (Table 3). An overestimation caused by interference by free nucleic acids is unlikely in hot springs due to their probable denaturation at high temperatures (although to our knowledge this has not been examined per se). The most probable cause for an overestimation of prokaryotic and VLP abundances with EFM is likely related to the high background noise, which was also visible in TEM and on FCM charts. This was particularly the case for VT1 samples, that made enumeration arduous in general. An examination of the variances in our abundance data (Table 5) suggests that FCM produced more consistent results than EFM, making it a more reliable enumeration method. Our study thus extends the use of FCM to underwater and terrestrial hot springs in viral ecology and appears to be a trustworthy method for enumeration. We stress, nevertheless, the importance of preliminary analyses to adjust enumeration protocols to new types of ecosystems, thus avoiding agents such as colloids, which can interfere with counts (Brussaard et al. 2010). Enumeration results are also known to vary from one laboratory to another (Suttle and Fuhrman 2010) and so, using more than one enumeration method can help to obtain more reliable results. *In fine*, the most realistic estimation of prokaryotic and/or viral abundance of our samples probably lies somewhere between the results obtained by EFM and those observed with FCM.

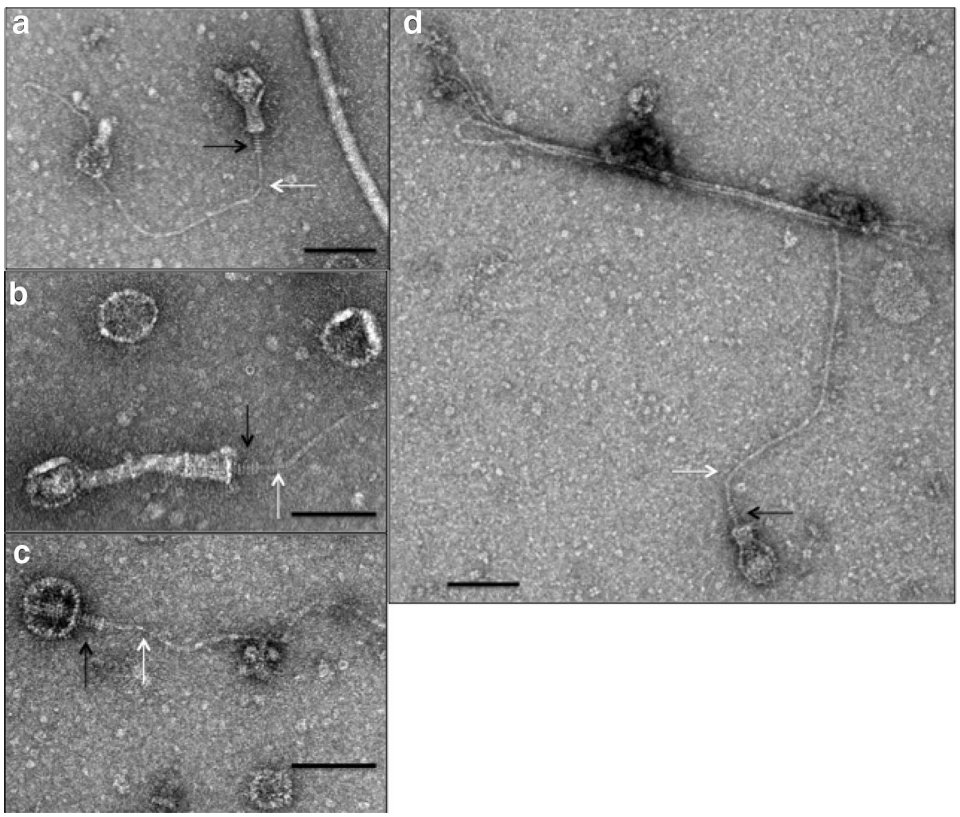

Fig. 9 Transmission electron micrographs of observations of virus-like particles made in supernatants of an enrichment culture of Saint Paul Island (SP11, incubated at 70 °C) (**a, b**) and the isolate MC6 of *Thermosipho africanus* (**c, d**). Arrows show structures common to all the observed elements: black arrows show ring-like structures and white arrows filaments attached to these ring-like structures. Scale bar = 100 nm

Table 5 Variances of microbial and virus-like particle abundances in studied samples

Site	PA (EFM) $\times 10^9$ cells mL^{-1}	PA (FCM) $\times 10^9$ cells mL^{-1}	VLPA (EFM) $\times 10^9$ particles mL^{-1}	VLPA (FCM) $\times 10^9$ particles mL^{-1}
VT 1	18.31	ND	743.79	0.048
VT 2	3.37	0.41	25.35	0.054
VT 3	139.04	15.69	26.45	9.80
SP1 I	30.66	0.36	26.37	1.00
SP1 II	4.32	2.79	67.44	29.01
SP1 III	8.66	4.29	162.64	111.80
SP2 I	7.15	5.52	379.45	241.89
SP2 II	2.13	1.83	27.95	20.01
SP2 III	9.34	91.69	113.05	704.83
SP2 bis	6.06	10.30	456.55	494.84

EFM epifluorescence microscopy, *FCM* flow cytometry, *ND* not determined, *PA* prokaryotic abundance, *SP* Saint Paul, *VLPA* virus-like particle abundance, *VT* Val Travers

Prokaryotic and VLP abundances

Few studies have highlighted microbial and viral abundances and thus their ecology in high temperature ecosystems or other "extreme" ecosystems. Most studies have been conducted on deep-sea hydrothermal vents (Juniper et al. 1998; Wommack et al. 2004; Ortmann and Suttle 2005;; Williamson et al. 2008; Yoshida-Takashima et al. 2012; Peduzzi et al. 2013), and only one on coastal marine hot springs (Manini et al. 2008) and a few on terrestrial hot springs (Chiura et al. 2002; Breitbart et al. 2004; Lee et al. 2007; Schoenfeld et al. 2008). Within these ecosystems, the average prokaryotic abundances have been calculated to be 7.4×10^5 cells mL^{-1} and the VLP abundance around 5.6×10^6 particles mL^{-1} (Parikka et al. 2017). Our study is the first to report on the thermophilic prokaryotic and viral communities from hot systems situated in the Southern Hemisphere. It is also unique in that it is completely isolated geographically from other hot spring systems and human activities. Results reveal that their abundances are of the same order of magnitude as those obtained in previous investigations on thermophilic communities. In these sites, as in other similar ecosystems, gene transfer activity is likely to be carried out by at least viruses and membrane vesicles. It is possible that gene transfer activity is also likely carried out by plasmids although this hypothesis was not addressed in this study.

The highest recorded prokaryotic abundance was measured in the Saint Paul Island beach hot spring SP13 (with 2.8×10^6 cells mL^{-1}), the lowest in the Kerguelen terrestrial spring VT1 (7.0×10^4 cells mL^{-1}). The highest VLP numbers were found in the Saint Paul submarine vent of SP2 (7.1×10^6 particles mL^{-1}), the lowest in the Saint Paul beach hot spring SP12 (1.1×10^5 particles mL^{-1}). We also found the lowest reported virus-to-prokaryote ratios in hot springs, but a lack of further information on prokaryotic and VLP dynamics prevents any extensive speculation on their meaning. It is possible that the oligotrophic nature and the high temperatures of the studied springs could lead to low viral production, which in turn would be linked to the instability of viral particles at high temperature (and, therefore, high decay rates) and/or to certain low-producing viral lifestyles (such as the "carrier-state", which is common within archaeal viruses (Pina et al. 2011)).

The surrounding temperate seawater in the vicinity of the Saint Paul hot vent SP1 had higher numbers of prokaryotes and VLPs, with the highest recorded VLP abundance of 7.5×10^6 particles mL^{-1}. However, no clear trend could be observed in the studied transects SP1 and SP2. As prokaryotic and VLP abundances are generally lower in hot ecosystems as compared to temperate ones (Parikka et al. 2017), we expected to see growing numbers of prokaryotes and VLPs when moving from the centre of the hot vents towards the surrounding seawater (i.e. from SP1 I to SP1 III and SP2 I to SP2 III). Although this was partly the case (i.e. for SP1), no clear conclusions can be drawn.

A positive correlation ($r = 0.72$, $p = 0.012$, $n = 13$ with FCM; $r = 0.62$, $p = 3.6 \times 10^{-3}$, $n = 23$ with both EFM and FCM) between prokaryotic and VLP numbers has also been reported in previous studies (Weinbauer et al. 1994; Maranger and Bird 1995; Anesio et al. 2007; Danovaro et al. 2008; Personnic et al. 2009; Helton et al. 2012; Pradeep Ram et al. 2014) suggesting that most viruses detected are infectious to prokaryotic microbes and that factors influencing host productivity and abundance affect viral abundance (Wommack and Colwell 2000; Weinbauer 2004; Parikka et al. 2017). A lack of correlation between prokaryotic and VLP numbers with data obtained with EFM, however, was here more probably linked to the robustness of the enumeration method. As explained above, variances of our data on abundance (Table 5) suggest that FCM produced more reliable results with less variability than EFM, which could explain the statistical differences observed, when comparing abundance data.

When analysing the Saint Paul FCM charts, cellular populations/communities of phytoplankton (not shown here) could be identified in all marine samples, with detections even in the centres of the hot vents' output. Three different phytoplanktonic groups could be identified, one comprising of marked levels of phycoerythrin-like pigment, with a FCM signature typical of *Synechococcus spp.* The global autotrophic cellular abundance varied from 1.3×10^4 cells mL^{-1} at both hot vents SP1 I and SP2 I to ca. 1.0×10^5 cells mL^{-1} in the surrounding seawater. Signatures of what seemed to be picocyanobacteria could be identified in the hot springs at 1.2×10^4 cells mL^{-1} in SP1 I and at 6.5×10^4 cells mL^{-1}

in SP2 I, with only slightly higher numbers in the surrounding seawater (7.1×10^4 cells mL^{-1}). *Synechococcus spp* are ubiquitous in all marine ecosystems in numbers varying between ca. 5.0×10^2 and 1.5×10^6 cells mL^{-1} (Partensky et al. 1999). The presence of thermophilic *Synechococcus* have already been reported in terrestrial hot springs reaching up to 75 °C (Klatt et al. 2011; Ward et al. 2012). Although some observations have also been made in the proximity of coastal marine hot springs (Tarasov et al. 1999, 2005; Tarasov 2006; Maugeri et al. 2013), the presence of *Synechococcus* is more common in alkaline terrestrial hot springs and remains uncommon in hot marine ecosystems. It is possible that the Saint Paul Island coastal hot springs harbour thermophilic picocyanobacteria, but is unlikely due to their high temperature. A more plausible explanation of autotrophic cells present in the dischargers of these vents is the mixing of the hot spring water with that of the surrounding environmental marine seawater, prior to their emanation from the bottom of the sea (e.g. seepage through the seafloor). This would not only explain the similarities between the autotrophic numbers of the hot vent fluids and the surrounding seawater, but also of the (heterotrophic) prokaryotic and viral populations. Membrane vesicles were probably present amongst viral particles, but the nature of their size and shape would have made them indistinguishable from other VLPs (both on EFM and FCM).

Observations using transmission electron microscopy

The morphological diversity of VLPs within our samples was less rich than in many previous reports on hot springs. However, it is not uncommon to face difficulties in the hunt for VLPs in hot environments (Prangishvili 2006). Our observations of tailed phages associated to Thermotogales suggests both their ubiquitous presence within the order, but more importantly, it highlights the significance of improved protocols that aim to isolate viruses of anaerobic thermophiles. The difficulties met during the pursuit of thermophilic VLPs (especially within anaerobic and thermophilic hosts) have probably prevented the discovery of new viruses. The problems emerge already in the culture of hosts, which remains complicated.

Whereas several tailed phages from hot springs belonging to thermophilic hosts such as *Geobacillus* (Liu et al. 2006, 2009, 2010; Wang and Zhang 2008, 2010), *Thermus* (Sakaki and Oshima 1975; Yu et al. 2006) and recently Thermotogales (Lossouarn et al. 2015) have been discovered, observations of icosahedral phages in hot environments have been rarer. Sightings of putative members of the *Tectiviridae* family have been reported (Yu et al. 2006; Jaatinen et al. 2008) and the archeaovirus *Sulfolobus* turreted icosahedral virus (STIV) has been isolated and studied in more detail (Rice et al. 2004). Though it is possible that we observed an icosahedral virus in Saint Paul SP1 hot vent, it is also possible that the particle represented the capsid of a tailed phage. Heads lacking tails can be observed when tails break off during centrifugation (Ackermann and Heldal 2010).

The unusual morphology of some of the observed particles related to Thermotogales cast doubt on the possibility of them being *bona fide* viruses. However, some factors put forward the possibility of them being VLPs rather than cellular structures. First, all the supernatants had positive signals on epifluorescence microscopy, suggesting the presence of nucleic acids, possibly contained by these elements. The cultured strain *T. africanus* MC6 was induced with Mitomycin C to check for the presence of potential prophages, as this viral lifestyle has also been reported for thermophilic prokaryotes (Lee et al. 2007; Schoenfeld et al. 2008; Prangishvili 2013; Lossouarn et al. 2015). A strong signal after induction was indeed detected, suggesting a possible release of virions. Second, the filament attached to the ring-like component is likely to be composed of nucleoprotein. Descriptions of filaments of nucleoproteins originating from (hyper)thermophilic archaeal viruses *Pyrobaculum* spherical virus (PSV) (Häring et al. 2004) and *Aeropyrum* coil-shaped virus (AVC) (Mochizuki et al. 2012) (the former having a genome composed dsDNA and the latter of ssDNA) have been previously reported. The electron micrographs that revealed the disrupted virions of both aforementioned viruses display very similar filaments as the ones we observed in our elements. The filaments of the PSV even had roughly the same width (6 nm *vs* 7 nm in our case) (Häring et al. 2004).

The possibility that the sighted elements would be organelles cannot be ruled out. However, this seems less likely, as these organelles would have to contain nucleic acids, thus explaining the observations made by epifluorescence microscopy. The electron micrographs also contained flagella surrounded by a sheath, a previously unreported feature of the *Thermotogales* (to the best of our knowledge) (Online Resource Fig. 5a–c), but a described property of other bacteria such as *Vibrio cholera* (Bari et al. 2012), *Helicobacter pylori* (Kostrzynska et al. 1991) and *Pseudomonas rhodos* (Schmitt et al. 1974) for example. The flagellar sheath of *P. rhodos* has a similar aspect as the fishnet-looking sheath of *T. africanus*. The dimensions of the flagella are incompatible with the ones of the filaments attached to the ring-structure of our particles. The flagellum is ca. 20 nm width with ca. 10 nm of width extension due to the sheath. Its anchor point (Online Resource Fig. 5c) has three visible rings of 43 nm, 37 nm and 27 nm, respectively. Other possibilities for the observed elements could be secretion systems, which are anchored into cell membranes and walls, such as those found in Gram-negative bacteria. Though several secretion systems are currently well described within Gram-negative bacteria, especially pathogens (see the review by Costa et al. (2015)),

relatively little is still known on the secretion systems of thermophilic bacteria. Furthermore, Abby et al. (Abby et al. 2016) recently identified secretion systems in a variety of bacterial genomes and reported the total absence of any secretion systems in *Thermotogae*, although they found some in other thermophilic bacteria. Authors speculate this to be the result of the presence of the sheath (toga), which could have prevented the presence of secretion systems described so far in literature. However, Thermotogae could have evolved specific ones adapted to their cellular structure, analogous to Mycobacteria, which have peculiar secretion systems adapted to their mycolate outer membrane (Abby et al. 2016). The particles we observed seem to contain nucleic acids and would then have to be structures similar to type IV pili, although this appears unlikely as systems currently described as morphologically different in shape and size. The observed particles could be also attached to cellular pili (i.e. the filament), as was hypothesised by Janekovic et al. (Janekovic et al. 1983) when they discovered the *Thermoproteus tenax* virus 1 (TTV1); a hyperthermophilic archeal virus associated to a filament. Finally, variability in the morphology of viruses has been described in the case of the hyperthermophilic *Acidianus* two-tailed virus (ATV), consisting of the budding of two tails outside the cell (Häring et al. 2005) *Pyrococcus abyssi* virus 1 (PAV1), as a result of changes in osmolarity (Geslin et al. 2003) or simply as feature of myoviruses, related to host recognition (Lavigne and Ceyssens 2012).

Altogether, the results of our study have revealed thermophilic prokaryotic and viral communities in abundances that fall within the previously reported lower ranges found in the literature, along with the presence of both previously described and possibly novel virion types and membrane vesicles. However, further studies are needed to confirm the viral nature of the observed VLPs, which could lead to a new family of bacterial viruses (the last novel family having been described in the 1970′s (Ackermann and Prangishvili 2012)).

Acknowledgments This study benefited of the Ministère de l'Enseignement Supérieur et de la Recherche doctoral-grant, (a CAREX-grant allowing a visit to the University of Bergen, Norway, and an IPEV (the French Polar Institute Paul- Emile Victor) fundings in the HOTVIR programme no 408. The sampling expedition was organised by the Institut Paul Emile Victor. The authors would like to thank the Territoire des Terres Australes et Antarctiques Françaises (TAAF), IPEV and its logistical staff for assistance in the field. The scientific diving protocol was initiated by Stéphan Jacquet. At field, diving was designed and coordinated by Patrick Le Chevalier and carried out by himself, Gilles Sarragoni and Christine David-Beaussire. Authors would also like to thank Dr. Mikal Heldal for his help in making electron microscopic observations of raw samples, Gérard Sinquin and Philippe Eliès of the Plateforme d'Imagerie et de Mesure en Microscopie (UBO) for their help in making electron microscopic observations of enrichment and pure culture supernatants, Valerie Chavagnac, Philippe Besson, Stéphanie Mounic and Carole Causserand (GET) for the chemical analyses of hydrothermal fluids, and Dr. Julien Farasin for his help in sample preliminary analyses. Authors are also grateful for Malcolm O'Toole for his valuable comments on the manuscript.

References

Abby SS, Cury J, Guglielmini J, Neron B, Touchon M, Rocha EP (2016) Identification of protein secretion systems in bacterial genomes. Sci Rep 6:23080. https://doi.org/10.1038/srep23080

Ackermann H-W, Heldal M (2010) Basic electron microscopy of aquatic viruses. In: Suttle CA, Wilhelm SW, Weinbauer MG (eds) Manual of Aquatic Viral Ecology. ASLO, Waco, pp 182–192

Ackermann H-W, Prangishvili D (2012) Prokaryote viruses studied by electron microscopy. Arch Virol 157:1843–1849

Adams MD (1959) Bacteriophages. Interscience Publishers Inc, New York

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Anesio AM, Mindl B, Laybourn-Parry J, Hodson AJ, Sattler B (2007) Viral dynamics in cryoconite holes on a high Arctic glacier (Svalbard). J Geophys Res. https://doi.org/10.1029/2006jg000350

Armórsson S, Andrésdóttir A (1995) Processes controlling the distribution of boron and chlorine in natural waters in Iceland. Geochim Cosmochim Acta 59:4125–4146

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol Rev 43:260–296

Bari W, Lee KM, Yoon SS (2012) Structural and functional importance of outer membrane proteins in *Vibrio cholerae* flagellum. J Microbiol 50:631–637. https://doi.org/10.1007/s12275-012-2116-3

Bize A et al (2009) A unique virus release mechanism in the Archaea. PNAS 106:11306–11311. https://doi.org/10.1073/pnas.0901238106

Børsheim KY, Bratbak G, Heldal M (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. Appl Environ Microbiol 56:352–356

Breitbart M, Wegley L, Leeds S, Schoenfeld T, Rohwer F (2004) Phage community dynamics in hot springs. Appl Environ Microbiol 70:1633–1640

Brum JR, Steward GF (2010) Morphological characterization of viruses in the stratified water column of alkaline, hypersaline Mono Lake. Microb Ecol 60:636–643. https://doi.org/10.1007/s00248-010-9688-4

Brum JR, Sullivan MB (2015) Rising to the challenge: accelerated pace of discovery transforms marine virology. Nat Rev Microbiol 13:147–159. https://doi.org/10.1038/nrmicro3404

Brum JR, Schenck RO, Sullivan MB (2013) Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. ISME J 7:1738–1751. https://doi.org/10.1038/ismej.2013.67

Brussaard CP (2004) Optimization of procedures for counting viruses by flow cytometry. Appl Environ Microbiol 70:1506–1513

Brussaard CP, Marie D, Bratbak G (2000) Flow cytometric detection of viruses. J Virol Method 85:175–182

Brussaard CPD, Payet JP, Winter C, Weinbauer MG (2010) Quantification of aquatic viruses by flow cytometry. In: Suttle CA, Wilhelm SW, Weinbauer MG (eds) Manual of Aquatic viral ecology. ASLO, Waco, pp 102–109

Canganella F, Wiegel J (2014) Anaerobic Thermophiles. Life 4:77–104. https://doi.org/10.3390/life4010077

Charvis P, Recq M, Operto S, Brefort D (1995) Deep structure of the northern Kerguelen Plateau and hotspot-related activity. Geophys J Int 122:899–924

Chavagnac V, Monnin C, Ceuleneer G, Boulart C, Hoareau G (2013) Characterization of hyperalkaline fluids produces by low temperature serpentinization of mantle peridotites in the Sultanate of Oman and in Liguria (Northern Italy). Geochem Geophys Geosyst 14:2496–2522

Chen F, Lu JR, Binder BJ, Liu YC, Hodson RE (2001) Application of digital image analysis and flow cytometry to enumerate marine viruses stained with SYBR gold. Appl Environ Microbiol 67:539–545. https://doi.org/10.1128/AEM.67.2.539-545.2001

Chiura HX, Yamamoto H, Koketsu D, Naito H, Kato K (2002) Virus-like particles derived from a bacterium belonging to the oldest lineage of the domain bacteria. Microb Environ 17:48–52

Costa TR, Felisberto-Rodrigues C, Meir A, Prevost MS, Redzej A, Trokter M, Waksman G (2015) Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat Rev Microbiol 13:343–359. https://doi.org/10.1038/nrmicro3456

Cunningham BR, Brum JR, Schwenck SM, Sullivan MB, John SG (2015) An inexpensive, accurate, and precise wet-mount method for enumerating aquatic viruses. Appl Environ Microbiol 81:2995–3000. https://doi.org/10.1128/AEM.03642-14

Danovaro R, Dell'Anno A, Corinaldesi C, Magagnini M, Noble R, Tamburini C, Weinbauer M (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. Nature 454:1084–1087. https://doi.org/10.1038/nature07268

Delorme H, Verdier O, Cheminee JL, Giret A, Pineau F, Javoy M (1994) Etude chimique et rapports isotopiques du carbone des fumerolles de la péninsule Rallier du Baty (îles Kerguelen). Mém Soc Géol France 166:5–30

Duhamel S, Jacquet S (2006) Flow cytometric analysis of bacteria- and virus-like particles in lake sediments. J Microbiol Methods 64:316–332. https://doi.org/10.1016/j.mimet.2005.05.008

Ewert DL, Paynter MJ (1980) Enumeration of bacteriophages and host bacteria in sewage and the activated-sludge treatment process. Appl Environ Microbiol 39:576–583

Gagnevin D, Ethien R, Bonin B, Giret A (2003) Open-system processes in the genesis of silica-oversaturated alkaline series of the Rallier du Baty peninsula, Kerguelen archipelago (Indian Ocean). J Volc Geotherm Res 123:267–300

Gaudin M, Gauliard E, Schouten S, Houel-Renault L, Lenormand P, Marguet E, Forterre P (2013) Hyperthermophilic archaea produce membrane vesicles that can transfer DNA. Environ Microbiol Rep 5:1–8

Gaudin M et al (2014) Extracellular membrane vesicles harbouring viral genomes. Environ Microbiol 16:1167–1175. https://doi.org/10.1111/1462-2920.12235

Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, Prieur D (2003) PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote, "Pyrococcus abyssi". J Bacteriol 185:3888–3894

Giret A (1983) Le plutonisme océanique intraplaque. Exemple de l'archipel Kerguelen. Terres Australes et Antarctiques Françaises. Thesis, Université Pierre et Marie Curie, Paris, France

Hara S, Terauchi K, Koike I (1991) Abundance of viruses in marine waters: assessment by epifluorescence and transmission electron microscopy. Appl Environ Microbiol 57:2731–2734

Häring M, Peng X, Brugger K, Rachel R, Stetter KO, Garrett RA, Prangishvili D (2004) Morphology and genome organization of the virus PSV of the hyperthermophilic archaeal genera *Pyrobaculum* and *Thermoproteus*: a novel virus family, the *Globuloviridae*. Virol 323:233–242. https://doi.org/10.1016/j.virol.2004.03.002

Häring M, Vestergaard G, Rachel R, Chen L, Garrett RA, Prangishvili D (2005) Virology: independent virus development outside a host. Nature 436:1101–1102. https://doi.org/10.1038/4361101a

Helton RR, Wang K, Kan J, Powell DH, Wommack KE (2012) Interannual dynamics of viriobenthos abundance and morphological diversity in Chesapeake Bay sediments. FEMS Microbiol Ecol 79:474–486. https://doi.org/10.1111/j.1574-6941.2011.01238.x

Itoh T (2003) Taxonomy of nonmethanogenic hyperthermophilic and related thermophilic archaea. J Biosci Bioeng 96:203–212

Jaatinen ST, Happonen LJ, Laurinmaki P, Butcher SJ, Bamford DH (2008) Biochemical and structural characterisation of membrane-containing icosahedral dsDNA bacteriophages infecting thermophilic *Thermus thermophilus*. Virol 379:10–19. https://doi.org/10.1016/j.virol.2008.06.023

Jacquet S, Dorigo U, Personnic S (2013) A few tests prior to flow cytometry and epifluorescence analyses of freshwater bacterio- and virioplankton communities. In: Papandreou S (ed) Flow cytometry: principles, methodology and applications, chapter 1. Nova Science Publishers, New York, pp 1–30

Janekovic D, Wunderl S, Holz I, Zillig W, Gierl A, Neumann H (1983) TTV1, TTV2 and TTV3, a family of viruses of the extremophilic, anaerobic, sulfur reducing archaebacterium *Thermoproteux tenax*. MGG 192:39–45

Juniper SK, Bird DF, Summit M, Vong MP, Baker ET (1998) Bacterial and viral abundances in hydrothermal event plumes over northern Gorda Ridge. Deep Sea Res Part II 45:2739–2749

Klatt CG et al (2011) Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. ISME J 5:1262–1278. https://doi.org/10.1038/ismej.2011.73

Kostrzynska M, Betts JD, Austin JW, Trust TJ (1991) Identification, characterization, and spatial localization of two flagellin species in *Helicobacter pylori* flagella. J Bacteriol 173:937–946

Kott Y (1965) Estimation of low numbers of *Escherichia coli* bacteriophage by use of the most probable number method. Appl Microbiol 14:141–144

Lavigne R, Ceyssens P-J (2012) Family—*Myoviridae*. In: King AMQ, Adams MJ, Carstens EB, Efkowitz EJL (ed) Elsevier Academic Press, San Diego, pp 46–62

Lee MH et al (2007) Evaluation of viral and prokaryotic community dynamics in Alvord Desert hot springs, Oregon, USA. Aquat Microb Ecol 48:19–26. https://doi.org/10.3354/ame048019

Liu B, Wu S, Song Q, Zhang X, Xie L (2006) Two novel bacteriophages of thermophilic bacteria isolated from deep-sea hydrothermal fields. Curr Microbiol 53:163–166. https://doi.org/10.1007/s00284-005-0509-9

Liu B, Zhou F, Wu S, Xu Y, Zhang X (2009) Genomic and proteomic characterization of a thermophilic *Geobacillus* bacteriophage GBSV1. Res Microbiol 160:166–171. https://doi.org/10.1016/j.resmic.2008.12.005

Liu B, Wu S, Xie L (2010) Complete genome sequence and proteomic analysis of a thermophilic bacteriophage BV1. Acta Oceanol Sin 29:84–89

Lossouarn J et al (2015) 'Menage a trois': a selfish genetic element uses a virus to propagate within Thermotogales. Environ Microbiol 17:3278–3288. https://doi.org/10.1111/1462-2920.12783

Manini E et al (2008) Prokaryote diversity and virus abundance in shallow hydrothermal vents of the Mediterranean Sea (Panarea Island) and the Pacific Ocean (north Sulawesi-Indonesia). Microb Ecol 55:626–639. https://doi.org/10.1007/s00248-007-9306-2

Maranger R, Bird DF (1995) Viral abundance in aquatic systems: a comparison between marine and fresh waters. Mar Ecol Prog Ser 121:217–226

Marie D, Brussaard CPD, Thyrhaug R, Bratbak G, Vaulot D (1999) Enumeration of marine viruses in culture and natural samples by flow cytometry. Appl Environ Microbiol 65:45–52

Maugeri TL, Lentini V, Spanò A, Gugliandolo C (2013) Abundance and diversity of picocyanobacteria in shallow hydrothermal vents of Panarea Island (Italy). Geomicrobiol J 30:93–99

Mehta R, Singhal P, Singh H, Damle D, Sharma AK (2016) Insight into thermophiles and their wide-spectrum applications. 3. Biotech 6:81. https://doi.org/10.1007/s13205-016-0368-z

Mochizuki T, Krupovic M, Pehau-Arnaudet G, Sako Y, Forterre P, Prangishvili D (2012) Archaeal virus with exceptional virion architecture and the largest single-stranded DNA genome. PNAS 109:13386–13391. https://doi.org/10.1073/pnas.1203668109

Noble RT, Fuhrman JA (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113–118. https://doi.org/10.3354/ame014113

Nougier J, Ballestracci R, Blavoux B (1982) Les manifestations post-volcaniques dans les îles australes françaises (T.A.A.F.): zones fumeroliennes et sources thermo-minérales. CR Acad Sc Paris 295:389–392

Okutan E et al (2013) Novel insights into gene regulation of the rudivirus SIRV2 infecting *Sulfolobus* cells. RNA Biol 10:875–885. https://doi.org/10.4161/rna.24537

Ortmann AC, Suttle CA (2005) High abundances of viruses in a deep-sea hydrothermal vent system indicates viral mediated microbial mortality. Deep Sea Res Part I 52:1515–1527

Parikka KJ, Le Romancer M, Wauters N, Jacquet S (2017) Deciphering the virus-to-prokaryote ratio (VPR): insights into virus-host relationships in a variety of ecosystems. Biol Rev 92:1081–1100. https://doi.org/10.1111/brv.12271

Partensky F, Blanchot J, Vaulot D (1999) Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review. Bulletin de l'Institut océanographique, Monaco, no spécial 19:457–475

Patel A, Noble RT, Steele JA, Schwalbach MS, Hewson I, Fuhrman JA (2007) Virus and prokaryote enumeration from planktonic aquatic environments by epifluorescence microscopy with SYBR Green I. Nat Protoc 2:269–276. https://doi.org/10.1038/nprot.2007.6

Payet JP, Suttle CA (2008) Physical and biological correlates of virus dynamics in the southern Beaufort Sea and Amundsen Gulf. J Mar Syst 74:933–945. https://doi.org/10.1016/j.jmarsys.2007.11.002

Peduzzi P, Agis M, Luef B (2013) Evaluation of confocal laser scanning microscopy for enumeration of virus-like particles in aquatic systems. Environ Monit Assess 185:5411–5418. https://doi.org/10.1007/s10661-012-2955-8

Personnic S, Domaizon I, Sime-Ngando T, Jacquet S (2009) Seasonal variations of microbial abundances and virus- versus flagellate-induced mortality of picoplankton in three peri-alpine lakes. J Plankton Res 31:1161–1177

Pina M, Bize A, Forterre P, Prangishvili D (2011) The archaeoviruses. FEMS Microbiol Rev 35:1035–1054

Postec A, Ciobanu M, Birrien JL, Bienvenu N, Prieur D, Le Romancer M (2010) *Marinitoga litoralis* sp. nov., a thermophilic, heterotrophic bacterium isolated from a coastal thermal spring on Ile Saint-Paul, Southern Indian Ocean. Int J Syst Evol Microbiol 60:1778–1782. https://doi.org/10.1099/ijs.0.017574-0

Pradeep Ram AS, Palesse S, Colombet J, Thouvenot A, Sime-Ngando T (2014) The relative importance of viral lysis and nanoflagellate grazing for prokaryote mortality in temperate lakes. Freshw Biol 59:300–311. https://doi.org/10.1111/fwb.12265

Prangishvili D (2006) Hyperthermophilic virus-host systems: detection and isolation. In: Rainey F, Ohren A (eds) Extremophiles. Academic Press, Cambridge, pp 331–347

Prangishvili D (2013) The wonderful world of archaeal viruses. Annu Rev Microbiol 67:565–585. https://doi.org/10.1146/annurev-micro-092412-155633

Rice G et al (2004) The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. PNAS 101:7716–7720. https://doi.org/10.1073/pnas.0401773101

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101. https://doi.org/10.1038/35059215

Sakaki Y, Oshima T (1975) Isolation and characterization of a bacteriophage infectious to an extreme thermophile, *Thermus thermophilus* HB8. J Virol 15:1449–1453

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning—A Laboratory Manual. In: Ausubel M, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Second Edition, Cold Spring Harbor Laboratory Press (CSH), New York

Schmitt R, Raska I, Mayer F (1974) Plain and complex flagella of *Pseudomonas rhodos*: analysis of fine structure and composition. J Bacteriol 117:844–857

Schoenfeld T, Patterson M, Richardson PM, Wommack KE, Young M, Mead D (2008) Assembly of viral metagenomes from yellowstone hot springs. Appl Environ Microbiol 74:4164–4174. https://doi.org/10.1128/AEM.02598-07

Sime-Ngando T, Mignot J-P, Amblard C, Bourdier G, Desvilettes C, Quiblier-Llobéras C (1996) Caractérisation des particules virales planctoniques dans un lac du Massif Central français: aspects méthodologiques et premiers résultats. Annls Limnol 32:259–263

Soler N, Marguet E, Verbavatz JM, Forterre P (2008) Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. Res Microbiol 159:390–399. https://doi.org/10.1016/j.resmic.2008.04.015

Suttle CA (2005) Viruses in the sea. Nature 437:356–361. https://doi.org/10.1038/nature04160

Suttle CA (2007) Marine viruses-major players in the global ecosystem. Nat Rev Microbiol 5:801–812. https://doi.org/10.1038/nrmicro1750

Suttle CA, Fuhrman JA (2010) Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy. In: Suttle CA, Wilhelm SW, Weinbauer MG (eds) Manual of aquatic viral ecology. ASLO, Waco, pp 145–153

Tarasov VG (2006) Effects of shallow-water hydrothermal venting on biological communities of coastal marine ecosystems of the western pacific. Adv Mar Biol 50:267–410

Tarasov VG et al (1999) Effect of shallow-water hydrothermal venting on the biota of Matupi Harbour (Rabaul Caldera, New Britain Island, Papua New Guinea). Cont Shelf Res 19:79–116

Tarasov VG, Gebruk AV, Mironov AN, Moskalev LI (2005) Deep-sea and shallow-water hydrothermal vent communities: two different phenomena? Chem Geol 224:5–39

Truesdell AH (1975) Geochemical techniques in exploration. In: Proceedings of 2nd UN Symposium Development and Use of Geothermal Resources 1:53–86

Valentine DL (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat Rev Microbiol 5:316–323. https://doi.org/10.1038/nrmicro1619

Vaulot D (1989) CYTOPC: processing software for flow cytometric data. Signal Noise 2

Wang Y, Zhang X (2008) Characterization of a novel portal protein from deep-sea thermophilic bacteriophage GVE2. Gene 421:61–66. https://doi.org/10.1016/j.gene.2008.05.015

Wang Y, Zhang X (2010) Genome analysis of deep-sea thermophilic phage D6E. Appl Environ Microbiol 76:7861–7866. https://doi.org/10.1128/AEM.01270-10

Ward DM, Castenholz RW, Miller SR (2012) Cyanobacteria in geothermal habitats ecology of cyanobacteria II: their diversity in space and time. Whitton BA (ed) Springer, Netherlands, pp 39–65

Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28:127–181. https://doi.org/10.1016/j.femsre.2003.08.001

Weinbauer MG, Fuks D, Puskaric S, Peduzzi R (1994) Diel, seasonal, and depth-related variability of viruses and dissolved DNA in the Northern Adriatic Sea. Microb Ecol 30:25–41

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Williamson SJ, Cary SC, Williamson KE, Helton RR, Bench SR, Winget D, Wommack KE (2008) Lysogenic virus-host interactions predominate at deep-sea diffuse-flow hydrothermal vents. ISME J 2:1112–1121. https://doi.org/10.1038/ismej.2008.73

Wommack KE, Colwell RR (2000) Virioplankton: viruses in aquatic ecosystems. MMBR 64:69–114

Wommack KE, Williamson SJ, Sundbergh A, Helton RR, Glazer BT, Portune K, Cary SC (2004) An instrument for collecting discrete large-volume water samples suitable for ecological studies of microorganisms. Deep Sea Res Part I 51:1781–1792

Yoshida-Takashima Y et al (2012) Spatial distribution of viruses associated with planktonic and attached microbial communities in hydrothermal environments. Appl Environ Microbiol 78:1311–1320

Yu MX, Slater MR, Ackermann HW (2006) Isolation and characterization of *Thermus* bacteriophages. Arch Virol 151:663–679. https://doi.org/10.1007/s00705-005-0667-x