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From Environmental Sequences to Morphology: Observation and Characterisation of a Paulinellid Testate Amoeba (*Micropyxidiella edaphonis gen. nov. sp. nov.* Euglyphida, Paulinellidae) from Soil using Fluorescent in situ Hybridization

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High microbial diversity is revealed by environmental DNA surveys. However, nothing is known about the morphology and function of these potentially new organisms. In the course of an environmental soil diversity study, we found for the first time environmental sequences that reveal the presence of Paulinellidae (a mostly marine and marginally freshwater family of euglyphid testate amoebae) in samples of forest litter from different geographic origins. The new sequences form a basal, robust clade in the family. We used fluorescent in situ hybridization (FISH) to detect the organisms from which these sequences derived. We isolated the cells and documented them with light and scanning electron microscopy. Based on these observations, we described these organisms as *Micropyxidiella edaphonis* gen. nov. sp. nov. The organisms were very small testate amoebae (generally less than 10 μm) with an irregular proteinaceous test. This suggests an unknown diversity in testate amoebae, and calls for extending this type of investigations to other protist groups which are known only as environmental DNA sequences.

Key words: Cercozoa; molecular diversity; soil; novel clades; evolutionary transitions.

Introduction

Recent culture and isolation independent environmental DNA surveys based on sequencing the small subunit ribosomal RNA gene of eukaryotes

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have revealed several new clades whose existence was unsuspected (Lara et al. 2010; Lopez-Garcia et al. 2001; Massana et al. 2004), bringing a more complete picture of the overall diversity of domain Eukaryota (Epstein and Lopez-Garcia 2008). However, the existence of these "orphan" sequences remains of limited interest if no further information is obtained on the morphology, lifestyle and traits of the organisms. The correspondence between environmental sequences and protist morphotypes is therefore an important goal in modern protistology (Pawlowski 2013). One of the most common approaches to link environmental sequences and organisms consists in designing a specific FISH probe to be hybridized selectively to the cells from which the sequence of interest derived. Once the organism is located, this method is generally coupled to other approaches to document cell morphology such as light or scanning electron microscopy. In this way, cells of the uncultured environmental MAST-12 were documented from a sub-oxic enrichment culture. These cells were demonstrated to have and showed typical heterokont morphology and to be bacterivorous (Kolodziej and Stoeck 2007). Also, novel deep sea Acantharea and their different life-stages have been detected and documented (Gilg et al. 2010). This approach opens therefore the way for a better knowledge of uncultured microbial eukaryotic forms.

Paulinellidae are a family of filose testate amoebae that belong to the larger clade Euglyphida (Cercozoa, Rhizaria). To date, it comprises only two genera, *Paulinella* and the monospecific *Ovulinata* (Adl et al. 2012; Howe et al. 2011). While the first genus includes species harbouring self-secreted silica scales on their tests, thus presenting a typical Euglyphida morphology, *Ovulinata* secretes a hyaline proteinaceous test (Anderson et al. 1996, 1997). To date, Paulinellidae have been found mostly in marine environments (Hannah et al. 1996; Nicholls 2009; Vørs 1993), and marginally in freshwater (Hasler et al. 2008; Pankow 1982), but never in soils. *Paulinella chromatophora* is by far the best studied species because of its symbiotic association with a cyanobacterium, considered as the only reported case of recent primary endosymbiosis (Marin et al. 2005). In the course of an environmental survey on the diversity of Euglyphida in forest litters, we obtained unexpectedly sequences belonging to Paulinellidae in samples originating from environments as different as Switzerland, Southern Morocco and West Coast Canada. Because testate amoebae are large and conspicuous protists in comparison to e.g. nanoflagellates and naked amoebae, the possibility that these organisms remained unnoticed seemed unlikely. Therefore, and in order to document better the diversity of forms and features of this group, we aimed at revealing these organisms at their features using fluorescent in situ hybridization (FISH).

Results

Clone sequences related to paulinellids branched robustly within this family, which comprised the two described species (*Paulinella chromatophora* and *Ovulinata parva*), as well as several environmental clones, mostly from marine sediment except MPE2_30 (AB695524), which was retrieved from submerged freshwater mosses from Antarctica. The three soil sequences branched robustly together, forming a relatively deep clade within family Paulinellidae (Fig. 2). These sequences have been deposited in GenBank under the accession files KP892886-KP892888.

At the described conditions for hybridization, cells from the close-related species *Ovulinata parva* showed only a weak Cy3 signal (Fig. 3, subfig. 3b), if any, as the probe binding site had a single mismatch. In contrast, small cells present in the filters gave a strong signal (Figs 3, 4-7), indicating a specific hybridization. DAPI counterstain showed a large nucleus in *O. parva* situated at the back of the cell (Fig. 3, subfigs 2a and 3a). Hybridization of the environmental samples revealed fluorescing small testate amoebae cells in samples from both litter and moss, however the high level of autofluorescence in soil samples did not allow to take good pictures; only samples derived from mosses are shown here (Fig. 3, subfigs 4a-b, 5a-b, 6a-b, 7a-b).

In the soil Paulinellids, nucleus was less conspicuous than in *O. parva*, perhaps because of the small size and possibly a different test composition. Cells were also visualised under classical light microscopy, which permitted us to detect similar organisms in the sample suspension extracts.

Discussion

M. edaphonis is the first member of family Paulinellidae found in a strictly edaphic environment (sampling site was far from any river or pond, and was situated on a slope). Life in soils demands a series of adaptations for a protist, including the capacity of forming resistant structures (cysts) against desiccation or frost. As a result, it has been evidenced that soil communities are significantly