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Fine structure of *Leptomyxa ambigua* n. sp. CCAP 1546/2 strain, formerly known as "*Rhizamoeba flabellata*" (Amoebozoa, Tubulinea, Leptomyxida)

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Abstract

The species *Leptomyxa flabellata* was described by Goodey in 1915 and re-isolated by Pussard and Pons in 1976. It seems that it was never seen (or never recognized) again since that time. The strain designated as "*Leptomyxa flabellata* CCAP 1546/2" was studied by Cann in 1984, however the quality of the electron microscopic images of that time was poor. Based on the cyst structure and size characters, Page in 1988 suggested that this strain is not co-specific with Goodey's *Leptomyxa flabellata*, but represents a species 'Ripidomyxa' *australiensis* Chakraborty and Pussard, 1985, nowadays known as *Rhizamoeba australiensis*. In the present paper light- and electron-microscopic images of CCAP 1546/2 strain, which is now lost, are provided. Based on the morphological evidences it is suggested to establish it in a rank of a new species, *Leptomyxa ambigua* n. sp. Neither "true" *L. flabellata* Goodey, 1915 nor original *R. australiensis* Chakraborty et Pussard, 1985 are nowadays represented in the culture collections, and no original type material is available on both these species.

Keywords: Amoebozoa; Leptomyxida; Systematics; Ultrastructure

Introduction

An amoeba species, *Leptomyxa flabellata*, was described by Goodey (1915). His description was rather detailed and illustrated with high-quality line drawings, showing a typical leptomyxid amoeba with adhesive uroidal structures and a body varying in its shape from nearly monopodial, clavate to flattened, overall expanded, sometimes with pronounced "tail". These amoebae formed rounded, double-walled cysts. Pussard and Pons (1976) isolated an amoeba strain fitting this description from a soil habitat in France and provided light-microscopic data; they also documented feeding and mitosis in this organism. Page (1988) transferred this species to the genus *Rhizamoeba*. Further Smirnov et al. (2017), during the revision of the order Leptomyxida, re-defined the genus *Rhizamoeba* and argued that *R. flabellata*, according to descriptions of Goodey (1915) and Pussard and Pons (1976), does not fit the diagnosis of this emended genus. In that paper it was transferred back to the renewed genus *Leptomyxa*, restoring the taxonomic position proposed by Goodey.

Neither Goodey, nor Pussard and Pons left any strains that they studied, so the only way to continue studies of this organism is to re-isolate it. The Culture Collection of Algae and Protozoa (CCAP, nowadays a part of UK National Culture Collection, UKNCC) for a long time held a strain CCAP 1546/2 labelled as *Leptomyxa flabellata*. It was isolated in 1974 from the leaf litter in Wandlebury Wood, Cambridgeshire, UK. This strain was studied with transmission electron microscopy by Cann (1984); however, the quality of EM images of that time was relatively poor. He noted close similarity of this strain to amoebae of the genus *Rhizamoeba* Page, 1972 and transferred it into this genus under the

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name *R. flabellata*. However, Page (1988) noted that it had single-walled cysts, which is not characteristic for *L. flabellata sensu* Goodey (1915) and Pussard and Pons (1976) and suggested that it may be co-specific with another amoeboid organism, first isolated by Chakraborty and Old (1982) and described under the name *Ripidomyxa australiensis* three years later (Chakraborty and Pussard 1985). Page (1988) transferred this species to the genus *Rhizamoeba* as *Rh. australiensis*, but kept the name *Rh. flabellata* for Goodey's organism. With this transfer, the genus *Ripidomyxa* was *de facto* invalidated (Page 1988 p. 89). Nevertheless, two sequences of amoebae designated as "*Ripidomyxa* sp." appeared in GenBank in 2007, which is an incorrect application of the abandoned generic name (see Smirnov et al. 2008); they belong to the organism, now known as *Leptomyxa variabilis* (Smirnov et al. 2017).

Among the materials, prepared by the author during the studies of CCAP amoebae collections in the year 1999 there were TEM embeddings of CCAP 1546/2 strain. Now this strain is lost. This is the same strain that Cann (1984) studied and illustrated, but not that, which was studied by Pussard and Pons (1976). These embeddings were sectioned and examined. The quality of fixation was adequate, so the proper ultrastructure of this strain has become available. In the present paper it is argued that this strain is co-specific neither with *L. flabellata sensu* Goodey, 1915, nor with *R. australiensis sensu* Chakraborty and Pussard, 1985. Based on the light- and electron-microscopic evidences it is proposed to describe this strain as a new amoeba species.

Material and Methods

Amoebae of CCAP 1546/2 strain were maintained in 90 mm Petri dishes on non-nutrient agar (NN, Page 1988) with *E. coli* and accompanying bacteria as a food source. Cultures were maintained in darkness, under 20 °C and transferred once in two-three months.

Light-microscopic observations, imaging and videorecords were done on the glass object slides under room conditions; cells were washed off from the agar surface and placed in the drops of PJ medium (Prescott and James 1955). Observations were performed using Olympus BH2 microscope equipped with the phase contrast and DIC optics; videorecords were done using JVC sVHS camera.

For electron microscopy cells were fixed individually, in glass wells, with 2.5% glutaraldehyde prepared with phosphate buffer (pH 7.4) for 40 min under the room temperature and postfixed with osmium tetroxide made on the same buffer at the final concentration of ca. 2% for one hour (tapered-tips, glass Pasteur pipettes were used to handle cells). Amoebae were washed 3 × 5 min with the same buffer between fixation steps and prior to dehydration. Further, cells were dehydrated in ethanol series followed by propylene oxide and embedded in Spurr's resin according to the manufacturer instructions. Sections were double-stained using 2% aqueous solution of uranyl acetate followed by Reynolds' lead citrate.

Results

CCAP 1546/2 strain — light microscopy

The locomotive form of amoebae was pronouncedly clavate, with large frontal area of the hyaloplasm and a well-developed bulbous uroid, usually covered with short adhesive filaments (Fig. 1A–D). The length of the locomotive form varied from 85 to 156 μm, breadth (in the central part of the cell) from 25 to 34 μm (measured in videoprints and videorecords, n = 8). Large contractile vacuole was frequently located next to the frontal hyaline area, but it could move posteriorly as well (Fig. 1A–C). It was formed by fusion of smaller vacuoles. Most of the cells had one nucleus, some were binucelate, according to my records from 1999, few contained three to four nuclei, but no images of such cells are left. The nucleus was of vesicular type with a compact central nucleolus. The size of the nucleus was 8–12 μm across (measured in photographs, n = 6).

Moving amoebae often show pronounced eruptions of the hyaloplasm in the direction, opposite to that of the locomotion (Fig. 1E). If this happened, a cell became irregular for some time and then formed a pronounced pseudopodium. The rest of the cell mass was rapidly absorbed with this pseudopodium and the cell became monopodial. Slowly moving cells were rather wide and frequently formed eruptive waves of the cytoplasm (Fig. 1F). Irregularly moving cells were flattened and often formed numerous adhesive lobes and filaments, surrounding the major part of the cell periphery (Fig. 1H). Immobile (or nearly immobile) cells were flattened, with numerous hyaline lobes and short adhesive filaments (Fig. 1G). A developed floating form was never observed in this strain. Cells floated as irregular masses; often they started to adopt a clavate monopodial form while suspended in the water, without contacting the substratum. Such cells often show eruptions of the cytoplasm in different directions, an eruption often resulted in the formation of a leading pseudopodium in this direction and respective change in the direction of the cell progressing. When cells contacted the substratum, they rapidly started active locomotion, but the contact with the substratum in locomotive cells was weak. They easily detached and continued movement over the substratum, in the layer of medium. Because of this process, amoebae rarely moved in one and the same direction for a long time. The encystment in CAAP 1546/2 strain was never seen. According to my records, in old, drying culture amoebae degraded and died but did not form cysts.

CCAP 1546/2 strain — electron microscopy

Uninucleate and binucleate cells were observed in TEM sections. The nucleus was of vesicular type, oblong, with pronounced central nucleolus (Fig. 2A–B). Inside the nucleolus there were characteristic small transparent areas surrounded with patches of electron-dense material, 1–2 μm across, the