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Testate amoeba *Arcella vulgaris* (Amoebozoa, Arcellidae) is able to survive without the shell and construct a new one

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Summary

Testate amoeba *Arcella vulgaris* normally reproduces by binary fission, resulting in formation of two shelled specimens. However, the process of a new shell construction may not result in the cell division. Formation of an empty shell and construction of a new shell to fill it in, leaving the old one empty (exuviation) are known (both not followed by the division of the cytoplasmic body). In the present study the ability of *A. vulgaris* deprived of the shell to construct a new one was demonstrated. The approximate time until the detection of the newly constructed shell ranged from 4 to10 days; in most cases the newly formed shell enclosed the entire amoeba cell, but in two cases amoebae formed smaller shells enclosing only a portion of the cytoplasm. Newly formed shells usually had aberrant shape, but cultured descendants of such specimens restored normal shell morphology in successive generations. We presume a certain stabilizing role of the mother shell in the normal division process. The cell damage in our experiments may have contributed to structural aberrations as well.

Key words: testate amoebae, *Arcella vulgaris*, shell regeneration

Introduction

Testate lobose amoeba *Arcella vulgaris* Ehrenberg, 1830 belongs to the order Arcellinida Kent, 1880, family Arcellidae Ehrenberg, 1832. This organism is known from different freshwater habitats, such as periphyton or upper layer of the bottom sediment of ponds and lakes (Leidy, 1879; Pénard, 1902; Deflandre, 1928; Escobar et al., 2008). It can be found in river plankton (Lansac-Tôha et al., 2000) and sphagnum bogs (Corbet, 1973; Bobrov et al., 2002). Normally *A. vulgaris* is a binucleate amoeba with a dome-shaped shell; an aperture is situated at the top of a funnel-like ventral depression and is encircled by a collar, which continues the shell wall (Fig. 1, a, b). The shell wall is composed of a monolayer of proteinaceous alveoli. The major component of these alveoli is a keratin-like protein (Moraczewski, 1969).

Arcella vulgaris reproduces by binary fission, resulting in the formation of two shelled specimens. Prior to division, the cell accumulates in the cytoplasm the predecessors of alveoli – organic membrane-bounded building units, so-called

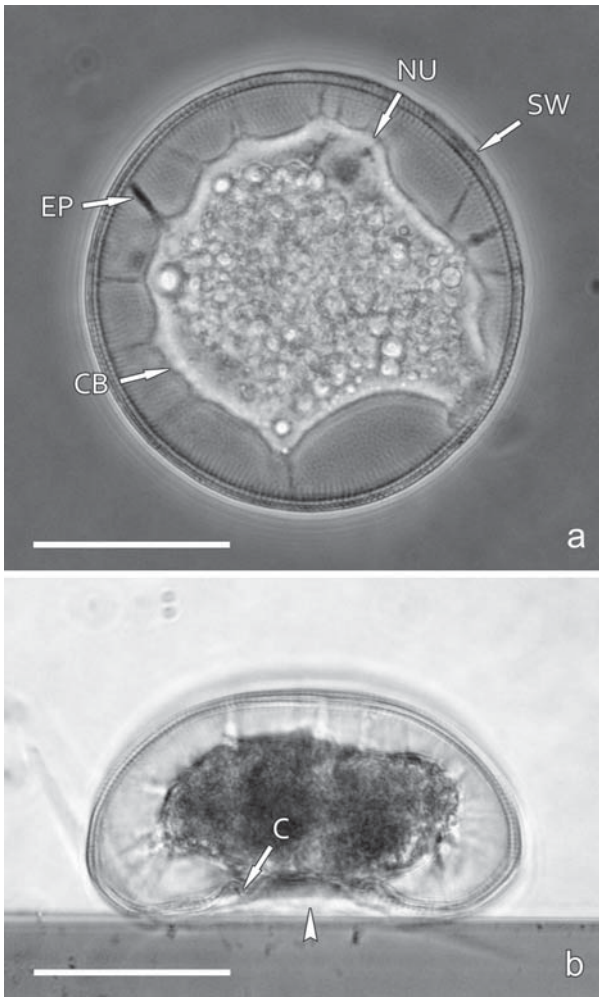

Fig. 1. Testate amoeba *Arcella vulgaris*. a – Dorsal view, b – lateral view. *Abbreviations*: C – collar, CB – cell body, EP – epipodium, NU – nucleus, SW – shell wall; arrowhead marks an aperture (opening in the shell). Scale bars = 50 μm.

thecagenous granules (Netzel, 1975). At the beginning of cell division, the cell withdraws pseudopodia and produces a large cytoplasmic protrusion called the thecagenous bud, through the aperture. Thecagenous granules migrate into this bud and form a monolayer beneath its membrane. The cell secretes the material of thecagenous granules by exocytosis; on the surface of the bud this material converts into alveoli. About four minutes later, the cell produces a circular dome-like pseudopodium, surrounding the thecagenous bud. This pseudopodium takes part in the shaping of the shell wall from the outer side, cooperating with the thecagenous bud. The newly formed shell and the old one are connected to each other with the tubular clasp. This clasp passes through both apertures and anchors their margins (Mignot and Raikov, 1990). Nuclear division (closed orthomytosis) takes place only after formation of the new shell (Raikov and Mignot, 1991). Two of four nuclei migrate into the daughter cell and the clasp between shells breaks. The entire process takes ca. 26 min. (Netzel and Heunert, 1971); of this time, the shell construction takes ca. 10 minutes (Netzel, 1975).

In testate amoebae, two ways of shell construction by intact specimens independently of cell division were described (Pénard, 1902; Hegner, 1919; Hegner, 1920). The organism can form a new shell in conventional manner and fill it in leaving the old one empty. This phenomenon is referred to as exuviation (Pénard, 1902). During this process nuclei do not divide (Charret, 1963; 1964). Alternatively, the new shell may be casted off empty. Hegner (1919) mentions empty shell formation in about 1% of cells in the culture of *A. dentata*. He noted also the formation of empty shells by descendants of dissected *A. dentata* specimens, which was accompanied by the nuclear division (Hegner, 1920).

During his studies of the relation between the nuclear number, chromatin mass, cytoplasmic mass, and shell characteristics in some species of the genus *Arcella*, Hegner (Hegner, 1920) dissected *A. dentata, A. polypora* and *A. discoides* (Fig. 2, b). He found that dissected specimens containing at least one nucleus were able to produce a vital clone of amoebae with normal shell morphology. The study of the multiplication of dissected amoebae was not among Hegner's objectives. His description of this phenomenon was limited to collocation "the offspring of the (dissected) specimen" (Hegner, 1920). It is not clear from his records what happened with the dissected mother cell during multiplication. Thus, the ability of *Arcella* species to restore the shell after dissection was not proved.

In the present study we show the ability of *A. vulgaris* to construct a new shell after depriving of the old one and describe some details of this phenomenon.

Material and methods

Arcella vulgaris was isolated on 28th August, 2006 from the Lake Leshevoe (Valamo Island, the Lake Ladoga, North-West Russia). The sample was collected from the upper layer of the bottom sediment at the depth of about 40 cm. The strain was maintained in 90 mm Petri dishes filled with

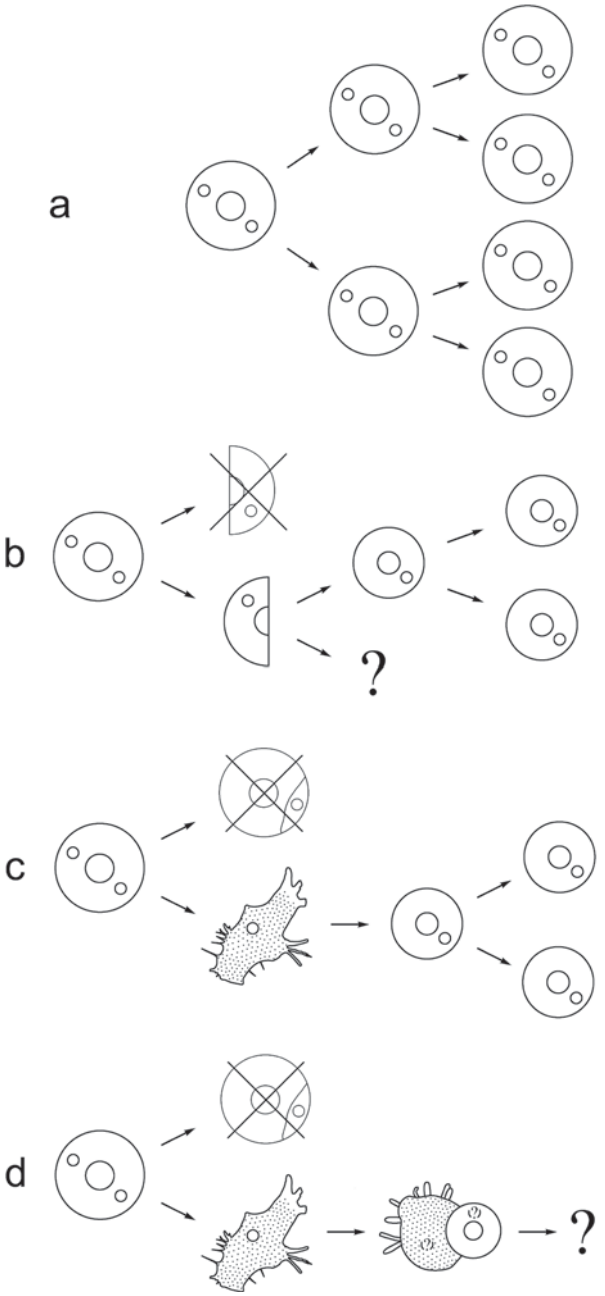

Fig. 2. Comparative diagram of normal binary fission (a), division of dissected amoeba with one nucleus in Hegner's (Hegner, 1920) experiment (b), and construction the shell anew in our experiments (c, d); in our experiments either the cell body covered itself by the shell entirely (c), or significant amount of cytoplasm could be found outside of the newly formed shell (d).

boiled tap water, with two rice grains per dish as a source of nutrients, at room temperature and light according to Raikov et al. (1989). Operations on 300

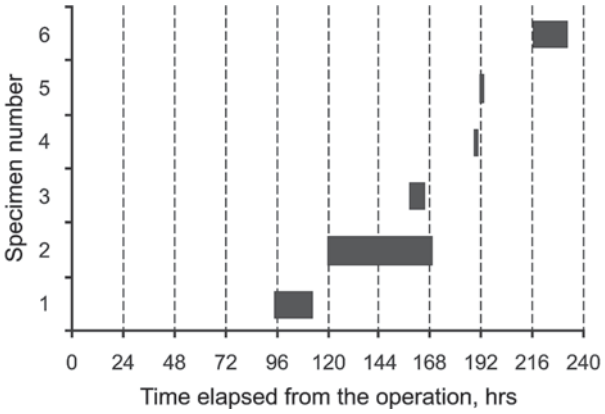

Fig. 3. Time required for treated *Arcella vulgaris* amoebae to prepare themselves to the shell construction. The start time corresponds to the last observation of the cell in the naked condition and the end time – to the detection of the newly formed shell.

amoebae were performed under MBS-9 dissecting microscope (Russia) at 28× magnification. During the procedures, 247 specimens were destroyed and 53 were taken in the experiment. To liberate the cytoplasmic body of amoeba from the shell, the cell was oriented perpendicularly to the bottom of the culture dish, and gently squashed with a fine glass needle. The major part of the cytoplasm was squeezed out from the shell; however, some amount unavoidably remained in the crumpled shell, so the squeezed substance represented only a fragment of the amoeboid cell (Fig. 2, c, d). Cell fragments were transferred to 40 mm Petri dishes and further monitored using inverted phase contrast microscope Nikon Eclipse TS100-F (Japan) and photographed using Nikon DS-Fi1 digital camera.

Results

From the 53 operated specimens nineteen contained at least one nucleus. Ten of these specimens with nuclei died for unknown reasons or were lost; the formation of new shells was traced for six fragments. The approximate time until the detection of the newly constructed shell ranged from 95 hours (4 days) to 233 hours (10 days) (Fig. 3). Two fragments persisted without constructing shell during 14 (Fig. 4, i) and 28 days respectively. Specimens successful in shell construction as well as those persisted for 2-4 weeks, possessed 1-2 nuclei. Details cannot be provided because of numerous cytoplasmic inclusions that hampered observations.

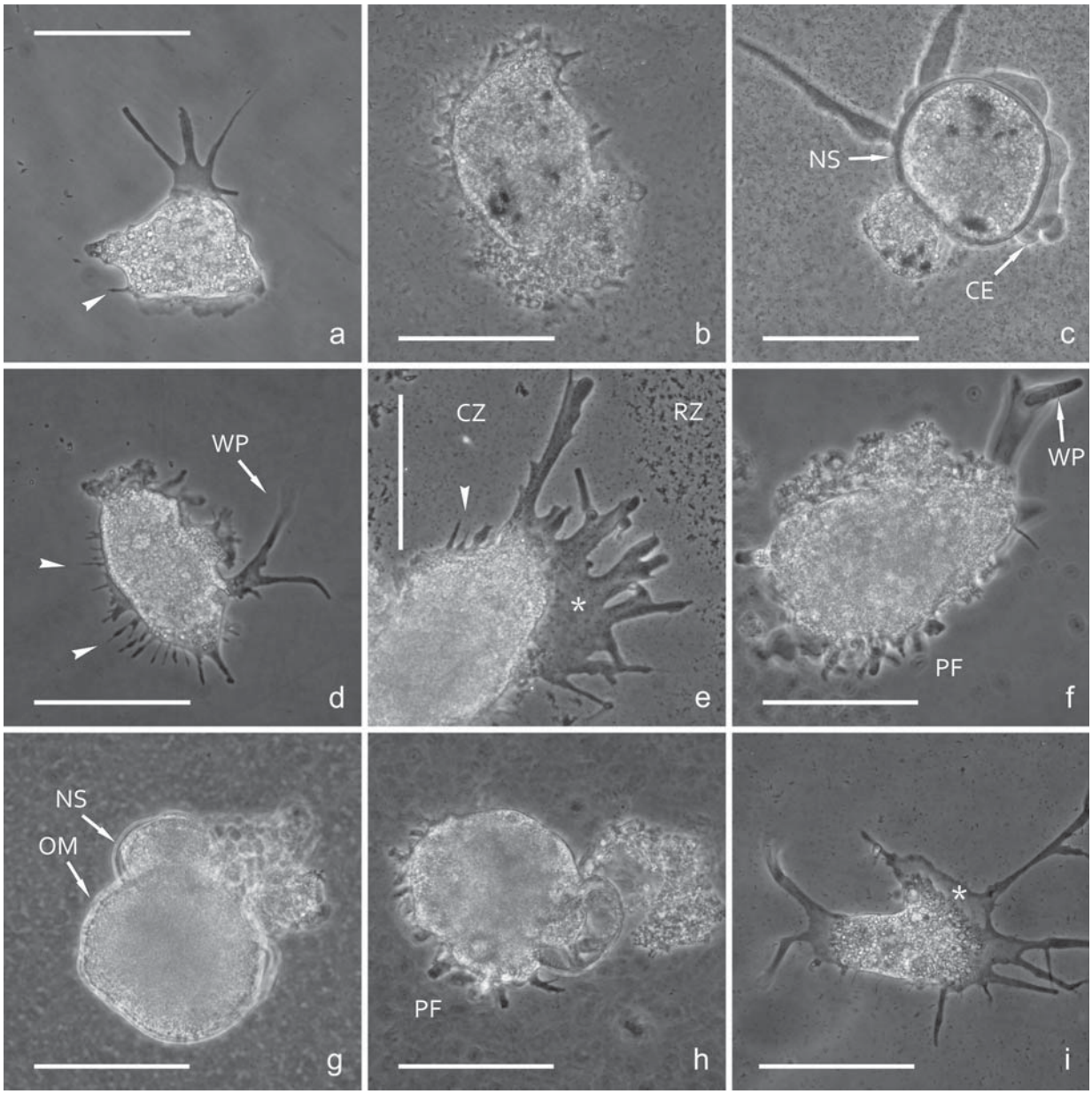

Fig. 4. Shell restoration by *Arcella vulgaris*. a-c – The first type, resulted in complete covering of cell body by the newly formed shell; a – cell fragment 14 hours after operation, b – 6 days 14 hrs after operation and 7,5 hr before the new shell detection, c – newly formed shell; d-h – the second type, resulted in incomplete covering of the living organism by the newly formed shell, d – 17,5 hours after operation, e – 7 days 17 hrs after operation, f – contracted form 7 days 22,5 hrs old and 1,5 hr before the new shell detection, g – the first detection of the newly formed shell, note the absence of pseudopodia, h – 2,5 hr after the shell detection, i – fourteen-day fragment that did not develop a test (was removed from the experiment). *Abbreviations*: CE – cytoplasmic eruption, CZ – the zone free from the microorganisms, NS – the newly formed shell, OM – outer cytoplasmic mass, PF – pseudopodial fringe, RZ – microorganism-rich zone, WP – waving projection. Arrowheads mark epipodia-like projections. Asterisks mark lamellipodium-like structures. Scale bars = 100 μm.

Soon after squeezing out of the shell, the naked cell fragments became flattened and expanded (Fig. 4, a, d). The majority of them formed remarkable hyaline lobes on the common lamellipodium-like base (Fig. 4, e, i). These lobes were rather dynamic; cells continuously retracted and protruded them. The bottom of the Petri dish was free from bacteria at the area that was within reach of these lobes,

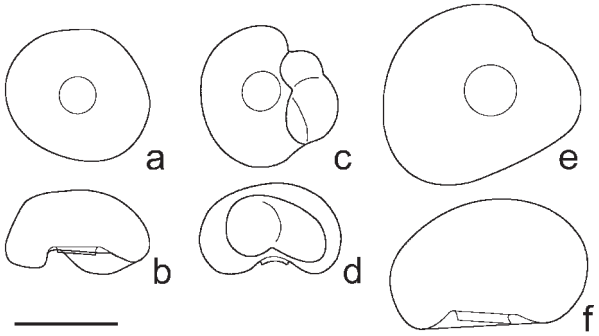

Fig. 5. Shells formed anew by the treated *Arcella vulgaris* – dorsal view (a, c, e) and lateral view (b, d, f), respectively. Scale bar = 50 μm.

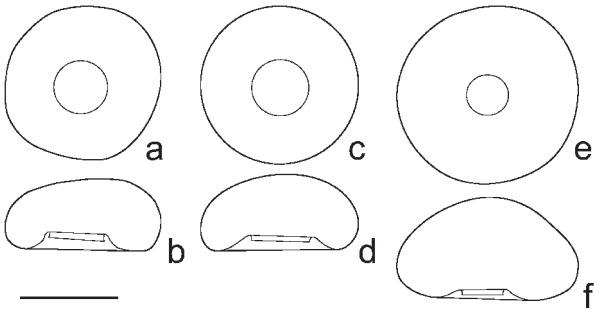

Fig. 6. *Arcella vulgaris*, three descendants of the specimen that constructed the shell anew (alcohol fixation of the 11th day culture); dorsal view (a, c, e) and lateral view (b, d, f), respectively. Scale bar = 50 μm.

though we did not observe any relocation of the main cytoplasmic mass of cells with such a pseudopodial activity (Fig. 4, e). Besides hyaline lobes oriented in the plane of the Petri dish bottom, we observed projections that performed waving movements in the culture medium (Fig. 4, d, f). The adhesion of floating microorganisms by waving projections was not observed. Fragments contracted at the bottom of the Petri dish (Fig. 4, b, f) presented distinct pseudopodial activity. These fragments might have a pseudopodial fringe consisting of a group of short pseudopodia, apparently not taking part in the movement or feeding (Fig. 4, f, h). Like intact cells of *A. vulgaris,* the treated ones withdrew pseudopodia before the shell construction and formed them after it (Fig. 4, g, h). After the construction of a new shell, amoebae could show cytoplasmic eruptions (Fig. 4, c). Our efforts to capture the process of the shell formation were unsuccessful, possibly due to the effect of excess illumination disturbance on amoebae.

The formation of a new shell could follow two different ways. In five cases the newly formed shell enclosed the entire cell body – "way (1)" (Figs 2, c; 4, a-c). However, in two cases, the newly formed shell was relatively small, and the main portion of cytoplasm remained outside of it – "way (2)" (Figs 2, d; 4, d-h).

Both construction methods resulted in aberrant shell forms (Fig. 5). Shell 1 (Fig. 5, a, b) has lobe-like projections on the ventral side. Shell 2 (Figs 4, h; 5, c, d) has an additional structure formed by alveolar material. Shells 2 (Figs 4, h; 5, c, d) and 3 (Fig. 5, e, f) are not circular in the apertural view. Amoebae following the way (1) were able to divide and form a vital culture. In these cultures normal morphology of the shell was eventually restored (Fig. 6). Amoebae following the way (2) never divided further and died.

Discussion

The ability of naked *Arcella* cells to construct new shells was supposed by many authors and was attributed to different stages of the suggested complex life cycle of *Arcella* (Awerintzew, 1906; Swarczewsky, 1908; Cavallini, 1926). The existence of such a complex life cycle (and ability of naked cells to construct a shell *de novo*) has never been confirmed by modern studies. Hence, this report is the first one describing the shell construction by naked *A. vulgaris* cells.

Some pseudopodial structures formed by the naked cells of *A. vulgaris*, were similar to those of intact amoebae. For example, hyaline lobes (which apparently are feeding structures) were documented by Netzel (1971) for *A. vulgaris* var. *multinucleata.* Furthermore, pseudopodial fringe was a common trait in the movement of untreated amoebae in our cultures. On the contrary, we did not observe cytoplasmic eruptions in intact amoebae of the strain under consideration, but found them in those shelled specimens, that constructed the shell anew. Deflandre (1928), enumerating types of *Arcella*'s pseudopodia, did not mention anything similar to these eruptions as well. Diverse and sometimes specific pseudopodial activity of the treated *A. vulgaris* cells probably indicated different cytoskeletal alterations as compared to the intact amoebae.

Many researchers observed aberrant shells in *Arcella* cultures (Reynolds, 1923; Jollos, 1924). In Hegner's experiments, first descendants of dissected specimens had shells of deviant morphology (Hegner, 1920). Newly formed shells in our experiments exhibited morphological shifts as well. Chardez (1956) considered the culture medium depletion as a reason for aberrations of the shell morphology in

cultured *Arcella hemispherica*. As to abnormalities in the shells formed anew, we can presume a certain stabilizing role of the mother shell in the normal division process. Mechanical stress is known to cause wide variety of biological consequences (Braam and Davis, 1990; Barbee, 2005; Wang and Thampatty, 2006). For example, in response to mechanical stretch human trabecular meshwork cells alter their actin cytoskeleton organization (Tumminia et al., 1998) and cytoskeletal gene expression (Luna et al., 2009). Thus, we can suggest malfunction of the molecular cell machinery due to harsh treatment as the cause of morphological aberrations in our experiments.

Our results indicate that the shell is not essential for testate amoebae survival since some treated specimens were able to reproduce. Nevertheless, we did not observe multiplication of naked cells. Moreover, specimens successful in new shell construction divided only in the shelled stage. Thus, we presume shell presence to be crucial for the cell division. However, our data are insufficient for making firm conclusions. Further research is needed to clarify this issue.

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