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A Smiling Amoeboid Protist

How easy it was until the 1950s to assign a protist to higher taxonomic categories! A specimen of the genus *Nuclearia* was an amoeba and belonged to the animal kingdom. In the 1970s, bioscience began to see the tree of life in the area of the highest taxonomic units, the kingdoms, in a somewhat more differentiated way. Instead of the animal/plant dichotomy, the tree was now divided into protists without a nucleus, protists with a nucleus, plants, animals and fungi (Margulis et al., 1999). Even then, as far as amoeboid protists were concerned, everything was still relatively simple: whether lobose or filose amoeba, whether with or without a shell, all were united in the phylum Sarcomastigophora (Hausmann et al. 2003). The naked amoeba genus *Nuclearia* was one of them.

How Nuclearia is located in the present time (the article was written in 2023) in the taxonomic tree is discussed at the end of this small report. First of all, I want to describe an observation of life processes that I was able to make with material from my freshwater aquarium.

Habitat black beard algae

The aquarist is usually not happy when he finds brush algae in the tank. He feels like the (hobby) gardener: what he hasn't planted, he doesn't want to see grow. Most algae do not contribute to the beauty of the tank, and they tend to take over when the aquarist has problems with the water quality (e.g. due to too many nutrients caused by overstocking), or the plants are ailing because they have a problem with the water quality in general. Since I am both an aquarist and a microscopist, I can discover an interesting unicellular world in clusters of algae, so I have a somewhat more relaxed view on the occurrence of algae in the tank.

Audouinella belongs to the red algae and grows in freshwater aquariums in the form of small clusters on plants, stones and driftwood (Fig. 1 c, d). It does not appear red, but rather blue-black, and also likes to settle at the outlets of filters and flow pumps or on slow-growing plants such as the *Anubias* species. The sparsely branched cell filaments

are very thin, as I have observed, they are often used by choanoflagellates as a substrate for growth (Fig. 1 e, f). The growth of diatoms is sparse, in contrast to the cell filaments of the green alga *Cladophora*, which are often and sometimes massively overgrown by the diatom genera *Fragilaria*, *Gomphonema* and *Cocconeis* (Fig. 1 a, b). In addition, I regularly find a large number of amoeboids of the genus *Nuclearia* in my red algae clusters.

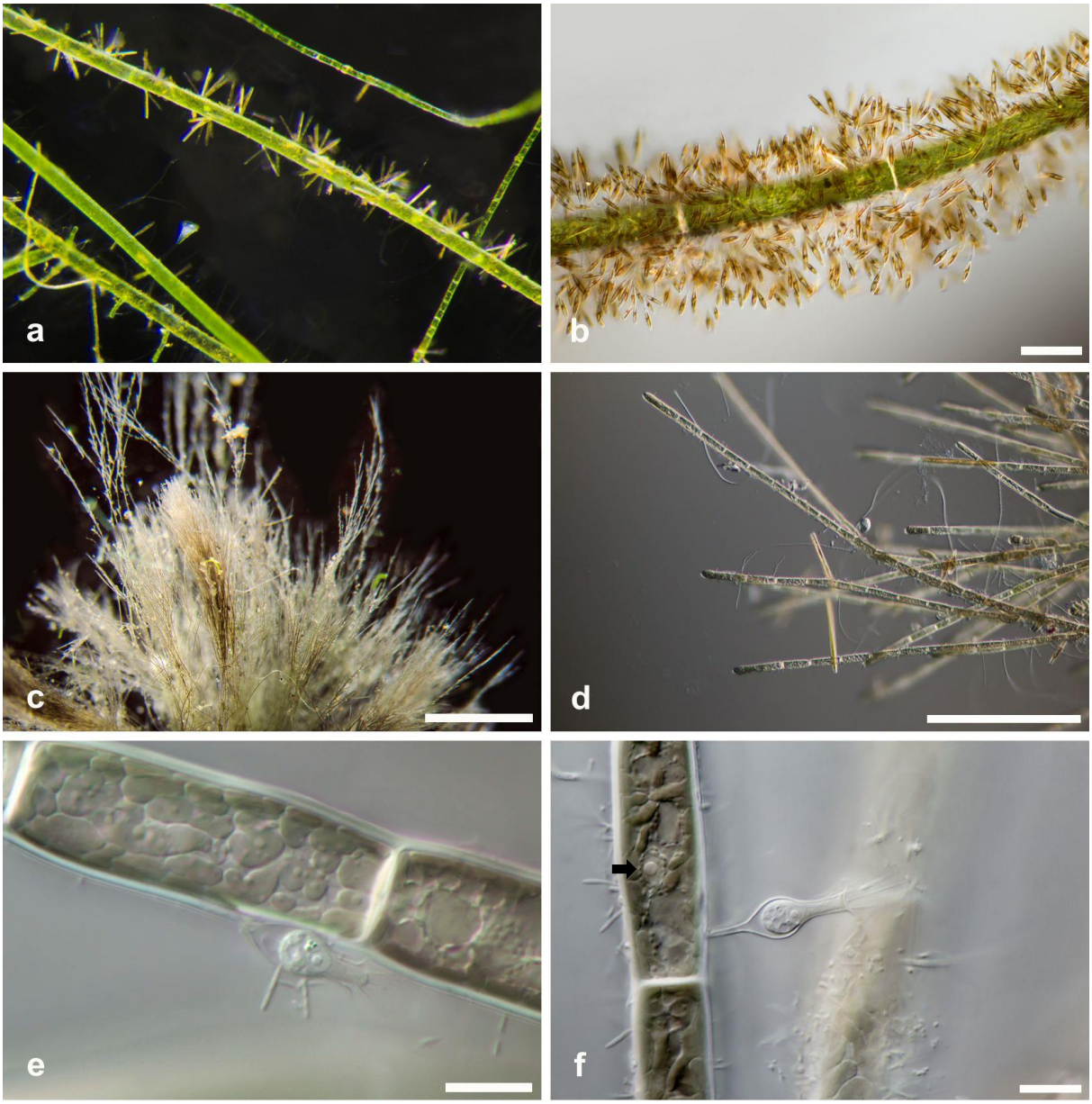

Fig. 1: Aufwuchs on the green alga *Cladophora* and the red alga *Audouinella*. **a** Diatoms of the genus *Fragilaria* on *Cladophora*. Image taken through dissecting microscope, without scale. **b** Dense stocking with *Gomphonema*. Scale bar indicates 100 µm. **c** Overview: A clump of *Audouinella* grown on a *Cryptocoryne* leaf. Scale bar indicates 2.5 mm. **d** Detail: The cell threads are very narrow; you can see some diatoms growing on them. Scale bar indicates 250 µm. **e** and **f** Choanoflagellates of the genus *Salpingoeca* growing on the cell filament. Scale bar indicates 10 µm. The plate-shaped, parietal chloroplasts, each with a pyrenoid body, can be seen at **e**, the optical cross-section at **f** shows a cell nucleus of the alga (arrow).

Defecation in *Nuclearia*

It turned out that a *Nuclearia* cell came into the field of view of my microscope objective, which had trapped a large piece of indigestible material in a vacuole. Its shininess in polarized light indicated its birefringent property. This and the fibrous structure led to the conclusion that it was cellulose. The series of pictures in Fig. 2 shows how the piece of cellulose was excreted. Unlike ciliates, for example, amoeboid beings have no special areas prepared for defecation openings, no cytoproct. Figure 2 f gives the impression that the large piece of waste affected the cell somewhat during the excretion process. In the genus *Nuclearia*, however, the formation of such cell appendages is not uncommon.

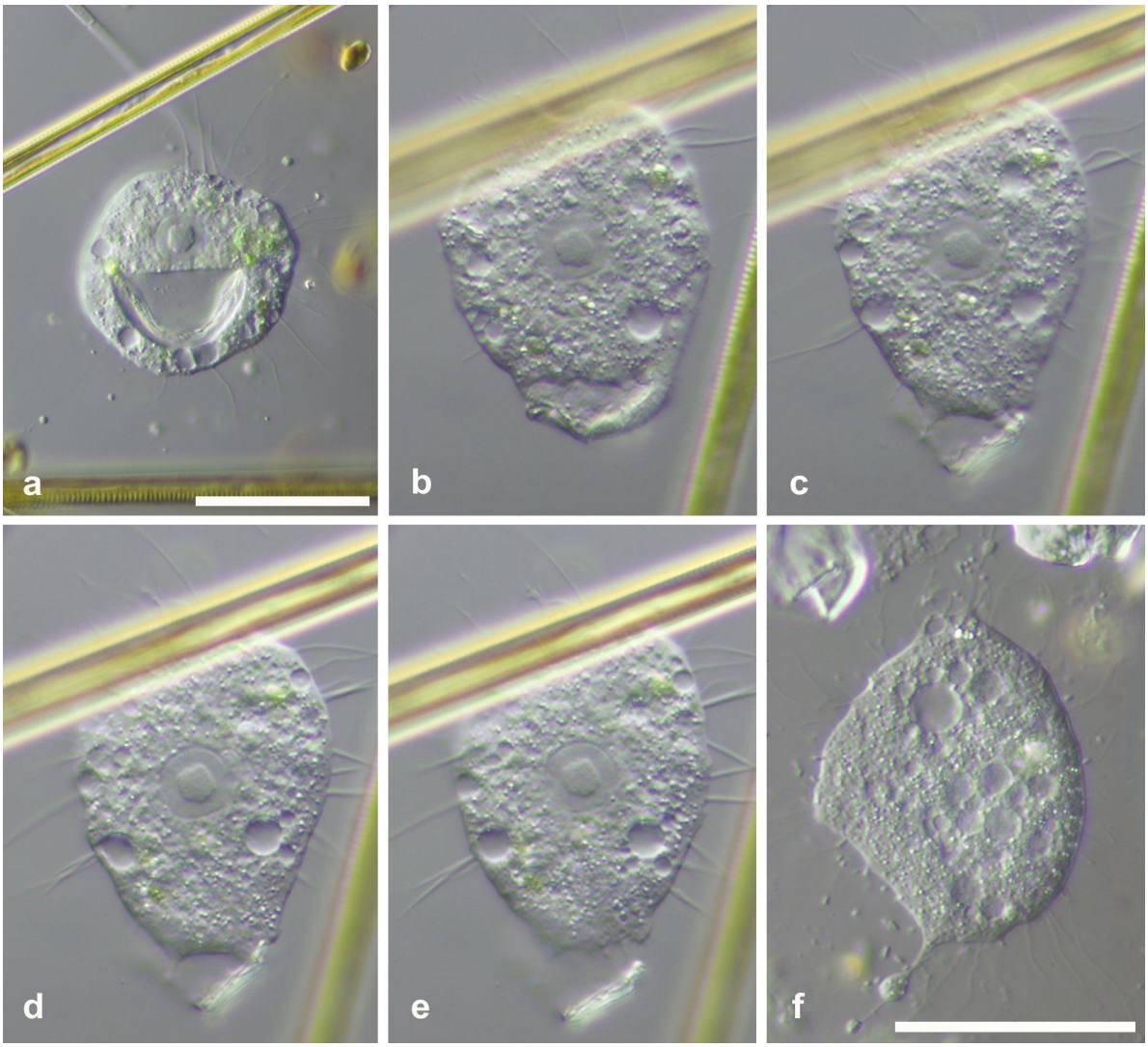

Fig. 2: Defecation in a *Nuclearia* cell. **a** Cell with large defecation vacuole. **b** The vacuole has migrated to the edge of the cell. **c** The vacuole has opened to the outside. **d** and **e** The indigestible material is being discharged. **f** End of the defecation process. Scale bar indicates 25 µm.

A multi-nucleated specimen

I usually focus carefully through the viewed objects to discover the cell organelles and to get an idea of the extension of the viewed beings in the third dimension. In one *Nuclearia* cell (Fig. 3) I discovered that it probably had more than one nucleus. At first it was still too mobile, so I could not be sure how many nuclei were actually in the cell. As the water under the coverslip progressively evaporated, mobility became restricted, I was able to take tomographic images and counted six large vesicular nuclei! The rod-shaped bacteria show very vividly the extent of the mucous membrane around the cell. The properties “mucilaginous coat” and “multinucleated, 4-12 nuclei” ensure the identification of the species *Nuclearia delicatula*.

Fig. 3: Three tomograms of a *Nuclearia delicatula* cell showing several nuclei. Scale bar indicates 10 µm.

The place of the genus *Nuclearia* in modern taxonomy

The taxonomic tree of all living beings was subdivided step by step in the area of its base (the kingdoms) in the years after 1970. The number of five kingdoms mentioned at the beginning of this report was no longer sufficient to represent the complex relationships between the many different groups of organisms that had been identified by genetic analysis. According to Adl et al. (2019) the genera collected in the **former** phylum Sarcomastigophora are placed in the following phyla:

- Amoebozoa
    - Tubulinea
    - Evosea
        - Archamoeba
        - …
    - Discosea
    - …
- SAR (Stramenopiles, Alveolata, Rhizaria)
    - …
    - Rhizaria
        - Cercozoa
        - …
- Opisthokonta
    - Holozoa (including Metazoa, i.e. animals)
    - Nucletmycea or Holomycota (including Fungi)
        - *Nuclearia*

This division of the amoeboid protozoa is based on fundamental molecular-biological differences; the genetic distance between the lobose and filose shell amoeba (as an example) is immense, greater than between animals and plants! More details can be found in Lahr et al. (2014).

Distribution of amoeboid protists in the taxonomic tree

Amoebozoa

In the **Tubulinea** group, the amoebas with lobe-like pseudopodia (lobopodia) gather, such as the naked amoeba genera *Amoeba*, *Chaos*, *Hartmanella*, *Saccamoeba* and the shell amoeba genera *Arcella*, *Difflugia*, *Nebela*, *Heleopera*. The **Discosea** group includes genera such as *Cochliopodium*, *Vanella*, *Mayorella* or *Thecamoeba*, and the **Archamoeba** group includes genera such as *Entamoeba*, *Mastigamoeba* or *Pelomyxa*.

SAR

The phylum with the collective name SAR (= Stramenopiles, Alveolata, Rhizaria) contains the **Cercozoa** in the Rhizaria supergroup, which include the amoebae with finely drawn out pseudopodia, so-called filopodia. Among them are, for example, the Euglyphida, the Vampyrellidae, *Gromia* or *Clathrulina*.

Opisthokonta

In the phylum Opisthokonta, which also contains the taxonomic subtrees of fungi and animals, the genus *Nuclearia* is now found in the subtree of fungi (Nucletmycea).

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