

# Morphotypes of virus-like particles in two hydrothermal vent fields on the East Scotia Ridge, Antarctica

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**Abbreviations:** VLP, virus like particle; ESR, East Scotia Ridge; LSS, light scattering sensor; TEM, transmission electron microscopy

Viruses from extreme environments are still largely unexplored and may harbor unseen genetic potential. Here, we present a first glance at the morphological diversity of virus like particles (VLPs) from an environment that is extreme in more than one respect: two recently discovered hydrothermal vent fields on the East Scotia Ridge in the Southern Ocean near Antarctica. They are the southernmost hydrothermal sites found to date and have been shown to present a new biogeographic province, containing several new macrofaunal species and associated microbial organisms. Transmission electron microscopy revealed a range of tailed and untailed VLPs of various morphologies as well as an unusual long rod-shaped VLP with three long filaments. Based on its distant similarity with several known archaeal viruses, we hypothesize that this presents a new viral morphology that most likely infects an archaeon. Notably absent in the samples we analyzed were lemon- or spindle-shaped VLPs that have previously been described in other hydrothermal vent settings.

## Introduction

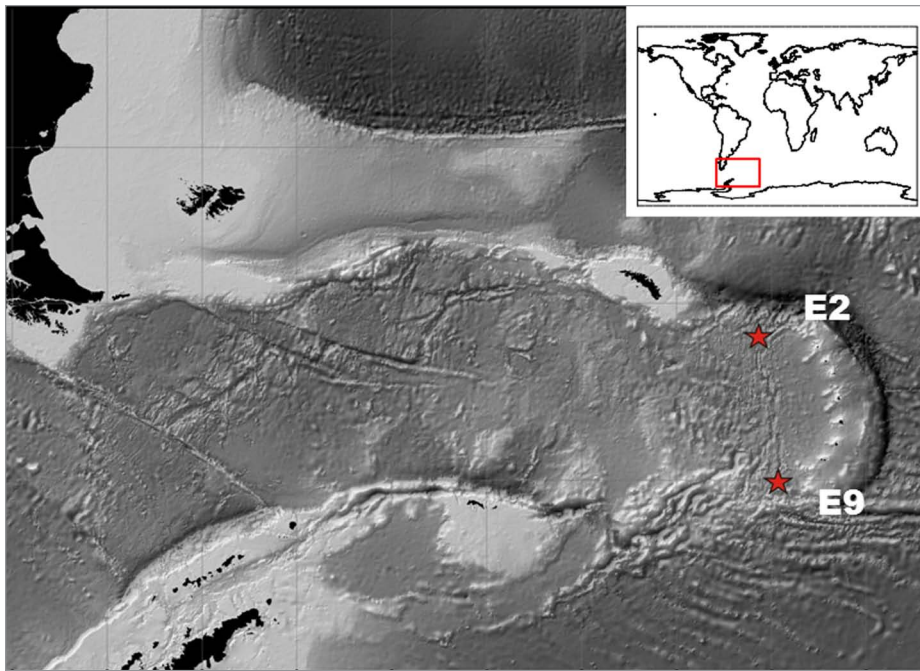
A large part of the world of viruses is still an undiscovered country. Particularly extreme or unusual environments still hold many secrets about their viral diversity. Hydrothermal vents were only discovered as a biological habitat within the past 30 years. A lot of research has since focused on the vent sites along the Mid-Atlantic and Pacific Ridges, which are characterized by completely different, endemic, macro- and microflora. In 2010, the first hydrothermal vents in the Antarctic sub-polar region were discovered on the East Scotia Ridge (ESR).<sup>1</sup> Due to ocean currents, these vent sites were hypothesized to represent stepping stones connecting the Pacific and Atlantic hydrothermal vents. However, this was found to be only partially true and although the ESR vent fields contain some biological features of both Atlantic and Pacific sites, they are nevertheless unique enough to present a new biogeographical province in their own right. A first characterization of the macro- and microbiology of these new vent sites is beginning to emerge.<sup>1–4</sup> Here, we present a first glimpse at the viral diversity of water samples influenced by both the hydrothermal vent fluids and Antarctic deep sea conditions.

Vent fluids emanating from hydrothermal vent chimneys can reach temperatures above 400 °C at the top of the chimney and contain high concentrations of particulate matter as well as a range of dissolved chemicals, including sulfides, methane, metals and rare earth elements. Temperatures drop rapidly as the hot vent fluid mixes with the surrounding sea water and temperatures within the buoyant plume within a few meters above the vent orifice are generally only one or two degrees above the background water. Due to the sharp temperature drop, sulfides, metals and other chemicals dissolved in the vent fluid precipitate, creating the “smoke” of the eponymous black smoker chimney. The plume moves upwards and (depending on currents) vertically in the water column until it reaches neutral buoyancy, several hundred meters above the vent site. In contrast to the black plume immediately above the vent orifice, which presents a very volatile environment due to strong mixing forces, the neutrally buoyant plume forms a distinct and stable water mass, which is characterized by elevated levels of particulate matter and chemicals derived from the vent fluid and can thus favor specific microbiota and their associated viruses. It can easily be detected and sampled with a CTD equipped with a light-scattering sensor (LSS) and an  $E_h$  probe measuring the redox potential.<sup>5</sup>

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**Figure 1.** Map of the East Scotia Ridge showing the location of the E2 and E9 vent fields.

The aim of this study was to examine for the first time the diversity of virus like particles at two recently discovered hydrothermal vent fields on the ESR. Water samples from the neutrally buoyant plume at the two vent fields were analyzed by transmission electron microscopy (TEM).

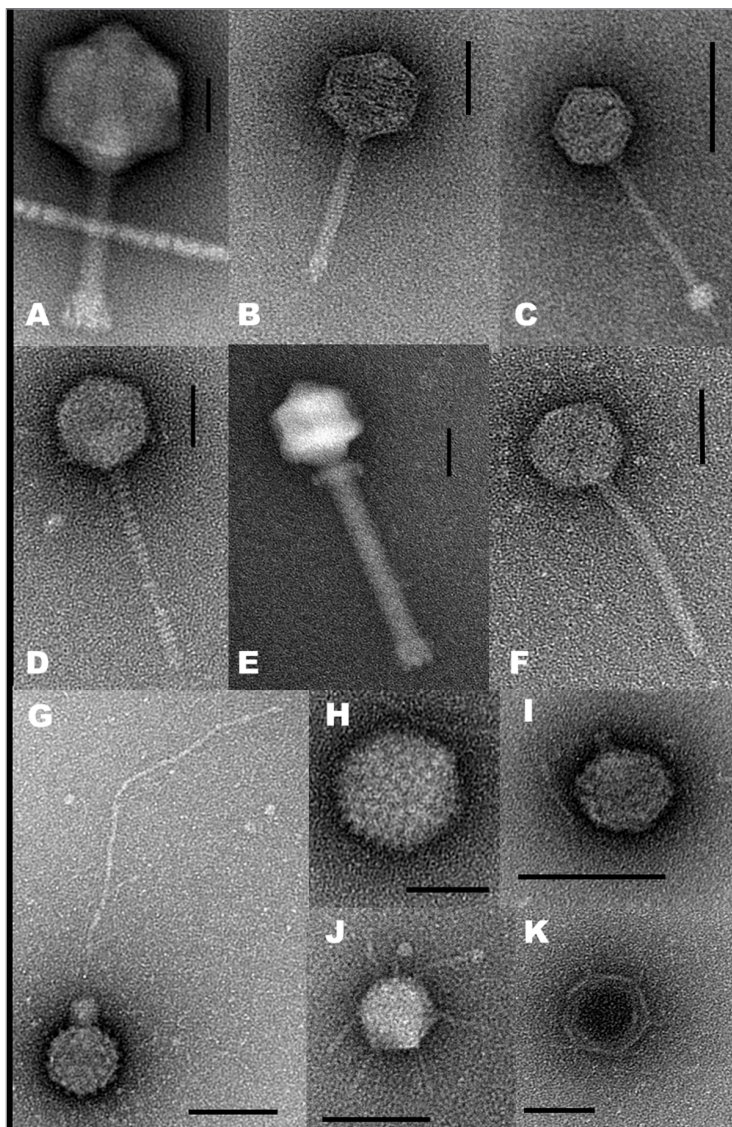
## Results and Discussion

Vent fields E2 and E9 are located on the East Scotia Ridge (Fig. 1), at  $56^{\circ}05.351'S/30^{\circ}19.131'W$  and  $60^{\circ}02.498'S/29^{\circ}59.992'W$ , at a depth of ca. 2500 m and have several actively venting black smoker chimneys with

temperatures of up to  $380^{\circ}C$ .<sup>1</sup> The vent fields are ca. 450 km apart and exhibit subtle, but distinct differences in their chemistry, micro- and macrofauna.<sup>1,3,6</sup> Samples were taken within the neutrally buoyant plume, ca 300 m above the chimneys. The water temperature at this depth was  $0^{\circ}C$ . In spite of this low temperature, 16S rRNA analysis of the samples showed a high proportion of bacteria and archaea with high similarity to species that have been characterized as thermophilic and vent-related in the literature, confirming the influence of the vent fluids on these water masses (Zwirgmaier, unpublished). It is unknown, whether these organisms are metabolically active at these low temperatures or whether they are in fact thermophiles.

TEM on samples from both vent fields revealed a range of tailed and untailed polyhedral VLPs, including several *Myoviridae*, *Siphoviridae* and *Podoviridae* (Fig. 2; Table 1). An unusual *Myovirus* with a very large head (135 nm) of morphotype A1<sup>7</sup> was found at both vent fields (Fig. 2A). A further putative *Myovirus*, also of morphotype A1 (Fig. 2E), with a prominent tail and wide collar, was found only at E2. A range of *Siphoviruses* of the morphotype B1 were also observed (Fig. 2B, C, D, and F), along with *Podoviruses* (Fig. 2I). The observance of these morphologies is not unexpected, as ~96% of phages are of the order *Caudovirales*.<sup>8</sup>

However, a number of unique and unusual morphologies were observed within these samples. The E9 sample contained VLPs of a morphotype that, as far as we are



**Figure 2.** TEM images of virus-like particles with a range of different morphologies found in the neutrally buoyant plume over the two vent fields. Images (A, B, C, D, H, I, and K) are from the E2 sample, images (E, F, G, and J) from E9. Scale bars: (A, B, D, E, F, H and K) 50 nm; (C, G, I, and J) 100 nm. See Table 1 for further details.

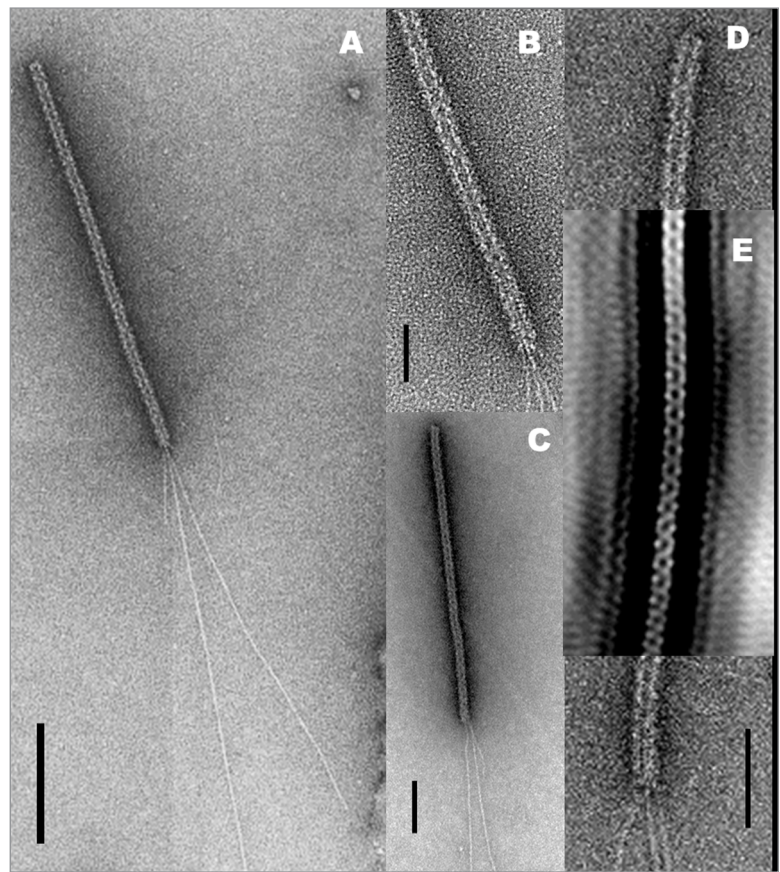
**Table 1.** Virus capsid measurements in nm

Fig.	Virus family	Sample from	Capsid size <sup>a</sup>	Tall length (excl base plate)	Tall width	Base plate width	Base plate length	Full-length	
2A	<i>Myoviridae</i>	E2	135	114	25/20 <sup>b</sup>	49	40	285	
2B	<i>Siphoviridae</i>	E2	59	108	11	n/a	n/a	170	
2C	<i>Siphoviridae</i>	E2	80	140	14	31	25	245	
2D	<i>Siphoviridae</i>	E2	74	150	15	n/a	n/a	229	
2E	<i>Myoviridae</i>	E9	90	180	30	35	30	320	Collar length/ width 53/20
2F	<i>Siphoviridae</i>	E9	60	132	14	n/a	n/a	192	
2G	potential new family	E9	90	34	34				Tail fiber length 427
2H	resembles <i>Adenoviridae</i>	E2	74	n/a	n/a	n/a	n/a	n/a	
2I	<i>Podoviridae</i>	E2	65	18	14	n/a	n/a	83	
2J	<i>Tectiviridae</i>	E9	68	n/a	n/a	n/a	n/a	n/a	
2K	<i>Corticoviridae</i>	E2	60	n/a	n/a	n/a	n/a	n/a	

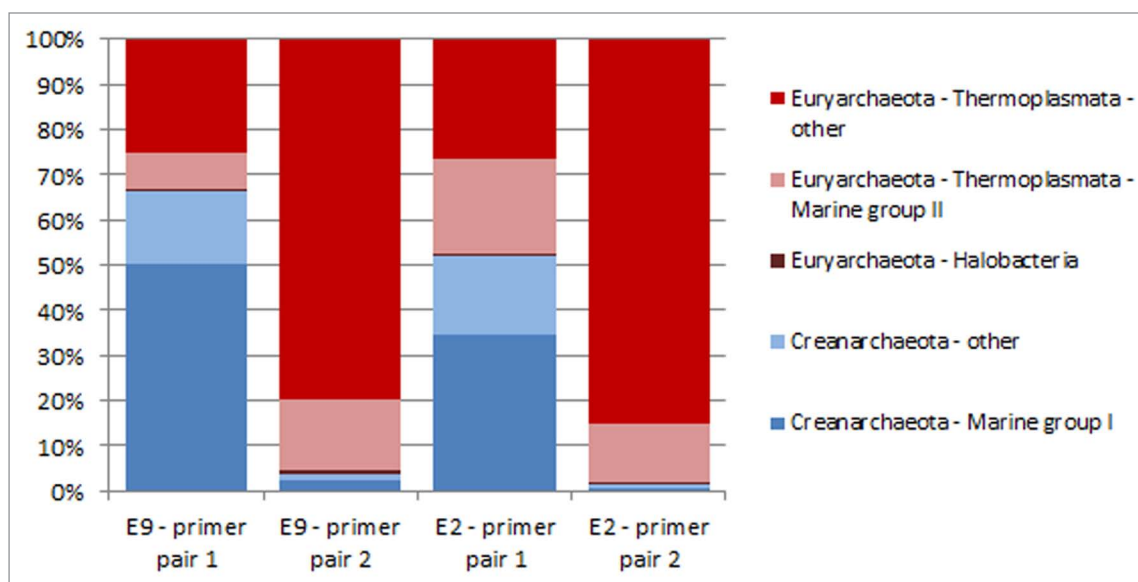
<sup>a</sup>Capsid measured at the flat sides; <sup>b</sup>Near base plate/near head; n/a, not applicable.

aware, has not been described before and could therefore be a new virus morphotype—a polyhedral non-enveloped capsid (diameter 90 nm) with a short thick tail ( $34 \times 34$  nm), with transverse striations on the tail and one long protruding filament (427 nm) (Fig. 2G). Additionally, a putative tectivirus was also observed, similar to those first detected by Ackermann et al. 1978<sup>9</sup> (Fig. 2J). A putative corticovirus was observed (Fig. 2K), currently there is only one type species within the *Corticoviridae*, but genomic data suggests they are widespread in aquatic bacteria.<sup>10</sup> Intriguingly, viruses similar in morphology to Adenovirus were observed (Fig. 2H). Adenoviruses are well known for causing respiratory infections in mammals, but also reptiles and fish.<sup>11</sup> The presence within plume samples is puzzling, suggesting they persist within the marine environment and may infect eukaryotic organisms around hydrothermal vents.

The most striking VLP morphotype in the sample from the E2 vent field was a long, non-enveloped, rod-shaped VLP with a helical appearance and three filaments attached (Fig. 3). To get a clearer idea of the capsid shape, a Fourier transformation of the image was taken, spots picked out to approximately 4 nanometers and used to generate an inverse Fourier (Fig. 3E). The pitch of the helix was 19 nm. The measured length of the rod ranged between 515–830 nm ( $644 \pm 144$  nm), with a diameter of  $34 \pm 1$  nm. The inconsistent length may be due to fractured VLPs. We frequently observed shorter, presumably fractured fragments without any filaments attached. Intact VLPs had three tail filaments, two long ones ( $664 \pm 6$  nm and  $564 \pm 55$  nm) and one short ( $115 \pm 7$  nm).



**Figure 3.** Filamentous VLP with a new morphotype found at the E2 vent field, showing a long rod with two long and one short filament attached at one end. (E) Fourier transformation, overlay of the VLP in picture (D), showing the helical structure of the rod. Scale bars: (A and C) 200  $\mu$ m; (B) 50 nm; (D) 100 nm.



**Figure 4.** 454 sequence analysis of archaeal 16S rRNA gene sequences at E2 and E9 using two different primer pairs. Number of sequences analyzed: primer pair 1: 1563 sequences at E2, 1790 sequences at E9, primer pair 2: 1123 sequences at E2, 849 sequences at E9.

The structure has similarities with the *Rudiviridae*<sup>12</sup> and the recently described *Spiraviridae*.<sup>13</sup> The observed rod structure is similar in size of that to that of rudivirus SIRV1 at 830 nm in length, although wider than the 23 nm for SIRV1.<sup>12</sup> Both rudiviruses SIRV1 and SIRV2 have 3 tails fibers in common with the particles we observed.<sup>12</sup> However, the observed VLPs had far longer filaments all greater than 100 nm in length and were consistently found to have 2 larger filaments and one short filament. In comparison to other helical viruses such as the spirivirus ACV, the observed particles are again longer than 220 nm of ACV and the filaments substantially larger than the 20 nm found on ACV.<sup>13</sup> The estimation of a the pitch of the helix of 19 nm is far larger than that observed for SIRV2 and ACV at ~2.4 nm and ~4.8 nm respectively. These observations indicate the VLP has a novel morphotype that may fit within the *Rudiviridae* family.

The known hosts of *Rudiviridae* are archaea, more precisely Crenarchaeota, and all currently described representatives have been isolated from high temperature environments. Based on these similarities, we hypothesize that the hosts of the rod-shaped ESR viruses are also archaea. 16S rRNA analysis of the ESR water samples showed that at least 50% of all E2 and 20% of E9 sequences are archaeal (Zwirgmaier, unpublished). This is a conservative estimate, since the “universal” primers used for sequencing (see Material and Methods, primer pair 1) have a higher coverage for bacteria than archaea.<sup>14</sup> Therefore, the actual proportion of archaea in the samples is likely to be higher. A 16S rRNA survey using archaea-specific primers (primer pair 2) showed 99% (at E2) and 97% (at E9) of the sequences to be Euryarchaeota. The dominance of Euryarchaeota in this data set is due to the forward primer, which has been shown to have a strong bias for this phylum of archaea.<sup>14</sup> Within the Euryarchaeota, 98% (at E2) and 97% (at E9) were assigned to the Thermoplasmatales (Fig. 4). All known members of the Thermoplasmatales are acidophilic thermophiles.<sup>15</sup> The pH of

ca. 3 and temperatures of up to 380 °C of the vent fluids at both E2 and E9 suggest that the Thermoplasmatales found in the neutrally buoyant plume originated from the vents, rather than being native to the surrounding water, which has a temperature of ca. 0 °C and pH of ca. 7.5.

In samples taken above the neutrally buoyant plume or several kilometers off-site from the vents (sample volume 80–150 L) hardly any VLPs were found with TEM and none of the new rod-shaped morphotype (data not shown). This would suggest that the concentration of VLPs within the neutrally buoyant plume is slightly higher compared with the surrounding Antarctic deep sea water, although we do not have any data on VLP counts/ml to confirm this. A possible explanation is a switch from lysogenic to lytic cycle in infected cells derived from the vent, which are under stress due to the low temperatures in this environment. Although unfavorable conditions for the host are more commonly associated with lysogeny, allowing the virus to take refuge,<sup>16</sup> the opposite has also been found to occur, i.e., increased lytic events, with the virus “abandoning the sinking ship” in order to preserve its genome.<sup>17</sup>

Lemon-, spindle- or droplet-shaped (archaeal) viruses that have frequently been observed in various hydrothermal vents and hot springs<sup>18</sup> were notably absent in the ESR samples.

## Conclusions

Viruses of the order *Caudovirales* were the most commonly observed morphotype within the samples collected from a novel vent field. The hosts of these virus-like particles cannot be ascertained from their morphology, given viruses infecting both archaea and bacteria are part of the *Caudovirales* order. The finding in this small scale study of at least one novel viral morphotype, suggest that this newly described biogeographic province

may harbor many other previously undescribed viruses. This highlights how little is known about this environment and how further work is needed to fully determine the diversity of viruses that are capable of infecting both the micro and macro organisms within this environment.

## Material and Methods

### Sampling and sample processing

Samples were collected on cruise JC42, Jan–Feb 2010 on board the RRS James Cook. The position of the neutrally buoyant plume above the vent fields was determined with a light scattering sensor (LSS) and a sensor to measure the redox potential, both mounted on the Conductivity-Temperature-Depth (CTD) frame. At vent field E2, 185 L of seawater were sampled at a depth of 2262 m (300 m above the seafloor), at E9, 80 L were sampled at 2100 m (also 300 m above the seafloor). The water was filtered through 3  $\mu\text{m}$  and then 0.2  $\mu\text{m}$  cellulose nitrate filters (Whatman) to remove larger particles and bacterial cells. VLPs were then concentrated by tangential flow filtration (TFF) using a 10 kDa membrane (Pellicon Biomax 10, Merck-Millipore, Billerica, MA, USA). VLPs in the TFF concentrate (ca. 150 ml) were precipitated with PEG-8000 and further purified and concentrated by ultracentrifugation at 35 000  $\times$  g for 2 h (Optima L-80XP, Beckman Coulter, Buckingham, UK) in a caesium chloride gradient (1.45 g L<sup>-1</sup>/ 1.5 g L<sup>-1</sup>/ 1.7 g L<sup>-1</sup>) as described previously.<sup>19</sup>

### Transmission electron microscopy (TEM)

Samples were negatively stained with 2% uranium acetate. Briefly, Formvar-carbon grids were glow discharged, 5–10  $\mu\text{l}$  sample was applied for 60 s, blotted and 5–10  $\mu\text{l}$  of 2% uranium acetate (pH 4.5) was added for another 60 s and then blotted dry. The images were taken on an JEOL (Welwyn Garden City, England) 2010F 200 kv electron microscope using a Gatan Ultrascan 4000 camera. The microscope was calibrated with a commercially available lattice of bovine liver catalase crystals (PSI, West Chester, Pennsylvania, USA). Measurements and image analysis were performed using Gatan Digital Micrograph software.

### Extraction of cellular DNA and 454 sequencing of archaeal 16S rRNA gene

DNA from cellular organisms concentrated on 0.2  $\mu\text{m}$  filters (see above) was extracted with phenol/chloroform as described

previously.<sup>20</sup> The 16S rRNA gene was amplified by PCR and sequenced using a 454 FLX Titanium at LGC Genomics (Berlin, Germany). PCR primers and conditions were as follows: Primer pair 1: 786Fm<sup>21</sup>/1492R,<sup>22</sup> PCR conditions 94 °C, 3 min, followed by 30 cycles of 94 °C, 45 s, 50 °C, 30 s, 72 °C, 60 s and a final extension of 5 min at 72 °C; primer pair 2: A344F1<sup>23</sup>/A912R,<sup>24</sup> PCR conditions 94 °C, 3 min, followed by 30 cycles of 94 °C, 45 s, 52 °C, 45 s, 72 °C, 45 s and a final extension of 5 min at 72 °C using MyTaq polymerase (Bioline, UK).

Sequence data was analyzed with mothur v.1.31<sup>25</sup> and arb.<sup>26</sup> For quality control, sequences were trimmed based on the qfile, with a qwindowaverage of  $\geq 30$  and a window size of 50. Sequences with any ambiguities or homopolymers longer than 10 nt or a length < 100 nt were discarded. Chimeric sequences were detected and discarded with chimera slayer within mothur. The trimmed set of sequences was aligned in arb using SINA<sup>27</sup> and a distance matrix was created within arb using the Jukes-Cantor correction. Sequences were then clustered with mothur using the furthest neighbor method. OTUs were defined at the level of 0.03. Sequences were classified with mothur to assign each sequence to a taxonomic group using a reference database containing 16073 16S rRNA sequences, which is based on the living tree project database LTPv104,<sup>28</sup> containing 8545 sequences, plus 7528 hydrothermal vent related sequences downloaded from Silva.<sup>29</sup>

Sequence data have been submitted to GenBank, accession numbers SRR1179069, SRR1179071, SRR1179072, and SRR1179074.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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