

ORIGINAL ARTICLE

Rediscovery and redescription of *Pelomyxa quarta* (Gruber, 1884) comb. nov. (Archamoebae, Pelobiontida): another pelomyxa rescued from oblivion

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Summary

Pelomyxa quarta was re-isolated firstly after its description by A. Gruber in 1884 as *Amoeba quarta*. This pelomyxa species was found in samples of bottom sediments from the Osinovskoe Lake (North-West of Russia) and investigated using methods of light, immunofluorescent and transmission electron microscopy. *P. quarta* has a complex of features that are characteristic for representatives of the genus *Pelomyxa*: amoeboid movement, many immotile flagella, multinuclearity, vacuolized cytoplasm, and presence of prokaryotic endobionts and agglomerations of reserve nutrients in cells. This species clearly differs from the known pelomyxes in the organization of its nuclei, in which a compact nucleolar mass is located eccentrically.

Key words: Archamoebae, immunofluorescent staining, light microscopy, microtubular cytoskeleton, *Pelomyxa*, species diversity, ultrastructure

Introduction

Pelomyxae are common inhabitants of the anaerobic zone of bottom sediments in various freshwater bodies that may have natural or artificial origin and are often located in urban areas (Frolov, 2011). The greatest diversity of *Pelomyxa* spp. is observed in ponds and small lakes where the bottom is covered with an undisturbed and unmixed layer of plant residues, including leaf litter. At least half of the known *Pelomyxa* spp. have been found in stagnant ponds of various urban parks (Greeff, 1874; Frolov et al., 2005; Chistyakova and Frolov, 2011; Ptáčková

et al., 2013; Chistyakova et al., 2014; Berdieva et al., 2015; Chistyakova et al., 2021, 2022).

Fourteen valid *Pelomyxa* spp. are currently known. About twenty others have never been re-isolated after the first description (Goodkov et al., 2004), and the validity of some of them is doubtful. Meanwhile, the descriptions by some early authors are so complete and detailed that there are good reasons to suppose that these organisms really exist in nature. For instance, August Gruber in his *Studien über Amöben* described several amoeboid protists from various water bodies of southern Germany (Gruber, 1884), attaching primary importance to

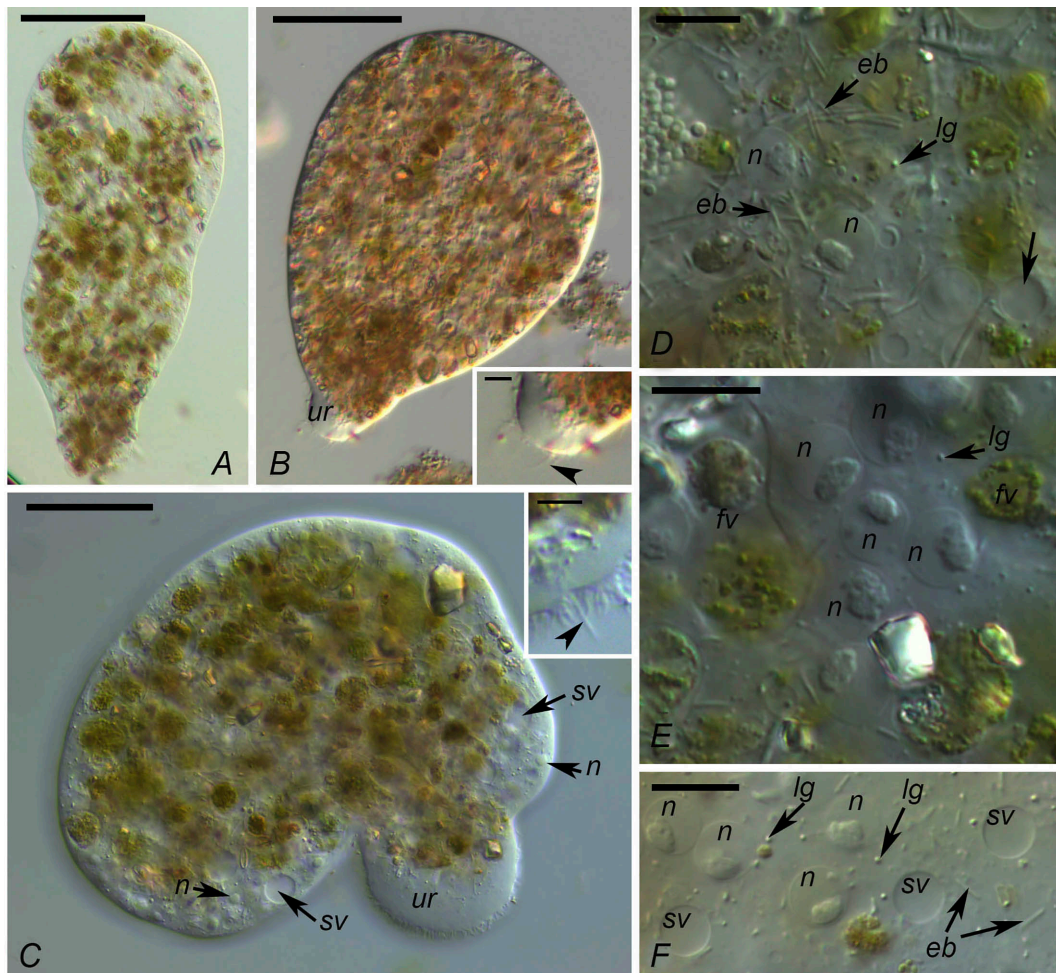


Fig. 1. *Pelomyxa quarta*, light microscopy (DIC). A–C – *Pelomyxae* during locomotion, inserts – uroid; D–F – details of the cell structure. Abbreviations: ur – uroid, sv – structural vacuoles, fv – food vacuoles, n – nucleus, lg – lipid globules, eb – prokaryotic endocytobionts, arrowheads – flagella. Scale bars: A–C – 50 μm ; D–F – 10 μm , inserts – 5 μm .

the shape of the cell, the organisation of the nuclear apparatus and the structure of the cytoplasm, i.e. the characters that are still being used to identify *Pelomyxa* spp. Although A. Gruber assigned these organisms to the genus *Amoeba*, he noted their similarity with *Pelomyxa palustris* Greeff, 1874 and *P. villosa* Leidy, 1879. Later, Eugène Penard transferred Gruber's *Amoeba prima*, *A. tertia* and *A. binucleata* to the genus *Pelomyxa* (Penard, 1902). Recently, we found "*Amoeba secunda*" described by A. Gruber in a pond in St. Petersburg and redescribed it as *Pelomyxa secunda* (Berdieva et al., 2015). This means that four out of the seven species of amoeboid protists featuring in *Studien über Amöben* (Gruber, 1884) are now considered as valid *Pelomyxa* spp.

Examining samples of bottom sediments from the Lake Osinovskoe (North-West of Russia, Lenin-

grad Region) we found an organism, which, judging from its morphology, undoubtedly belonged to the genus *Pelomyxa*. At the same time, it was strikingly similar to "*Amoeba quarta*" originally described in *Studien über Amöben* (Gruber, 1884) and never re-isolated since then. We studied this organism with the use of light, immunofluorescence and transmission electron microscopy and redescribed it as *Pelomyxa quarta* comb. nov. The results of our study are presented in this paper.

Material and methods

Bottom sediments of the Lake Osinovskoe (North-West of Russia, Leningrad Region) were sampled from spring to autumn in 2022 and 2023.

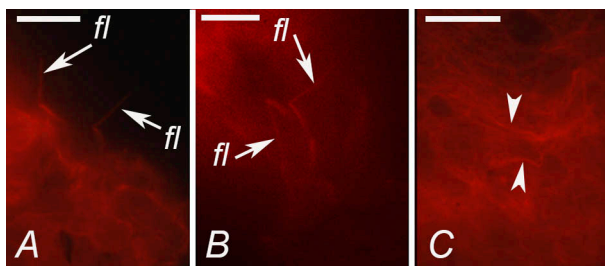


Fig. 2. *Pelomyxa quarta*, immunofluorescent staining for α -tubulines. Abbreviations: fl – flagella, arrowheads – microtubules. Scale bars: A – C – 10 μ m.

One-litre plastic bottles were filled with detritus (1/4 of the bottle volume) and topped up with lake water. The bottles were stored in the dark at 10° C. The samples were viewed in glass Petri dishes under a Nikon SMZ 1270 stereomicroscope (Nikon Corporation, Japan). The cells of pelomyxae were collected one by one using a glass pipette. Light optical observations and microphotography were performed using a Leica DM2500 microscope (Leica-Microsystems, Germany) equipped with a differential interference contrast and a Leica DFC495 (8.0MP) digital camera. Fixation and examination of samples for immunofluorescence microscopy and TEM were performed as described previously (Frolov et al., 2005; Chistyakova et al., 2020a).

Results

LIGHT MICROSCOPY

During examination of the bottom sediments in a Petri dish, the pelomyxae were often seen moving actively on the substrate. The cells were monopodial, tapering towards the posterior end, where a bulbous uroid with a broad hyaline rim was formed (Fig. 1, A, B, C). The surface of the uroid was covered with hyaline villi interspersed with short immobile flagella (Fig. 1, B, C).

The pelomyxae were 150–250 μ m long and 80–150 μ m wide. They became rounded when changing the direction of movement or stopping, with the uroid persisting for quite a long time (Fig. 1, C). Except in the uroid zone, no hyaline rim was detected in the periphery of the cell.

The cytoplasm was filled with digestive vacuoles mostly containing unicellular green algae and plant debris, so the cells were usually greenish-brown in

colour (Fig. 1, D–F). The cytoplasm also contained optically empty rounded vacuoles, 5–10 μ m in diameter (the so-called “structural” vacuoles), and numerous small light-refracting granules (Fig. 1, D, F). Symbiotic rod-shaped bacteria were present in the cytoplasm in large numbers (Fig. 1, D, F).

The cell contained from 20 to 30 rounded nuclei with a diameter of 6–7 μ m (Fig. 1, D–F). A compact mass of nucleolar material, 3–4 μ m in diameter, was clearly visible in the nucleoplasm; it was located eccentrically, close to the nuclear envelope (Fig. 1, D–F).

IMMUNOFLUORESCENCE MICROSCOPY

Immunofluorescence staining of the pelomyxae with labelled antibodies to α -tubulin revealed numerous short flagella concentrated mainly in the uroid zone (Fig. 2, A, B). A bundle of microtubules 4–5 μ m long extended at an angle from the flagellum base (Fig. 2, A, B). It could be seen at some preparations that this bundle was located directly under the plasma membrane (Fig. 2, A). In addition, a developed network of microtubular bundles with various orientation was present in the cytoplasm (Fig. 2, C).

ELECTRON MICROSCOPY

A very thin layer of amorphous glycocalyx was seen in some areas of the surface of the plasma membrane (Fig. 3, A). The cytoplasm was mainly filled with digestive vacuoles (Fig. 3, C). Rounded bodies, 300–500 nm in diameter, with a medium electron-density could also be seen (Fig. 3, C, G). We assumed that they corresponded to the light-refractive granules detected during light-optical examination and were accumulations of lipids.

Rod-shaped prokaryotic endocytobionts were located in the individual symbiontophoric vacuoles that were surrounded by a distinct rim of filaments perpendicular to the vacuole’s membrane (Fig. 3, B, C, G). The bacteria measured 2.0–2.5 μ m in length and 0.3–0.4 μ m in width and had a dense bacterioplasm.

Flagella were frequently seen on the cell surface. It could be seen at transverse sections that both duplets and singletons of microtubules were present in the axoneme (Fig. 3, E, F). A bundle of 10–20 microtubules forming a cone-like structure started from the base of the flagellum (Fig. 3, A, D, E). Numerous variously oriented microtubules were present in the cytoplasm (Fig. 3, G).

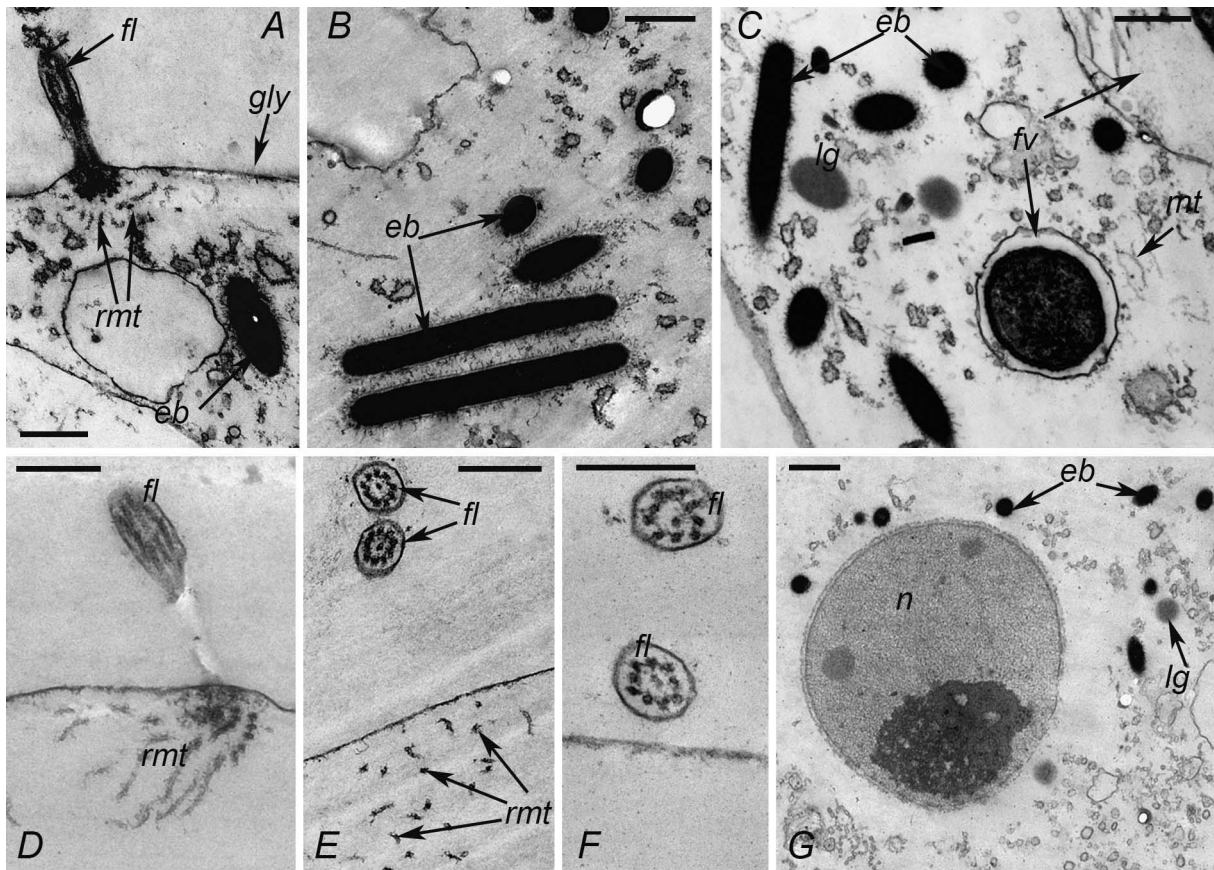


Fig. 3. *Pelomyxa quarta*, electron microscopy. Abbreviations: gly – glycocalyx, fv – structural vacuoles, n – nucleus, lg – lipid globules, eb – prokaryotic endocytobionts, rmt – cone of radial microtubules, fl – flagella, mt – microtubules in cytoplasm. Scale bars: A, D, E, F – 500 nm, B, C, G – 1 μ m.

The nuclei of pelomyxae were rounded, with an eccentric cluster, 3–4 μ m in size, of loose electron-dense material (Fig. 3, G). The nucleoplasm also contained a few small rounded bodies of a lesser electron density, 400–500 nm in diameter (Fig. 3, G). A layer of small vesicles could be seen outside the nuclear envelope (Fig. 3, G).

Discussion

The analysis of the characters of the organism examined in our study indicates that it belongs to the genus *Pelomyxa*. Members of this genus are amoeboid protists with multiple nuclei at least at one life-cycle stage (Chistyakova et al., 2013). The cell surface bears numerous, usually immobile flagella that are uninvolved in the movement. In general, the flagellar apparatus in these protists shows signs of reduction, which is probably associated with the loss of locomotor function of the flagella (Goodkov, 1989; Chistyakova et al., 2020a). The axoneme

structure often deviates from the standard 9(2)+2 microtubule pattern. The central pair of microtubules may be missing, while in the periphery singlet, microtubules may be found instead of duplets or additional microtubules may be present. There may be various deviations from the “classical formula” of the kinetosome, and sometimes the latter is almost entirely reduced. In different pelomyxae, the kinetosomes can be associated with the following microtubular derivatives: radial microtubules, basal microtubules, lateral rootlet, and the structure of the basal apparatus of the flagellum, which is one of the main characters used in identification of *Pelomyxa* spp. (Chistyakova et al., 2023). However, at least in several *Pelomyxa* spp., the same cell may have both flagella with a relatively well-developed set of rootlet microtubules and flagella with an almost completely reduced rootlet apparatus (Chistyakova et al., 2020a). The cytoplasm of pelomyxae is usually strongly vacuolated owing to numerous digestive and optically empty (“structural”) vacuoles (An-

dresen et al., 1968; Goodkov and Seravin, 1991; Frolov, 2011). In addition, the cytoplasm often contains accumulations of reserve nutrients in the form of glycogen granules (often concentrated in special glycogen bodies) as well as lipid droplets (Chistyakova et al., 2020b). Pelomyxae lack mitochondria but possess obligate prokaryotic endocytobionts (Chistyakova et al., 2016). From one to three morphologically different types of symbiotic bacteria can be found in different *Pelomyxa* spp.

In his *Studien über Amöben*, August Gruber considered the number, the size and the structure of nuclei as the main diagnostic characters (Gruber, 1884). He also used other features such as the cell shape and the cytoplasm organisation. According to his description of *Amoeba quarta*, this species is characterised by the presence of numerous nuclei about 10 µm in size. Their distinctive feature is an eccentric location of the compact chromatin substance, which lies close to the inner surface of the nucleus. A. Gruber mentioned the presence of small light-refracting granules in the cytoplasm as well as the fact that the digestive vacuoles of *A. quarta* mostly contained green algae and plant debris. He also noted the presence of numerous rod-like structures, assuming them to be fungal hyphae. It cannot be doubted, however, that they were actually rod-shaped bacteria.

A. Gruber noted that non-moving cells of *A. quarta* were often round, about 250 µm in diameter, and formed a hyaline border with short hyaline pseudopodia pointed in different directions. He also noted that a zone covered with numerous wrinkles was formed at the posterior end during directed locomotion. We did not observe the formation of pronounced hyaline border and pseudopodia in stationary cells. In all other respects (firstly, nuclear morphology, structural features of the cytoplasm, size of the cell, etc.), the characteristics of pelomyxae examined in our study correspond well to the features of the organism described by A. Gruber as *A. quarta* (Gruber, 1884). Therefore, we confidently accept that we have re-isolated this protist in Russia for the first time since its original description from a pond in Stuttgart (Germany) in 1884.

The structure of nuclei and the flagellar apparatus are primary characters used for identification of *Pelomyxa* spp. Other diagnostic characters are cell shape during locomotion, the features of cytoplasm organisation, the structure of glycocalyx and the composition of prokaryotic endocytobionts. Concerning cell shape during locomotion, *P. quarta*

is similar to *P. palustris*, *P. stagnalis*, *P. binucleata*, *P. belevskii*, *P. flava*, *P. tarda* and *P. doughnuta* (Greeff, 1874; Frolov et al., 2005a, 2011; Chistyakova and Frolov, 2011; Ptáčková et al., 2013; Chistyakova et al., 2021, 2022). In these pelomyxae, no lateral pseudopodia are formed during directed locomotion, and the uroid zone, bearing hyaline villi and often bulbous in shape, is formed at the posterior end. However, *P. quarta* is distinguished by its characteristic greenish-brown colouration, which is due to the presence of numerous green algae and plant remains in the digestive vacuoles.

The cytoplasm of *P. quarta*, in contrast to most other known pelomyxae (Chistyakova et al., 2020b), lacks glycogen accumulations, with reserve nutrients being represented by lipid droplets only. Glycogen accumulations were also not discovered in *P. paradoxa*, *P. prima*, *P. gruberi* and apparently also in *P. schiedti* (Frolov et al., 2005b, 2006; Chistyakova et al., 2014; Zadrobilková et al., 2015). There are few structural vacuoles, which in most other pelomyxae are so numerous as to give the appearance of a “foamy” cytoplasm (Chistyakova et al., 2013). Few structural vacuoles are characteristic of only one other pelomyxa, *P. binucleata* (Frolov et al., 2005a).

A very thin layer of amorphous glycocalyx was only occasionally observed on the surface of the plasma membrane in *P. quarta*. A thin amorphous glycocalyx is present in *P. stagnalis*, *P. binucleata*, *P. paradoxa* and *P. secunda* (Frolov et al., 2005a; Chistyakova and Frolov, 2011; Chistyakova et al., 2014; Berdieva et al., 2015).

Several different morphotypes of symbiotic bacteria have been found in the cytoplasm of *Pelomyxa* spp. (Chistyakova et al., 2016). In most of them, the so-called Af-symbionts are present: rod-shaped bacteria with a dense bacterioplasm capable of autofluorescence when irradiated by light with a wavelength of 420 nm. Symbiontophoric vacuoles containing bacteria of this morphotype are surrounded by a “brush” of short filaments oriented perpendicularly to the vacuole’s membrane. We failed to detect any distinct autofluorescence in prokaryotic endocytobionts of *P. quarta* but showed that they were similar to Af-symbionts of other pelomyxae in their morphology and the localization in the cytoplasm.

Numerous flagella are present on the cell surface of *P. quarta*. The structure of their axoneme often differs from the typical 9(2)+2 formula. A bundle of 10-20 microtubules parallel to the cell surface lies at the base of the flagellum. A similar organisation of the basal flagellar apparatus is

characteristic of *P. palustris*, *P. stagnalis*, *P. pilosa* and *P. binucleata* (Goodkov, 1989; Frolov et al., 2005a, 2007; Chistyakova and Frolov, 2011; Chistyakova et al., 2024). The bundle of microtubules associated with the kinetosome is a derivative of the cone of radial microtubules, which is present, in one form or another, in all members of the Archamoebae that possess flagella (Chistyakova et al., 2023).

The structure of nuclei is a key feature in identification of *Pelomyxa* spp. Many of them demonstrate an increased complexity of the nuclear envelope due to additional layers outside the nuclear membrane (Chistyakova et al., 2013). A layer of small vesicles, which surrounds the nuclei of *P. quarta*, is also present in *P. palustris* and *P. corona* (Frolov et al., 2004, 2007). At the same time, the organisation of nucleolar material in the form of a compact mass, which always lies eccentrically and is closely adjacent to the nuclear membrane, is a unique feature of *P. quarta*.

To conclude, in this study we re-isolated an amoeboid organism originally described in the 19th century and never seen since that time. We showed that it belonged to the genus *Pelomyxa* but was easily distinguished from the known congeners by the set of morphological characters. *Pelomyxa quarta* (Gruber, 1884) comb. nov. is a valid species and another pelomyxa rescued from oblivion by this discovery.

DIAGNOSIS OF *PELOMYXA QUARTA* (GRUBER, 1884) COMB. NOV.

Cells monopodial in locomotion, slightly widened at anterior end, with bulbous uroid with a broad hyaline rim bearing hyaline villi. Digestive vacuoles contain green algae and plant debris. Numerous rod-shaped prokaryotic endocytobionts enclosed in symbiontophoric vacuoles. Numerous short, immobile flagella; rootlet apparatus represented by bundle of 10–20 microtubules passing in parallel to plasma membrane. Cell with 20–30 rounded nuclei surrounded by thin layer of small vesicles. Nucleolar material forming compact mass lying eccentrically, very close to the nuclear membrane.

Differential diagnosis. *Pelomyxa quarta* differs distinctly from the other *Pelomyxa* spp. in the structure of the nuclei, which contains compact nucleolar mass lying eccentrically.

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