

Short Communication

Unusual *Vermamoeba Vermiformis* Strain Isolated from Snow in Mount Teide, Tenerife, Canary Islands, Spain

María Reyes-Batlle¹, Maryam Niyiyati², Carmen M. Martín-Navarro¹, Atteneri López-Arencibia¹, Basilio Valladares¹, Enrique Martínez-Carretero¹, José E. Piñero¹, Jacob Lorenzo-Morales^{1*}

¹ University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Avda. Astrofísico Fco. Sánchez, S/N, 38203 La Laguna, Tenerife, Canary Islands, Spain

² Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background: Free-living amoebae (FLA) are protozoa that are widely distributed in the environment mainly in water and soil related habitats. Thermophilic amoebae are among the most common FLA in water bodies, being *Vermamoeba vermiformis* one of the most common species reported worldwide from these sources. Interestingly, *V. vermiformis* has often been reported to survive at high temperatures and osmotic pressure worldwide.

Materials and Methods: In this study, snow samples were collected from Mount Teide, Tenerife, Canary Islands during the winter season of 2014. The samples were culture on non nutrient agar plates and checked daily for the presence of FLA. After a week, *V. vermiformis* amoebae were observed in the plates incubated at room temperature and 37°C.

Results: Molecular characterization was carried out by amplifying the 18S rDNA gene and DNA sequencing, confirmed that the isolated strain belonged to *Vermamoeba vermiformis* species.

Conclusion: To the best of our knowledge, this is the first report of *Vermamoeba vermiformis* isolation from such an unusual habitat (low temperatures and high altitude) and the first report of these species in the Canary islands.

Keywords: *Vermamoeba vermiformis*, snow, Tenerife, Canary Islands, Spain

***Corresponding Author:** Jacob Lorenzo-Morales, University Institute of Tropical Diseases and Public Health of the Canary Islands, University of La Laguna, Avda. Astrofísico Fco. Sánchez, S/N, 38203 La Laguna, Tenerife, Canary Islands, Spain. Phone:(+34) 922316502 ext 6111, Fax: (+34) 922318514. Email: jmlorenz@ull.edu.es

Please cite this article as: Reyes-Batlle M, Niyiyati M, M. Martín-Navarro C, López-Arencibia A, Valladares B, Martínez-Carretero E, et al. Unusual *Vermamoeba Vermiformis* Strain Isolated from Snow in Mount Teide, Tenerife, Canary Islands, Spain. Novel Biomed. 2015;3(4):189-92.

Introduction

Free-living amoebae are ubiquitous protozoa commonly found in water and soil environments. Among them, *Acanthamoeba* and *Vermamoeba* are the most common genera presented in water bodies¹. Amoebae belonging to various genera, including *Acanthamoeba*, *Vahlkampfia*, *Naegleria* and *Vermamoeba*, can cause pathologies such as keratitis or meningoencephalitis². Amoebae have also been

reported to be associated with bacterial or viral Pathogens such as *Legionella* and mimivirus³⁻⁵. The historical and best-known of those amoeba associated pathogen is *Legionella pneumophila*. This bacterium uses the amoeba as a Trojan horse to survive, multiply and acquire high virulence factors, and its ecological association with amoebae also protects it from adverse environmental conditions^{2,6}.

Vermamoeba vermiformis has been reported from many locations worldwide and has been described to

be a thermotolerant amoeba^{6,7}. The locations that *V. vermiformis* strains have been isolated from many habitats but of importance to be highlighted is geothermal springs in countries such as Iran or Mexico among others^{8,9}. Interestingly, in the study from Bonilla-Lemus et al. (2014), samples were collected from spring located at 2000m above sea level and at temperatures between 5°C to 17°C.

In this study, snow samples from Mount Teide National park were collected and checked for the presence of FLA in such an extreme habitat with temperatures below 0°C and altitude ranging from 2800 to 3718 m (top of the volcano peak) above sea level. Isolated amoebae were identified using morphological and molecular tools.

Methods

Sample collection and location

Snow samples were collected (in triplicate) 5cm below surface for avoiding contamination in the National Park of Mount Teide in Tenerife, Canary Islands, Spain (28° 16' 23" N, 16° 38' 22" W) using sterile glass bottles during the winter season of 2014. Samples were kept at 4°C until processing in the laboratory. Mount Teide is a volcano on Tenerife which is an island in the Canary Islands, Spain. This volcano is the highest point in Spain (3718m) and the highest point above sea level (from 2700m to 3718 m) in the islands of the Atlantic. The volcano and its surroundings comprise Teide National Park, which has an area of 18,900 hectares and was named a World Heritage Site by UNESCO in 2007 (<http://whc.unesco.org/en/list/1258>).

Culture and identification of the amoebae

Samples were cultured directly on 2% non-nutrient agar (NNA) plates at 22°C and 37°C and were

monitored daily for the presence of free-living amoebae as previously described³ with a layer of *Escherichia coli* suspension that had been heat inactivated (2h at 60°C). Plates that were positive for amoebic growth were subcultured until was reached a clean plate.

DNA isolation and molecular identification by PCR

DNA from cultures identified as positive for FLA by microscopy was extracted by placing 1-2 ml of amoebae cultures directly into the Maxwell® 16 Tissue DNA Purification Kit sample cartridge (Promega, Madrid, Spain). Amoebic genomic DNA was purified using the Maxwell® 16 Instrument as described in the Maxwell® 16 DNA Purification Kits Technical Manual #TM284 (Promega, Madrid, Spain). DNA yield and purity were determined using the NanoDrop® 1000 spectrophotometer (Fisher Scientific, Madrid, Spain)¹⁰.

After DNA extraction, PCR amplification of FLA 18S rDNA was performed using a universal primer pair as previously described^{11,12}. The PCR products were purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using a MEGABACE 1000 automatic sequencer (Healthcare Biosciences, Barcelona, Spain) in the University of La Laguna Sequencing Services (Servicio de Secuenciación SEGAI, University of La Laguna). Sequences were aligned using Mega 5.0 software program¹¹. Species identification was based on sequence homology analysis by comparison to the available *Vermamoeba vermiformis* DNA sequences in Genbank database.

Results and Discussion

Upon observation of the snow samples cultured in NNA plates microscopically, the presence of amoebic

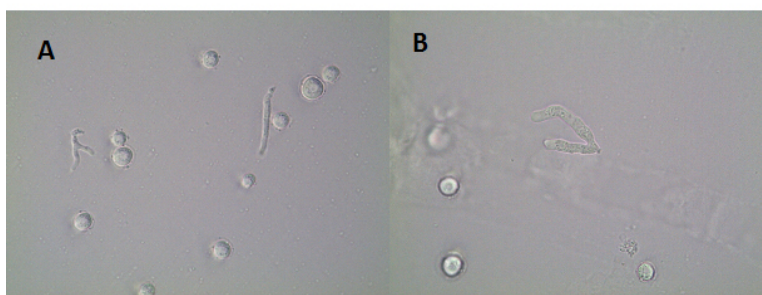


Figure 1. *Vermamoeba vermiformis* trophozoites and cysts at 20X (A) and 40X (B) of magnification

trophozoites was revealed. After that, pieces of agar from the initial plates were cultured in 2% NNA plates for the isolation of the amoebae. The morphological analysis revealed that the isolated amoebae belonged to *Vermamoeba vermiformis* species by morphology of the trophozoites and cysts (Figure 1A and 1B) (Page, 1988).

After morphological identification, DNA was extracted from the amoebic cultures and 18s rDNA FLA PCR was carried out to verify the microscopy observations. PCRs were also positive for these cultures and thus were sequenced. BLAST analysis revealed homology of more than 98% when compared to the available *Vermamoeba vermiformis* sequences in Genbank.

Interestingly, the isolated amoebae were able to grow at temperatures up to 37°C in the laboratory even when it was initially isolated from snow and at high altitude (more than 2700m, see sample location section). It is important to mention that our laboratory is located in the city of San Cristóbal de La Laguna (28° 29' 14" N - 16° 18' 52" W) also on Tenerife island which is around 549 m above sea level and thus the tolerance of the isolated *V. vermiformis* strain to altitude and temperatures conditions was proven to be wide. However, to the best of our knowledge this is the first report of this species surviving at temperatures below 0°C and altitudes above 2700 m above sea level. Most of the reported isolations of *V. vermiformis* have described this species to be tolerant to high temperatures and osmotic pressure conditions. Only in the study from Bonilla-Lemus et al. (2014), altitude was taken into account since the collected samples in this study were located at 2000 m above sea level⁹.

To the best of our knowledge, this is the first report of a species of *Vermamoeba vermiformis* strain which is tolerant to temperatures below 0°C and altitudes of more than 2700m above sea level. Moreover, this is the first report of *V. vermiformis* in the Canary Islands.

Acknowledgments

This work was supported by the grants RICET (project no. RD12/0018/0012 of the programme of Redes Temáticas de Investigación Cooperativa, FIS),

Spanish Ministry of Health, Madrid, Spain, the Project PI13/00490 “Protozoosis Emergentes por Amebas de Vida Libre: Aislamiento, Caracterización, Nuevas Aproximaciones Terapéuticas y Traslación Clínica de los Resultados” from the Instituto de Salud Carlos III, and Project ref. AGUA3 “Amebas de Vida Libre como Marcadores de Calidad del Agua” from CajaCanarias Fundación. ALA and MRB were funded by Becas de Investigación Obra Social La Caixa-Fundación Cajacanarias para Postgraduados 2014”. JLM was supported by the Ramón y Cajal Subprogramme from the Spanish Ministry of Economy and Competitiveness RYC-2011-08863.

Conflict of Interest

The authors have no financial interest in the products discussed in this article.

References

1. Fouque E, Yefimova M, Trouilhé MC, Quillard N, Fernandez B, Rodier MH, et al. Morphological Study of the Encystment and Excystment of *Vermamoeba vermiformis* Revealed Original Traits. *Journal of Eukaryotic Microbiology*. 2015;62(3):327-37.
2. Pagnier I, Valles C, Raoult D, La Scola B. Isolation of *Vermamoeba vermiformis* and associated bacteria in hospital water. *Microbial pathogenesis*. 2015;80:14-20.
3. Lorenzo-Morales J, Ortega-Rivas A, Foronda P, Martínez E, Valladares B. Isolation and identification of pathogenic *Acanthamoeba* strains in Tenerife, Canary Islands, Spain from water sources. *Parasitology research*. 2005;95(4):273-7.
4. Lorenzo-Morales J, Coronado-Álvarez N, Martínez-Carretero E, Maciver SK, Valladares B. Detection of four adenovirus serotypes within water-isolated strains of *Acanthamoeba* in the Canary Islands, Spain. *The American journal of tropical medicine and hygiene*. 2007;77(4):753-6.
5. Richards AM, Von Dwingelo JE, Price CT, Abu Kwaik Y. Cellular microbiology and molecular ecology of *Legionella*-amoeba interaction. *Virulence*. 2013;4(4):307-14.
6. Thomas V, Loret JF, Jousset M, Greub G. Biodiversity of amoebae and amoebae-resisting bacteria in a drinking water treatment plant. *Environmental microbiology*. 2008;10(10):2728-45.
7. Thomas V, McDonnell G, Denyer SP, Maillard J-Y. Free-living amoebae and their intracellular pathogenic microorganisms: risks for water quality. *FEMS microbiology reviews*. 2010;34(3):231-59.
8. Solgi R, Niyayati M, Haghghi A, Mojarad EN. Occurrence of thermotolerant *Hartmannella vermiformis* and *Naegleria* spp. in hot springs of Ardebil Province, Northwest Iran. *Iranian journal of parasitology*. 2012;7(2):47.
9. Bonilla-Lemus P, Villegas ASC, Jiménez JC, Vázquez AL. Occurrence of free-living amoebae in streams of the Mexico Basin. *Experimental parasitology*. 2014;145:S28-S33.
10. Reyes-Batlle M, Todd CD, Martín-Navarro CM, López-

Arencibia A, Cabello-Vilchez AM, González AC, et al. Isolation and characterization of Acanthamoeba strains from soil samples in Gran Canaria, Canary Islands, Spain. Parasitology research. 2014;113(4):1383-8.

11. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum

parsimony methods. Molecular biology and evolution. 2011;28(10):2731-9.

12. Smirnov AV, Chao E, Nasonova ES, Cavalier-Smith T. A revised classification of naked lobose amoebae (Amoebozoa: Lobosa). Protist. 2011;162(4):545-70.