ISSN 1990-519X, Cell and Tissue Biology, 2015, Vol. 9, No. 2, pp. 158–165. © Pleiades Publishing, Ltd., 2015.
Original Russian Text © M.A. Berdieva, L.V. Chistyakova, O.A. Miteva, A.O. Frolov, A.V. Goodkov, 2015, published in Tsitologiya, 2015, Vol. 57, No. 1, pp. 62–69.

A Light and Electron Microscopic Study of *Pelomyxa secunda* (Gruber, 1884) Comb. Nov. (Archamoebae, Pelobiontida)

M. A. Berdieva[a], L. V. Chistyakova[a], O. A. Miteva[b], A. O. Frolov[b], and A. V. Goodkov[c]

[a] *St. Petersburg State University, St. Petersburg, Russia*
[b] *Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia*
[c] *Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia*
e-mail: maria.berd4@yandex.ru, pelgood1@gmail.com

Received August 18, 2014

Abstract—The morphology of the *Pelomyxa secunda* (Gruber, 1884) comb. nov. pelobiont was studied at light and ultrastructural levels. The locomotive forms are oblong and cigar-shaped. The size range of moving specimens constitutes from 200 to 300 μm. Larger specimens reaching 400 μm are not capable of directed movement. At the sides of the body and at the frontal end, small hyaline pseudopodia could be formed most often that were finger-shaped. The cellular coat is represented by amorphous glycocalyx with a thickness of up to 300 nm. A thin layer of the peripheral cytoplasm without any organelles, vacuoles, endocytobionts, and other inclusions, which is separated from the rest of the cytoplasm by a layer of microfilaments, is below the plasmalemma. *P. secunda* has two species of obligate prokaryotic endocytobionts located in symbiontophoric vacuoles. Granular nuclei and nucleosomal material are represented by discrete structures of two types differing in size and electron density. The external membrane of the nuclear envelope has from two to three layers of short microtubules parallel to each other and to the nucleus surface. Undulipodia, kinetosomes, and their root derivatives have not been observed.

Keywords: Archamoebae, pelobionts, *Pelomyxa*, morphology, ultrastructure
DOI: 10.1134/S1990519X15020029

Pelomyxa secunda (Gruber, 1884) is a eukaryotic microorganism from the Pelomyxidae Schulze, 1877 family that is part of the composition of the Archamoebae group (Ptáčková et al., 2013). Representatives of the *Pelomyxa* genus are free-living anaerobic protists with amoeboid cellular organization possessing in most cases a varying number of immobile or slow-moving flagella, which are not involved in cellular locomotion; their life cycle, as a rule, includes a multinuclear stage (Griffin, 1988; Goodkov et al., 2004; Frolov, 2011; Ptáčková et al., 2013; Chistyakova et al., 2014).

In 1874, Greeff described a type species of the *Pelomyxa* genus, *P. palustris* (Greeff, 1874); numerous studies devoted to the description of other species of amoeboid organisms with similar organization related to have since *Pelomyxa* appeared. As a result, at the turn of the 20th century, the *Pelomyxa* genus contained more than 20 independent species (see Goodkov et al., 2004), with many descriptions having been made based on single and rather incomplete observations and having been low informative and vague. Thus, it is not surprising that, already at that time, researchers expressed doubts regarding the systematic validity of a number of such species (see, for example, Gruber, 1884; Penard, 1902).

In the second half of the 20th century, a tendency toward reduction in the number of species included in the composition of the genus prevailed due to their synonymization with the type species *P. palustris*. In the end, for a rather long time, the notion of the monotypic nature of the *Pelomyxa* genus prevailed in the literature (Page, 1976; Whatley, Chapman-Andresen, 1990), its inconsistency having been proven rather recently. During the last decade, ten *Pelomyxa* species have been found in northeastern Russia. Six of them (including the type species), *P. palustris*, *P. prima*, *P. belevskii*, *P. tertia*, *P. binucleata*, and *P. paradoxa*, are species known earlier that were reisolated, identified, and redescribed at the state-of-the-art level (Frolov et al., 2005a; Frolov et al., 2005b; Frolov, 2011; Chistyakova et al., 2014). The other four species, *P. corona*, *P. stagnalis*, *P. gruberi*, and *P. flava*, were found to be new (Frolov et al., 2004, 2006; Frolov et al., 2010; Chistyakova and Frolov, 2010). The results of these studies clearly indicate that there is an urgent need for a complete reinvestigation of the species diversity of representatives of the *Pelomyxa* genus.

The present study presents the results of light and electron microscopic studies of the morphology of *Pelomyxa secunda*. This organism was described by

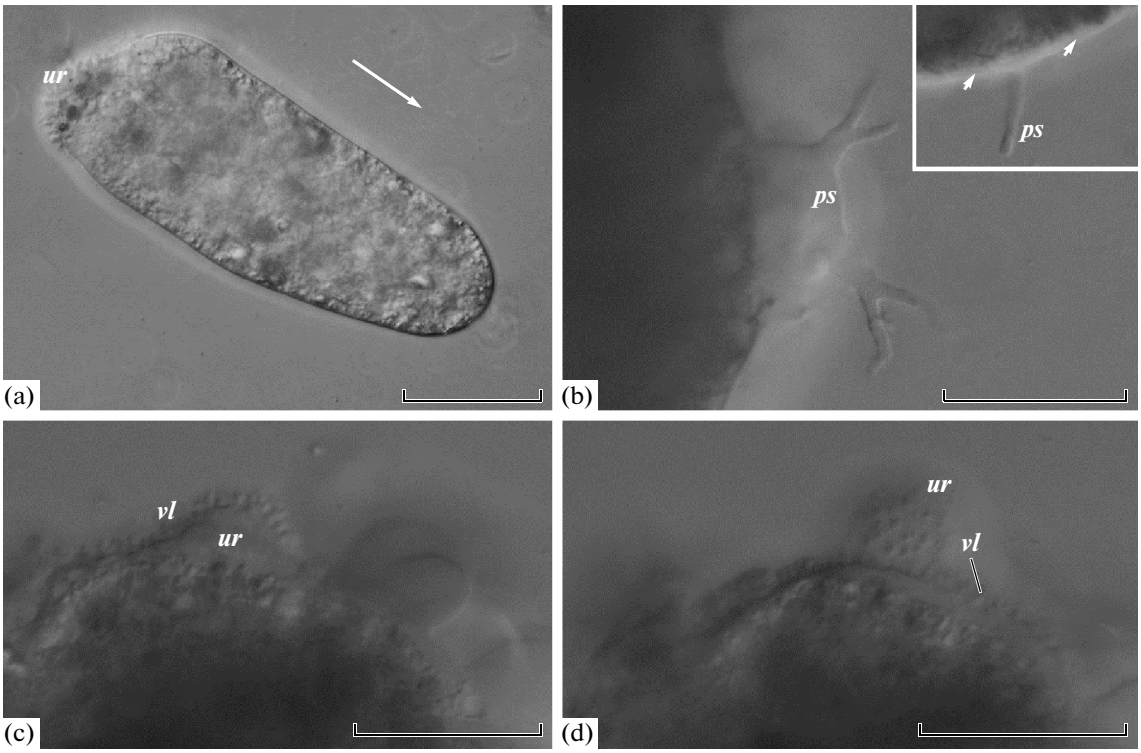

Fig. 1. Organization of locomotive forms of *Pelomyxa secunda* at light level.
(a) The cell during directed locomotion, the arrow indicates the direction of the movement; (b) small hyaline pseudopodia (*ps*) formed at the frontal end and in the sides of the body (insertion); (c) and (d) uroid (*ur*) and adjacent regions of the body surface covered with numerous hyaline villi (*vl*). Differential interference contrast. Scale bars: (a) 100 and (b–d) 50 μm.

Gruber in 1884 as *Amoeba secunda* and hardly mentioned in the subsequent literature.

MATERIALS AND METHODS

Material was collected in summer through autumn of 2010–2013. Samples were taken in the Tseratofillievyi pond (Sergievka park, Old Peterhof, St. Petersburg) and several small water bodies on the territory of Pskov oblast. The biotopes mentioned were chosen in accordance with the most typical habitat conditions for pelomyxes, i.e., freshwater stagnant water bodies with a large content of decomposing phytogenous organic material (see Goodkov et al., 2004).

Samples in glass and plastic vessels filled with water and containing detritus from the collecting ground were stored in a cooler at 4°C. Light microscopic studies of protists were conducted using a Leica DM2500 microscope with differential interference contrast and Nikon DS-Fi1 digital camera.

Samples for studying the ultrastructure were prepared in accordance with a protocol adapted for pelomyxes using glutaraldehyde-osmic fixation and Epon-Araldite embedding (Frolov et al., 2004; Chistyakova and Frolov, 2010). Sections were prepared using an Ultracut E Reichert ultramicrotome (England) and after staining examined under Jeol-1400, Tesla BS-500, and Zeiss Libra 120 microscopes.

RESULTS

Organisms that were undoubtedly related to the *Pelomyxa* genus, but, however, different from all the other *Pelomyxa* species recognized as systematically valid at present were found in the samples among sand and detritus particles.

Light microscopy. Cells actively moving, their shape is significantly oblong up to cigar-shaped (Fig. 1a). Uniform axial flows of the endoplasm are seen. The size of moving specimens is from 200 to 300 μm. Sometimes, larger cells are observed reaching 400 μm, but such specimens hardly move along the substrate. In the periphery of the cell, a narrow layer of transparent hyaline cytoplasm with a width of up to 3–5 μm is present (Figs. 1b, 1d). In the sides of the cell and in the anterior part, small finger-shaped hyaline pseudopodia, sometimes branching in the former case, could be formed (Fig. 1b).

In the posterior part of the cell, in actively moving organisms, an uroid with a bulblike shape is morphologically clearly observed (Figs. 1c, 1d). The uroid surface and quite often neighboring parts of the cell have

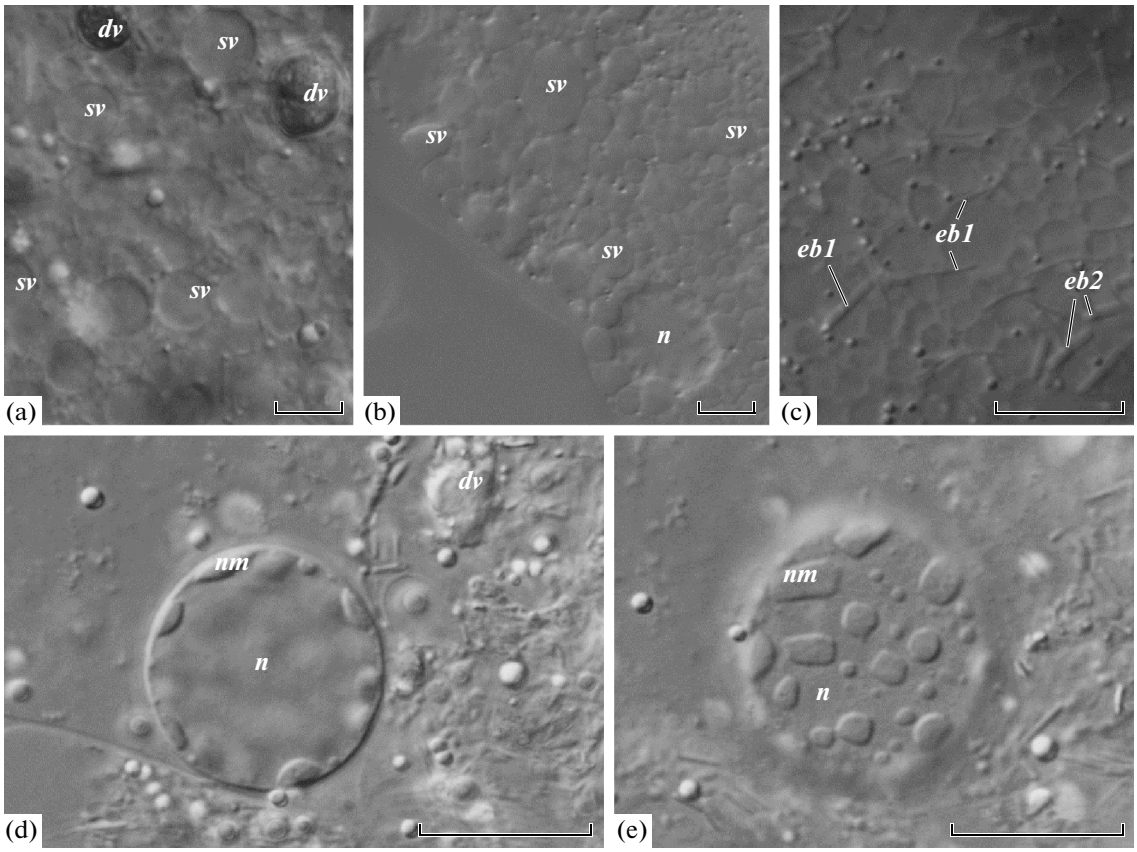

Fig. 2. Details of cell structure of *Pelomyxa secunda* at light level: (a–c) cytoplasm organization and (d, e) nuclei. Designations: *sv*, structural vacuoles; *dv*, digestive vacuoles; *eb1* and *eb2*, bacterial endocytobionts of two species; *nm*, nucleosomal material; and *n*, nucleus. Differential interference contrast. Scale bars: 10 μm.

numerous short branching hyaline protuberances, villi, present (Figs. 1c, 1d).

A significant part of the cytoplasm content is optically empty vacuoles, so-called structural vacuoles with a different diameter (see Andresen et al., 1968; Goodkov, Seravin, 1991) (Figs. 2a–2c). However, they often are barely discernible in intact cells—in particular, in small forms—since the majority of the inner volume of the cell is occupied by large and small digestive vacuoles containing detritus particles, different algae, and mineral granules (Fig. 2a). Due to the significant amount of detritus ingested, the cells of the organisms studied are brownish. The *Pelomyxa* cytoplasm has a great number of small (about 3 μm) rod-like prokaryote endocytobionts present—most probably, of two species (Fig. 2c).

The pelomyxes studied are multinucleated organisms. The number of the nuclei varies from several nuclei up to 20 depending on the cell size. The nuclei are spherical and of granular type, with the diameter being from 13 to 18 μm (Figs. 2b, 2d, 2e). Nucleosomal material is fragmented and represents relatively small oval and of irregular shape granules of different sizes located along the nucleus periphery (Figs. 2d, 2e).

Flagella have not been observed using light microscopy.

Electron microscopy. The outer surface of the plasma membrane of the cell has a thin layer of amorphous glycocalyx with a thickness reaching 0.3 μm (Figs. 3a, 3b). There is a zone of the peripheral cytoplasm without any organelles, endocytobionts, and other inclusions under the plasmalemma. The thickness of this zone is from 0.5 to 2 μm. It is clearly separated from the remaining cytoplasm by a layer of bundles of actin microfilaments parallel to the cell surface of the *Pelomyxa* (Figs. 3a, 3b).

The majority volume of the cytoplasm is occupied by electron transparent structural vacuoles of different sizes (from those smaller than 1 up to those with the diameter of 6–8 μm), numerous vacuoles filled with different inclusions, and digestive vacuoles (Figs. 3a, 3c, 3e).

Small rounded bodies with a diameter of 1–1.5 μm filled with homogeneous content of average electron density and resembling lipid inclusions are often found in the cytoplasm (Fig. 3c). Moreover, there are larger bodies of irregular shape with a characteristic granular structure with a size of about 2.5 μm (Fig. 3d). The latter most probably represent a form of so-

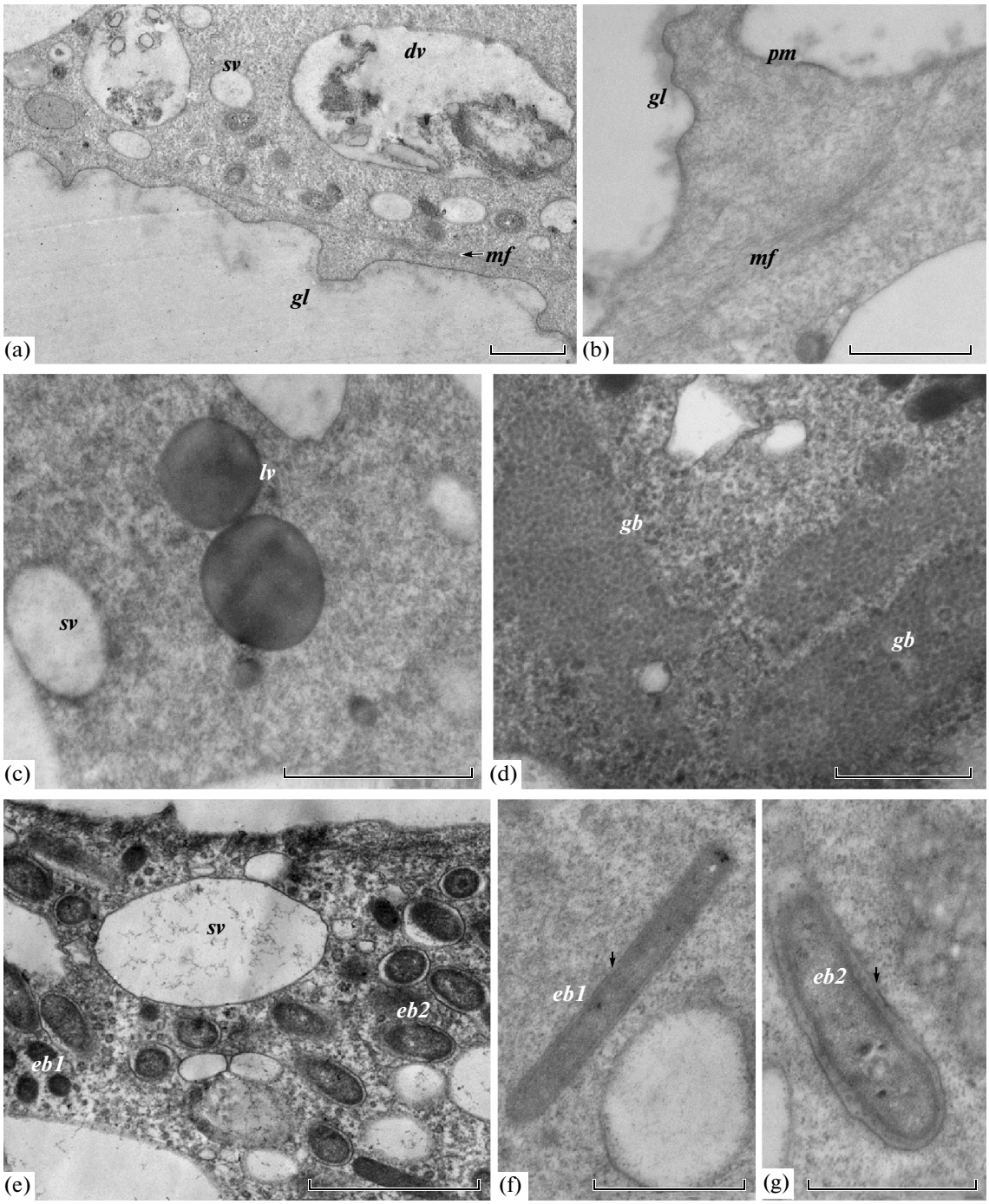

Fig. 3. Ultrastructure of *Pelomyxa secunda*.
(a, b) Peripheral regions of the body; (c), (d) characteristic cytoplasmic inclusions; (e–g) bacterial endocytobionts in the cytoplasm. Designations: *gl*, glycocalyx; *gb*, glycogen bodies; *lv*, lipid vesicles; *mf*, microfilament layer; *pm*, plasma membrane; *sv*, structural vacuoles; *dv*, digestive vacuoles; and *eb1* and *eb2*, bacterial endocytobionts of two species. Scale bars: (a), (c), (e) 2 and (b), (d), (f), (g) 1 μm.

called glycogen bodies typical for many representatives of the *Pelomyxa* genus that are storage compounds (see Andresen at al., 1968; Daniels, 1973; Whatley, 1976; Frolov, 2011).

The cytoplasm of the organisms studied has a sufficient amount of prokaryotic endocytobionts of two species located in individual symbiontophoric vacuoles (Figs. 3e–3g). The first species includes long thin

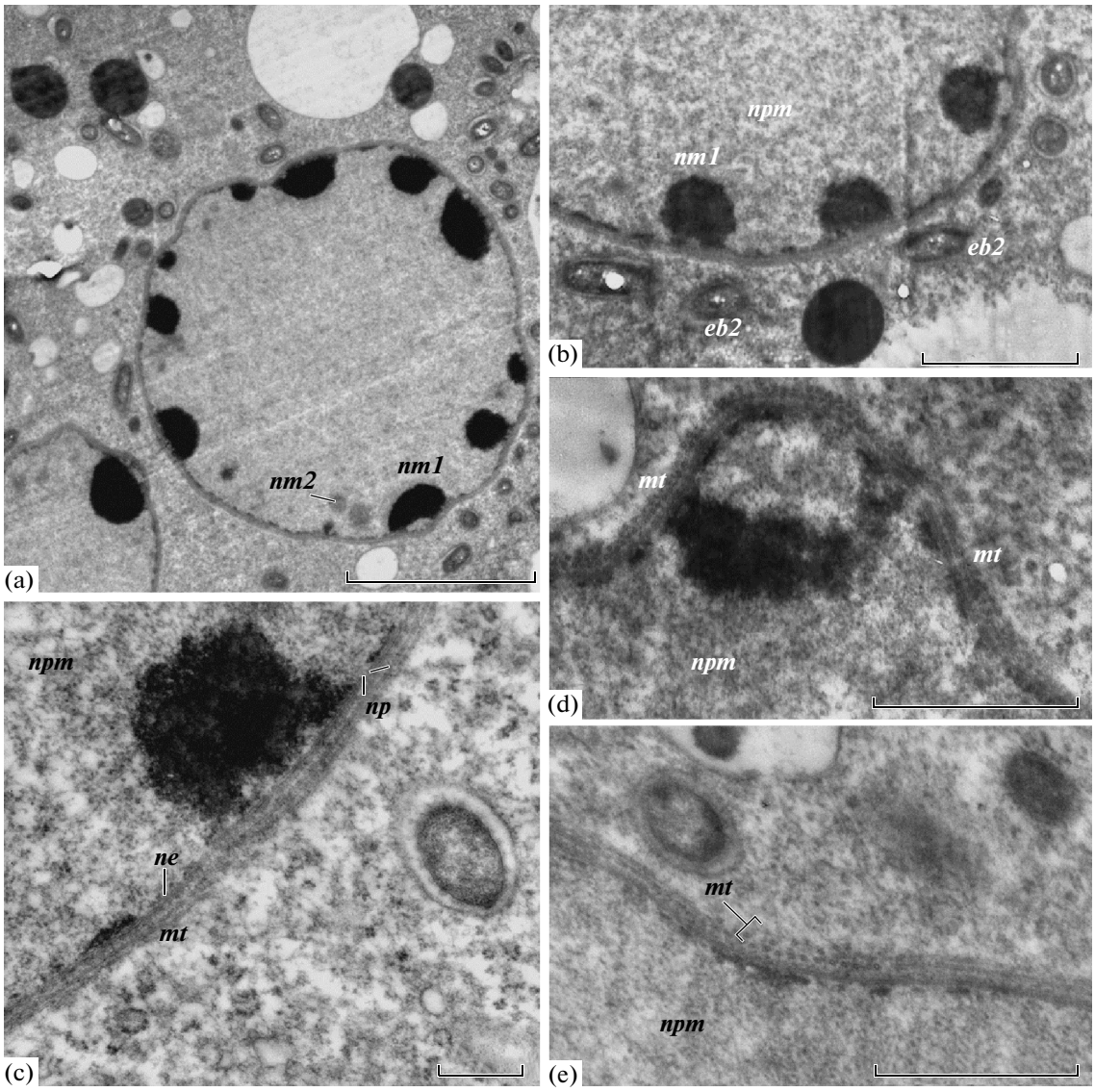

Fig. 4. Ultrastructural organization of *Pelomyxa secunda* nuclei.
(a) Cross section of the nucleus; (b) a peripheral region of the nucleus and aggregation of endocytobionts around the nuclear envelope; and (c–e) organization of the nuclear envelope. Designations: *nm1* and *nm2*, nucleosomal material of high and low electron density, respectively; *npm*, nucleoplasm; *mt*, microtubules; *eb2*, endocytobionts of the second species; *ne*, nuclear envelope; and *np*, nuclear pore (arrows). Scale bars: (a) 5, (b) 2, and (d, e) 1 μm and (c) 500 nm.

rodlike bacteria with dense bacterioplasm. They are 2–3 μm long with a diameter of the cross section of 0.2–0.3 μm and tightly adjacent to the membrane of the symbiontophoric vacuole (Fig. 3f). The morphology of these bacteria resembles that of methanogenic bacteria often found in the cytoplasm of pelomyxes and other species of free-living microaerobic protists (see van Bruggen et al., 1983, 1988; Vogels et al., 1984).

The other species of the endocytobionts represents less oblong and thicker rodlike forms (1–2 μm long with a diameter of up to 0.8 μm) (Fig. 3g). These bacteria are relatively freely distributed within the symbiontophoric vacuole, and their bacterioplasm has lower electron density; therefore, it is sometimes possible to observe a structure resembling a nucleoid. As a rule, they are more or less uniformly distributed in the cell cytoplasm; however, small agglomerations of them are sometimes found around the nuclei (see Fig. 4b).

The entire nucleosomal material in the nuclei of the organism studied is concentrated along the nucleus periphery and represented by discrete structures of two types (Fig. 4a). First, these are bodies with high electron density of more or less oval shape with the size of about 1 μm, which are partially adjacent to the inner membrane of the nuclear envelope (Figs. 4a–4c). Second, the nuclei have spherical bodies of significantly smaller sizes and lower electron

density located in the nucleoplasm at a small distance from the nuclear envelope (Fig. 4a). Moreover, there is a thin layer of electron dense material with nonuniform thickness tightly adjacent from inside to the nuclear envelope (Figs. 4b–4e).

Numerous short microtubules having two, and at some regions three, layers located strictly along the nucleus surface are tightly adjacent to the outer membrane of the nuclear envelope (Figs. 4c, 4d, 4e). It should be noted that, except for the perinuclear region, no organized microtubule structures were observed in the cell cytoplasm.

DISCUSSION

The results obtained clearly indicate that the amoeboid organism studied in the present work without a doubt belongs to the *Pelomyxa* genus. At the same time, it could not be identified as one of the ten species of the *Pelomyxa* genus acknowledged to date as systematically valid. All pelomyxes have typical peculiarities of the morphology, which often makes it possible to identify them at light microscopical level (Frolov et al., 2004, 2005a, 2006; Frolov et al., 2005b, 2009, 2010; Chistyakova, Frolov, 2010; Frolov, 2011; Chistyakova et al., 2014). The species-specific features include, in particular, the morphology and sizes of the locomotive form, details of the structure of the nuclear apparatus, a set of the obligate prokaryotic endocytobionts, the presence or absence of flagella, the structure of their basal apparatus, peculiarities of the cytoskeleton organization, etc. The locomotive form of the organism studied is oblong, cylindrical, and, on the whole, typical for many other representatives of the *Pelomyxa* genus; however, there are certain differences in the details. In particular, the organization of the uroid structures and localization and character of formation of the finger-shaped hyaline pseudopodia at the body of the protozoan differ from the corresponding variants found in other *Pelomyxa* species. This organism has amorphous glycocalyx with the thickness reaching 300 nm. Representatives of the *Pelomyxa* genus have three types of the glycocalyx structure including amorphous one (Frolov, 2011). It is present in *P. binucleata* and *P. stagnalis*. However, its thickness in these species does not exceed 5–7 and 15–20 nm, respectively (Frolov et al., 2005a; Chistyakova, Frolov, 2010).

As has been mentioned earlier, relatively large inclusions of irregular shape with a characteristic granular structure sometimes found in the cytoplasm of the organism studied most likely represent glycogen bodies typical for many representatives of the *Pelomyxa* genus (see Andresen et al., 1968; Daniels, 1973; Whatley, 1976; Frolov, 2011). However, in this case, these formations do not have the irregular spherical shape observed in other *Pelomyxa* species. They are not surrounded by cisternae of the ER, and their surface does not contain agglomerations of bacterial endocytobionts, which is typical for some other representatives of the genus.

The presence of obligate bacterial endocytobionts is typical for all representatives of the *Pelomyxa* genus, and their species set is often specific for one or another *Pelomyxa* species. For a long time, it was believed that the cytoplasm of all pelomyxes has large bacteria with a rectangular shape, so-called large-type bacteria with a typical slit-like invagination of the cell wall (Whatley, 1976; van Bruggen et al., 1988; Whatley, Chapman-Andresen, 1990). However, it has been recently shown that the two species *P. stagnalis* and *P. paradoxa* do not have these prokaryotes (Chistyakova, Frolov, 2010; Chistyakova et al., 2014). They are also absent in the species studied in the present work. At the same time, the rodlike endocytobionts of the second species are not observed in other pelomyxes, at least, in such significant amounts.

A surprising peculiarity of representatives of the *Pelomyxa* genus is extreme diversity and species-specific organization of their nuclei. Pelomyxes are observed to have both granular and vesicular nuclei (Daniels et al., 1966; Daniels, Breyer, 1967; Frolov et al., 2004, 2005a, 2006; Frolov et al., 2005b, 2009, 2010; Chistyakova, Frolov, 2010; Frolov, 2011; Chistyakova et al., 2014). However, even in cases in which the nuclei are of the same type, the details of their structure differ significantly. This rule is also applied to the nuclei of the species considered in this study. They are granular, but their characteristic pattern of organization and distribution of the nucleosomal material and other peculiarities are not observed in any known *Pelomyxa* species.

Structural complications of the outer surface of the nuclear envelope are no less diverse and specific in species with granular nuclei (Frolov, 2011). Thus, additional layers, derivatives of channels and vesicles of the endoplasmic reticulum, could be found above the outer nuclear membrane; this could be a layer of short microtubules located directly in the perinuclear region or small vesicles with an electron-dense content. The species that we consider is quite in accordance with this rule: its nuclei are surrounded by two or three tightly adjacent layers of short microtubules parallel to each other and to the surface of the nucleus. *P. flava* also has a layer of ordered microtubules surrounding the nucleus; however, this is the only layer, and, moreover, this species has additional layers of electron-dense material and cisternae of the endoplasmic reticulum (Frolov et al., 2010).

The last thing that should be specially mentioned is the absence of flagella in the species discussed in this study. Neither light optical nor ultrastructural studies revealed undulipodia, basal bodies, and their root derivatives. Moreover, except for the perinuclear region, no traces of organized microtubule structures have been found in the cell cytoplasm.

Only one *Pelomyxa* species without flagella, *P. corona*, has been known to date. Nevertheless, the

cortical region of the cytoplasm of these organisms has large arranged bundles of microtubules located along the body surface of the protozoan, which makes it possible to suggest the existence in them of some microtubule-organizing centers. (Frolov et al., 2004).

Thus, as has been mentioned above, the organism studied in the present work could not be identified as one of the ten *Pelomyxa* species known at present. Nevertheless, the thorough analysis of literature that we performed makes it possible to clearly identify it as the species described by Gruber in the second half of the 19th century and referred to as *Amoeba secunda* (Gruber, 1884). This conclusion could be drawn based on the quite accurate description of this protozoan including characteristic of its locomotive form, peculiarities of formation of the pseudopodia, organization of the nuclear apparatus, and protoplasm content. Observations and images of amoebas and Gruber's data, on the whole, correspond to ours (Figs. 1, 2). It should be noted that the author considered some similarities of this species with pelomyxa-like organisms, which were described by Leidy (Leidy, 1879) as different forms of *Pelomyxa villosa*.

As could be seen from the analysis of the literature, *A. secunda* subsequently was only casually mentioned in two particular publications (Blochmann, 1894; Hollande, 1945) and, in further studies, including ones devoted to analysis of the composition and taxonomic revision of the *Pelomyxa* genus, this species has been completely overlooked by researchers. We reisolated it from nature and identified it for the first time since it was first described in 1884. Thus, *Pelomyxa secunda* is the fourth species of the amoebas described by Gruber (Gruber, 1884) after *P. prima*, *P. binucleata*, and *P. tertia*, which were reisolated and redescribed during the last decade (see Frolov et al., 2005a; Frolov et al., 2005b; Frolov, 2011).

Description of the *Pelomyxa secunda* species (Gruber, 1884) comb. nov. During directed movements, specimens are oblong cigar-shaped, with uniform axial flows of the central endoplasm being noticeable. The length of the locomotive form is from 200 to 300 μm. Larger cells reaching 400 μm are sometimes found, but such specimens hardly move along the substrate. In the sides of the body and at the frontal end, small conic or finger—shaped, sometimes branching, hyaline pseudopodia are formed from time to time. In the posterior part of the cell, a hemispherical uroid covered with numerous branching hyaline villi is morphologically clearly observed. The number of nuclei varies from a few up to 20 depending on the cell size. They are spherical with a diameter of from 13 to 18 μm (on average, 15 μm). The granular nuclei and nucleosomal material concentrated in the nucleus periphery are represented by discrete structures of two types differing in size and electron density. Two, and in some regions three, tightly adjacent layers of short microtubules parallel to each other and to the nucleus surface are present in the outer side of the nuclear envelope. The cytoplasm contains obligate rodlike prokaryotic endocytobionts of two species occupying symbiont-containing vacuoles. Undulipodia, kinetosomes, and their root derivatives have not been observed.

ACKNOWLEDGEMENTS

This study was performed using equipment of the Research Resource Center for Molecular and Cell Technologies, Center for Culture Collection of Microorganisms, and Chromas Core Facility of St. Petersburg State University.

REFERENCES

Andresen, N., Chapman-Andresen, C., Nilsson, J.R., The fine structure of *Pelomyxa palustris*, *Compt. Rend. Trav. Labor. Carlsberg, Ser. Chim.*, 1968, vol. 36, pp. 285–320.

Blochmann, F., Kleine mitteilungen liber protozoen, *Biol. Zentralbl. Bd.,* 1894, vol. 14, pp. 82–91.

Chistyakova, L.V. and Frolov, A.O., Light and electron microscopic study of *Pelomyxa stagnalis* sp. n. (Archamoebae, Pelobiontida), *Cell Tissue Biol.*, 2011, vol. 5, no. 1, pp. 90–97.

Chistyakova, L.V., Berdieva, M.A., Frolov, A.O., and Goodkov, A.V., Reisolation and redescription of pelobiont *Pelomyxa paradoxa* Penard, 1902 (Archamoebae, Pelobiontida), *Cell Tissue Biol.*, 2014, vol. 8, no. 6, pp. 504–512.

Daniels, E.W. and Breyer, E.P., Ultrastructure of the giant Amoeba *Pelomyxa palustris*, *J. Protozool.*, 1967, vol. 14, pp. 167–179.

Daniels, E.W., Breyer, E.P., and Kudo, R.R., *Pelomyxa palustris* Greeff. II. Its ultrastructure, *Zeit. Zellforsch.*, 1966, vol. 73, pp. 367–383.

Daniels, E.W., Ultrastructure, in *The Biology of Amoeba,* New York: Acad. Press., 1973, pp. 125–169.

Frolov, A.O., Order Pelobiontida (Page, 1976) Griffin, 1988, in *Protists*, St. Petersburg: Nauka, 2007, vol. 3, pp. 270–307.

Frolov, A.O., Chistyakova, L.V., and Goodkov, A.V., A new pelobiont protist *Pelomyxa corona* sp. n. (Peloflagellatea, Pelobiontida), *Protistology*, 2004, vol. 3, pp. 233–241.

Frolov, A.O., Chistjakova, L.V., and Goodkov, A.V., Light- and electron-microscopic study of *Pelomyxa binucleata* (Gruber, 1884) (Peloflagellatea, Pelobiontida), *Protistology*, 2005a, vol. 4, pp. 57–73.

Frolov, A.O., Chistyakova, L.V., Malysheva M.N., and Goodkov, A.V., Light and electron microscopic study of *Pelomyxa prima* (Gruber, 1884) (Peloflagellatea, Pelobiontida), *Tsitologiia*, 2005b, vol. 47, no. 1, pp. 89–98.

Frolov, A.O., Goodkov, A.V., Chistjakova, L.V., and Skarlato, S.O., Structure and development of *Pelomyxa gruberi* sp. n. (Peloflagellatea, Pelobiontida), *Protistology*, 2006, vol. 4, pp. 227–244.

Frolov, A.O., Chistyakova, L.V., and Malysheva M.N., Light and electron microscopic study of *Pelomyxa flava* sp. n. (Archamoebae, Pelobiontida), *Cell Tissue Biol.*, 2011, vol. 5, no. 1, pp. 81–89.

Goodkov, A.V. and Seravin, L.N., Ultrastructure of the 'giant Amoeba' *Pelomyxa Palustris*. III. The vacuolar sys-

tem; its nature, organization, dynamics and functional significance, *Tsitologiia*, 1991, vol. 33, no. 9, pp. 17–25.

Goodkov, A.V., Chistyakova, L.V., Seravin, L.N., and Frolov, A.O., The concept of pelobionts (class Peloflagellatea): brief history and current state, *Zool. Zh.*, 2004, vol. 83, no. 6, pp. 643–654.

Griffin, J.L., Fine structure and taxonomic position of the giant amoeboid flagellate *Pelomyxa palustris*, *J. Protozool.*, 1988, vol. 35, pp. 300–315.

Gruber, A., Studien uber Amoben, *Zeitschr.Wissenschaft. Zool.*, 1884, vol. 41, pp. 186–225.

Hollande, A., Biologie et reproduction des rhizopodes des genres pelomyxa et amoeba et cycle evolutif de *Amoeboiphilus destructor* nov. gen. nov. spec, Chytridinee (?) parasite de *Pelomyxa palustris* Greeff. Bui, *Biol. France et Belg.*, 1945, vol. 79, pp. 31–66.

Leidy, J., Freshwater rhizopods of North America, *Rept. US Geol. Surv. Territ.*, 1879, vol. 12, pp. 1–324.

Page, F.C., A revised classification of the Gymnamoebia (Protozoa: Sarcodina), *Zool. J. Linn. Soc.*, 1976, vol. 58, pp. 61–77.

Penard, E., *Faune Rhizopodique du Bassin du Leman*, Genèva: Henry Kündig, Librarie de l'Institut, 1902.

Ptáčková, E., Kostygov, A.Y., Chistyakova, L.V., Falteisek, L., Frolov, A.O., Walker, G., Patterson, D.J., and Cepicka, I., Evolution of Archamoebae: morphological and molecular evidence for pelobionts including *Rhizomastix, Entamoeba, Iodamoeba*, and *Endolimax*, *Protist*, 2013, vol. 164, pp. 380–410.

Van Bruggen, J.J.A., Stumm, C.K., and Vogels, G.D., Symbiosis of methanogenic bacteria and sapropelic protozoa, *Arch. Microbiol.*, 1983, vol. 136, pp. 89–95.

Van Bruggen, J.J.A., van Rens, G.L.M., Geertman, E.G.M., Stumm, C.K., Zwart, K.B., and Vogels, G.D., Isolation of a metanogenic endosymbiont of the sapropelic amoeba *Pelomyxa palustris* Greeff, *J. Protozool.*, 1988, vol. 35, pp. 20–23.

Vogels, G.D., Van, der, Drift, C., Stumm, C.K., Keltjens, J.T.M., and Zwart, K.B., Methanogenesis: surprising molecules, microorganisms and systems, *Antonie Van Leeuwenhoch*, 1984, vol. 50, pp. 557–567.

Whatley, J.M. and Chapman-Andresen, C., Phylum Karyoblastea, in *Handbok of Protoctista,* Boston: Jones and Bartlett Publishers, 1990, pp. 167–185.

Whatley, J.M., Bacteria and nuclei in *Pelomyxa palustris*: comments on the theory of serial endosymbiosis, *New Phytol.*, 1976, vol. 76, pp. 111–120.

Translated by E. Berezhnaya