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Reservoirs of human pathogens: amoeba-associated microorganisms in the environment

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Microorganisms, that evolve to acquire resistance to environmental amoeba, are likely to become human pathogens due to the physiological similarities of free-living amoebae to human macrophages. In this sense free-living amoebae can be regarded as nurseries of pathogenic microorganisms. Due to the widespread distribution of amoebae in the environment and their resistance to current disinfection procedures, they may constitute important reservoirs of pathogenic microorganisms [1, 2]. Striking examples are *Legionella pneumophila* and several mycobacteria including *Mycobacterium tuberculosis*. This work represents the first attempt to detect and characterize amoebae and their associated microorganisms in Portugal.

Detection, isolation and *in vitro* culture of amoebae from several environmental sources, including lagoon and estuarine water and sediments, were performed according to previously developed methods. Identification of the isolated amoebae was done by optical microscopy based on morphological criteria and by DNA sequence analysis of PCR products amplified with one of the following two sets of primers: EUK [3] and ITS [4]. Transmission electron microscopy (TEM) studies and PCR/sequencing approaches were used to detect and identify amoeba-associated microorganisms (AAMs). Bacterial 16S region was amplified with the primers 616V/630R [5]. TEM studies were performed according to standard procedures. In short, samples were fixed sequentially in glutaraldehyde, osmium tetroxide and uranyl acetate, dehydrated in ethanol and embedded in Epon-Araldite. Thin sections contrasted with uranyl acetate and lead citrate were observed with a JEOL 100-SX electron microscope.

Several species of amoeba (*Acanthamoeba lenticulata*, *A. polyphaga*, *A. rhyzodes*, *Platyamoeba oblongata*, *Saccamoeba limax*, *Vannella simplex*, *Vannella* sp. and other unidentified amoebae) were found and AAMs were detected both by TEM and PCR/sequencing. The bacteria species *Variovorax paradoxus*, previously found in association with *Saccamoeba* and *Arcella* [6, 7], were isolated and identified by the PCR/sequencing approach, which also allowed the detection of an unidentified species with about 85% identity with *Marivirga tractuosa*, a species not yet associated with amoebae. These results point to the existence of AAMs in the environments subjected to this study and the need to evaluate their pathogenic potential. This is an important issue particularly in environments related to human recreational, nutritional and public health activities.

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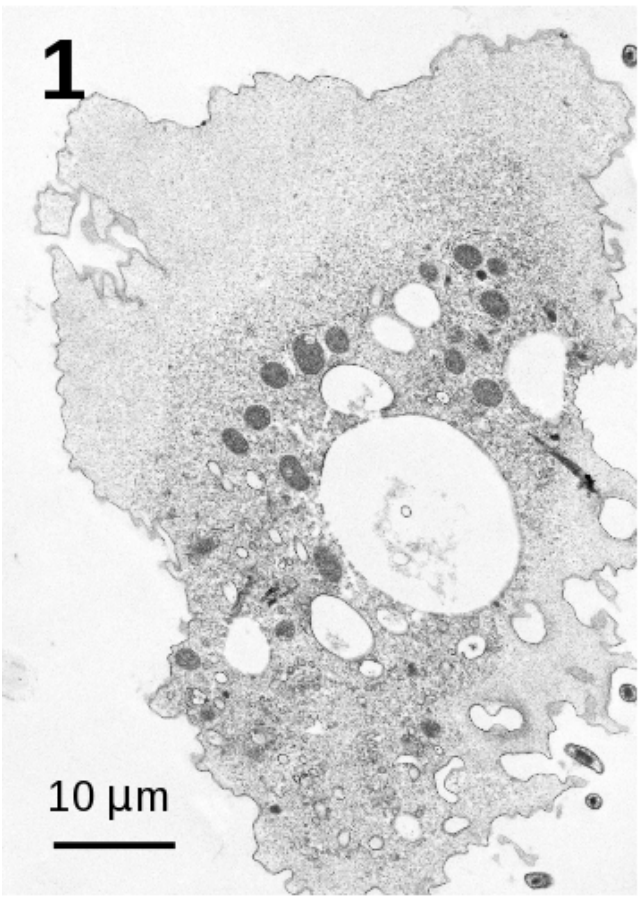

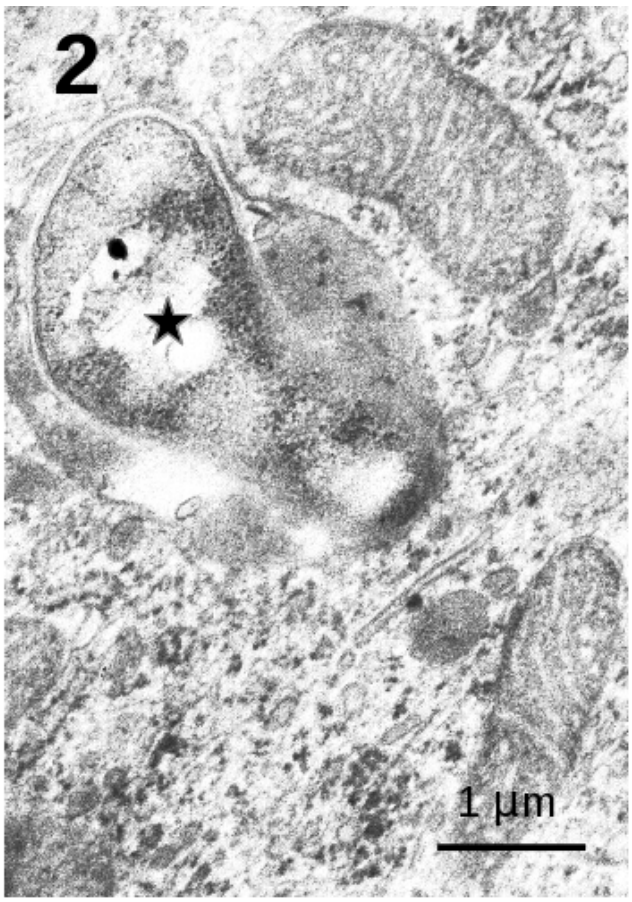

Fig. 1. *Vannella* sp. from a lagoon (Granjal) identified by PCR.
Fig. 2. *Platyamoeba oblongata* from the Tagus estuary. Morphologically intact bacteria (*) were found inside a lysosome-like structure.

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