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Squamamoeba japonica n. g. n. sp. (Amoebozoa): A Deep-sea Amoeba from the Sea of Japan with a Novel Cell Coat Structure

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Squamamoeba japonica **n. g. n. sp. was isolated and described from marine bottom sediments collected at a depth of ca. 2700 m in the Sea of Japan. Trophic amoebae of this species are elongated and flattened, with a wide anterior hyaloplasm producing numerous ventral subpseudopodia for adhesion to the substratum. The cell coat consists of flat oval scales tightly packed together to form a continuous layer separated from the plasma membrane. Amoebae can form cytoplasmic projections protruding through the scale layer and having tips covered only with the plasma membrane. Small subunit ribosomal RNA gene phylogeny shows that *S. japonica* forms a long branch in the amoebozoan tree, robustly grouping with the marine strain '*Pessonella*' sp. PRA-29. Morphological data available for the latter, although scarce, give additional support for the relatedness of both species. The resulting clade comprising the two taxa shows no close relationships to other Amoebozoa and seems to be a novel lineage that developed an ability to temporarily liberate local areas of the plasma membrane from the cell coat independently from Himatismenida, Trichosida, Pellitida and *Dermamoeba*.**

Key words: Amoebae; cell coat; deep-sea protists; phylogeny; SSU rRNA; taxonomy; ultrastructure.

Introduction

In spite of recent efforts (Arndt et al. 2003; Atkins et al. 2000; Hausmann et al. 2002a, b; Pawlowski et al. 2011; Scheckenbach et al. 2005), very little is known about the diversity of protozoa in deep-sea communities, apart from Foraminifera and Komokiacea which are slightly better characterised (e. g. Gooday 1994, 2002; Gooday and Bowser 2005; Gooday et al. 2007). There are especially few data on lobose amoebae (phylum Amoebozoa). Apart from three published records (Hausmann et al. 2002a; Kudryavtsev et al. 2011b; Moran et al. 2007), no information on amoebae inhabiting the ocean bottom deeper than 200 m is available. During the last few years we have been investigating the diversity of amoebozoans isolated from deep-sea bottom sediments. Among other findings an unusual amoeba with a novel set of morphological characters was found in bottom sediments collected from the Sea of Japan (Pacific Ocean),

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representing a new genus and species of Amoebozoa. The purpose of this paper is to describe this new amoeba and analyse its phylogenetic position using microscopic and molecular tools.

Results

Morphology and Ultrastructure of the Studied Strain

The newly isolated amoeba could be cultured easily, and readily adopted a locomotive form in culture dishes or when placed in plankton observation chambers with a glass bottom. Locomotion occurred less frequently in wet mounts on coverslips, and many cells remained stationary during most of the time of observation. The rate of locomotion at +18 °C was 3-8.7 μm/min (average 5.3 μm/min) (n=21) or about 0.5-1 times the cell length per minute. The locomotive form was flattened and elongated (Fig. 1A-C), with length greater than breadth (all measurements are given in the diagnosis). Most of the cells when viewed from above had an irregularly triangular shape, being broader anteriorly with a pointed posterior end, while others were oval, with a rounded posterior end. A flat anterior hyaline area occupied about one-third to one-half of the cell length. The anterior and lateral edges of the hyaline area always produced short mammiliform subpseudopodia that moved after formation towards the ventral surface of the hyaloplasm (Fig. 1A-C). These subpseudopodia remained stationary with respect to the substratum as the cell advanced, and looked like rounded spots when the ventral surface of the cell was in focus. Non-directionally moving and stationary amoebae (Fig. 1B, D) were more flattened and occupied a larger area of the substratum than in the locomotive forms. They produced digitiform subpseudopodia that were usually longer than the subpseudopodia formed during locomotion. Sometimes the cell produced a single tapering hyaline projection terminating with a pointed tip, or several tiny projections. Cells rarely adopted a differentiated floating form. When detached from the substratum, amoebae had the same shape as when remaining stationary on the substratum, usually with several asymmetrically formed hyaline pseudopodia. Some of the cells after a long time on slides or in culture detached from the substratum and adopted a spherical shape with short hyaline projections. These looked rather like dying cells than the normal floating forms; they were never seen to settle down back to the substratum and resume normal locomotive activity. Amoebae were uninucleate; the nucleus was usually very difficult to see in the living cells due to its small size. It was spherical, vesicular, with an inconspicuous central nucleolus (Fig. 1E). During locomotion the nucleus was usually located in the anterior part of the granuloplasm, which was filled with minute spherical granules and food vacuoles containing bacteria. There was no contractile vacuole. Encystment was never observed.

Scanning electron microscopy (Fig. 1F-G) usually demonstrated non-directionally moving or stationary amoebae with long digitiform pseudopods, often with very fine tips. Short conical subpseudopodia used for adhesion to the substratum were clearly seen in some cells (Fig. 1G). The surface of every cell observed was uneven and carried minute papillate structures (Fig. 1G), but no other remarkable surface features were seen. Transmission electron microscopy (Fig. 1H, 2) revealed a pronounced flexible cell coat consisting of a single layer of flat, electron dense scales having an oval shape in tangential sections, and with the margins slightly bent distally (Fig. 2A, B, D). Thin filamentous structures were seen arising vertically from the center of each scale (Fig. 2A). In favorable tangential sections the scales were seen to possess denser points in their centers that probably correspond to the bases of these filamentous structures (Fig. 2B). The scales were connected to each other with a material of medium electron density (Fig. 2B). The size of the scales was 95-145(125)x 60-90(75)x10 nm (n=17), and the measured length of the distal filaments was 160-420(258) nm (n=20). The plasma membrane underlying the scale layer was folded (Fig. 2A), and an electron transparent space 25-85 nm wide was always present between the plasma membrane and the scale layer (Fig. 1H, 2A-D). All the cells observed in the sections carried eventual local expansions of the scale layer where it was widely separated from the plasma membrane (Fig. 2C). These expansions always contained membranous material of unclear origin. No structures that might correspond to these expansions could be seen with light microscopy. Several points of disruption of the scale layer were seen in some cells (Fig. 2B, D). In these points the neighboring scales were separated from each other and small tapering cytoplasmic projections were seen to expand beyond the scale layer (Fig. 2D). These projections were filled with the electron dense cytoplasm. The nucleus in sections (Fig. 1H) was rounded or lobate, with an inconspicuous central nucleolus of irregular shape. The nuclear envelope was underlain with a layer of electron dense material (Fig. 2A). Most of the