

## Case Report

## First Report of *Vermamoeba vermiformis* in the Island of El Hierro, Canary Islands, Spain

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### Abstract

**Background:** Free-living amoebae (FLA) are group of protozoa distributed worldwide in many habitats mainly water and soil related sources. Some members of FLA are able to act as opportunistic pathogens and are environmental carriers of other pathogenic agents such as bacteria and viruses. *Vermamoeba vermiformis* is a highly abundant FLA species in water bodies and has recently gained environmental importance as it acts as a vehicle of many pathogenic bacteria such as *Legionella pneumophila*.

**Cases Report:** In this study, water samples were collected from the island of El Hierro, Canary Islands, Spain during 2015. El Hierro island was designated by UNESCO as a biosphere reserve and it is currently the less populated of the Canary Islands. The water samples were culture on 2 % Non-Nutrient Agar (NNA) plates covered with a thin layer of heat killed *E. coli* 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. Molecular characterization was carried out by amplifying the 18S rDNA gene and DNA sequencing, confirming 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 in the island of El Hierro and the second report of this species in the Canary Islands.

**Keywords:** *Vermamoeba vermiformis*, water, Canary Islands, Spain

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### Introduction

Free-Living Amoebae (FLA) are of medical importance since they could act as opportunistic

pathogenic agents, but also as vehicles for other pathogens such as bacteria and viruses<sup>1,2</sup>. Regarding the abundance in water bodies, FLA of *Acanthamoeba* and *Vermamoeba* genus are among the most common

ones<sup>3-6</sup>.

In the case of *Vermamoeba vermiformis* species, it is important to mention that it has been reported in many areas worldwide and has recently gained medical relevance since it is a vehicle of highly important pathogenic agents such as *Legionella pneumophila*<sup>7,8</sup>. Furthermore, this amoebic species present a wide distribution and has been described from many habitats including geothermal springs and even snow<sup>9-11</sup>.

In this study, water samples from the island of El Hierro were collected and checked for the presence of *V. vermiformis*. Isolated amoebic strain was identified using morphological and PCR/sequencing tools. To the best of our knowledge, this is the first report of *V. vermiformis* species in the island of El Hierro and the second report in the Canary Islands.

## Case Report

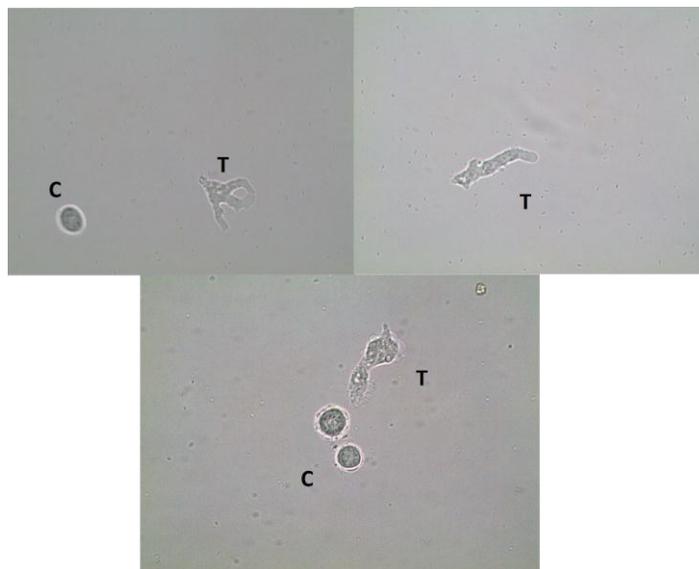
**Sample collection and location:** Water samples were collected (triplicate) in the island of El Hierro, Canary Islands, Spain (27° 45' N 18° 00' W) using sterile glass bottles during the summer season of 2015. The samples were kept at 4°C until further processing in the laboratory. The island of El Hierro, nicknamed *Isla del Meridiano* (the Meridian Island), is the smallest and farthest south and west of the Canary Islands in the Atlantic Ocean off the coast of

Africa, with a population of around 10.000 inhabitants. Moreover, the island was designated by UNESCO as a Biosphere Reserve in 2000.

**Culture and identification of the amoebae:** Water samples were filtered using a vacuum manifold system and 0.45 µm nitrocellulose filters (Pall, Madrid, Spain). Filters were then cultured inverted onto 2% non-nutrient agar (NNA) plates with a layer of heat killed *E. coli* at room temperature and 37 °C and were monitored daily for the presence of FLA as previously described<sup>11,12</sup>. Plates that were suspicious for amoebae were subcultured until a clean plate was obtained.

**DNA isolation and molecular identification by PCR:** DNA from cultures identified as positive for FLA by microscopy was extracted as previously described<sup>11,12</sup> 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).

After that, PCR amplification of the FLA 18S rDNA gene was carried out using a universal primer pair FLA-F and FLA-R<sup>13</sup>. The resulting PCR products were purified using the Qiaquick PCR purification kit



**Figure 1.** *Vermamoeba vermiformis* trophozoites (T) and cysts (C) at 100X of magnification isolated in the island of El Hierro.

(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 program<sup>14</sup>. Species identification was based on sequence homology analysis by comparison to the available *Vermamoeba vermiformis* DNA sequences in Genbank database. One of the cultured samples in NNA plates was positive for FLA growth under the inverted microscope. After that, pieces of agar from the initial positive plate were subcultured in 2% NNA plates for the isolation of the amoebae until a clean plate was obtained as it was described in the material and methods section. Morphological characterization of the strain using the currently available identification key from Page<sup>15</sup>, yielded the identification of this amoebic strain as a member of *V. vermiformis* species (Figure 1A and 1B). Furthermore, molecular characterization of the strain after PCR/sequencing of the 18S rDNA gene confirmed the strain to be a member of *V. vermiformis* species (99% homology when compared to the available *Vermamoeba vermiformis* sequences in Genbank).

## Discussion

This is the first report of FLA species in the island of El Hierro from water samples. The positive sample was collected in the area of La Dehesa in Frontera municipality that is a widely visited area with locals and tourists all over the year. The presence of this species in water bodies in the island should raise awareness to health authorities. Moreover and to the best of our knowledge, this is the first report of *Vermamoeba vermiformis* species in the island of El Hierro and the second report of this amoebic species in the Canary Islands.

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