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Neuston protists

On the island of Hiddensee in the Baltic Sea near the island of Rügen there is a small fen called Suploch. The water body is abounded with amoebae.

If sample water is left to rest in Petri dishes in the laboratory for a few days or weeks, a small, natural habitat can develop there. Samples from oligotrophic waters are particularly suitable for this method. With the dissecting microscope one can observe the protistic life in such microhabitats very well and transfer interesting objects with the pipette to a slide for more detailed examination. The following report deals with protists that are part of neuston (Preston, 2003), as they live on the surface membrane of the water body.

Tiny watch glasses

The overview in Fig. 1 shows a bacterial lawn, a number of circular structures, which on closer inspection are hourglass-shaped, and naked amoebae. The small watch glasses turned out to be shell amoebae (testaceans) from the group of Arcellinida, genus *Pyxidicula* (Meisterfeld, 2002). The cited article notes that the number of contractile vacuoles is relevant to the genus identification. Regarding the genus of *Pyxidicula*, it is stated: “one contractile vacuole”. However, Ralf Meisterfeld, the author of this section in the guide, confirmed my identification and qualified the number of two contractile vacuoles as an identifier for this special case (personal communication).

Figures 2 to 5 show several optical sections through the observed cells. In the group of four in Fig. 2, the focus is on the level of the bacterial layer. In Fig. 3 the focus is on the nuclei. Figure 4 presents the vesicular (ellipsoidal) nucleus with its large central nucleolus together with the contractile vacuoles, next to it an empty shell, where one can even see the fine granulation of the shell material (pseudochitin with sandpaper structure) and its beaded rim. In order to be able to display the surface structure of the shell material and the beaded rim equally sharply, several optical sections were combined using DOF image technology.

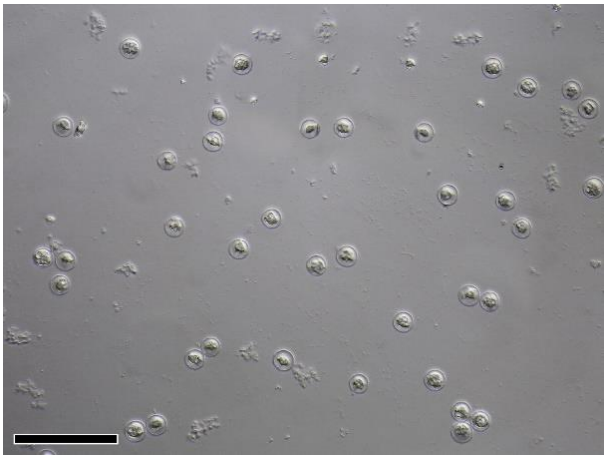

Fig. 1: Overview photo of the surface membrane with bacterial lawn, shell and naked amoebae. Scale bar indicates 100 µm.

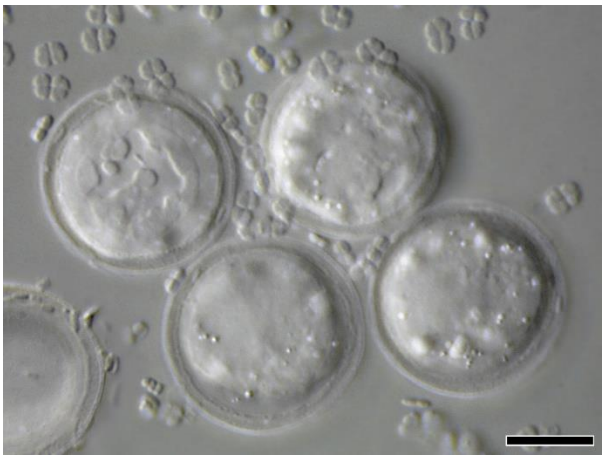

Fig. 2: *Pyxidicula operculata* amoungst bacteria. Scale bar indicates 10 µm.

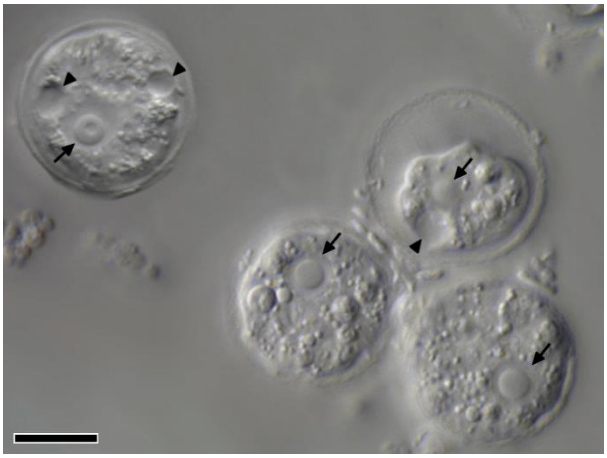

Fig. 3: *Pyxidicula operculata*, focused on the level of the nuclei (arrow) and the contractile vacuoles (arrowhead). Scale bar indicates 10 µm.

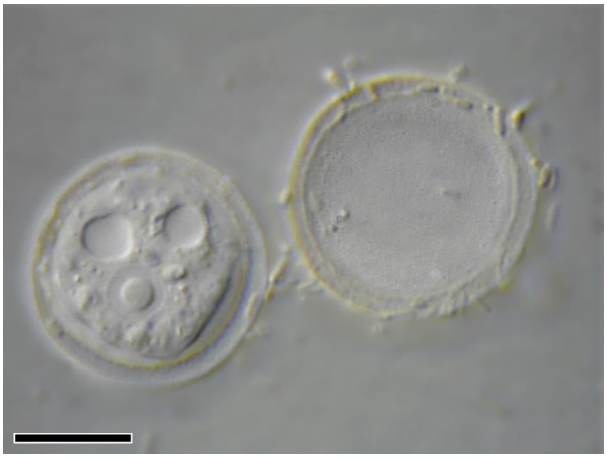

Fig. 4: *Pyxidicula* with the typical vesicular nucleus and the two contractile vacuoles next to an empty shell (DOF image). Scale bar indicates 10 µm.

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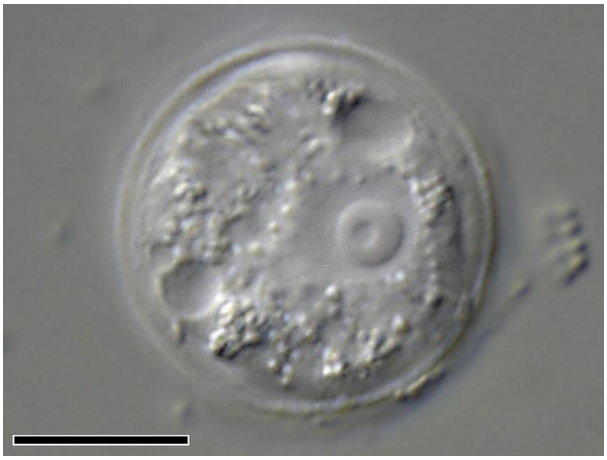


Fig. 5: Detail from Fig. 3. Nucleus with extraordinary structured nucleolus. Scale bar indicates 10 µm.

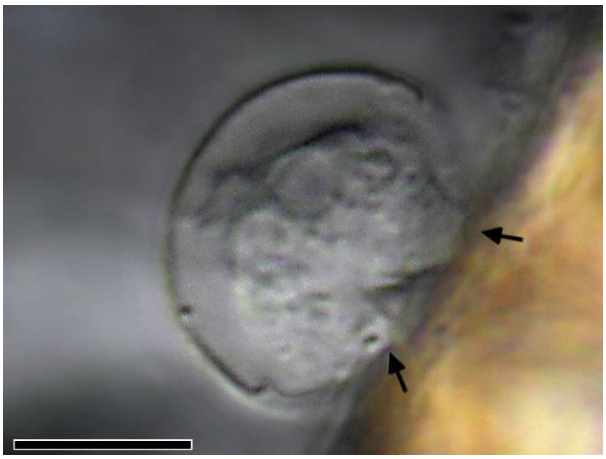


Fig. 6: Lateral view of a *Pyxidicula* showing the conical (v-shaped) pseudopodia (arrow). Scale bar indicates 10 µm.

The aperture (the pseudostome, the shell opening) is almost as large as the shell diameter. The shells are shaped like a flattened hemisphere. Fig. 5 shows a rarely

observed nucleus figure with the additional zone of aberrant optical density in the center of the nucleolus.

The larger dots in the cell plasma are mitochondria. A piece of chitin from a mosquito that hatched in the sample container and then accidentally fell into the water and died opened up the opportunity to photograph a *Pyxidicula* cell in lateral view (Fig. 6). The shell cross section and the aperture are clearly visible. The observer can discover the way of attachment to the substrate and the shape of the pseudopodia.

Naked amoebae hunting for Pyxidicula

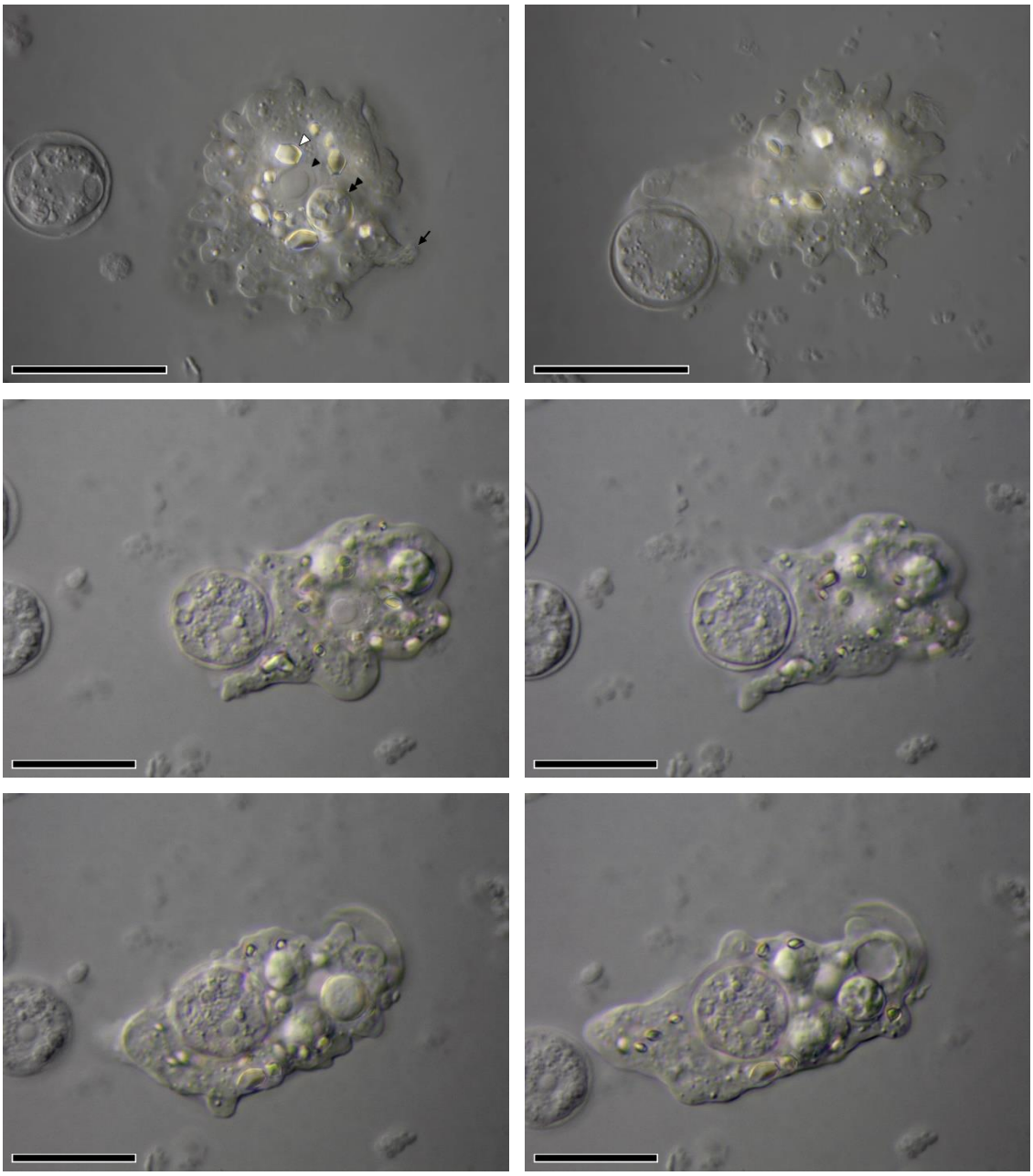


Fig. 7: *Saccamoeba limax* in floating form with vesicular nucleus (arrowhead), crystals (arrowhead outline), food vacuole (double arrowhead) and the uroid (arrow).
Fig. 8–12: Phagocytosis of *Pyxidicula operculata* by *Saccamoeba limax*. Scale bar indicates 25 µm.

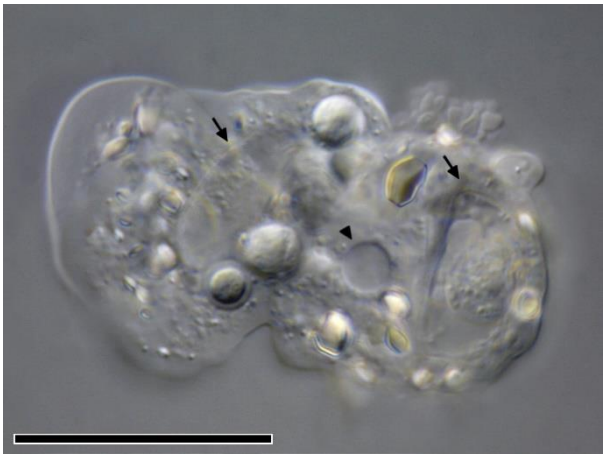


Fig. 13: *Saccamoeba limax* with two ingested *Pyxidicula* cells (arrows). The contractile vacuole (arrowhead) is surrounded by large crystals typical for *Saccamoeba limax*. Scale bar indicates 25 µm.

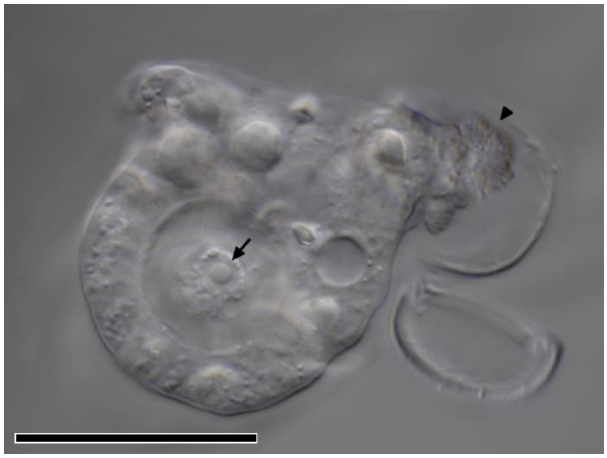


Fig. 14: *Saccamoeba limax*. Display of contractile vacuole and uroid (arrowhead). The phagocytosed *Pyxidicula* has a nucleus that still appears intact (arrow). Scale bar indicates 25 µm.

In addition to the testaceans, a large number of approximately 60 µm elongated naked amoebae of a certain species moved in the surface membrane of the water body. Strictly speaking, it is the hyponeuston, the community just below the surface of the water, to which the amoebae belong. It is distinguished from epineuston, the symbiotic community upon the surface membrane; the creatures there (e.g. certain species of golden algae) are not surrounded by water (Fott, 1959).

The naked amoebae showed very different silhouettes, which at first glance I could not associate with one and the same species. After a longer period of observation, however, I had the opportunity to witness the transformation of the star-shaped resting form into the rounded and elongated locomotive form. The sequence Fig. 7–12 shows this as well as the phagocytosis of a *Pyxidicula* cell by a naked amoeba.

Like *Pyxidicula operculata*, this species has a vesicular nucleus with a relatively large central nucleolus. Both phases of the karyoplasm show no granules. Other larger deposits can be identified in the cytoplasm of the naked amoeba: rhombic and bipyramidal crystals and food vacuoles. Fig. 12 also shows the contractile vacuole and at the right end of the cell one can guess an empty *Pyxidicula* shell, which had been excreted a short time before the exposure was made.

Figures 13 and 14 show other cells. In the cell body of the amoeba from Fig. 13, two *Pyxidicula* shells can be seen (arrows). The individual in Fig. 14 has phagocytosed a *Pyxidicula* cell, the digestion is apparently not very far advanced, at least the cell nucleus of the *Pyxidicula* still appears intact (arrow). On the uroid (arrowhead), the shaggy cell region that is dragged along during movement, two shells excreted shortly before the picture was taken are attached to plasma threads.

Fig. 15: *Saccamoeba limax* in its elongated locomotive form. Scale bar indicates 25 µm.

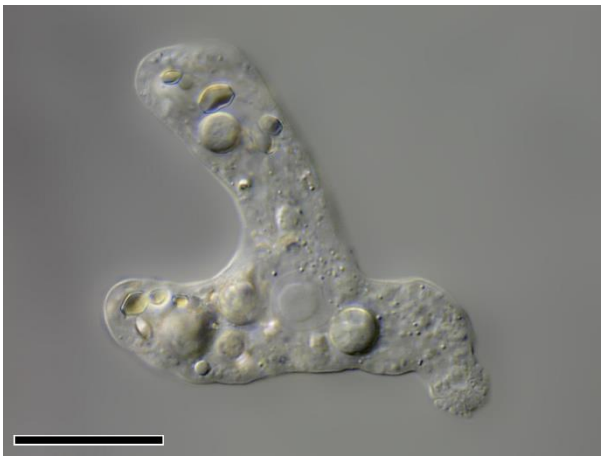


Fig. 16: *Saccamoeba limax* when changing moving direction. Scale bar indicates 25 µm.

The different forms of appearance of *Saccamoeba limax*

When the naked amoebae persented here are in the phase of locomotion on substrate, they show an elongated, monopodial shape with a round cross section and a fine warty uroid. When changing direction, a Y-shape often results. According to Alexander Kudryavtsev (kind mediation by Prof. Klaus Hausmann, Free University Berlin), it is a species of the genus *Saccamoeba* (on *Saccamoeba* see also Mrva, 2007). The description of *Saccamoeba limax* in Page (1976) and Siemensma (1987) fits well, both in terms of overall size, size and shape of the crystals (Page describes them as 'truncate bipyramidal', meaning "shaped like two truncated pyramids placed one on top of the other"), and the shape of the uroid.

Smirnov's (1999) key is a valuable aid for classifying the forms of movement of the lobose naked amoeba. After that, the form observed here falls into the "monotactic" category:

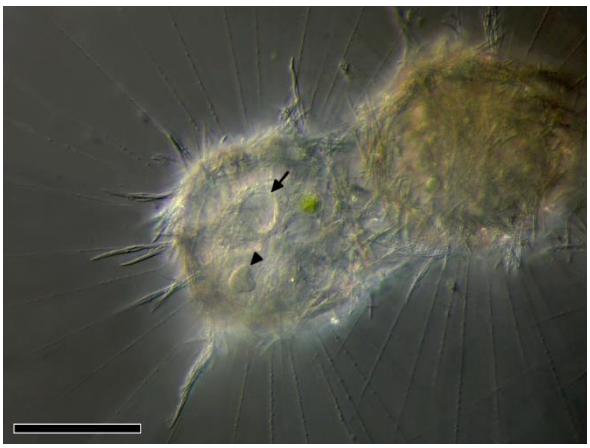
Monopodial. Body more or less cylindrical with round cross section, no lateral wrinkles. No species with adhesive uroid.

This categorization also leads to a group of genera, among which is also *Saccamoeba*. The other genera in this category can be excluded by criteria such as size, uroid shape, and shape as well as size of the hyaline front cap.

As the above images show, *Saccamoeba limax* can change shape from star-shaped (which also represents the floating shape in open water) to oblong up to pear-shaped. It should also be noted that in some manifestations the uroid is only partially visible.

Heliozoans can also be neuston members

After the interesting discoveries in the water surface membrane in my Petri dishes described above, I was curious to see if anything more unusual could be found there. Small bright dots were visible to the naked eye near another chitinous part of a dead mosquito. Observing with the dissecting microscope, the spots turned out to be two feeding communities of *Raphidiophrys pallida* (Kreutz and Foissner, 2006; Page and Siemensma, 1991).



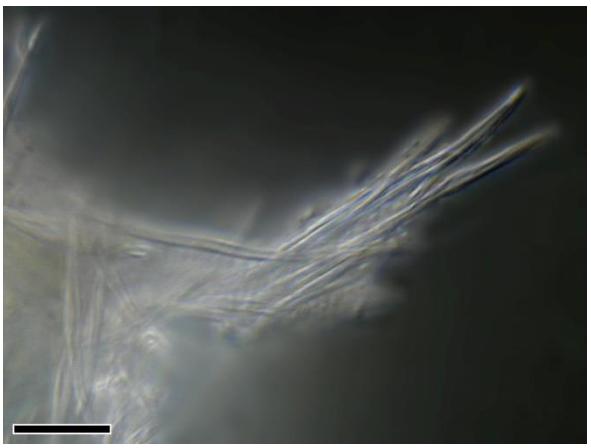


Fig. 17: Feeding community of *Raphidiophrys pallida*. Scale bar indicates 100 µm.
Fig. 18: Picture detail showing the nucleus (arrowhead) and the contractile vacuole (arrow). Scale bar indicates 50 µm.
Fig. 19: In *Raphidiophrys* the spiculae also adhere a short distance along the axopodia. Scale bar indicates 10 µm.

Typical for *Raphidiophrys*, many 10–30 µm long needle-like silicate scales (spiculae) stick tangentially in a mucous cell sheath. This thick cuticle scatters light a lot, so it was not easy to get a sufficiently clear view of the centroplast and nucleus. The centroplast is the origin and organizing center of the microtubules, which in bundles stabilize the axopodia, the radiating pseudopods of the heliozoans. This structure is determinative for the order Centrohelidae, to which the genus *Raphidiophrys* belongs (Fig. 17–19).

Vorticella cell in binary fission

In the immediate vicinity of the *Raphidiophrys* groups, some ciliates of the group Peritrichia, genus *Vorticella*, were attached to the chitinous part of the mosquito mentioned above.

They dangled their heads between the axopodia of the heliozoans and sometimes folded their peristome field for a short time after collision with the axopodia. I assumed that the *Vorticella* cell heads were only inside the heliozoan axopods because of the narrowness under the coverslip, and thus did not feel comfortable between these rods armed with dangerous extrusomes (nodular prey-catching organelles).

Figure 20 shows that the peristome field is fully retracted. This is a familiar sight for me as the cell prepares to develop a telotroch (column of cilia) to detach from the stalk and escape from an uncomfortable area.

I assumed that was the case here, and I turned back to the *Raphidiophrys* and *Pyxidicula* cells, which were my main concern at that moment. I had already seen several times a transformation of a *Vorticella* trophont into the swarmer state, along with

Fig. 20: *Vorticella* spec. shortly before binary fission. Macronucleus and contractile vacuole. Scale bar indicates 25 µm.

Fig. 21: *Vorticella* spec. shortly after binary fission. Scale bar indicates 25 µm.

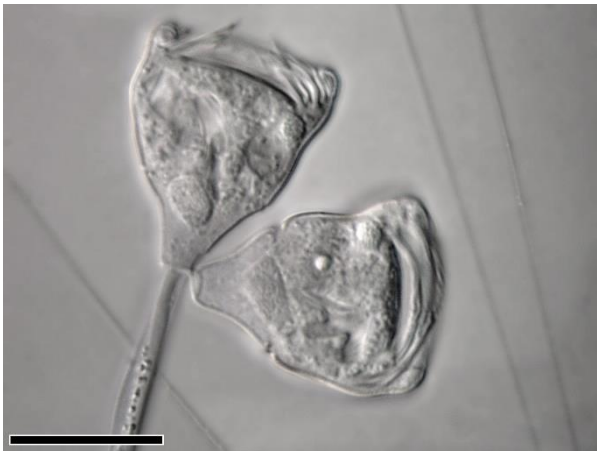


Fig. 22: *Vorticella* spec., the adoral zone of membranelles arises from the newly formed cell. Scale bar indicates 25 µm.

Fig. 23: *Vorticella* spec. The new cell has balled up and is already showing the appearance of a swarmer. Scale bar indicates 25 µm.

detachment from its stalk, and had already documented this with photographs. But I was wrong. Peritrichia also fold their peristome when they want to undergo binary fission.

When I accidentally came back into the zone of this *Vorticella* cell with the lens about 40 minutes later, I only had a short time to get angry because I saw that most of a cell division had already taken place. Now it was my job to at least document the rest of the binary fission process. It still took about 90 minutes for the swarmer to leave the stalk of the mother cell like a rocket.

The conversion to the swarmer state

In Fig. 21 and Fig. 22 you can already see the approach of the transitory ciliary girdle (telotroch), the notches at the end of the outline of the conical part of the cell body. A bulging constriction in the cortex (the complex cell cover of the ciliates) evolves, from which lots of cilia are slowly sprouting, which then deliver the propulsion in the migration phase. In Fig. 23, the ring-shaped bead can be seen clearly. The positioning of the *Vorticella* cells amidst the axopod region of the *Raphidiophrys* group can also be seen. It was astonishing to observe how both cells were synchronized to extend their adorale zones of membranelles (AZM) of their peristomes. While this makes sense for the cell that keeps the stalk, the other cell, which has to swim away, has to retract the AZM later again.

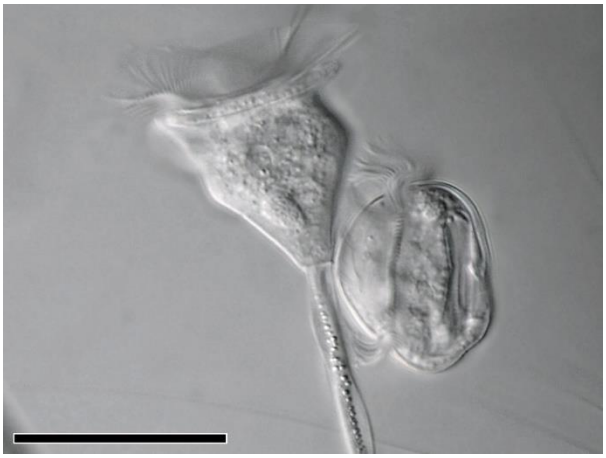
In the course of further observation, I could see the swarmer getting a spherical outline, and retracting its AZM. Fig. 23 already shows the telotroch clearly developed, the cilia are almost 5 µm long. At this stage, the ciliary ring undulated slowly, sometimes twitching, with a period of four to five seconds. At the end of this process, the cilia had grown

up to a length of approx. 8 µm. The initially quite atactic movements of the cilia (very jerky undulation of the ciliary ring) became increasingly coordinated and faster over time, the undulation frequency rose to once per second.
Suddenly it was as if the engine ran warm. The fast movements of the cilia could no longer be observed separately, the cell tore violently on the thread-shaped connection, and a short time later it had loosened.

A symbiotic community?

I have observed the group of *Vorticella* cells in the vicinity of the two feeding communities of *Raphidiophys* for about 2 hours, not just the cell that had divided. After the impressions that I have collected during this observation time, I can well imagine that *Vorticella* actively get the spatial connection with the *Raphidiophrys*, because they came across the axopodia relatively often, but their extrusomes have never attacked the cells.
In space between the axopodia of the heliozoans, the *Vorticella* cells enjoy protection. The water current that their AZM create is potentially advantageous for the heliozoans. This could be a symbiotic community for the benefit of both species.

When a swarmer hits roots



The landing of a *Vorticella* swarmer with subsequent development of the stalk is also an interesting spectacle. In the swarmer state, the cell moves so that the retracted peristome shows backwards. If the cell during its underwater flight encounters a substrate that is suitable for the adhesion, it begins to press – stabilized by the propulsion of the telotroch. In this phase, the swarmer looks like a humming-top, which has a slightly too little momentum and therefore begins to stagger. After a few minutes, the initial of the stalk has formed. As soon as the attachment is stable, the cilia ring of the telotroch stops undulating. Its retrofitting begins very quickly, but it takes some time for the ciliary girdle to be fully degenerated. Figures 25 to 30 show the entire cycle from landing to the adhesion, the subsequent development of the stalk, up to a renewed conversion into the swarmer status, and finally the detachment from the stalk.

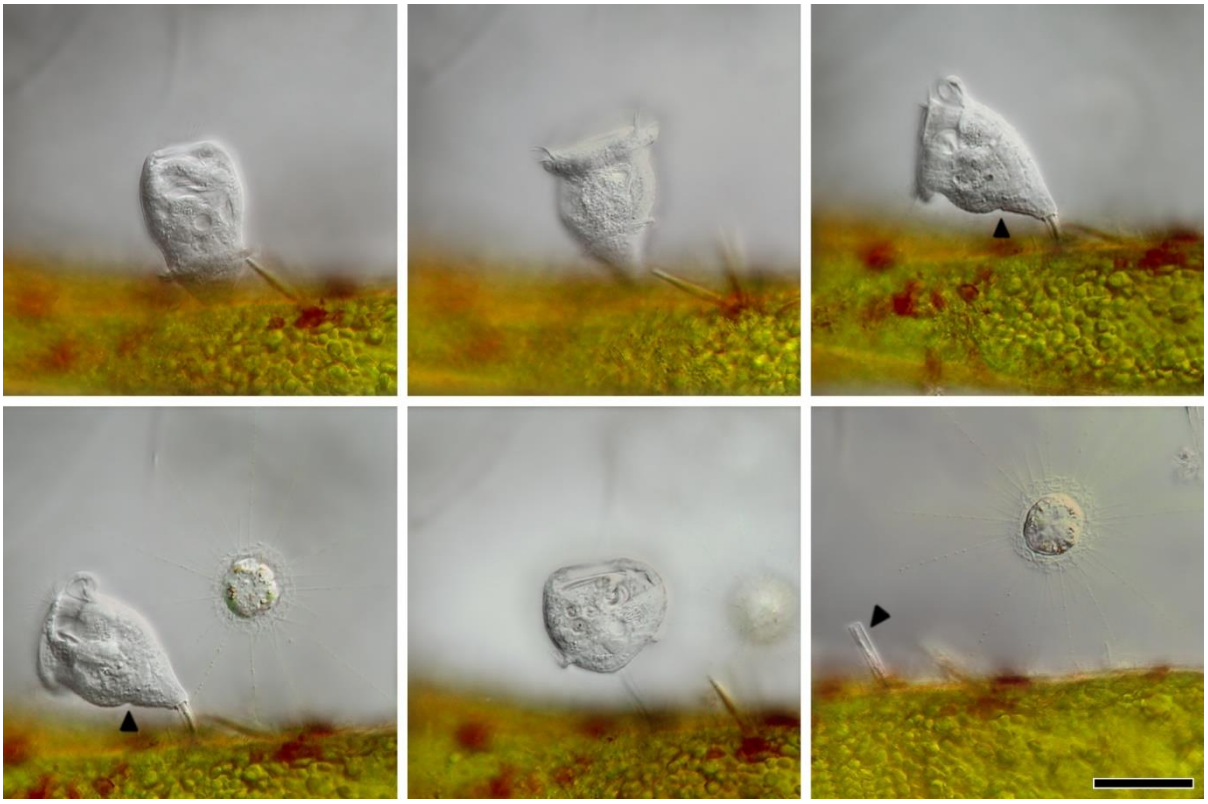


Fig. 25: *Vorticella* spec. shortly after fastening on the substratum. Fig. 26: Eight minutes later: The initial stalk has been formed, the telotroch is being degenerated. Fig. 27: 17 minutes later: The notch (arrowhead) represents the rest of the telotroch. Fig. 28: 50 minutes later: The initial notch of the newly forming telotroch (arrowhead) is visible again.
Fig. 29: 90 minutes later: The development of the new telotroch has been completed. Fig. 30: 100 minutes later: The remaining stalk (arrowhead), and the heliozoan *Raphidocystis tubifera*. Scale bar indicates 25 µm.

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