Protozoological Monographs, Vol. 4, 211 – 219

Soil Ciliates

Wilhelm Foissner

INTRODUCTION

Of the 10,000 ciliate species described, about 1000 are known to occur in terrestrial habitats, such as mosses, leaf litter, soil, and the mixture of mud and soil at the bottom of puddles. However, the real number of terrestrial ciliates is much higher because new species are continuously being described. The earlier view that the same ciliate species occur in soil and freshwater is outdated. We now know that most soil ciliates are autochthones, that is they occur only, or preferably, in terrestrial habitats.

Soil ciliates have interesting morphological and functional adaptations for the optimal use of the resources of the soil. These adaptations demonstrate important ecological principles. The morphological peculiarities are caused by the spatial constraints of the narrow soil pores and the film-like distribution of the water. Thus, most soil ciliates are small, their average length, width, and biomass being significantly smaller than in limnetic communities. The diminution is reached either by a general size reduction (Fig. 1, 2, 7, 9, 10, 12, 13) or by the reduction of body width, causing the vermiform shape typical for many soil organisms (Fig. 3 – 6, 8, 11). Usually, soil ciliates are flat and ciliated only on the ventral (oral) surface and exploit the thin films of water covering the soil particles and walls of soil pores. Most soil ciliates are mobile, while sessile Suctoria and Peritricha are frequent in limnetic biotopes. The ability to move freely is necessary to escape food depletion in the soil pores.

Free water is indispensable for protozoan life. Thus, most functional and physiological peculiarities of the soil ciliate community are related to the changing availability of water in the habitat; most soils even dry out from time to time. The second main factor is the special food resources, for example, the abundance of fungi that are used by mycophagous species (see *Grossglockneria acuta*). Dry periods are survived in a so-called resting cyst. When encysting, the ciliate rounds up, loses water, secretes a thick, waterproof wall, and then strongly reduces its metabolism. The cyst is left when good environmental conditions are available again. Obviously, it is advantageous to have a wide ecological range and fast reproduction to use the short periods of optimal moisture and food supply. Such organisms are called r- (<reproduction>) strategists, of which the colpodid ciliates described below are typical examples. Some of these ciliates are so moderate in their requirements that they can reproduce in very extreme habitats in which water is present for only a few hours, such as dew drops and antarctic soils.Today, such specialists are also called a-(<adversity>) strategists.

Active ciliates occur mainly during the very early stages of soil formation, especially in the fresh leaf litter. Thus, vertical distribution of active ciliates shows a sharp break (Table 1): the abundance abruptly decreases to almost zero in 3–9 cm soil depth, i. e., in the fermentation layer (= zone with intense decomposition processes).

This phenomenon is called "ciliatostasis". It has been supposed that certain substances, for example antibiotics, accumulate in evolved (older) soils and arrest excystment and reproduction of ciliates, as they are known to do for fungi (fungistasis). This inhibition can be partially released, for example, by drying the soil. When dry soil is rewetted, great numbers and intense reproduction of ciliates occur, at least in the laboratory, because many specimens, which were encysted for years, excyst and commence to reproduce (see the first four habitats in Table 1). The following method uses this phenomenon for obtaining rich raw cultures of ciliates and other protists.

Table 1: Number of active ciliates (per g dry mass of soil) in various soils.

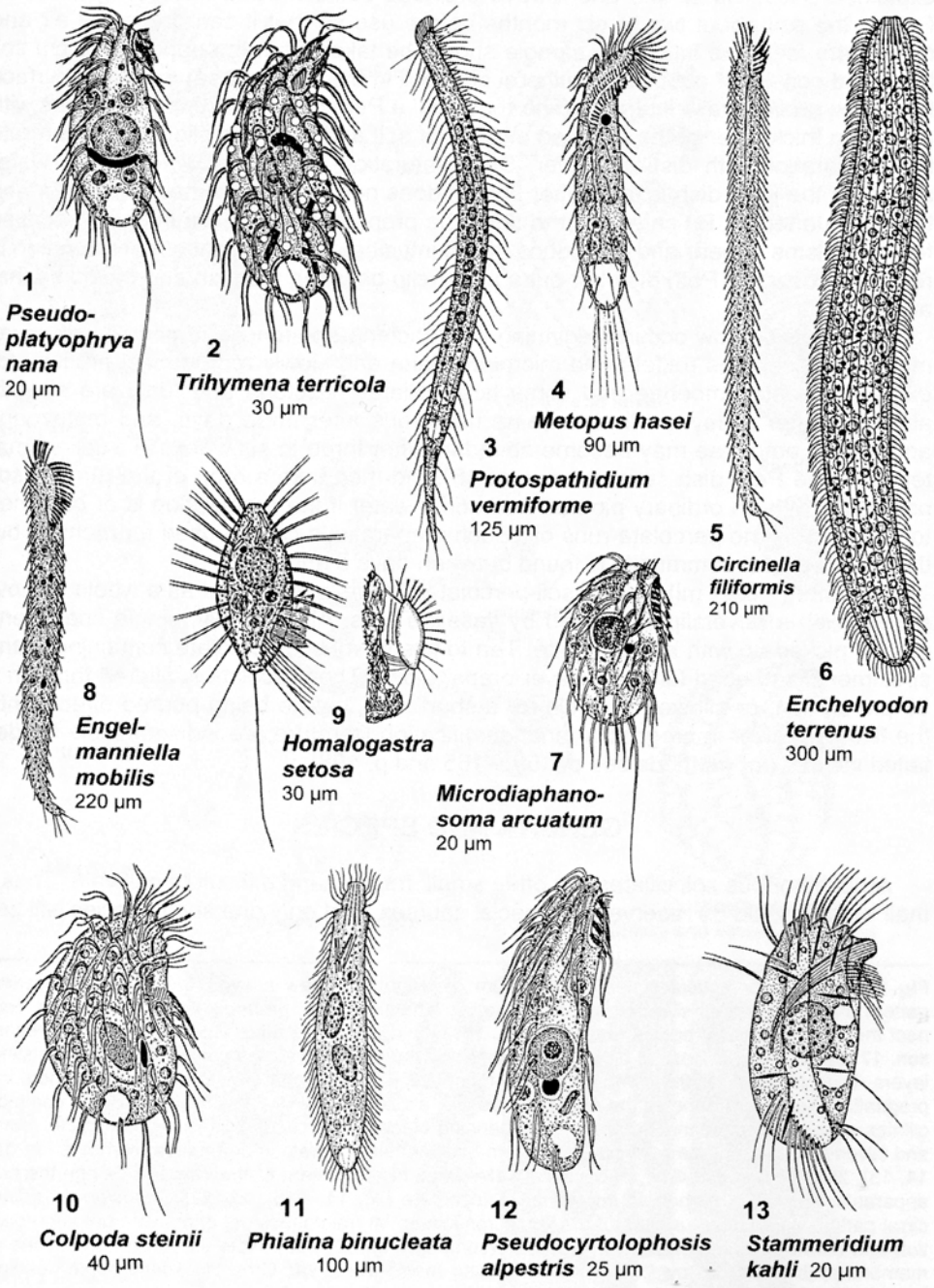
Habitat	fresh soil	dried, rewetted soil
Field	1	379
Meadow	3	520
Needle litter	468	17,390
Leaf litter	3326	104,340
Needle litter		
O_L 0 – 1 cm	350	not investigated
O_L 1 – 3 cm	109	not investigated
O_F 3 – 9 cm	14	not investigated

PRACTICAL TECHNIQUES

Soil ciliates can be collected 24–48 h after rain by rinsing some wet leaves and/or needles with a few ml of rain water or tap water. A drop of the resulting suspension can be investigated either as a whole, or single specimens can be picked out with a fine pipette under the stereomicroscope or other microscope at a magnification of x 40. The investigation of fresh, wet forest litter is strongly recommended for an introductory demonstration; it is impressive over and over again to see how rich natural forest litter is populated with bacteria, fungi, protists, and small metazoans.

Pure cultures of protists are often both difficult and time-consuming to obtain. However, the species discussed here are rather easily cultivated. The methods are given as the individual species are described. Material from water-saturated soil cultures usually suffice, and show diversity much better than a series of pure cultures.

Fig. 1 – 13. Small size or vermiform shape as adaptations to the soil habitat. **1:** *Pseudoplatyophrya nana* (20 µm), a mycophagous, flattened colpodid. **2:** *Trihymena terricola* (30 µm), a bacterivorous colpodid. **3:** *Protospathidium vermiforme* (125 µm), a raptorious holotrich. **4:** *Metopus hasei* (90 µm), a bacterivorous spirotrich indicating oxygen depletion. **5:** *Circinella filiformis* (210 µm), a bacterivorous, very slender hypotrich. **6:** *Enchelyodon terrenus* (300 µm), a large, holotrichous predator. **7:** *Microdiaphanosoma arcuatum* (20 µm), a bacterivorous, flattened colpodid. **8:** *Engelmanniella mobilis* (220 µm), a bacterivorous, slender hypotrich. **9:** *Homalogastra setosa* (30 µm), a hymenostome ciliate; at right, a specimen attached to soil particles and feeding on bacteria. **10:** *Colpoda steinii* (40 µm), a bacterivorous colpodid (compare Fig. 20). **11:** *Phialina binucleata* (100 µm), a holotrichous predator. **12:** *Pseudocyrtolophosis alpestris* (25 µm), a bacterivorous, flattened colpodid. **13:** *Stammeridium kahli* (20 µm), a bacterivorous, strongly flattened, microthoracid ciliate.


1
Pseudo-
platyophrya
nana
20 µm
2
Trihymena terricola
30 µm
3
Protospathidium
vermiforme
125 µm
4
Metopus hasei
90 µm
5
Circinella
filiformis
210 µm
6
Enchelyodon
terrenus
300 µm
7
Microdiaphano-
soma arcuatum
20 µm
8
Engel-
manniella
mobilis
220 µm
9
Homalogastra
setosa
30 µm
10
Colpoda steinii
40 µm
11
Phialina binucleata
100 µm
12
Pseudocyrtolophosis
alpestris 25 µm
13
Stammeridium
kahli 20 µm

This so-called "non-flooded Petri dish method", which uses the release of ciliatostasis explained above, is simple and always provides sufficient and interesting material. Collect the soil about two to six months before use so that it can dry in the air and remain dry for some time. The sample should be taken from the upper 0–10 cm soil layer and consist of about 70% mineral soil (soil in the strict sense) and 30% surface litter (tree and/or grass litter) and fine roots. Fill a Petri dish (10–20 cm diameter) with a 1–3 cm thick layer of the air-dried mixture of soil and litter and slightly oversaturate the preparation with distilled water. Supersaturation is reached when some water runs off if the Petri dish is tilted after 5h; if it does not then add some more water and test again after 1h. Be careful not to flood the preparation as for an infusion because few organisms appear and reproduce in soil infusions. When slight supersaturation is reached, cover the Petri dish but put a paper clip between the dish and the lid so that air can enter.

A succession now occurs beginning with bacteria and fungi and ending with small metazoans, such as rotifers and microturbellaria and slowly reproducing protists, for example testate amoebae and some large ciliates. Bacteria and fungi are already abundant after a day, protists become numerous after three days, and metazoans and testate amoebae may become abundant after three to six weeks. To collect material, tilt the Petri dish several times at 45° and then take a drop of the run off (soil percolate) with an ordinary pipette. Add some water if the preparation is or becomes too dry, that is, no percolate runs off. Such preparations can be used for months, but the richest protist community is found between days 5 and 20.

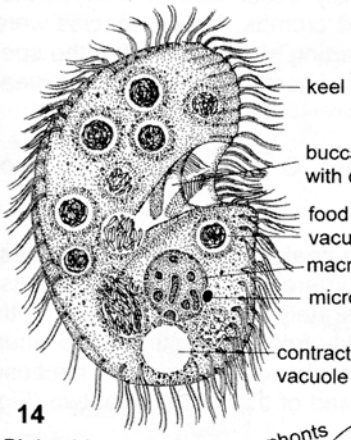
A minute (~ 0.01 ml) drop of soil percolate can be investigated as a whole by covering it with a coverslip supported by vaseline tabs. Alternatively, single specimens can be picked up with a fine pipette. Ten to twenty ml soil percolate containing many specimens is needed for some silver preparations. The percolate is filtered through a 50 µm screen, or allowed to settle for a short time, before being poured directly into the fixative. Silver impregnation and identification literature are indispensable for detailed studies (for methods see p. 159 – 165 and p. 198).

GENERA AND SPECIES

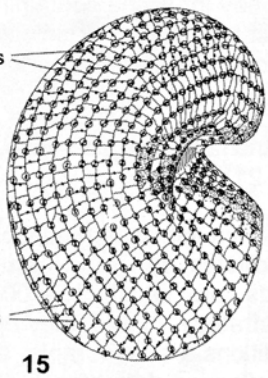
Autochthonous soil ciliates are often small, fragile, and difficult to cultivate. Thus, their study should be reserved for special courses, and only one such species will be

---

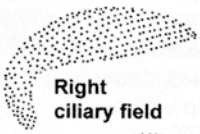
Fig. 14 – 19. *Colpoda cucullus*, length 60–80 µm. **14:** Right side view in vivo. **15:** Ciliary and silverline pattern of right side after silver nitrate impregnation. The silverlines, probably some sort of fibre, connect the individual basal bodies and their cilia. **16:** Oral ciliary fields after silver carbonate impregnation. **17:** Life cycle. **18:** Resting cyst, living, diameter 50 µm. The cyst wall consists of several distinct layers. The cell proper is the compact, central mass. **19:** Nuclear apparatus after silver carbonate impregnation. The arrow denotes the micronucleus with a size of 3 x 2 µm. **Fig. 20 – 24.** Some common ciliates after silver impregnation and in the scanning electron microscope. **20:** *Colpoda steinii*, ciliary and silverline pattern of the right side after Klein-Foissner silver nitrate impregnation (compare Fig. 10, 14, 15). **21:** *Colpoda cucullus*, macronucleus (MA) and ciliary pattern of the ventral side and the oral apparatus after silver carbonate impregnation (compare Fig. 14 – 16). **22, 23:** *Gonostomum affine*, cirral pattern and nuclear apparatus (MA, macronucleus; MI, micronucleus) after protargol impregnation and in the scanning electron microscope (compare Fig. 30 – 32). Note the large adoral zone of membranelles (AZM). Arrows mark the undulating membranes. **24:** *Grossglockneria acuta*, feeding tube (arrow) in the scanning electron microscope (compare Fig. 25 – 27).



14
Right side view
in vivo



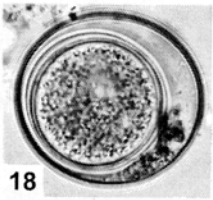
15
Ciliary and silver-
line pattern



Right
ciliary field

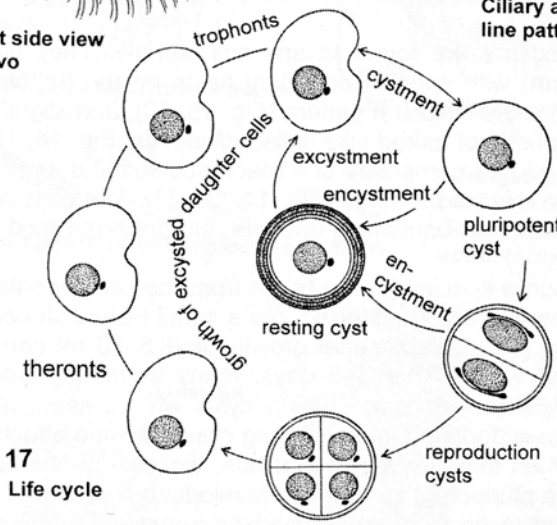
16

Left
ciliary field



18
Resting cyst

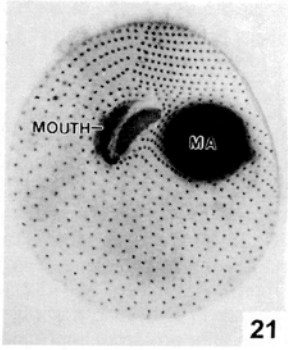
19
Nuclear apparatus



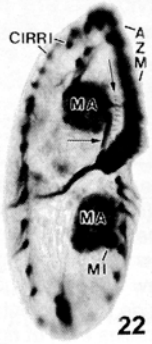
17
Life cycle

Colpoda cucullus (14 – 19, 21)

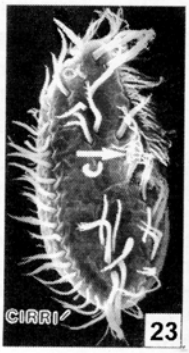
20
Colpoda steinii
Ciliary and silverline pattern



21



22



23

24

described here, namely the obligate mycophage *Grossglockneria acuta*. The other species chosen are more robust and may occasionally occur also in limnetic biotopes, especially in hay infusions containing some soil crumbs. These species were selected with respect to survival (r/k selection) and feeding strategies. All of the species described are common in non-flooded Petri dish cultures of forest and/or meadow soils.

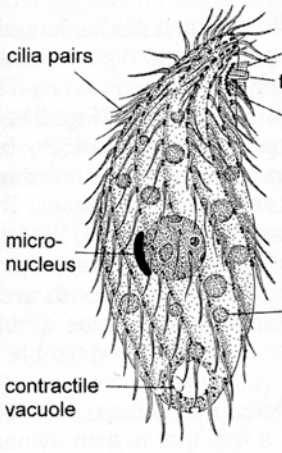
Colpoda spp. Holotricha in a broad sense, Cl. Colpodea, O. Colpodida, Fam. Colpodidae (Fig. 10, 14 – 21)

The soil ciliate community is termed Colpodetea (in analogy to plant communities) because colpodid ciliates are very frequent and numerous in terrestrial habitats. In general, colpodids are small (30–100 µm), moderately flattened ciliates with r-selected survival strategy, that is, they emerge quickly from the resting cysts when environmental conditions are favourable, and then soon become numerous because they generate four offspring during each division, instead of the more usual two (Fig. 17).

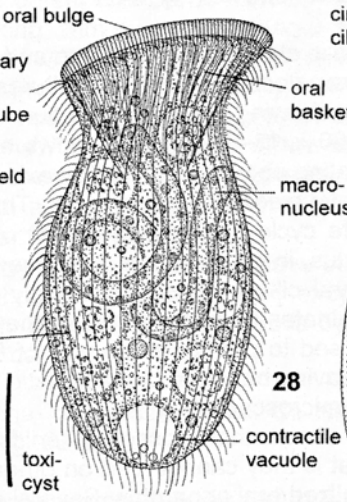
Species of the family Colpodidae are found in any soil sample. They have a subapical oral funnel (vestibulum) with specialized ciliary fields on the bottom and roof (Fig. 14 – 16, 20, 21), a reticulate silverline pattern (Fig. 15, 20), and slightly spirally extending ciliary rows composed of paired cilia called dikinetids (Fig. 14, 15, 20, 21). As usual, the nuclear apparatus is composed of a macronucleus and a micronucleus, both located in the middle third of the body (Fig. 14, 19, 21). The cells are often studded with globular food vacuoles containing bacteria, the preferred food (Fig. 14).

For studying the life cycle, some specimens are taken from the raw (non-flooded Petri dish) culture with a micropipette and transferred into a small Petri dish containing a squashed wheat grain (to stimulate bacterial growth) and 5–10 ml non-chlorinated tap water or still bottled water. After 3–5 days, many swimming *Colpoda* specimens and, on the bottom, some globular division cysts will be seen; after 2 weeks, when the culture medium is depleted, many resting cysts become attached to the bottom of the Petri dish. When they are ready to divide, the cell rounds up and secretes a thin wall, producing a pluripotent cyst which develops to a division cyst or thick-walled resting cyst, depending on favourable or adverse environmental conditions (Fig. 17, 18). The first division within the cyst produces two offspring, which then divide once more. Thus, four individuals are produced in each cycle (Fig. 17). Occasionally, the offspring divide several times generating 8 or 16 individuals.

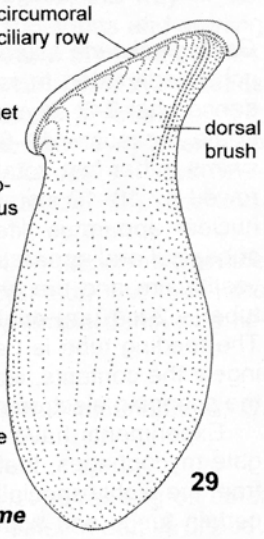
Fig. 25 – 27. Morphology and cultivation of the mycophagous *Grossglockneria acuta*. **25:** Right side view in vivo, length 40–80 µm. **26:** Cultivation (see text). **27:** A fungal hypha with a small hole (arrow) produced by the feeding tube of *Grossglockneria acuta*. **Fig. 28, 29.** Morphology of the raptorious *Epispathidium amphoriforme*. **28:** Left side view from life, length 130 µm; left a toxicyst from the oral bulge at higher magnification. **29:** Left side view after protargol impregnation. **Fig. 30 – 32.** Morphology of *Gonostomum affine*. **30** and **31:** Right side (about ventral) views in vivo, length about 100 µm. **32:** Infraciliature (cirral pattern, adoral membranelles etc.) of right side after protargol impregnation.



25 ***Grossglockneria acuta***

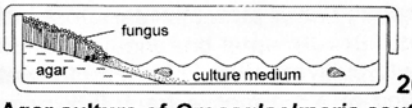


28



29

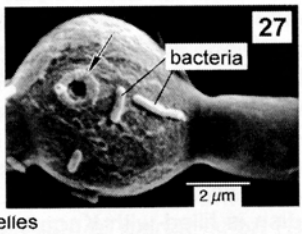
Epispathidium amphoriforme (28, 29)

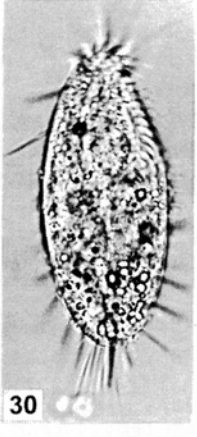


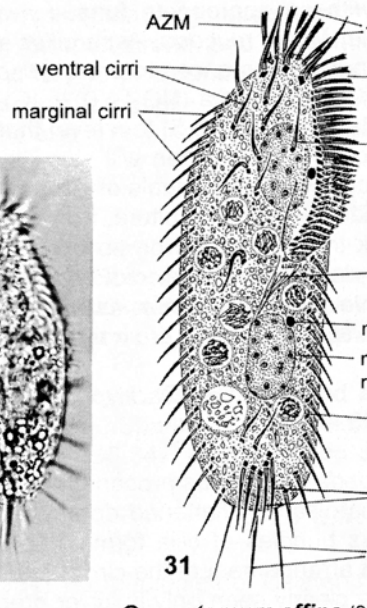
26

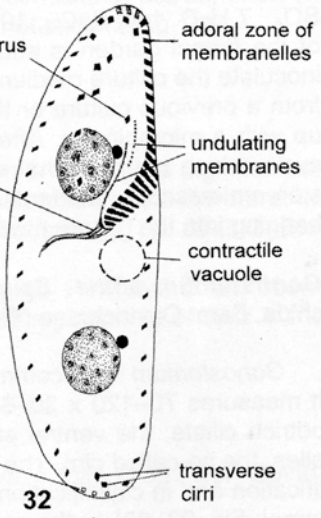
Agar culture of *Grossglockneria acuta*

A fungus hypha perforated by *Grossglockneria acuta* (arrow)









Gonostomum affine (30 – 32)

Grossglockneria acuta Holotricha in a broad sense, Cl. Colpodea, O. Grossglockneriida, Fam. Grossglockneriidae (Fig. 24 – 27)

Grossglockneria acuta is also a colpodid ciliate and is very frequent in acidic, fungal-rich needle litter. In non-flooded Petri dish cultures, it develops to its highest abundance after 2–3 days, showing the r-selected survival strategy described above. The ciliate measures 40–60 x 15–35 µm and shows an acute anterior end (Fig. 25). It swims rather fast, rotating about its main body axis. The pellicle is often distinctly furrowed by 10–12 spirally extending ciliary rows. The general organization (ciliature, nuclear apparatus, life cycle etc.) matches that of *Colpoda* described above. The subapical oral apparatus, in contrast, has a unique organization (Fig. 24, 25, 27): the vestibulum and the oral ciliary fields are strongly reduced, while a minute feeding tube (~ 2 x 1 µm) originates from the centre of the very small (3–5 µm) mouth area. The feeding tube is used to penetrate the wall of fungal hyphae and spores and to ingest the contents, leaving behind a characteristic hole that is easily recognizable in the scanning electron microscope (Fig. 24, 27).

Experiments have shown that grossglockneriids (7 species are known) are obligate mycophages, that is they cannot feed on other items, a fact that is also evident from the highly specialized oral apparatus. Several species of the family feed only on certain fungi, and some feed on plant pathogens, for example, *Ustilago maydis*, a very damaging pathogen of maize. Thus, mycophagous ciliates offer the possibility for biological control of soil-borne fungal diseases.

Grossglockneria acuta is comparatively easy to cultivate (Fig. 26). Half of a Petri dish is obliquely filled with nutrient agar consisting of 3.5 g malt extract, 0.5 g peptone, 3 g agar, and 93 ml tap water (sterilized by steaming for 3 x 20 min). During solidification, the agar is inoculated with an appropriate fungus available in most microbiological laboratories, for example, *Mucor mucedo*, *Aspergillus* spp., or *Absidia orchidis*. After some days, when the fungus commences to produce spores, the Petri dish is filled with Knop´s solution (1g KNO_3, 0.1g Ca $(NO_3)_2$, 0.2g K_2HPO_4, 0.1g Mg $SO_4 \cdot 7\ H_2O$, 1mg $FeCl_3$, 1000 ml distilled water, pH 6.5) to a level that only the edge of the fungal garden is submerged (flooding the garden will stop its growth!). Now inoculate the culture medium with one or several individuals of *Grossglockneria acuta* from a previous culture or the non-flooded Petri dish culture, from which it is picked up with a micropipette. After one week the ciliates will be so abundant that the life cycle, which matches that of *Colpoda* described above, can be studied. Under the stereomicroscope, students can observe *Grossglockneria acuta* feeding on hyphae hanging into the culture medium and on spores lying on the bottom of the Petri dish.

Gonostomum affine Spirotricha in a broad sense, Cl. Hypotrichea, O. Stichotrichida, Fam. Oxytrichidae (Fig. 22, 23, 30 – 32)

Gonostomum is as common as *Colpoda* and is thus present in most soil samples. It measures 70–120 x 30–60 µm and is distinctly flattened dorsoventrally. As a hypotrich ciliate, the ventral surface bears bundles of cilia forming compound organelles, the so-called cirri. The very varied arrangement of the cirri is important for identification and in classification but can be clearly seen only in silver preparations (protargol; Fig. 22, 32) or the scanning electron microscope (Fig. 23). On the dorsal sur-

face, there are three ciliary rows each composed of 3 µm long, inconspicuous bristles. The lack of a functional dorsal ciliature is associated with the way of life: most hypotrichs are grazers "walking" with the cirri on various substrates and swirling food, often bacteria, into the mouth with the help of the adoral zone of membranelles (AZM), which is usually the most prominent feature in hypotrichs. The AZM extends along the left anterior half of the body and is composed of about 27 ciliary plates, each consisting of four rows of cilia. The right border of the buccal cavity bears two inconspicuous undulating membranes. The nuclear apparatus consists of two ellipsoidal macronucleus nodules with a micronucleus each (Fig. 22, 31, 32). The contractile vacuole is in mid-body near the left body margin.

Most hypotrichs are k-(capacity) strategists, that is, have other ecologies than the r-selected colpodids discussed above. For example, they excyst later and reach the abundance maximum more slowly because they reproduce by simple transverse fission producing only two daughter cells.

Spathidium sensu lato. Holotricha in a broad sense. Cl. Kinetofragminophora, O. Spathidiida, Fam. Spathidiidae (Fig. 28, 29)

The Spathidiidae are holotrichous (completely ciliated) predators feeding on other ciliates. There are several genera and over 200 species which differ in details of body shape, nuclear apparatus, and ciliary pattern (Fig. 3, 28). The species shown here, is rather frequent and large: 90–150 x 35–65 µm. *Epispathidium amphoriforme* has an amphoriform body with the entire anterior margin modified to a mouth bulge. This bulge contains hundreds of 7 µm long toxicysts that kill the prey, which is transported into the cell along the conical oral basket (Fig. 28, 29). The basket is made of many fine rods (nematodesmata) originating from the circumoral ciliary row. The macronucleus is a long, tortuous strand, and the contractile vacuole is in the rear of the body (Fig. 28). The ciliary rows extend meridionally and three dorsal rows are anteriorly modified to a special organelle, the dorsal brush, which consists of paired, short (≤ 5µm), bristle-like cilia. The function of the dorsal brush is not known (Fig. 29).

BIBLIOGRAPHY

Foissner, W. (1987): Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. In: Corliss, J. O. & Patterson, D. J. (eds.), *Progress in protistology*, pp. 69-212. Biopress Ltd., Bristol.

Foissner, W. (2004): Protozoa. In: Hillel, D., Rosenzweig, C., Powlson, D. S., Scow, K. M., Singer, M. J., Sparks, D. L., Hatfield, J. (eds.), *Encyclopedia of soils in the environment*, pp. 336-347. Elsevier, Oxford.

FILM

Grell, K. G. (1964): Morphologie der Ciliaten I – Holotricha. – Film C 881 IWF Göttingen (spoken commentary in German), 9 ½ min.