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# Study of a rediscovered large freshwater multinucleate amoeba *Chaos illinoisense* (Kudo, 1950)

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**Summary**

A large freshwater multinucleate amoeba *Chaos illinoisense* (Amoebidae), previously only recorded in natural collection by Kudo (1950, 1951) from a small pond in Illinois, Central North America, has been rediscovered in backwaters of the River Luga, North-West Russia. Amoebae were cultured, cloned, and studied using light- and electron-microscopic techniques. This species is the only currently known cyst-forming representative of the genus. Numerous cytoplasmic crystals is a conspicuous characteristic of the species; they appear in two forms: platelike and bipyramidal. Trophozoites contain several hundred spherical nuclei of granular type. The fibrous inner nuclear lamina with loosely organised honeycomb-like structure was revealed as well as distinct mitochondrial heteromorphism. Two-layered cell surface coat with filamentous appearance is demonstrated. Current justification of the specific diagnosis is presented.

**Key words:** amoebae, Lobosea, Amoebidae, *Chaos illinoisense*, ultrastructure, trophozoites, cysts, systematics

## Introduction

A large freshwater multinucleate amoeba *Chaos illinoisense* was discovered and briefly described under the name *Pelomyxa illinoisensis* by Kudo in 1950. A more detailed description of this species was published a year after (Kudo, 1951). Current generic home of this amoeba was accepted after a long period of confusions with taxonomy and nomenclature of the genus *Chaos* (see Bovee and Jahn, 1973; Lorch , 1973). At last, the correction of the adjectival ending, as *Ch. illinoisense,* was proposed by Page (1976, 1986).

Amoeba was initially collected from a small pond located about 25 miles to the east of the University of Illinois. Its laboratory cultures was established and intensively studied (McClellan, 1958, 1959; Daniels, 1955, 1958, 1962, 1964; Daniels and Breyer, 1965, 1966; Daniels and Roth, 1961, 1964; etc.), but in subsequently they became extinct. All attempts at re-isolating this species from natural habitat failed (Daniels, 1973). Daniels (op. cit.) wrote: «I have searched extensively for this amoeba, but failed to find it» (P. 148). Just twenty years later Fishbeck and Bovee (1993) has noted: «*Chaos illinoisense* was discovered... by Kudo (Kudo, 1950)... [and] has not... been reported from field collections since» (P. 134).

Now we can report that this species has been rediscovered in North-West of Russia (Luga River, St. Petersburg Region). The results of its light- and electron-microscopic investigation are presented here as well as brief systematic account and current justification of the specific diagnosis.

## Material and methods

*Chaos illinoisense* was collected from backwater of the Luga River near the village Lemowga, St.Petersburg Region, North-Western Russia. Amoebae were isolated and maintained in polyxenic culture in Prescott-James solution (Prescott and James, 1955) with wheat grains at room temperature. Cloned cultures were established and main-

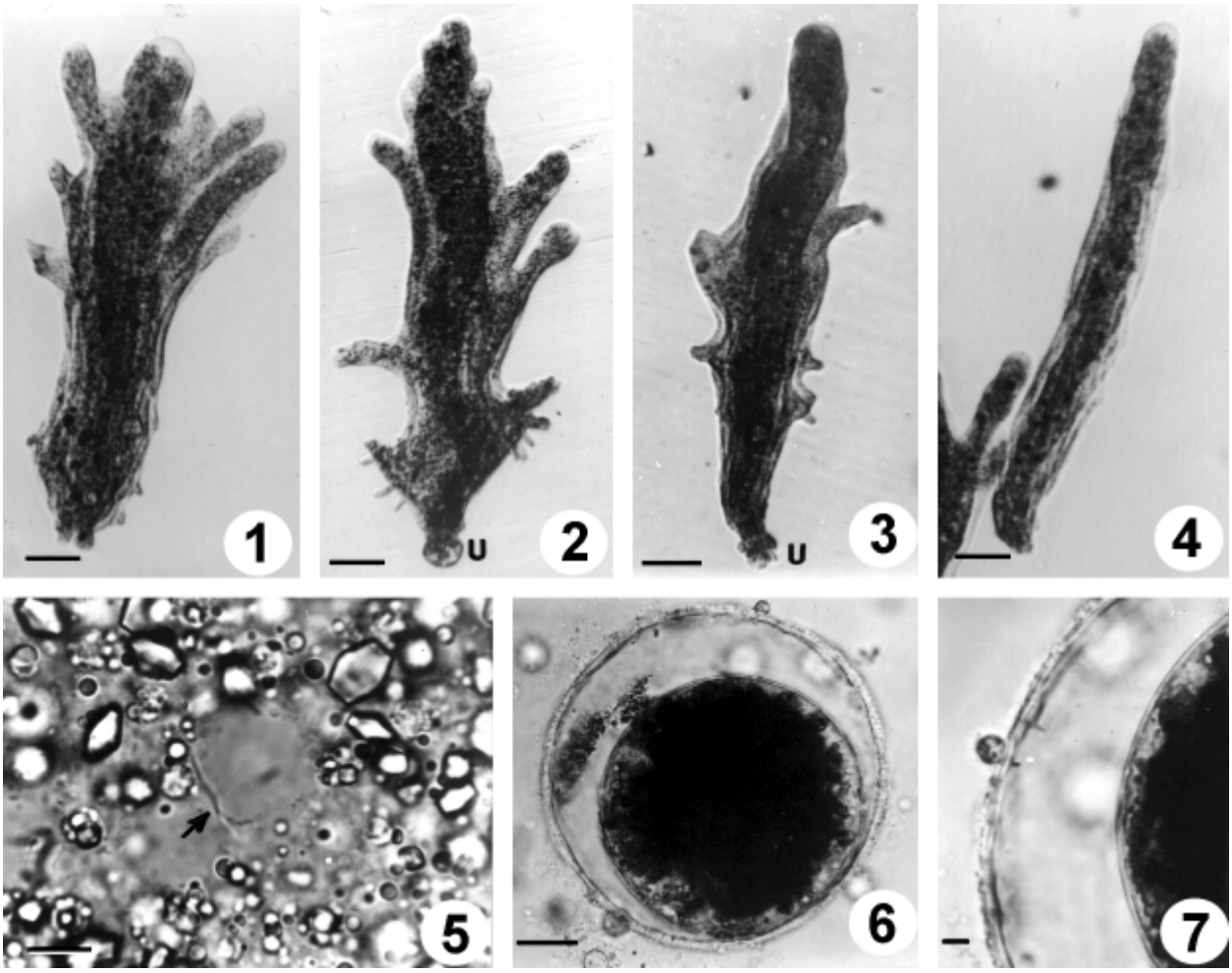


**Fig. 1–7.** Light microphotographs of living trophozoites and cysts of *Chaos illinoisense*. **1, 2.** Polytactic and **3, 4.** orthotactic locomotive forms. **5.** Cytoplasmic crystals (arrow indicate a large platelike form). **6.** Cyst. **7.** Portion of cyst periphery. U – uroid. Scale bars: 1–4 – 100 μm; 5, 7 – 10 μm; 6 – 50 μm.

tained under the standard methods for large freshwater amoebae (Prescott and James, 1955; Prescott and Carrier, 1964; Yudin, 1975).

For electron microscopy we used slightly modified conventional glutaraldehyde fixation with an osmium tetroxide postfixation procedure (for details see Smirnov and Goodkov, 1997, 1998), which has proven to give a good preservation of material by our previous studies of various species of large freshwater amoebae, i.e. *Ch. carolinense*, *Ch. glabrum* and *Polychaos annulatum*. Standard strain of *Chaos carolinense* maintained in the culture collection of the Laboratory of Cytology of Unicellular Organisms, Institute of Cytology, RAS (Gromov, 1986a; Smirnov and Goodkov, 1997) was used in the present study as a control organism for the fixation procedures adequacy.

## Results and discussion

1. Light-microscopical observations

Amoebae in locomotion adopted either “polytactic” or “orthotactic” forms (Figs 1–4), – terms created by Grebecki and Grebecka (1978) for the recognition of different morphodynamic forms of locomoting large lobose amoebae. Polytactic specimens of *Ch. illinoisense* had a few anteriorly directed pseudopodia, each of them possessed a small frontal hyaline cap (Figs 1, 2). The development of very elongated orthotactic form in continuous rapid locomotion (Figs 3, 4) was characteristic for this species, as well as for *Ch. glabrum* (Smirnov and Goodkov, 1997). Bulbous or (sometimes) compact morulate uroid frequently presented in advancing specimens (Figs 2, 3). Kudo (1951) also noted orthotactic forms of *Ch. illinoisense*, and his schematic drawing (Fig. 3g, op. cit.) shows such a form with bulbous uroid. Very distinct, numerous, nearly parallel longitudinal surface wrinkles, running from rear end up to anterior subregion of the body were a common feature of all amoebae in active locomotion. According to our measurements the length of locomoting specimens was 650–1300 μm (including orthotactic forms), agreeing well with previous data for this species (Kudo, 1951).

Numerous interphase nuclei of *Ch. illinoisense* were spherical in shape, contrary to all other known species of this genus possessing biconvex disc-shaped nuclei. The details of their structure were hardly distinguishable in the living amoebae due to the presence of large numbers of crystals and food vacuoles in the cytoplasm.

The presence of cytoplasmic crystals is a characteristic feature of the majority of freshwater species of Amoebidae (Andersen, 1973; Bovee and Jahn, 1973; Page, 1986), but *Ch. illinoisense* stand out against other members of the genus by the number and dimensions of its crystals (Fig. 5). Kudo (1951) noted that this feature "can be recognised as one of the specific characteristics" of *Ch. illinoisense* (P. 153). In our isolate crystals appeared in two main morphological forms: truncate bipyramidal and platelike rectangles. The first ones ranged in size up to 7 µm in long axis (average length is 4 µm approximately) while the latter in some cases measured up to 10x10 µm. Quantitative relationships among the two types of cytoplasmic crystals could be different, which is thought to be dependent on the physiological condition of the cell. Both types of cytoplasmic crystals were located inside special vacuoles that could be detected only with electron microscope.

*Ch. illinoisense* is a typical polyphage. Various bacteria, unicellular algae, ciliates and even some small multicellular organisms, for instance rotifers (Rotatoria), were found in the digestive vacuoles of the same amoeba (Fig. 13). The engulfment of small rotifers was also noted by Kudo (1951). Clonal cultures of this species were successfully maintained on the ciliates *Colpidium* or *Tetrahymena*.

Cysts of *Ch. illinoisense* were spherical and distinctly double-walled, with two walls widely separated (Figs 6, 7). Cysts were always single and never clumped together. The average diameters of the ectocyst (outer wall) and endocyst (inner wall) were 250 µm and 200 µm respectively. The ectocyst was less regular structurised than endocyst, and its layered organisation was frequently seen even at the light-microscopical level. The upper surface of the ectocyst was apparently sticky and various particles were often adhered to it. The cytoplasm of encysted amoeba was opaque, but it appeared more clear and transparent at the periphery, and particular large cytoplasmic crystals were sometimes visible here. Kudo (1951) noted in his paper that he saw a remarkable differences in nuclear morphology of encysted amoebae and trophic ones.

2. Electron-microscopical study

Nuclei of *Ch. illinoisense* were of granular (ovular) type with irregular nucleolar pieces distributed mainly, but not exclusively in a parietal layer (Fig. 8). Fibrous inner nuclear lamina was present, although it was rather weakly expressed and loosely structurised. It has honeycomb-like organisation not clear visible in transverse sections (Fig. 10), but only in tangential sections of the nuclei (Fig. 9). In general it resembled those in *Ch. carolinense*.

The nuclear pore complexes were numerous and had typical structure. Intranuclear helices, which are characteristic for many species of Amoebidae, were often observed in the caryoplasm of *Ch. illinoisense* nuclei (Fig. 10). These features were already mentioned for this species in previous studies (Daniels and Roth, 1964; Daniels, 1973).

Compact dictyosomes of *Ch. illinoisense* (Fig. 11) were numerous and dispersed over the whole cytoplasm of the cell. They usually contained fewer flattened cisterns (four, rarely five) than in most other representatives of the family Amoebidae (as many as eight or ten sacculus). In this respect they are similar to *Amoeba leningradensis* (Page and Kalinina, 1984; Page, 1986) and *Polychaos annulatum* (Smirnov and Goodkov, 1998). At the electron micrographs of "mature" dictyosomes of *Ch. illinoisense* presented by Daniels (1964, 1973) also no more then 4–5 cisterns were recognised.

*Ch. illinoisense* possessed a typically organised complex of contractile vacuole with well expressed vesiculate spongiom and associated mitochondria (Fig. 12). A large number of digestive vacuoles was observed in amoebae cytoplasm. These vacuoles included various food objects at different stages of digestion. Sometimes even whole engulfed small multicellular organisms could serve as food for these large amoeba, e.g. small rotifers (Fig. 13).

Mitochondria of two different morphological types could be observed in the cytoplasm of *Ch. illinoisense* simultaneously (Figs 14, 15). They were well distinguishable by the difference in matrix density. Mitochondria of the first type were highly electron dense, so that their cristae at the longitudinal sections looked like clear branching tubes or "channels" in the dark surrounding. Mitochondria of the second type possessed considerably less electron dense matrix. However all mitochondria exhibited branching cristae of the tubular type. In addition, there was no essential difference in their size and shape. Mitochondria of both types had irregular oval or elongate profiles at the sections. Sometimes they were located very closely to each other (Figs 14, 15) and we did not find any area preferably occupied by mitochondria of the one or another type in amoeba cytoplasm. Usually mitochondria of both types could be observed in the equal amount.

Numerous rod-like bacterial endobionts lying freely in amoeba cytoplasm were observed in all studied specimens of *Ch. illinoisense* (Figs. 14, 15). Daniels (1973) also mentioned that endobionts were occasionally observed, but at the electron micrograph published by him single endocytobiont was enclosed in the symbiontophorous vacuole. Now it is known that the presence of endobiotic bacteria in the cytoplasm of different species of large freshwater amoebae is rather regular than an exception (Sopina and Fokin, 1993; Smirnov et al.,

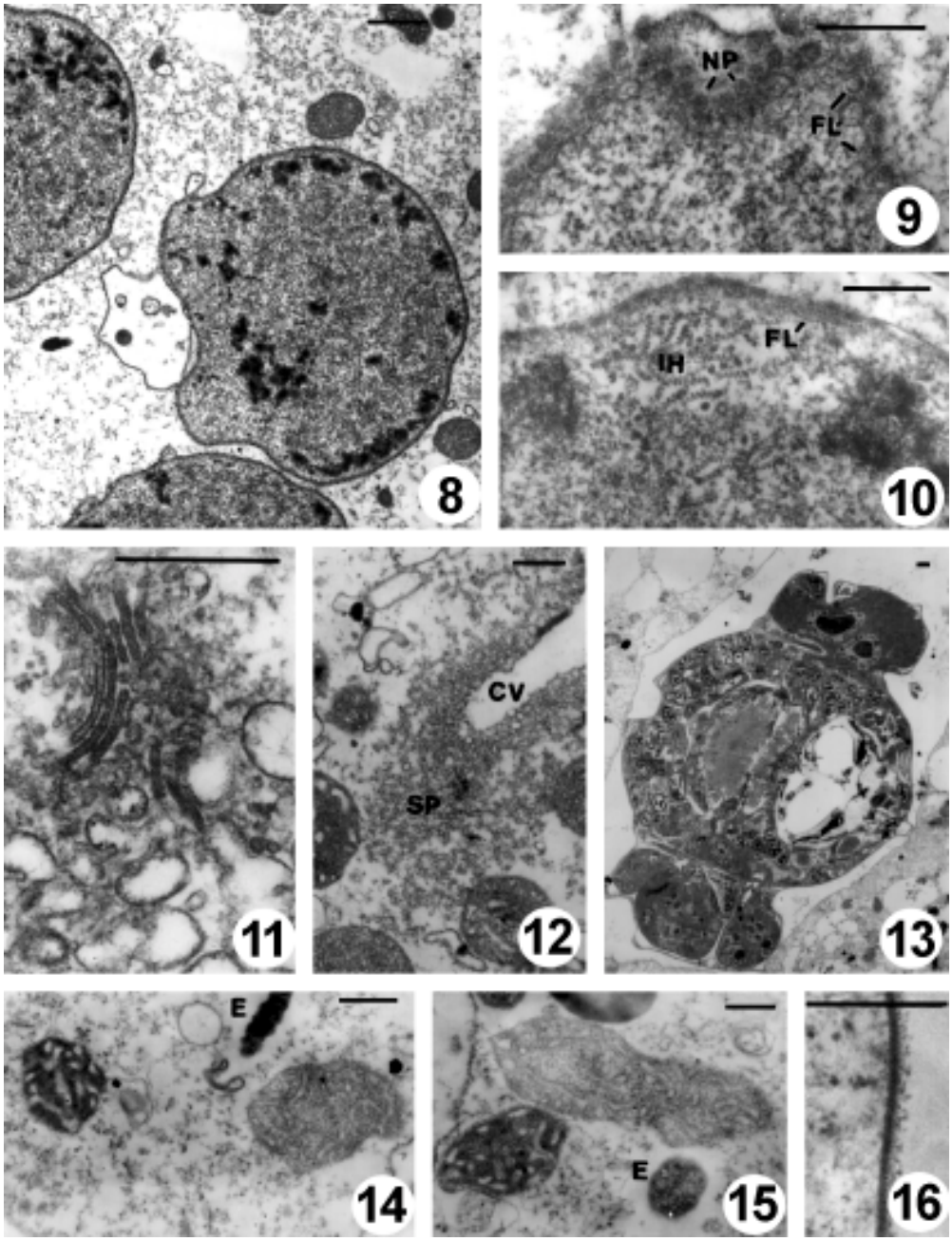


**Fig. 8–16.** Electron microphotographs of trophozoites of *Ch. illinoisense*. **8.** Nuclei. **9, 10.** Nuclear periphery. **11.** Dictyosome. **12.** Portion of contractile vacuole complex. **13.** Digestive vacuole with engulfed rotifera. **14, 15.** Mitochondria of two different morphological types. **16.** Cell surface. CV – contractile vacuole, E – endocytobionts, FL – fibrous inner nuclear lamina, IH – intranuclear helices, NP – nuclear pore complexes, SP – spongiom. Scale bars: 0.5 μm throughout.

1995; Ossipov et al., 1997). Moreover, bacterial endocytobionts lying freely in host cytoplasm and enclosed in symbiontophorous vacuoles may inhabit one and the same amoeba (Page, 1986).

The cell coat of *Ch. illinoisense* was about 50 nm thick and consisted of two layers (Fig. 16). The inner layer was thin amorphous, about 10 nm in thickness, and the outer one appeared to be filamentous. Filamentous nature of glycocalyx can be also recognised at the electron micrographs presented by Daniels (1964; 1973). The comparison with cell coat of the other familiar species shows the following. Well discernible discrete filaments were not distinguishable in *Ch. illinoisense* in contrast with that of *Ch. carolinense* and *Ch. nobile* (Pappas 1959; Daniels, 1973; Page 1986), although its outer layer was not as amorphous as that in *Ch. glabrum* (Smirnov and Goodkov, 1997) and *Amoeba leningradensis* (Page and Kalinina, 1984; Page, 1986). It is something resembling that of *Deuteramoeba*

*algonquinensis* (Baldock et al., 1983, as *Amoeba algonquinensis*; also see Page, 1986).

The fine structure of the cysts of *Ch. illinoisense* has never been studied yet. Our preliminary data on the young cysts (not illustrated here) show that the ectocyst was composed of finely fibrous material with the peripheral layer more densely packed than the major portion of the wall. Fibrils were arranged in a longitudinal or a reticular array. In contrast with the nuclei of trophic cells, these of encysted specimens have irregular outlines. The caryoplasm become less compact, the number of nucleoli decreased and they are irregularly distributed within the nucleus. These data correspond to the Kudo's (1951) notification about considerable changes in nuclear morphology in cysts.

The cytoplasm contained numerous large vacuoles filled with the degraded electron-dense material. These vacuoles differed from the routine phagosomes of trophic amoebae and included bacterial endobionts, which were also numerous in the surrounding cytoplasm. Evidently these were autophagous vacuoles. One of the most notable peculiarities was the presence of numerous large vacuoles, each containing two or even more crystals.

In this connection it is interesting to note the following. Daniels (1973) briefly reported that newly excysted amoebae contain a lot of large crystals and "sometimes two crystals appeared within a single vacuole", while "only single crystals were seen in the crystal vacuoles of normally growing amoebae" (P.154). Besides that, "the nuclei in the newly excysted trophozoites were much more irregular in outline than those in cultured amoebae" (op. cit.).

## Discussion

All characteristic features – locomotive morphology, organisation of nuclear apparatus, morphometry data, cytoplasmic crystals, presence and organisation of cysts, biological affinities, etc. – indicates beyond any reasonable doubt that the presently studied amoeba is the same species that was described by Kudo (1950, 1951) in Illinois as *Pelomyxa illinoisensis* and in subsequently transferred into the genus *Chaos*, as we already mentioned earlier.

The ability to cyst formation is highly uncommon among large free-living amoebae belonging to the family Amoebidae. In this respect *Ch. illinoisense* is an exclusive multinucleate species, although encystment of *Ch. carolinense* has been reported in some early publications (Wenstrup, 1945; Musacchia, 1947), but these data at least must be proved. The light-microscopic morphology of *Ch. illinoisense* cysts resembled in general those of *Deuteramoeba mycophaga* (Chakraborty and Old, 1986, as *Trichamoeba mycophaga*; also see Page, 1988), another rare member of the family Amoebidae possessing the ability to encystment.

Of great importance is that the fibrous inner nuclear lamina with honeycomb-like organisation was found in *Ch. illinoisense*. When Page prepared new diagnosis of the genus *Chaos* (Page, 1986), he wrote: "A re-examination of *Ch. illinoisense*, in which Daniels and Roth (1964) found no honeycomb lamina, would be important ... if that species could be re-isolated" (P. 312). As it has been thought earlier (Pappas, 1959; Daniels and Roth, 1964; Daniels, 1973) that the inner nuclear lamina is absent in *Ch. carolinense* also, but modern electron microscopic fixations revealed this structure (Gromov, 1986a; Page, 1986). In this context, Gromov (1986a) notes especially that the fixation methods that have been used previously do not preserve this structure in *Ch. carolinense*. We come to a similar conclusion concerning *Ch. illinoisense*. This structure was founded also in *Ch. nobile* (Gromov, 1986b; Page, 1986) and in recently described *Ch. glabrum* (Smirnov and Goodkov, 1997). Therefore, all known species of the genus *Chaos* possess a fibrous inner nuclear lamina with more or less developed honeycomb-like structure.

The presence of different morphological forms of mitochondria in the cytoplasm of *Ch. illinoisense* was not found in earlier studies, and this can be explained by the same reasons that are specified above concerning the inner nuclear lamina. Now we only ascertain this fact, and more detailed consideration of this phenomenon in large freshwater amoebae, which we termed as "mitochondrial heteromorphism", will be a purpose of special report. However, it is necessary to note here, that such a phenomenon was already mentioned in some earlier studies of *Amoeba proteus* (Flickinger, 1974; Ord, 1976; Smith and Ord, 1979). Recently it has been found also in *Chaos glabrum* (Smirnov and Goodkov, 1997).

## Diagnosis

***Chaos illinoisense* (Kudo, 1950)**

Polypodial (polytactic) amoeba, with strong tendency to adopt elongated orthotactic form in continuous rapid locomotion. Bulbous or sometimes compact morulate uroid frequently presents. Average length of locomoting specimens is 500–1000 µm, seldom reach 1500 µm when orthotactic. Several hundred nuclei per amoeba. Nuclei are spherical in shape, about 14–16 µm in diameter. Nuclei of granular (ovular) type with irregular nucleolar pieces distributed mainly, but not exclusively in a parietal layer. Fibrous inner nuclear lamina with loosely organised honeycomb-like structure. Numerous large bipyramidal and plate-like cytoplasmic crystals. Surface coat about 50 nm thick and consisted of two layers: thin amorphous inner layer and outer one which appeared to be filamentous.

Mitochondrial heteromorphism. Double-walled cysts, with two walls often widely separated.

Known habitat: Fresh water. Central North America, North-Western Russia.

Note: Slides of North-Western Russia isolate are deposited with the museum of preparations of the Laboratory of Invertebrate Zoology, Biological Research Institute, St. Petersburg State University: NN 912–914.

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