Is there an active hydrothermal flux from the Orca seamount in the Bransfield Strait, Antarctica?

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ABSTRACT. The rifting zone of Bransfield Strait, Antarctica, is tectonically and geologically unique. It is a back-arc basin that was opened by extensional forces associated to roll-back subduction after cessation of spreading activity of the Phoenix Ridge, and the transtension of the westward ending of Scotia-Antarctica Plate boundary. The Bransfield Rift/Ridge is still active generating volcanism or magma rise to force hydrothermal activity. During the ANT-XXV/4 cruise onboard R/V "Polarstern", standard CTD and beam transmission measurements were done to determine temperature anomaly and turbidity. Water sampling was performed to determine δ^3He and to find thermophilic microorganisms to examine the Orca seamount hydrothermal activity. A temperature anomaly of ~0.08 °C, a pick of turbidity, and high value of δ^3He (>10%) were found inside Orca seamount. Results are consistent with a hydrothermal flux coming from the seamount. The report of the first observation of thermophilic and hyperthermophilic microorganisms in cold deep Antarctic waters is part of this study. Inside Orca seamount these microorganisms were found at three different depth levels close to the bottom. We suggest that the fluid migration from the volcano resulted from recent magmatic activity and provided the required elemental nutrients for microbial growth. Besides some thermophiles were found outside the seamount in a small quantity close to the seafloor. These would probably be related to subsidiary structures of the Orca seamount, or were transported by currents from other active volcanic sites as Deception Island. The finding of these thermophilic and hyperthermophilic microorganisms raise questions about the dispersal and their resistance in these extreme environments.

Keywords: Hydrothermal, Helium isotope, Thermophiles, Seamount, Volcanism, Rift system, Antarctica.

RESUMEN. ¿Existe un flujo hidrotermal activo desde el monte submarino Orca en el estrecho Bransfield, Antártica?. La zona de rifting del estrecho Bransfield, Antártica, es tectónica y geológicamente única. Es una cuenca de trasarco originada por fuerzas de extensión asociadas a *roll-back* después del cese de la subducción y expansión de la dorsal Fénix, y la transtensión relacionada con la actividad del límite oeste de la Placa Scotia-Antártica. El rift/dorsal del estrecho de Bransfield, actualmente activo, provoca volcanismo o ascenso de magma al que se asocia actividad hidrotermal. Durante el crucero ANT-XXV/4 del R/V "Polarstern", se realizaron mediciones estándar de CTD y transmisión de luz (haz) para determinar anomalías de temperatura y turbidez, y también muestreo de agua para establecer su valor de δ^3He y presencia de microorganismos termofílicos y así evaluar la potencial actividad hidrotermal en el monte submarino Orca. Una anomalía de temperatura de ~0.08 °C, un pico de turbidez y un alto valor de δ^3He (>10%) se encontraron dentro del cráter de Orca. Los resultados son consistentes con un flujo hidrotermal proveniente del monte submarino. También se encontraron, por primera vez, microorganismos termófilos e hipertermófilos en aguas frías profundas de la Antártica. Dentro del cráter del monte submarino Orca, estos microorganismos se localizaron en tres niveles de profundidad diferentes cerca del fondo. Nosotros sugerimos que la circulación de fluidos hidrotermales, producto de la actividad magmática reciente, proporcionaron los nutrientes y el ambiente propicio para el crecimiento de estos microorganismos. Además, se encontraron fuera del volcán, cerca del lecho marino, microorganismos termófilos en una pequeña cantidad. Estos estarían relacionados, probablemente, con estructuras subsidiarias del monte submarino Orca o fueron transportadas por corrientes desde otros centros volcánicos activos como la isla Decepción. El descubrimiento de estos microorganismos termófilos e hipertermófilos genera nuevas preguntas sobre su dispersión y resistencia en estos ambientes extremos.

Palabras clave: Hidrotermal, Isótopo de helio, Termófilos, Monte submarino, Volcanismo, Sistema de rift, Antártica.

1. Introduction

The seafloor hydrothermal activity has a great impact on the chemistry of the oceans and is responsible for extensive alteration of the oceanic crust (Herzig and Hannington, 1995). The water from vents also plays an important role in the heat budget and circulation patterns of the oceans (Baker and Massoth, 1986). Typical submarine hydrothermal vents support unusual ecosystem communities where primary production, which is the basis for the local food chain, depends on chemosynthetic microorganisms (Baross and Deming, 1983). This phenomenon is not unique to mid-ocean ridges since it also occurs in volcanic arcs, back-arc spreading centers and intra-plate volcanoes (*e.g.,* German *et al.*, 2000). Every mentioned site has its own associated tectonics, geodynamics and geological characteristics that produce distinctive geophysical and geochemical signatures, which are reflected in the variability in composition, size or volume, spatial distribution, frequency and transience of the deep-sea hydrothermal vents (Lupton *et al*., 1998; Baker *et al.*, 2001; Hey *et al*., 2004; Hannington *et al.*, 2005).

The rifting zone of the Bransfield Strait, located between the South Shetland Islands and the Antarctic Peninsula (Fig. 1) is tectonically and geologically unique (Lawyer *et al.*, 1996). Subduction and extension processes occur simultaneously and there is currently no clarity in their geological evolution

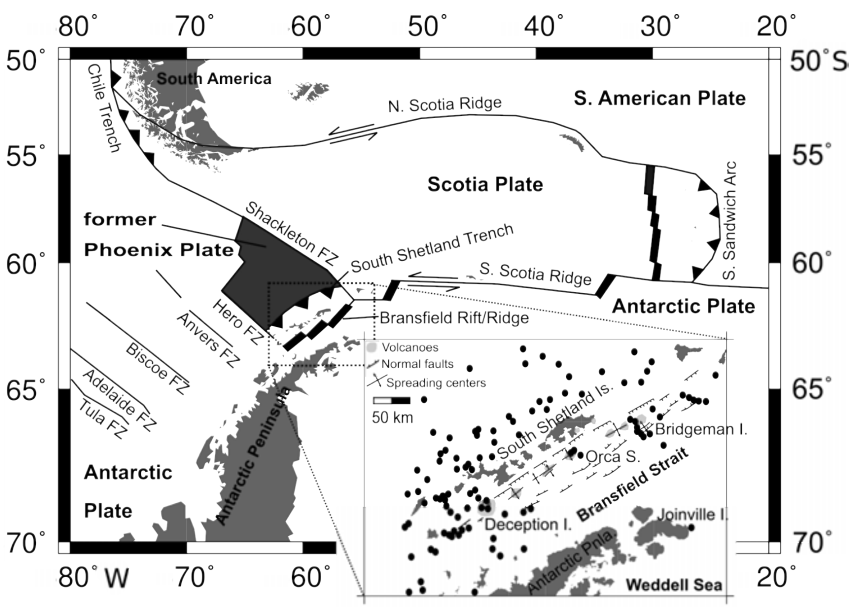

FIG. 1. Regional tectonic setting of the area studied based on Barker and Austin (1998). The location of former Phoenix Plate, other plates and tectonic structures are indicated. Zoom of the Bransfield Strait area with its tectonic structures, volcanic edifices are showed (modified after Pedrera *et al*., 2012), and also seismicity from Robertson Maurice *et al*. (2003) (epicenters in black dots).

(Barker and Austin, 1994, 1998; Fretzdorff *et al.*, 2004; Solari *et al.*, 2008; Poblete *et al.*, 2011). The former Phoenix Plate, located between the Hero and Shackleton Fracture Zones (Fig. 1), was formed by seafloor spreading on the Antarctic-Phoenix Ridge (APR) since the Jurassic (Barker, 1982). Oblique subduction under the Antarctic Plate ceased progressively from SW to NE during the Cenozoic as a result of successive ridge crest-trench collisions (Herron and Tucholke, 1976; Barker, 1982). Since the Pliocene the APR spreading stopped, and subduction slowed and roll-back started at the South Shetland Trench (Fig. 1) (Livermore *et al.*, 2000; Jabaloy *et al.*, 2003; Galindo-Zaldívar *et al.*, 2004). These mechanisms and the transtension associated with the westward ending of Scotia-Antarctica Plate boundary, enable the opening of the Bransfield Basin and associated Quaternary volcanism (Galindo-Zaldívar *et al.*, 2004; Solari *et al.*, 2008; Pedrera *et al.*, 2012). In this way, the Bransfield Rift/Ridge is still active producing magma rise and hydrothermalism (Barker and Austin, 1994, 1998; Rey *et al.*, 1995; Somoza *et al.*, 2004).

The Bransfield Strait area is a solitary Antarctic zone with its peculiar hydrothermal activity associated to submarine volcanic edifices (Klinkhammer *et al.*, 1995; Bohrmann *et al.*, 1998; Dählman *et al.*, 2001; Klinkhammer *et al.*, 2001; Petersen *et al.*, 2004). As part of the most important volcanic edifices of the Bransfield Strait, is the Deception Island (Fig. 2). It has showed recent volcanic events occurred during the last decades producing subaerial fumaroles and hot springs, but also submarine hydrothermal vents inside the submerged caldera of the island (Rey *et al.*, 1995; Somoza *et al.*, 2004). Hydrothermal activity may persist up to decades after volcanic eruptions helped by geological structures under the seafloor (Somoza *et al.*, 2004).

Another prominent volcanic structure on the Bransfield Ridge is the Orca seamount, an apparently inactive volcano, about 500 m of elevation above the seabed, with 3 km wide crater. (Fig. 2) (Hatzky, 2005;

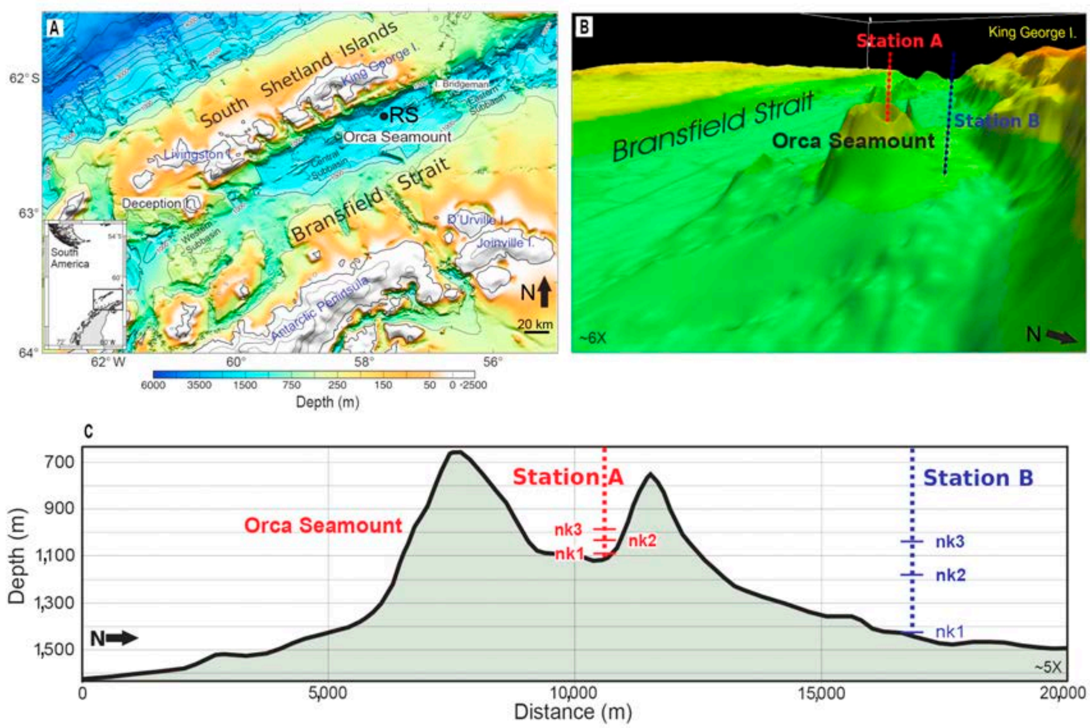

FIG. 2. Topography model of the studied area using multibeam bathymetry data available from Marine Geoscience Data System (hosted by LDEO-Columbia University). **A**. Location of the Orca Seamount in the Bransfield Strait and the "Reference Station" for δ^3He data (RS); **B**. 3D submarine topography model of Orca seamount and the location of Stations A (red line) and B (blue line); **C**. Bathymetric profile that cross stations A and B, showing depth levels of Niskin bottle sampling.

Schreider *et al*., 2014). Oceanographic measurements were performed within the Orca seamount to check the hydrothermal activity, however, no evidences were found (Lawver *et al.,* 1996). The seismic activity observed in the vicinity of Orca seamount has been interpreted as associated with magmatic activity (Fig. 1) (Kaminuma, 2001; Robertson Maurice *et al*., 2003; Dziak *et al*., 2010; Kanao, 2014), led us to begin an exploration of the volcano to check for its hydrothermal activity. This study was made considering that hydrothermal activity may persist long-time after magmatic discharges. The use of a multidisciplinary approach (oceanography, geochemistry and microbiology) would serve as background for future studies on the interaction of submarine volcanic hydrothermal flux on very cold water.

2. Methodology

2.1. Oceanographic cruise

During the ANT-XXV/4 cruise on board R/V "Polarstern" in April 2009 in the Drake Passage and the Bransfield Strait, Antarctica, a hydrographic station was performed inside the crater of Orca seamount (Station A), and another station was setup immediately to the north of Orca seamount (Station B) (Fig. 2A and 2B), to examine its potential hydrothermal activity. Seawater was collected using 12 l Niskin bottles mounted on a Sea-Bird SBE 32 Rosette equipped with a CTD (Conductivity, Temperature and Depth) Sea-Bird Electronics SBE911 plus. Deeper samples were taken 10 m from the seafloor (nk1 samples in table 1). The bathymetric profile in figure 2C, shows the shape and size of the seamount, the interior of the crater, and the location of the stations and depths (levels) of bottle sampling (nk). The CTD was supplemented by an oxygen sensor SBE 43, a transmissiometer (WetLabs C-Star, 660 nm wavelength), and a chlorophyll-sensitive fluorimeter (Chelsea Aquatracka). Details of the cruise methods and data analysis can be consulted in Provost (2010).

2.2. Oceanographic and gas analyses

Temperature, salinity, oxygen and light beam transmission data were taken by the CTD and converted to workable oceanographic variables using standard procedures (Provost *et al*., 2011). The processing of the data for plotting consisted in manual spike removal and the application of a low pass filter using a time of 0.5 s and, for beam transmission, 2 s. The potential temperature anomaly ($\Delta\theta$) calculation was performed graphically according to Baker *et al.* (2002) using a first order fit of the potential temperature as function of the potential density.

Helium-3 is an isotope that has been used as tracer of hydrothermal activity in mid-ocean ridges (*e.g.,* Baker *et al*., 2002; Jean-Baptiste and Fourré, 2004), and it has a solely magmatic origin. Thus its presence provides unequivocal evidence of magmatic activity (Lupton *et al*., 1977). Ocean water samples for noble gas analysis were stored from the Niskin bottles into gas tight copper tubes (50 ml). The noble gas samples were analysed in the Institute of Environmental Physics (IUP, Bremen) and in the noble gas mass spectrometry laboratory (Sültenfuß *et al*., 2009). They were processed in a first step with a UHV (Ultra High Vacuum) gas extraction system. Sample gases were transferred via water vapour into a glass ampoule were kept at liquid nitrogen temperature. Quality checks of

TABLE 1. SAMPLED LEVELS FOR THE ANALYSIS OF MICROORGANISMS AND ASSOCIATED OCEANOGRAPHIC DATA FOR STATIONS A (ORCA) AND B.

Station	Bottle	Latitude (S)	Longitude (W)	Station depth (m)	Bottle depth (m)	Temp. (°C)	Salinity (psu)	Oxygen (µmol/kg)	pH	δ^3He (%)
A	nk1	62°25.378'	58°24.352'	1,094	1,082	-1.14	34.53	304.8	7.69	11.52
A	nk2	62°25.378'	58°24.352'	1,094	1,033	-1.05	34.53	301.7	-	13.94
A	nk3	62°25.378'	58°24.352'	1,094	988	-1.04	34.54	301.7	7.71	13.91
B	nk1	62°22.153'	58°23.6'	1,444	1,435	-1.50	34.53	314.3	7.70	-
B	nk2	62°22.153'	58°23.6'	1,444	1,186	-1.40	34.53	312.3	-	-
B	nk3	62°22.153'	58°23.6'	1,444	1,036	-1.30	34.53	309.7	7.61	-

this sample preparation line were done on a routine basis. This includes special vacuum checks with the Quadrupole Mass Spectrometer (QMS) and preparation of accurately defined samples for internal control measurement. For analysis of the noble gas isotopes the glass ampoule is connected to a fully automated UHV mass spectrometric system equipped with a two stage cryo system. Every 2 to 4 samples the system is calibrated with standard atmospheric air measurements. Measurements of line blanks and linearity were also performed. Calibration of the data has to be done to obtain consistent data sets. For δ^3He the error was 0.2% accuracy and 0.1% precision from replicates.

2.3. Microbiological analysis

For the microbiological analysis, at both stations, samples were taken at three levels from the bottom (Table 1 and Fig. 2C) using Niskin bottles and were stored at 4 °C in sterile containers. Microorganisms were grown using techniques similar to those used by Summit and Baross (1998), emulating the conditions of mid-ocean ridge environments. The first step was isolation and culture of samples. They were incubated in various rich media (see Muñoz *et al.*, 2011). The microorganisms in the enrichment cultures were grown at temperatures from 70-97 ºC. Solid media were prepared at 1% solid agar. Colonies formed on the solid media were taken individually and incubated at 70 °C in liquid media to observe the generation of pure cultures. Growing conditions were improved with the determination of the optimal conditions of pH, temperature, NaCl, sources of carbon and oxygen. Gram stain reaction was determined using a Merck gram stain kit according to the manufacturers recommended protocol. In order to identifiy the microorganosms isolated we performed DNA isolation and Polymerase Chain Reaction (PCR) amplification. Genomic DNA was extracted from cultures grown for 48 h at 80 °C and 85 °C using two different cellular disruption methods followed by Phenol:Chloroform:Isoamyl alcohol precipitation (PCI). Gene Clean Purification kit (Bio 101) was used according to manufacturer's instructions to clean the DNA samples. The PCR amplification of 16S rDNA gene was performed using Taq DNA polymerase, universal primer 1492R and the domain Bacteria-specific primer 27F. Archaeal 16S rDNA was amplified with the universal primer 1492R and the domain Archaea-specific primer 21F. A ~1,500 kb fragment of the 16S rDNA gene was expected to be amplified. Verification of DNA extraction and PCR amplification was carried out by running the samples on a 1% agarose gel. Fluorescent microscopy was performed, 0.1 ml of sample of each cell suspension was removed from the main culture and immediately stained with the commercial kit LIVE/DEAD BacLightTM (invitrogen). Samples were incubated in a dark room at the temperatures for at least 15 minutes, before analysis. Samples also were visualised using an electronic microscope JEOL JSM-T300.

3. Results and discussion

3.1. Oceanographic and noble gas tracers

In general, the profiles of temperature (T) and salinity (S) for stations A (Orca) and B show similar patterns and represent the typical hydrographic characteristics of the area (Fig. 3A) (*e.g.,* Gordon and Nowlin, 1978; Wilson *et al.*, 1999; García *et al.*, 2002; Sangrà *et al.*, 2011). Both stations show a flow of warmer and saltier water between 200 and 450 m below the sea level (b.s.l.) that corresponds to the Modified Circumpolar Deep Water (CDW), which centres around σ_θ=27.75 (Fig. 3B). This flow has its maximum in T and S at ~400 m b.s.l., which coincides with the oxygen minimum, and it is more marked at the Orca Station profiles, indicating weaker influence of the Southern Ocean waters near the slope of the King George Island. The profiles also show a colder, fresher and more oxygenated layer at ~200 m depth. Deeper waters for Station B show uniform salinity below 1,000 m b.s.l., and they are colder and fresher relative to water at the same depth outside the strait in the Weddell Sea (Wilson *et al.*, 1999), reflecting the local formation of bottom water in this basin (Gordon and Nowlin, 1978; Wilson *et al.*, 1999).

Below the seamount summit level (~600 m) oceanographic variables for A Station (Orca) are uniform with depth, which is considered normal inside a seamount (Figs. 3A and B). In this station, close to the bottom, the water temperature is under -1 °C, that is very cold compared with mid-ocean ridge average temperature at ~2,000 m depth (~2 °C) (Baker *et al.*, 2002). There is a difference of 0.25 °C comparing the temperatures between stations A and B at

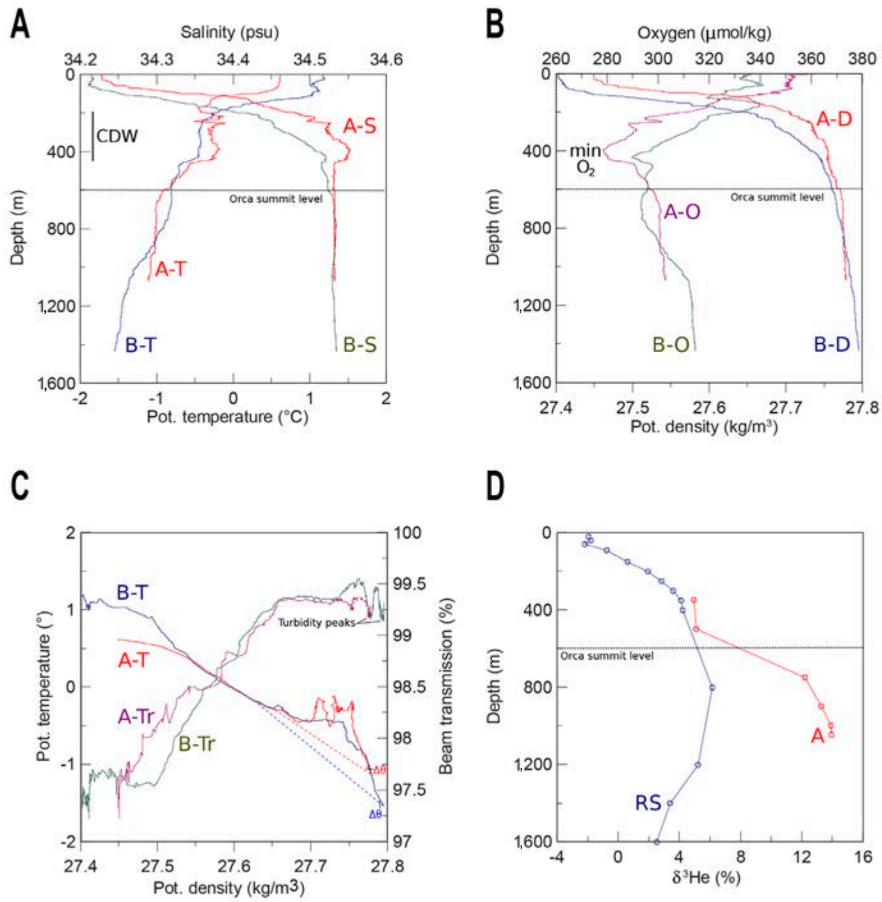

FIG. 3. Oceanographic profiles for Stations A (Orca) and B. **A**. Salinity (S) and Potential Temperature (T) profiles *versus* depth. The vertical position of the Modified Circumpolar Deep Water (CDW) is indicated; **B**. Oxygen (O) and Potential Density (D) *versus* depth; **C**. Potential Temperature (T) and Beam Transmission (Tr) *versus* Potential Density. Also is included temperature anomaly (Δθ) determination (curve fits: segmented lines); **D**. δ^3He *versus* depth. Reference Station for helium is indicated (RS).

1,033 m b.s.l. Both stations show a significant temperature increase at mid-depths due to CDW influence (right side of the D-T graphs, Fig. 3C) which coincides with a decrease in turbidity (transmission) (right side of the D-Tr graphs, Fig. 3C). To avoid confusion due to the influence of the warm CDW waters, the temperature anomaly (Δθ) was determined on the part of the curves that corresponds to the vicinity of the seabed, matching with turbidity picks (Fig. 3C). In this way, inside the crater of the volcano the temperature anomaly is ~0.08 °C, and for station B close to the bottom is ~0.03 °C. The Orca station value is higher than temperature anomalies observed by Klinkhammer *et al*. (1995) along the Hook and Middle Ridges, and Three Sisters (0.010-0.025 °C), meanwhile the anomaly for station B is similar. On the other hand, the determined values are lower than typical temperature anomalies for the East Pacific Rise (0.260 °C) (Baker *et al.*, 2002).

The transmission is taken as a relative value, and there is a higher peak inside the Orca crater (Fig. 3C). This is consistent with the idea that there is a hydrothermal venting present, demonstrating that the turbid environment perhaps is due to the dispersion and particle re-suspension due to hydrothermal activity. There is also a higher peak value close to the bottom of station B. Maybe there is a hydrothermal influence outside the volcano, or there is a particle flow due to reasons above explained.

The high values of δ^3He data also confirm the Orca hydrothermal activity (Fig. 3D). δ^3He is mainly the ratio between ^3He and ^4He (normalized to atmospheric values) in %. In equilibrium with the atmosphere and typical polar surface temperatures δ^3He is in the order of -1.8%. Only sources for ^{3}He (and thus for enhanced δ^3He ratios) are primordial helium (typically released by hydrothermal activities) or tritium decay (50 years after hydrogen bomb test negligible) (Huhn *et al.*, 2008). The Orca station shows below 500 m b.s.l. a value of δ^3He >10% (Fig. 3D). Previous measurements in the Bransfield Strait show a δ^3He maximum of ~7%, interpreted as a local injection of a ^{3}He-rich helium component into deep waters of the strait from the rift (Schlosser *et al.*, 1988). Tritiogenic ^{3}He and excess ^{3}He from mixing with CDW were excluded as possible sources. If it would be a CDW admixture, it could only come from the Weddell Sea (Transitional Zonal Water with Weddell Sea influence, TWW) via the northeastern entry of the Bransfield Strait. In the Weddell Sea the highest δ^3He values are in the order of <9.7%, and there the δ^3He maximum layer is on ~300-400 m depth. On a short section at the north eastern entry of Bransfield Strait the δ^3He values are even far smaller (<2%) (Huhn *et al.*, 2008), and the entry via Joinville Island and the Antarctic Peninsula is too shallow being the values mentioned lower than 9.7% inside the Weddell Basin. On the other hand, the TWW goes to the SW close to the Antarctic Peninsula and limited to the North by the Peninsula Front (PF) (Sangrà *et al.*, 2011), so there is not influence in shallow and mid waters. The influence of the TWW in depth reaches the Bransfield Front (BF) under the CDW, but also the influence should be minimum due to the predominance of local cold deep water formation on the Bransfield Basins (Wilson *et al.*, 1999).

Therefore, the maximum value of δ^3He inside the crater of Orca seamount (δ^3He=13.94%) is high and it is interpreted due to a source from the crater bottom. The position of the helium Reference Station is shown in figure 2A ("RS" point), whose data were obtained during the cruise ANT-XIII/4 on board R/V "Polarstern" in 1996, using same methodology of sampling and analysis. The RS station δ^3He profile shows similar structure and values compared with those of Schlosser *et al.* (1988), and also with Orca values up to ~500 m depth (Fig. 3D). The characteristics of the general vertical distribution of helium does not seem to have changed much over time in the Bransfield area, so, the hydrothermal influence at depths greater than 1,000 m coming from the rift is also maintained. On the other hand, in 2013 the helium measurements were repeated inside the Orca seamount (ANT-XXIX/3 cruise on board R/V "Polarstern", Dorschel *et al.*, 2015), and the values inside the volcano were very high (>27%), confirming the hydrothermal activity.

3.2. Microbiological tracers

The morphology and Gram stain of microorganisms isolated for samples taken in stations A and B are shown in table 2, meanwhile the characteristics of each type of microorganisms are shown in table 3. The information regarding the type of media used for culturing, oxygen, temperature, pH and

TABLE 2. MORPHOLOGY AND GRAM STAIN OF MICROORGANISMS ISOLATED AND TEMPERATURE OF GROWING.

Station	Bottle	Morphology and Gram stain	Temperature (°C)
A	nk1	Cocci, Gram negative	70
A	nk1	Cocci, Gram negative	80
A	nk1	Cocci, Gram negative	90
A	nk2	Cocci and Rods, Gram negative	70
A	nk3	Cocci and Rods, Gram negative Rods, Gram positive	70
B	nk1	Rods, Gram positive	70
B	nk2	Cocci, Gram positive	70
B	nk3	Cocci and Rods, Gram negative Rods, Gram positive	70

TABLE 3. CULTURING OF THERMOPHILIC AND HYPTHERMOPHILIC MICROORGANISMS.

Station	Bottle	MMM medium	TL medium	MMM Anaerobic medium	Temperature (°C)	pH	Domain
A	nk1	+	+	+++	70	5.8	Archaea/Bacteria
A	nk2	+	+	+++	70	5.8	Archaea/Bacteria
A	nk3	+	+	++	70	5.8	Not identified
A	nk1	+	+	+++	80	6.5	Archaea/Bacteria
A	nk2	+	+	+++	80	6.5	Not identified
A	nk3	+	+	+++	80	6.5	Archaea
B	nk1	+	+	++	70	5.8	Not identified
B	nk2	+	+	+	70	5.8	Not identified
B	nk3	+	+	+	70	5.8	Bacteria
B	nk1	+	+	+	80	6.5	Not identified
B	nk2	+	+	+	80	6.5	Bacteria
B	nk3	+	+	+	80	6.5	Not identified

Positive signs indicate positive growing of microorganisms at the different media. **MMM:** master medium that emulates seabed environment with trace elements; **TL:** specific medium to grow Thermococcales).

general phylogeny for the different cultures is also indicated in the tables 2 and 3. Thermophilic and hyperthermophilic microorganisms were found in both stations. Inside the crater of the Orca volcano the amount of microorganisms found was significantly higher for all levels from where samples were taken, specifically when anaerobic medium was used (Table 3). Fluorescent microscopy images (Fig. 4) clearly show the variations in the relative amounts of microorganisms and their morphology present in different samples. At station A the majority of microorganisms present in the sample are Gram negative with cocci and rod morphology (Fig. 5). Most of the microorganisms present in the sample grew between 70° and 80 ºC, but some of them were able to grow at 90 °C under identical experimental conditions (sample volume and time of growth). Information regarding use of substrates for cultivation, oxygen requirements, temperature, pH, NaCl requirements in conjunction with DNA analysis revealed that the microorganisms present in the samples belong to both Archaea and Bacteria Domains. Even more, hyperthermophiles and thermophiles (Table 3) as well as halophilic microorganisms could be identified in the samples analyzed. Most of these microbial strains grew in a wide range of temperatures (65-90 °C). Optimum growth temperatures are between 80 ºC and 90 °C and doubling times are about an hour. None of the strains grew at mesophilic temperatures or below.

The finding of high-temperature microorganisms at the bottom of station B, suggests a hydrothermal influence from the Orca area and its surroundings. Other possibility could be that some high temperature microorganisms were transported from the Central Rift or other active volcanic area, such as Deception Island (Somoza *et al*., 2004) (Fig. 6) or even transported by sediment re-suspension. In the case of Deception Island the transport of themophilic microoganisms could be helped by the Bransfield Current (BC) that goes to the NE close to South Shetland Islands coast (Fig. 6).

It is known that microorganisms thrive particularly under the surface of active oceanic ridges, where the circulation derived from volcanic activity and chemical energy sources have the ability to maintain a robust ecosystem, which could be potentially self-contained (Baross and Deming, 1983). The presence of microorganisms, thermophiles and hyperthermophiles, in an area which generally maintains low temperature flows, indicates a stable warm, anoxic habitat below the seafloor, where these microorganisms can multiply and proliferate. Although high temperature microorganisms are not the only components of subsurface microbial community, the presence of thermophiles and hyperthermophiles provides a biological indicator of submarine groundwater

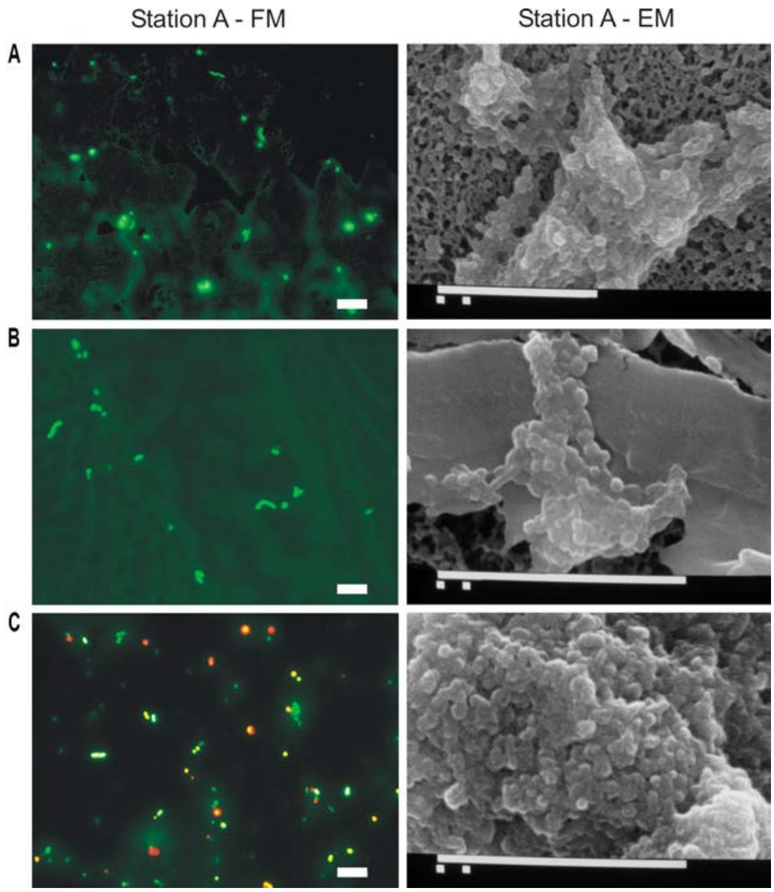

FIG. 4. Fluorescent (left side) and electronic microscopy (right side) of thermophilic and hyperthermophilic microorganisms for bottle samples of Station A (Orca seamount). Scale bars indicate 10 µm. **A.** nk 3: at 998 m depth; **B.** nk 2: at 1,033 m depth; **C.** nk 1: at 1,082 m depth.

conditions, as hyperthermophiles do not proliferate in cold water grounds (Summit and Baross, 1998).

4. Conclusions

The value of the temperature anomaly found (~0.08 °C), the distribution pattern and values of δ^3He (>13.9%), the presence of a turbidity (transmission) peak correlating with the other measurements and the finding of thermophilic and hyperthermophilic microorganisms inside the crater of the Orca Seamount growing in cultures at temperatures >70 °C and in a medium that emulated mid-ocean ridge environments, can confirm the existence of an active hydrothermal flux from the bottom of the seamount crater. Although the water inside the volcanic edifice is very cold (<-1°C), all measurements suggest a hydrothermal migration from the bottom. We suggest that this flux could result from past eruptions, in a similar way to submarine hydrothermal venting in Deception Island, or triggered by recent magmatic activity that facilitated the upward water flow through fractures or faults. Additionally, there is a hydrothermal influence outside of the volcano, perhaps related to subsidiary structures of the Orca seamount, or due to the dispersal of hydrothermal plumes from other volcanic structures of the Bransfield Rift and/or active volcanoes of the region (*e.g.,* Deception Island). The observations, inside and outside of Orca seamount, raise questions about the dispersal of microorganisms and their resistance in this cold environment. The biological results represent the first observations of thermophilic and hyperthermophilic microorganisms in deep cold Antarctic waters.

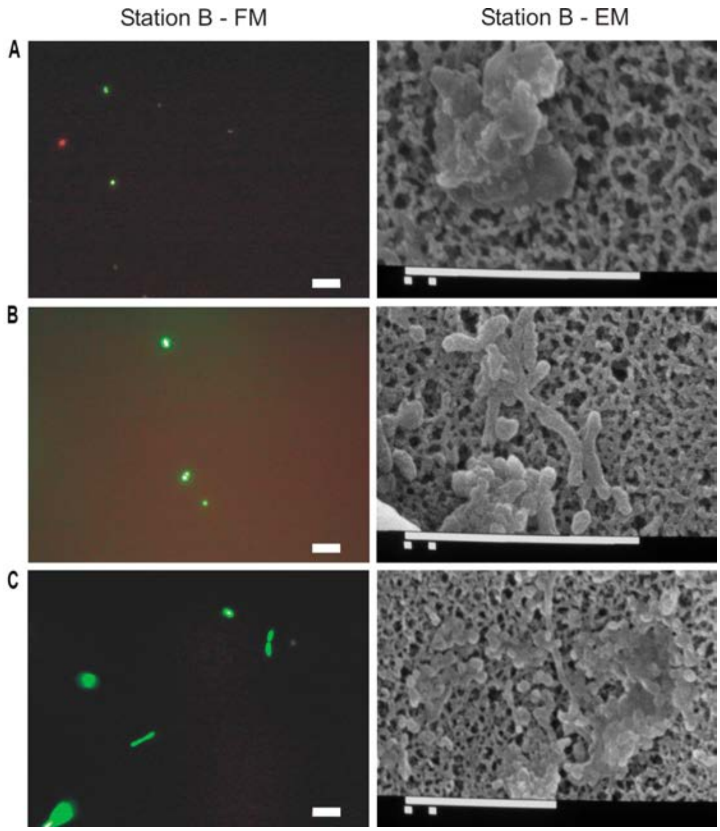

FIG. 5. Fluorescent (left side) and electronic microscopy (right side) of thermophilic and hyperthermophilic microorganisms for bottle samples of Station B. Scale bars indicate 10 µm. **A.** nk 3: at 1,036 m depth; **B.** nk 2: at 1,186 m depth; **C.** nk 1: at 1,036 m depth.

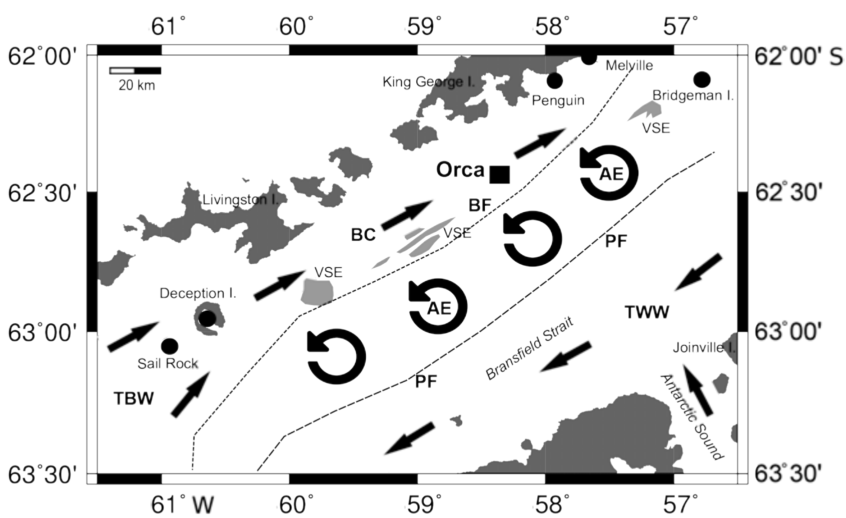

FIG. 6. Schematics of the main components of the Bransfield Current System based on Sangrà *et al*. (2011), and main submarine (VSE) and subaerial volcanic edifices (black circles) (Kraus *et al*., 2013). **PF:** Peninsula Front; **AE:** anticyclonic eddy; **BF/BC:** Bransfield Front/Current; **TWW:** Transitional Zonal Water with Weddell Sea influence; **TBW:** Transitional Zonal Water with Bellingshausen Sea influence.

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