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Fine structure of *Thecamoeba quadrilineata* strain CCAP 1583/10 (Amoebozoa, Discosea, Thecamoebida), the host of *Nucleophaga amoebae* (Opisthosporidia)

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Summary

Amoebae of the genus *Thecamoeba* (Amoebozoa, Discosea, Thecamoebida) are among the most widely distributed and best-known species of naked lobose amoebae. However, the number of available ultrastructural studies of these organisms remains low. The most of them are dated back to 1960th-1970th and virtually no modern data appeared in the literature, except for the newly described *Thecamoeba aesculea*. As a result, we have no complete reports on the fine structure of many widely distributed and most trivial *Thecamoeba* species. The present study provides the complete ultrastructure of *Thecamoeba quadrilineata* strain CCAP 1583/10. The studied strain is also known to serve as a host for *Nucleophaga amoebae* – an intranuclear parasite belonging to the Opisthosporidia clade, so the investigation of non-infected amoebae provides "reference" data for studying the infection process and intracellular development of this parasite.

Key words: Amoeba, Thecamoebidae, ultrastructure, Amoebozoa

Introduction

The genus *Thecamoeba* was established by Fromentel (1874) and nowadays includes 19 species, of which 11 are recognized as properly described and illustrated ones (see Mesentsev and Smirnov, 2018). All these amoebae belong to the striate or rugose morphotype (Smirnov and Goodkov, 1999; Smirnov and Brown, 2004). Most of them possess remarkable morphological characters – locomotive form, dorsal folds and wrinkles, the structure of the nucleus (Page, 1971; 1977; 1988; Smirnov, 1999; Kudryavtsev and

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Hausmann, 2009). Non-surprisingly, most of *Thecamoeba* species are described based on light-microscopy data. A comprehensive ultrastructure of the cell is known for *T. sphaeronucleolus* (Houssay and Prenant, 1970) and *T. aesculea* (Kudryavtsev and Hausmann, 2009). The structure of the cell coat has been studied in several more species: *T. munda, T. similis, T. quadrilineata, T. orbis* and *T. terricola*, but no general ultrastructure of the cell is provided for these organisms (Page and Blakey, 1979; Smirnov, 1999).

One of the most widely distributed species of the genus *Thecamoeba* is *T. quadrilineata*. This species was named by Carter (1856) as *Amoeba quadrilineata*. His publication included a single line drawing showing a striate *Thecamoeba* with vesicular nucleus (op. cit., Plate V, Fig. 3) but with no adequate text description. Lepşi (1960) formally transferred this species to the genus *Thecamoeba*. Page (1977) isolated two amoeba strains similar to that illustrated by Carter and suggested them to be co-specific with Carter's "*Amoeba quadrilineata*". He deposited to the Natural History Museum (London, UK) a permanent haematoxylin-stained preparation with the number 1975:8:4:11 that he designated as a neotype and preparation 1975:8:4:12, designated as paraneotype. He also deposited one of these strains with the Culture Collection of Algae and Protozoa (CCAP, UK) under the number CCAP 1583/7, but it was further lost.

In 1998, Rolf Michel isolated from roof gutter sediment in Melsbach (Germany) a strain that he identified as *T. quadrilineata* and deposited it with CCAP under the number CCAP 1583/10 (according to CCAP record its original designation is Dch-1). This strain also was lost, but resubmitted by R. Michel in 2007. The SSU sequence of this strain was obtained by Claudia Wylezich and deposited in GenBank under the number DQ122381, however, the original strain designation in GenBank is given as "Dach". Claudia Wylezich in private communication confirmed that this is the same strain. The first mentioning of this sequence is by Walochnik et al. (2003, as strain "DAC1"), in a regular journal and with GB number it was first used by Michel et al. (2006). So this strain and its sequence may be considered as the most reliable reference material on *T. quadrilineata*, which is available at the moment (hence the neotype by F.C. Page formally remains the type material of this species). The same strain (under the number ATCC PRA-259) was used by Tekle et al. (2016) for a multigene phylogeny. Rolf Michel in the year 2004 deposited with CCAP one more strain, designated as *Thecamoeba quadrilineata* CCAP 1583/15, (original strain designation was Tq-2). This strain is known as a host of an intranuclear parasite belonging to the genus *Nucleophaga*, however, the ability of this parasite to also infect 1583/10 strain was later demonstrated (Michel, 2008; Michel et al., 2009).

The species *T. quadrilineata* has been recorded from freshwater and soil habitats, including plant surface and leaf debris. It was mentioned in numerous faunistic studies (Grimm et al., 2001; Li et al., 2006; Jiang et al., 2006; Matis et al., 1997; Michel et al., 1995; Mrva, 2005; 2006; Mulec et al., 2015; Patcyuk and Dovgal, 2012; Tao et al., 2009). However, these records are based on light-microscopic identification only. Taken into account the potential existence of sibling species within the genus *Thecamoeba* (Kudryavtsev and Hausmann, 2009; Mesentsev and Smirnov, 2018), these faunistic records should be treated with care.

Thecamoeba quadrilineata was mentioned as a host for the parasitic fungus *Cochlonema euryblastum* Drechsler, 1942 and as a prey of another fungus, *Acaulopage tetraceros* Drechler, 1942 (Michel and Wylezich, 2005; Michel et al., 2014; Michel et al., 2015; Koehsler et al., 2007). It also is known to host the intranuclear parasite *Nucleophaga amoebae* Dangeard, 1895 (Opisthosporidia) (Michel et al., 2009; Corsaro et al., 2014). Besides this, it was mentioned that it could ingest oocysts of *Cryptosporidium parvum* (Apicomplexa, Eucoccidiorida) and thus be a potential carrier of this parasite (Scheid and Schwarzenberger, 2011). Recently it was isolated from the gut of the earthworm *Lumbricus terrestris*, which suggests that this species may be an amphyzoic one (Borovičková et al., 2019).

Despite a considerable amount of data and numerous records in the literature, the last detailed description of this species was given by Page (1977) and this was a light microscopic study. The cell surface of the strain CCAP 1583/7 was illustrated by Page and Blakey (1979), but no data on the general ultrastructure of the cell was provided. Certain illustrations of the general cell structure are available in the studies dedicated to the parasites of *Thecamoeba quadrilineata* (Koehsler et al., 2007; Michel et al., 2009; Corsaro et al., 2014), but in these papers, there are no data on non-infected cells. Hence, no «reference» ultrastructure is available for this amoeba species. In the present study, we provide new LM data and report the ultrastructure of *Thecamoeba quadrilineata* strain CCAP 1583/10.

Material and methods

The studied strain *Thecamoeba quadrilineata* CCAP 1583/10 was maintained by Rolf Michel as a host for *Nucleophaga amoebae* strain KTq2. A non--infected culture was maintained on NN agar (Panreac agar-agar, American Type QB, Spain) as described by Page (1988) made on PJ medium (Prescott and James, 1955). The eukaryote-free culture was initially fed by Rolf Michel on *Enterobacter cloacae*, but further the bacterial composition was not checked, so other bacteria might present as well.

Live cells were studied, measured and photographed on object slides (wet mounts in PJ medium) using a Leica DM2500 microscope equipped with DIC and Phase contrast. Special attention was paid not to press the cell with the coverslip as described by Mesentsev and Smirnov (2018). To get higher focal depth, stacks of optical sections were photographed from each specimen, later 5-9 optical sections were composed to get higher focal depth image using Helicon Focus 6.0 (Helicon Software Ltd).

For electron microscopy, cells were washed off from agar plates with PJ medium and concentrated by gentle centrifugation (1500 g) in 200 μl tubes for 90 sec. Further, the pellet consisting of cells was fixed in the same tubes (with centrifugation before the change of the solution at every step) using two different protocols:

(1) 2.5% glutaraldehyde made in 0.05M sodium cacodylate buffer (pH 7.1) for 30 min; postfixation with osmium tetroxide made on the same buffer at the final concentration of ca 2% for one hour. All procedures were performed under 4 °C (in the fridge). Amoebae were washed 3×5 min with the same buffer.

(2) The mixture of 2.5% glutaraldehyde and 1.6% formaldehyde prepared in 0.1M phosphate buffer (PH 7.4) for 1.5 hours under room temperature (rt); washed for 3×5 min in the same buffer (rt); postfixation with 1% osmium tetroxide (final concentration) for one hour at +4 °C. Amoebae were washed in the same buffer 3×10 min prior to dehydration (rt).

In both cases, the pellet consisting of cells was embedded in 2% agar before dehydration. Small pieces of agar (about 1 mm^3) containing amoebae were cut out and dehydrated in graded ethanol series followed by 100% acetone. Blocks were embedded in SPI-PON 812 resin (SPI, an analog of Epon 812) according to the manufacturer`s instructions. Sections were cut using a Leica Ultracut 7 ultramicrotome and double-stained using 2% aqueous solution of uranyl acetate and Reynolds' lead citrate.

To extract DNA, cells were washed off from the agar surface with sterile PJ medium and concentrated in 1.7 ml Eppendorf tubes by centrifugation for 90 sec under 1500 g. The genomic DNA from the cell sediment was extracted with guanidinium thiocyanate buffer (Maniatis et al., 1982). The 18S rRNA gene was amplified by PCR using universal eukaryote primers RibA (forward) and RibB (reverse) (Medlin et al., 1988). Thermal cycle parameters were: initial denaturation (10 min at 95 °C) followed by 39 cycles of 30 sec at 94 °C, 60 sec at 50 °C and 120 sec at 72 °C, followed by 10 min at 72 °C for final extension. Amplicons were purified in 1.5% agarose gel using Cleanup mini Purification Kit (Eurogene, Moscow, Russia). Amplicons were sequenced using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit using the RibA, s6F, s12.2, s12.2R and RibB primers (Medlin et al., 1988; Pawlowski, 2000; Adl et al., 2014). The sequence iden-tity level was calculated using the online tool "Ident and Sim" provided at http://www.bioinformatics.org/sms2/ident_sim.html website.

Results

Light microscopy and SSU sequencing

Locomotive cells were oblong, elongated, or nearly rounded (Fig. 1 A-D). Sometimes cells were narrowed to the posterior end; some cells were also narrower in the frontal part. They always had several well-pronounced longitudinal folds and a number of lateral wrinkles. The typical number of folds was four; in smaller cells – less, while in larger cells we have seen up to six folds. The frontal hyaline area occupied up to half of the cell and extended posteriorly along the lateral sides of the cell, forming an anterio-lateral hyaline crescent. The length of the locomotive cell was 25-65 μm (average 38.4 μm, n=50), the breadth of the cell was 20-36 μm (average 26.4 μm, n=50), and the Length/Breadth ratio (L/B) was 1-2.2 (average 1.5). Differentiated uroidal structures were not seen. Stationary cells were rounded, with a pronounced peripheral hyaline border. Floating cells formed several short, blunt hyaline pseudopodia. Cells readily adhered to the glass (object slide or coverslip). Amoebae had a spherical nucleus with a single central nucleolus (Fig. 1 E-I). The size of the nucleus (in maximal dimension) was 5.9-12.5 μm (average 9.3 μm,

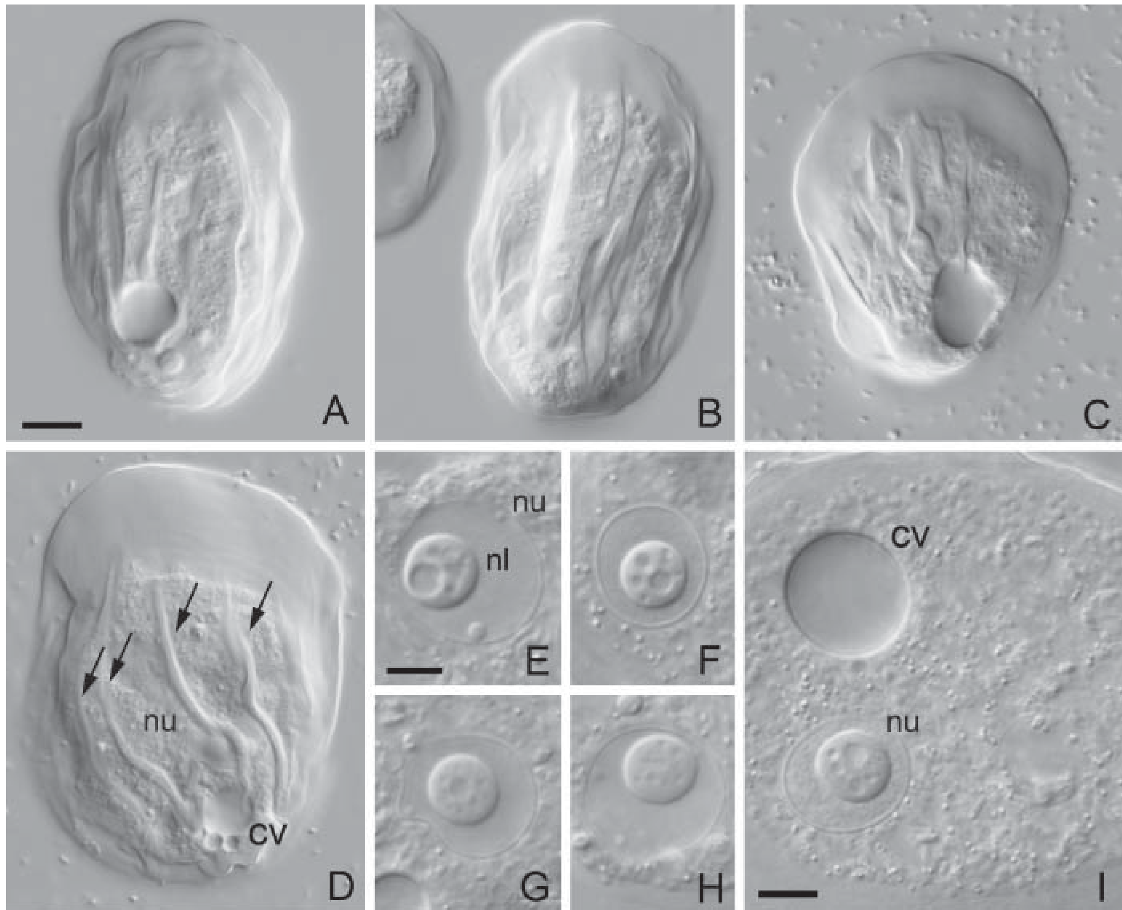

Fig. 1. Light-microscopic images of *Thecamoeba quadrilineata* strain CCAP 1583/10. A-D – locomotive forms; dorsal ridges are *arrowed* in D; E-H – organization of the nucleus. Cells are slightly pressed with the coverslip to make the nucleus better visible, so in some images the nucleolus became eccentrically located. Note that in most of nuclei there is a single central lacuna and several additional, smaller ones. I – Overview of the cytoplasm showing the nucleus, contractile vacuole and numerous granules located in the cytoplasm. *Abbreviations*: nu – nucleus, nl – nucleolus, cv – contractile vacuole. Scale bars: A-D – 10 µm, E-I – 5 µm.

n=25). The diameter of the nucleolus was 2.5-4.5 µm (average 3.5 µm, n=25). The nucleolus often had several lacunas. The nucleus was very labile and highly deformable. The granuloplasm was filled with small opaque granules; it contained food vacuoles and a single contractile vacuole, usually located at the posterior end of the cell (Fig. 1 I).

The SSU sequence of this strain obtained in our laboratory was almost identical to the sequence DQ122381 obtained from this strain in 2003 (99.9% identity, 2 bp difference, namely A->G in position 370 and C->G in position 1081, positions indicated in DQ122381 sequence). Both may be sequencing errors, probably in the DQ122381 sequence as the nucleotides are reliable in our phoregrams. Prove for this suggestion is that they both are identical to those in the sequence of our strain *T. quadrilineata* Ta24 (GB number MH628647) (Mesentsev and Smirnov, 2018). This check leaves no doubts that this is the same strain that was sequenced in 2003. The difference between this sequence and that of *T. quadrilineata* Ta24 strain in addition to these two nucleotides was the presence of a 4 bp insertion in the latter one (position 1907-1910 in MH628647 sequence).

Transmission electron microscopy

The fixation quality obtained with the use of protocol #2 (with formaldehyde) was generally higher than we have observed using the more traditional protocol #1. The present description is based on the results of protocol #2 if not otherwise mentioned. In the same time, results obtained by

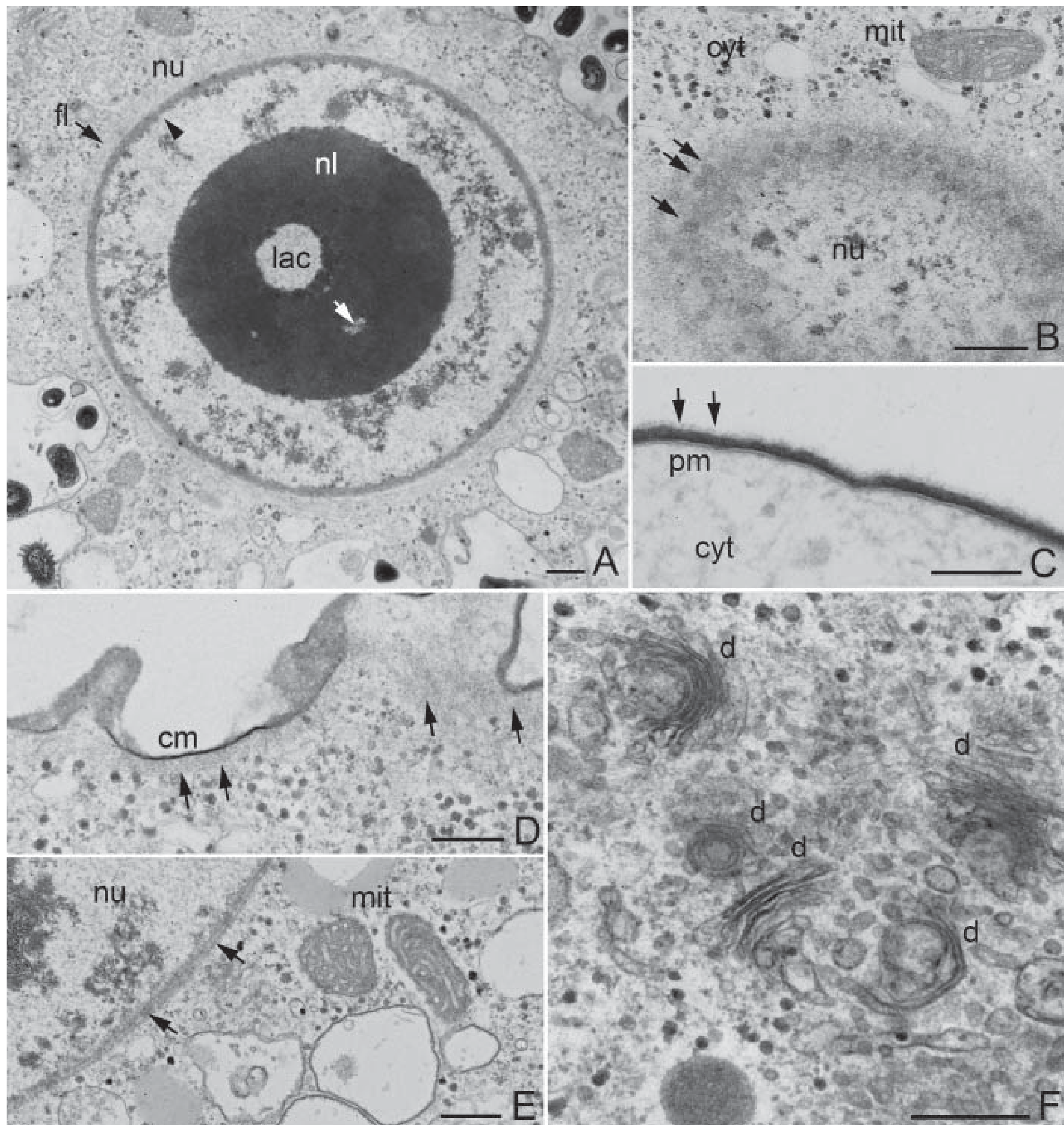

Fig. 2. Ultrastructure of *Thecamoeba quadrilineata* strain CCAP 1583/10. A – Cross-section through the nucleus (*white arrow* shows additional, smaller lacuna; *black arrowhead* inside the nucleus points to the layer of dense material located on the inner surface of the nuclear membrane); B – tangential section of the nucleus (*black arrows* indicate nuclear pore complexes); C – cell coat (*black arrows* point to the loose outer layer covering the dense, amorphous basal layer of the glycocalyx); D – layer of microfilaments underlying the cell membrane (*black arrows* point to the microfilaments); E – mitochondria and portion of the nucleus (*black arrows* point to the nuclear envelope); F – arrangement of dictyosomes. *Abbreviations*: cm – cell membrane, cyt – cytoplasm, d – dictyosomes, fl – fibrous layer surrounding the nucleus (pointed with *black arrow*), lac – the central lacuna, mit – mitochondria, nl – nucleolus, nu – nucleus, mit – mitochondrion, pm – plasma membrane. Scale bars: A-B, D-F – 500 nm; C – 100 nm.

both protocols revealed basically the same ultrastructural characters, so we cannot say that any of them resulted in evident artifacts.

The nucleus was spherical or nearly spherical. In our sections, it had a rounded, centrally or slightly eccentrically located electron-dense nucleolus (Fig. 2 A-B). In sagittal sections, the nucleolus usually had a small central lacuna filled with karyoplasm. The material forming the nucleolus was not entirely homogeneous, it was possible to dis-

tinguish slightly lighter and slightly denser areas (Fig. 2 A). A layer of a dense material, 120-200 nm in thickness, was associated with the internal side of the nuclear membrane. A granular material resembling chromatin formed loose patches in the karyoplasm. The nucleus from the outer side was surrounded with a layer of microfilaments (Fig. 2 A). Nuclear pore complexes were well visible in the tangential sections of the nucleus. They had a typical structure, showing the peripheral spoke ring assembly and the central plug (Fig. 2 B). Both tangential and sagittal sections showed no fibrous lamina inside the nucleus (Fig. 2 A-B, E).

The cell surface was covered with a layer of amorphous glycocalyx, approximately 20 nm in thickness. In the best sections, it appeared to be composed of two layers – a dense layer about 16 nm in thickness, located right over the plasma membrane and a loose outer layer, about 4 nm in thickness (Fig. 2 C). The appearance and thickness of the cell coat were almost identical under both protocols used.

The mitochondria were spherical, ovoid or (rarely) elongate in sections (Fig. 2 E). They had cristae of the tubular type and an electron-dense matrix. Dictyosomes of the Golgi complex were not numerous and were represented by stacks, consisting of 6-10 cisternae (Fig. 2 F). Usually, we saw several dictyosomes located nearby. No MTOCs were seen in the surrounding of the dictyosomes and no evident microtubules were found in the cytoplasm.

The granuloplasm was separated from the peripheral layer of the hyaloplasm by a layer of microfilaments. These filaments often formed bundles, tangentially arranged under the cell surface (Fig. 2 D). Small bundles of microfilaments were seen in the cytoplasm, crossing it in different directions. The filaments and the filamentous bundles were best preserved under the fixation procedure #2, and only moderately – under #1. The contractile vacuole was surrounded by numerous vesicles and tubular structures, forming the contractile vacuole complex (spongiome) (Fig. 3 A-B). Numerous mitochondria surrounded the area of the contractile vacuole. Bacteria, apparently located freely in the cytoplasm were seen in all cells (Fig. 3 C). Food vacuoles contained numerous bacteria. When amoebae were maintained with *Nucleophaga* spores, we sometimes saw ingested spores in the food vacuoles. Some of them appeared to be dead and half-digested (Fig. 3 D).

Discussion

The light-microscopic morphology of the studied strain generally corresponds to that of *T. quadrilineata* strains described by Page (1977) and *T. quadrilineata* Ta24 strain studied by Mesentsev and Smirnov (2018). The present strain in locomotion usually appears to be wider than other known strains of this species. However, the morphometric data do not support this impression. L/B ratio in the present strain varies from 1 to 2.2 with a mean value of 1.5. Page (1977) reported for his strains of *T. quadrilineata* L/B from 1.1 to 3.4 with mean value 1.4-2.0. So, despite some of his amoebae were considerably more oblong (maximal L/B values), our mean value is in the same range, hence on the "broader" side of the interval. The strain *T. quadrilineata* Ta24 described by Mesentsev and Smirnov (2018) has exactly the same L/B minimax values and the same mean value.

The nucleolus in the nucleus of the present strain had lacunae in almost every specimen. Many nuclei in addition to the pronounced central lacuna had several additional, smaller ones. This is not a characteristic for the original *T. quadrilineata* sensu Page (1977), and the strain Ta24 mentioned above also has fewer lacunae in the nucleolus. However almost complete 18S rRNA gene sequence identity between the *T. quadrilineata* strain CCAP 1583/10 and the *T. quadrilineata* strain Ta24 considered together with the large genetic distance to their nearest neighbor – *T. cosmophorea* leads to the conclusion that all these differences represent the range of intra-specific polymorphism of the species *T. quadrilineata* (see Mesentsev and Smirnov, 2018). Also, we cannot exclude that these differences are the result of the long maintenance of the strain CCAP 1585/10 in laboratory culture.

The nucleus in two *Thecamoeba* species studied by TEM, namely – *T. sphaeronucleolus* and *T. aesculea*, has a well-pronounced fibrous inner nuclear lamina (Houssay and Prenant, 1970; Kudryavtsev and Hausmann, 2009). In *T. quadrilineata*, we have seen a similar layer of material underlying the nuclear membrane, but no evidence for the filamentous lamina neither in sagittal nor in tangential sections (see Fig. 2 B, E). The material underlying the nuclear membrane always looked granular. This is congruent with the TEM images obtained during the studies of the parasites of *T. quadrilineata* (Michel et al., 2009 Figs 5, 7; Koehsler et al., 2007 Fig. 2).

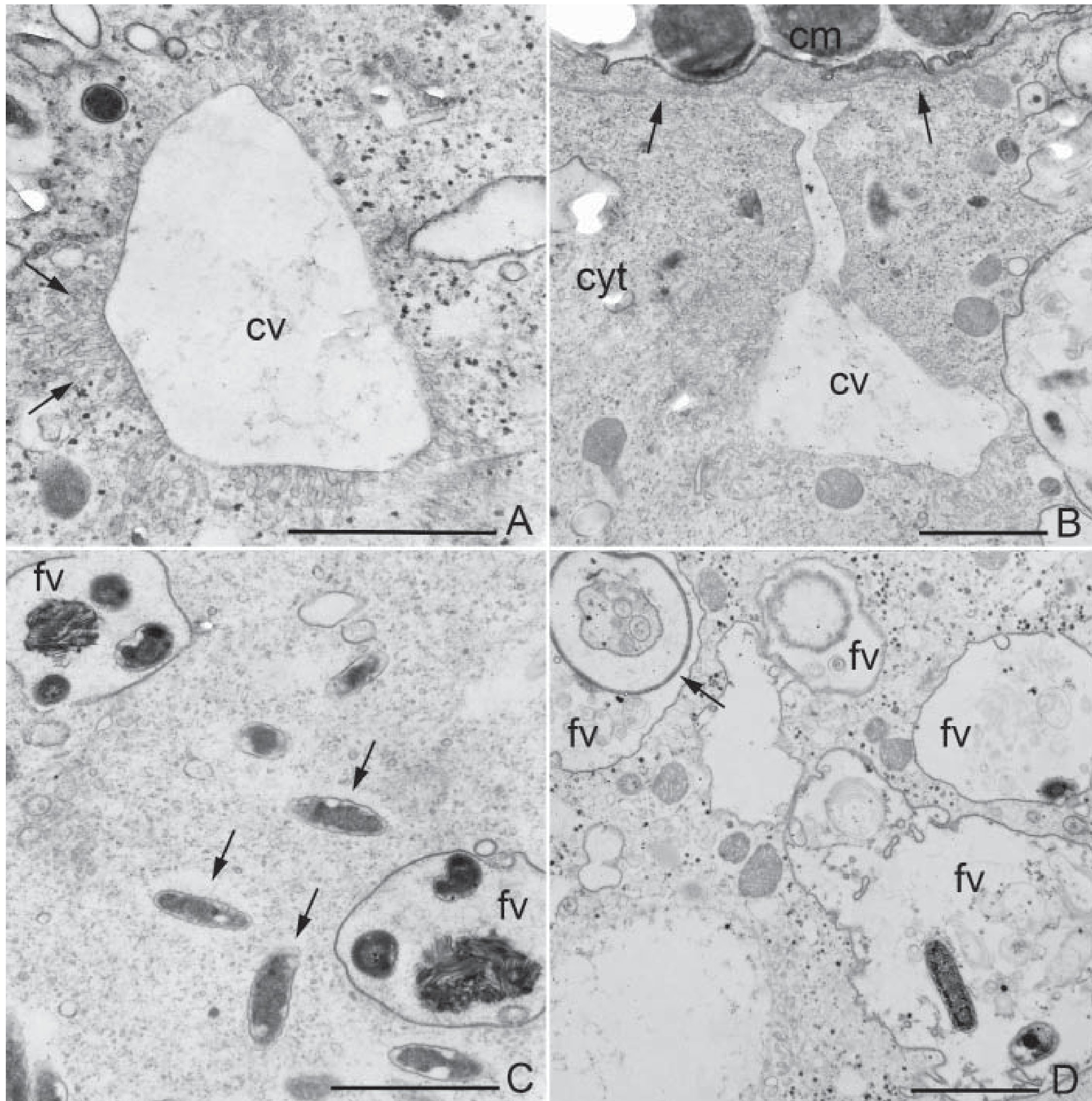

Fig. 3. Ultrastructure of *Thecamoeba quadrilineata* strain CCAP 1583/10. A – Section across the contractile vacuole, showing spongiom. Tubes and vesicles of the spongiom are *arrowed*; B – contractile vacuole soon after contraction, showing a projection toward the plasma membrane. *Arrows* point to the layer of microfilaments, underlying the plasma membrane; C – endocytobiotic bacteria (*arrowed*), located freely in the cytoplasm. This image also shows food vacuoles, containing bacteria; D – food vacuoles filled with various food objects. *Arrow* indicates the vacuole containing a eukaryotic food object (a half-digested spore of *Nucleophaga* sp.). *Abbreviations*: cv – contractile vacuole, cyt – cytoplasm, fv – food vacuole. Scale bars: 2 μm throughout.

This is an interesting point, which theoretically may be a fixation artifact. However, we observed a similar pattern under both fixation protocols used and simultaneously have seen pronounced bundles of microfilaments around the nucleus and in the cytoplasm (which provides evidence that microfilaments are preserved under both fixation protocols). One point, potentially favoring the hypothesis on the absence of the morphologically distinct nuclear lamina in this species is that the nucleus of *T. quadrilineata* is mentioned as "highly deformable" (Page 1977; Mesentsev and Smirnov, 2018). This may be related to mechanical properties of the nucleus, which in their turn are defined (among other matters) with the presence or absence of the nuclear lamina. It is believed that this morphologically distinct nuclear lamina is so widely distributed in large nuclei as (among other functions)

it gives them a mechanical support (Gruenbaum et al., 2003; Prokocimer et al., 2009; Ciska and Diaz de la Espina, 2014). The nucleus in *T. aesculea* is large, reaching 22 µm in diameter, with the nucleolus reaching about 15 µm in maximal dimension (Kudryavtsev and Hausmann, 2009). The size of the nucleus and of the nucleolus in *T. sphaeronucleolus* is also similarly large (Page 1977). The entire nucleus of *T. quadrilineata* is, in fact, smaller than the nucleolus in both above-mentioned species. This may explain the absence of the pronounced nuclear lamina in this species.

The appearance of mitochondria differed depending on the fixation; mitochondria looked best preserved under the fixation procedure #2 – clear outer membranes and those of cristae; dense matrix, regular outlines. Under the fixation procedure #1 they usually had a loose appearance of membranes and unclear inner content, not allowing us to characterize the matrix of the mitochondria. However, the general pattern of the mitochondrial cristae was the same under both fixation procedures used.

The cell coat in *T. quadrilineata* shows a typical structure, similar to that of other *Thecamoeba* species (Houssay and Prenant, 1970; Page and Blakey, 1979; Smirnov, 1999; Kudryavtsev and Hausmann, 2009). It consists of two layers – a dense inner one and a loose outer one. Page and Blakey (1979) report the overall thickness of the cell coat as 24 nm, which is nearly congruent with our results (about 20 nm). They mentioned a different structure of the cell coat, in particular, they did not note the upper loose layer and described it as a compact dense layer but this may depend on the fixation method (op. cit.; see also Page, 1988). Interestingly, a structure of the cell coat most similar to that obtained in the present study was shown for *T. terricola* (Page and Blakey, 1979 Fig. 2 F), but its overall thickness is much higher – 60-73 nm, and, according to their notes, “a compact inner and less dense outer layer… have approximately equal thickness”.

A similar structure of the cell coat – a thin layer of amorphous glycocalyx, sometimes with a loose outer layer is known in two other genera of Thecamoebida – *Stenamoeba* and *Sappinia*, hence in the first genus the cell coat is usually thinner and consist of only one layer, showing no vertical stratification, while in *Sappinia* it may have extra structures over the amorphous layer (Page and Blakey, 1979 Fig. 4 G-H; Dykova et al., 2010; Michel et al., 2006). All thecamoebids are known to have a similarly rigid cell coat; the cell envelope keeps its shape for some time even after the cytoplasm is squeezed out of the cell. As no specific submembranous structures are found in either of the studied species, we can suggest that it is the dense amorphous layer of the cell coat, which is mainly responsible for this.

The contractile vacuole complex in *T. quadrilineata* shows numerous tubules and vesicles surrounding the contractile vacuole. This is known for *T. aesculea* as well (Kudryavtsev and Hausmann, 2009) and represents a contractile vacuole complex of the type D sensu Patterson (1980), which is typical for many gymnamoebae. However, during the light-microscopic observations, we sometimes have seen the formation of a larger contractile vacuole by fusion of several smaller vacuoles.

The presence of bacterial endocytobionts is known for many protists, and amoebae are not an exception (Ossipov et al., 1997). Gram-negative bacteria, very similar (probably identical) to those that we found are visible in TEM images by Michel et al. (2009, Fig 6). In these images, they also appear to be located freely in the cytoplasm.

Food vacuoles in the studied strain contained bacteria when amoebae were kept in monoxenic culture, free from other eukaryotes. However, cells can phagocytize eukaryotic food as well. Michel et al. (2009, Fig. 9 B-C) show endocytosis of spores of *Nucleophaga* sp. In infected cultures, we also have seen the endocytosis of *Nucleophaga* spores. Interestingly, in Fig. 3 D it is possible to see a presumably dead, half-digested spore in the vacuole. This provides evidence that the infection via the phagocytosis is not 100% efficient and some spores of *Nucleophaga* may become just a food for the amoeba cell. These observations confirm the polyphagous nature of this species (Page, 1988).

Overall, *Thecamoeba quadrilineata* shows usual ultrastructure, similar to that of many other gymnamoebae species. It has no clear distinctive characters that can help to differentiate this species from the similar ones. The structure of the cell coat may depend on the fixation protocol and may be only side evidence in case of species identification. The nucleus in *T. quadrilineata* differs from that in two other studied *Thecamoeba* species, and this may be a distinctive character, but we have no information on the ultrastructure of many other *Thecamoeba* species, possessing a vesicular nucleus.

The present study raises the question on the type material of *T. quadrilineata*. An option might be to announce the strain CCAP 1583/10 a neotype of this species, as it is the only strain with published SSU sequence, and now even with transcriptomic data.

However, a live culture as a type strain is not reliable, as shown with the story of this and many other amoebae species, where type cultures have been lost. It is possible to prepare a new stained preparation of amoebae cells from CCAP 1583/10 strain, but it is hard to say why they should be better than Page's type slides of the strain CCAP 1583/7 deposited with the British Museum of Natural History. The strain CCAP 1583/10 theoretically may be lost in future as well – would this warrant establishing a new type material from some other available strain? So, the most parsimonious solution seems to be to keep Page's slides as a type material for this species and consider CCAP 1583/10 and Ta24 strains as reliable re-isolates of this species.

A brief taxonomic summary on the species *Thecamoeba quadrilineata* (Carter, 1856) Lepşi, 1960 thus looks as follows:

Diagnosis – Page (1977) p. 40. It does not require any revision.

Type material: permanent haematoxylin stained preparations by F.C. Page number 1975:8:4:11 (neotype) and 1975:8:4:12 (paraneotype), deposited with the Natural History Museum (London, UK).

Known strains: *Thecamoeba quadrilineata* strain CCAP 1583/7 (type) by F.C. Page – now lost; *T. quadrilineata* strain CCAP 1583/10 by R. Michel, original designation Dach, also was mentioned as DAC1 (alive, deposited with Culture Collection of Algae and Protozoa, UK); *T. quadrilineata* strain RC CCMAm0452 by Y. Mesentsev, original designation – strain Ta24 in Mesentsev and Smirnov (2018) (alive, deposited with the culture collection of the Core facility center "Culturing of Microorganisms" of the Science Park of St. Petersburg State University). One more available strain is *Thecamoeba quadrilineata* strain CCAP 1583/15 deposited by R. Michel, original designation Tq-2, identity with two other mentioned strains was not yet confirmed by molecular studies.

SSU sequence data: DQ122381 (strain CCAP 1583/10) and MH628647 (strain RC CCMAm0452).

Transcriptomic data: Bioproject PRJNA316025 assigned to ATCC PRA-259 strain (Tekle et al., 2016), which is by origin the same strain by Rolf Michel.

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