# CHAPTER 2-1

**PROTOZOA DIVERSITY**

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CHAPTER 2-1
PROTOZOA DIVERSITY

Figure 1. *Actinophrys sol*, a heliozoan that can sometimes be found among mosses in quiet water, with a diatom. Photo by Yuuji Tsukii, with permission.

Moss-Dwelling Micro-organisms

Bryophytes are truly an elfin world, supporting diverse communities of organisms that we often can't see without a microscope. As one might expect, micro-organisms abound (Figure 1) (e.g. Leidy 1880; Maggi 1888; Penard 1908; Heinis 1910; Sandon 1924; Bartos 1946, 1949a, b; Ramazotti 1958; Torumi & Kato 1961; Matsuda 1968; Smith 1974a, b; Schönborn 1977; Sudzuki 1978; Bovee 1979), traversing the crevices like fleas among a dog's hairs. Bovee (1979) reported 145 taxa of protozoa from bogs in the Lake Itasca region, Minnesota, USA. In fact, there are sufficient of these organisms associated with *Sphagnum* that there have been books published on their identification (e.g. Hingley 1993). From forest bryophytes, Bovee found only 68 taxa. Ciliates and testate amoebae dominate the protozoa in both habitats. Even floating liverworts like *Ricciocarpos natans* have their associated microfauna (Scotland 1934).

Gerson (1982) suggests that protozoa have evolved into the bryophyte habitat. Water that wets the mosses permits the protozoa to complete their life cycles. Moist bryophytes easily accumulate windborne dust, providing even epiphytic species with a source of nutrient matter to serve as food for bacteria and ultimately protozoa. Colonization of aerial bryophytes by micro-organisms could likewise be accomplished by wind. Dispersal of these small organisms may be similar to dispersal of spores of mosses, and the implications of their small size will be discussed later in this chapter.

Terminology

It has been a while since I examined the classification of the micro-organisms, so organizing this chapter turned out to be a bigger mire than I had bargained for. I am sure some of my classification is old-fashioned, but practicality has won out if I am ever to approach completion of this volume. I have tried to update where possible, but some things just don't fit there in my mind, or seem more appropriate to write about in a different place. I have decided to avoid kingdom arrangements completely, so you may find some traditional algae here and others in a chapter labelled algae.
Organisms living "firmly attached to a substratum," but not penetrating it, are known by the German term **Aufwuchs** (Ruttner 1953), introduced in 1905 by Seligo (Cooke 1956). Later the term **periphyton** (literally meaning "around plants") was introduced for organisms growing on artificial objects in water. This term was later expanded to refer to all aquatic organisms growing on submerged surfaces. Young (1945) restricted the definition to "that assemblage of organisms growing upon free surfaces of submerged objects in water and covering them with a slimy coat" (in Cooke 1956). The use of the term has varied, including not only **epiphytes** (those living on plants and algae), but also organisms on non-plant substrata. Although the term Aufwuchs has enjoyed a less confusing history of meanings, Americans tend to use periphyton more frequently to refer to those microorganisms living upon a substrate. By whatever term, this group of microorganisms often creates a rich community in association with bryophytes. This chapter will concentrate on the protozoa.

**Abundance**

One difficulty in describing the micro-organisms of bryophytes is the tedious task of sorting through and finding the organisms. Methods for finding and enumerating protozoa are discussed later in this chapter. Often identification and quantification requires culturing the organisms, which will bias the counts to those most easily cultured. Testate rhizopods are most easily located because the presence of the test permits recognition even after death. These limitations must be remembered in any discussion of abundance.

Tolonen and coworkers (1992) found up to 2300 individuals per cm³ among the bryophytes in Finnish mires. These include **rhizopods** — those with movement by protoplasmic flow, **ciliates**, and **flagellates** (Gerson 1982). The most abundant seem to be the rhizopods (Beyens et al. 1986b; Chardez 1990; Balik 1994, 2001), especially those with shells (**testate**) (Beyens et al. 1986a, b; Chardez & Beyens 1987; Beyens & Chardez 1994). Among these, *Diffugia pyriformis* (Figure 2), *D. globularis*, *Hyalosphenia* (Figure 3), and *Nebela* (Figure 4) are the most common among *Sphagnum* at Itasca, Minnesota, USA (Bovee 1979). In Pradeaux peatland in France, *Nebela tincta* (Figure 4) numbered an average of 29,582 L⁻¹ active individuals, with another 2263 in encysted form (Gilbert et al. 2003).

Schönborn (1977) actually estimated the production of protozoa on the terrestrial moss *Plagiomnium cuspidatum* (Figure 5) and found a yearly mean of $1.45 \times 10^6$ individuals per m² ($0.11 \text{ g m}^{-2} \text{ d}^{-1}$). Rainfall played an important role in the dynamics of protozoa among the mosses, contributing to dislocation and modifying production. Many of the protozoa were testate amoebae that carry sand houses around with them. Heavy rains easily knock these loose and carry them to deeper layers in the soil. On the other hand, the daily death rate of these testate amoebae is lower (only 3.0% per day) than in the river itself. Furthermore, the turnover rate in mosses is much lower than in the river. The higher drying rate (higher than in soil) decreases the number of generations to about half that in soil in the same time period.

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**Figure 2.** *Diffugia pyriformis* test. Photo by Yuuji Tsukii, with permission.

**Figure 3.** *Hyalosphenia papilio* showing test and ingested algae. Photo by Ralf Meisterfeld, with permission.

**Figure 4.** *Nebela tincta* test. Photo by Yuuji Tsukii, with permission.

In temperate forests of northeastern USA, Anderson (2008) identified 50 morphospecies of non-testate amoebae, averaging 17 per sample, based on lab cultures. Densities ranged $3.5 \times 10^3$ to $4.3 \times 10^4$ gdm⁻¹ of moss. As in other studies, numbers were highly correlated with
moisture content of the mosses ($p < 0.001$). These numbers exceeded those of soil, perhaps due to the heavier weight of soil per unit volume. As expected, number of encysted forms was inversely related to moisture content.

Figure 5. *Plagiomnium cuspidatum*, a terrestrial moss habitat. Photo by Michael Lüth, with permission.

**Peatlands**

Peatlands are unique habitats dominated by mosses. Because of their moist nature, they are home to numerous micro-organisms (Warner 1987; Kreutz & Foissner 2006) and will warrant their own sections as we talk about many of the groups of organisms that inhabit mosses.

In addition to the moist habitat of the peatland mosses, peatlands provide numerous small pools, hollows, channels, and small lakes that are ideal habitats for some micro-organisms. Using glass slides, Strüder-Kypke (1999) examined the seasonal changes in these micro-organisms in dystrophic bog lakes at Brandenburg, Germany. May brought ciliates and choanoflagellates and the highest degree of species diversity for the year. This community was replaced by one dominated by peritrich ciliates from August to October. Their decline coincided with early frost, yielding to a winter periphyton of small heterotrophic flagellates. The pioneers on the slides were bacterivorous ciliates.

Peatlands typically have vertical community differences, as will be seen as we discuss the various groups. Diminishing light restricts the photosynthetic organisms and those protozoa with *zoochlorellae* (algal symbionts) to the upper portion of the *Sphagnum*. In the German bog lakes, Strüder-Kypke (1999) found that this zone was characterized by autotrophic cryptomonads and mobile ciliates. Deeper portions were colonized by heterotrophic flagellates and sessile peritrich ciliates.

*Cyclidium sphagnetorum* (Figure 6) is known only from *Sphagnum* and is thus a bryobiont (Groliére 1978 in Gerson 1982). In fact, *Sphagnum* usually has the richest bryofauna of any moss, as shown by Bovee (1979) in Minnesota. In Canada, a single gram of *Sphagnum girgensohnii* (Figure 7) housed up to 220,000 individuals of protozoa, mostly flagellates, while *Campylium chrysophyllum* (Figure 8) had a maximum of only 150,000 in the same habitat (Table 1; Fantham & Porter 1945), suggesting there might be important microhabitat differences among bryophyte species. In Westmorland, the numbers translate to a mere 16 million of these animals in a single square meter of *Sphagnum* (Heal 1962).

*Sphagnum* is a particularly common habitat for micro-organisms (Chacharonis 1956; deGraaf 1957). It appears that even the surface of *Sphagnum* may offer a unique community. Gilbert *et al.* (1998, 1999) considered that these surface organisms might play an important role in recycling nutrients using the microbial loop, an energy/carbon pathway wherein dissolved organic carbon re-enters the food web through its incorporation into bacteria. Changes in these bryophyte protozoan communities could alter the return of nutrients through the microbial loop and indicate the degree of human disturbance.

Figure 6. *Cyclidium* sp. (Ciliophora). Photo by Yuuji Tsukii, with permission.

Figure 7. *Sphagnum girgensohnii*, a peatmoss that can house up to 220,000 individuals in 1 gram of protozoa. Photo by Michael Lüth, with permission.

Figure 8. *Campylium chrysophyllum*, a peatland species that may be less hospitable to protozoa than *Sphagnum*, but still can house 150,000 in just 1 gram. Photo by Michael Lüth, with permission.
Protozoa

Although Protozoa was once a recognized taxonomic unit, it is now only a convenient name used to describe the heterotrophic flagellates, ciliates, and amoebae. Of the now-recognized four major groups of protozoa, three can be found in association with bryophytes. These are Sarcodina – rhizopods (amoebae), Ciliophora – ciliates, and Mastigophora – flagellates (Chiba & Kato 1969; Gerson 1982). Bamforth (1973) described two nutritional protozoan groups associated with plant communities. The naked taxa are primarily bactriovores (consume bacteria) and depend on the decomposability of the litter (including bryophytes) where they live. The Testacea (those rhizopods living in a shell of their own making) are more slow growing, associate with humus and mosses, and live where the humus is of slow decomposability. These characteristics make bryophytes suitable substrates.

The most important factor in determining the habitation by the protozoa is moisture. This determines which species can occur there, what food is available, and whether the protozoan is active or dormant. Mosses act much like a sponge, absorbing water that is available from the soil, rain, and atmosphere, and retaining it. As such, they provide a moist safe haven for protozoans to continue an active life long after other surfaces are dry. But they also help to slow the drying of their underlying substrate and provide insulation against heat, cold, and wind, increasing the utility of the substrate, especially soil, as well (Das 2003).

Gerson (1982) has described four categories of bryophyte fauna, based on their occurrence among bryophytes: bryobionts – animals that occur exclusively in association with bryophytes; bryophiles – animals that are usually found among bryophytes but may survive elsewhere; bryoxenes – animals that regularly spend part of their life cycle on bryophytes; occasional – animals that may at times be found among bryophytes but do not depend on them for survival.

In a study of Polish peatlands, Mieczan (2006) named four categories of protozoa that inhabited the peatlands, based on percent presence: very constant species (in 61-100 percent of the samples), constant species (in 41-60 percent), accidental species (in 21-40 per cent), accessory species (in less than 20 per cent). Although this system aligns closely with that of Gerson (1982), it has the advantage that one does not need to know the occurrence of the species elsewhere and it is more quantitative. On the other hand, that quantification requires considerable time to determine.

As already noted, the richest protozoan habitat among the mosses is considered to be Sphagnum, with up to 16 million individuals m–2 (Richardson 1981). Whereas Sphagnum provides a moist habitat, Drepanocladus (sensu lato; Figure 9), a rich fen species, may be a better habitat by trapping more nutrients (Gerson 1982). In that habitat, the amount of available nutrients determined the numbers of protozoa, due to the greater availability of microbes and organic matter that served as food sources.

Table 1. Number of individuals occupying Sphagnum per gram dry moss. From Fantham & Porter 1945 in Hingley 1993.

<table>
<thead>
<tr>
<th>S. papillosum</th>
<th>S. subsecundum</th>
<th>S. palustre</th>
<th>S. girgensohnii</th>
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<tr>
<td>naked amoebae</td>
<td>testate rhizopods</td>
<td>flagellates</td>
<td>ciliates</td>
</tr>
<tr>
<td>440</td>
<td>3640</td>
<td>9920</td>
<td>1000</td>
</tr>
<tr>
<td>1344</td>
<td>1712</td>
<td>26672</td>
<td>2224</td>
</tr>
<tr>
<td>240</td>
<td>3360</td>
<td>5880</td>
<td>2080</td>
</tr>
<tr>
<td>over 220000</td>
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In their comparison of the protozoan groups and other small invertebrates on four Sphagnum species, Fantham and Porter (1945) found that Sphagnum girgensohnii supported the most protozoa, rotifers, and nematodes, and that flagellates were the most common on all four Sphagnum species (Table 1). Unfortunately, most extraction techniques do not work well for examining the flagellates, so it is likely that they are more common than most studies indicate.

We might well ask why Sphagnum girgensohnii was the preferred moss. This species tends to occur on higher ground and in forests where it is not submersed for significant periods of time and it is usually possible for protozoa and other small invertebrates to seek out higher parts of the plants to escape drowning. Water is not always a good thing.

The richness of the invertebrate fauna in peatlands is rather astounding in view of the antibiotic properties of Sphagnum. Its polyphenolic compounds could not only discourage herbivory on the moss, but reduce the availability of micro-organisms, especially bacteria, that might otherwise live there and serve as food for invertebrate inhabitants (Verhoeven & Lieveld 1997). Smirnov (1961) could find only one invertebrate species that ate the Sphagnum – Psectocladium psilopterus – a chironomid (midge) larva. Other fauna ate mostly algae from the surface. Nevertheless, microfauna seem to abound in a wide diversity of species and numbers among the Sphagnum (Smirnov 1961; Tolonen et al 1992; Gilbert et al. 1999), despite the fact they are on the menu at this mossy restaurant.

Figure 9. Drepanocladus (=Limprichtia) revolvens, a species among the brown mosses that live in rich fens. Photo by Michael Lüth, with permission.
In his study of Polish peatlands, Mieczan (2006) found 24 taxa of ciliates and 6 of testate amoebae among mosses. But he considered the majority of these to be accidental or accessory species.

Even dry cryptogamic crusts of prairies and deserts sport a diverse fauna of protozoa. In the Grand Canyon, Arizona, USA, 51 species of ciliates, 28 of amoebae, 17 of Testacea, 4 metazoan taxa, and a number of flagellate morphotypes were present in the water film among just 28 microbiotic crust samples (Bamforth 2003). These crusts were composed of Cyanobacteria, lichens, and bryophytes. In the predominating non-flagellated protozoan groups, r-selected (high level of reproduction, small body size, short generation time) bacterivores respond rapidly to wetting, quickly exploit resources, then encyst when unfavorable conditions return. It seems that these protozoan groups and bryophytes were made for each other (Kunz 1968).

**Zoomastigophora (Flagellates) and flagellated Chlorophyta**

Like Euglenophyta, flagellated green algae (flagellated Chlorophyta) are placed in this sub-chapter because of their movement capability and ecological relationships, especially with peat.

The flagellates, known as Zoomastigophora, swim by means of 1-4 long flagella and thus require at least a film of water. Fortunately, some are able to encyst, enabling them to become dormant when that film of water is absent.

As one might suspect, *Sphagnum* can provide long periods when leaves have a thin film of water. Numbers of flagellates can reach $10^7$ cells L$^{-1}$ (Gilbert & Mitchell 2006). For the green alga *Carteria sphagnicola* (Figure 10) *Sphagnum* provides an unique habitat, with its cation exchange making its surrounding water acid. This would be particularly true of a thin film of water that is not diluted by lake or fen water.

**Chlamydomonas** (Figure 11), a green alga, is a relatively common genus in peatlands. *Chlamydomonas acidophila*, as its name implies, lives at low pH and is common among *Sphagnum* plants with a pH of 2-6, where as many as 50,000 individuals may exist per cm$^2$ (Hingley 1993). Another *Chlamydomonas* species, known first from *Sphagnum*, has been named *C. sphagnicola*.

One advantage that the widely known genus *Chlamydomonas* shares with many of the bryophyte-inhabiting protozoa is the ability to form a palmelloid stage (Figure 12) – a stage that can remain dormant during dry spells (Rajan 202). This stage is named because of its resemblance to the green algal genus *Palmella*. In *Chlamydomonas*, to form the palmella stage, the cells lose their flagella, divide, and form a gelatinous ball in which the cells are embedded. Each cell is still capable of individual function. When favorable conditions return, individual cells are freed and continue an active life.

*Chlamydomonas reinhardtii* is known to form gelatinous masses or a palmelloid stage (Figure 13) when confronted by the predator *Brachionus calyciflorus*, a rotifer (Lurling & Beekman 2006). The reaction to form a palmelloid stage can occur within 25 hours and apparently affords some protection against rotifer grazing. The low pH of the *Sphagnum* habitat may contribute to this ability; calcium can cause the palmelloid stage to dissociate, but phosphorus can negate the dissociation (Iwasa & Murakami...
1969). Iwasa and Murakami suggest that organic acids (such as those produced by *Sphagnum*) chelate calcium and permit the formation of the palmelloid stage. Nakamura *et al.* (1976) have shown that there are other biochemical/chemical interactions that can inhibit the formation of the palmelloid stage in *Chlamydomonas eugametos*, suggesting that rotifers, and other organisms, could emit biochemicals that stimulate or interfere with palmelloid formation. Among bryophytes, cohabitation with rotifers is likely to occur frequently, so one should look for these special reactions.

*Euglena mutabilis* (Figure 15) can withstand pH as low as 1.8, numbering 50,000-70,000 per cm² of ground surface (Hingley 1993). Its numbers, like those of many other *Sphagnum* organisms, correlate positively with moisture content of the peat. *Euglena mutabilis*, common in the upper 2 cm of peat, lacks the flagellum that is typical of euglenoids and has only two chloroplasts. Of special interest is its ability to live inside hyaline cells of the *Sphagnum* leaves (Figure 16, Figure 17). *Sphagnum* species with hooded leaves seem to house more euglenoids than do other kinds of *Sphagnum*. The "hood" most likely helps to create a micro-basin for trapping water. Some of these tiny unicellular organisms, like *Euglena mutabilis*, enter through the *Sphagnum* leaf pores and live within the hyaline cells (these are non-living), dining on organic debris left by former residents.

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**Euglenophyta**

*Euglena* (Figure 14) is one of those organisms that caused consternation among early classifiers because of its combination of animal and plant traits. It can engulf food, but it also has chlorophyll and a flagellum. I have stubbornly used its algal name here but am writing about it with the protozoa because of its flagella. Additional Euglenophyta are listed in Table 2.

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Figure 13. *Chlamydomonas* close view of palmelloid stage. Photo by Jason Oyadomari, with permission.

Figure 15. *Euglena mutabilis*. Photo by Yuuj Tsukii, with permission.

Figure 16. Microscopic view of *Sphagnum* leaf showing hyaline cells and pores. Photo with permission from <http://www.botany.ubc.ca/bryophyte/LAB8.htm>.

Figure 17. SEM of *Sphagnum* hyaline cells, showing pores. Photo from <http://www.botany.ubc.ca/bryophyte/LAB8.htm>, with permission.
Despite their lack of a test, *Euglena acus* (Figure 18) and *Phacus longicaudatus* (Figure 19) can survive desiccation for more than seven years with no test to protect them (Hingley 1993).

![Figure 18. *Euglena acus* showing distinctive red eyespot that permits it to respond to light. Photo by Jason Oyadomari, with permission.](image)

![Figure 19. *Phacus longicaudatus*, a not-so-common member of the bryophytic protozoan fauna. Photo by Yuuji Tsukii, with permission.](image)

**Pyrrophyta (=Dinophyta)**

The name *Pyrrophyta* literally means fire plants, and these organisms are so-named because of the ability of some species to produce flashes of light through bioluminescence. Sadly, these spectacular show-offs are rarely known from bryophytes (Table 2). I have located only one Pyrrophyta species known commonly to inhabit bryophytes – *Hemidinium ochraceum* (Hingley 1993; Figure 20). But that gives me an excuse to write about these remarkable organisms, also known as dinoflagellates. *Hemidinium ochraceum* lives among the *Sphagnum* in hollows of peatlands where they give the *Sphagnum* a yellowish-rusty color (Hingley 1993).

![Figure 20. The dinoflagellate *Hemidinium* sp. Photo by Yuuji Tsukii, with permission.](image)

Whereas some dinoflagellates (so-named because of their twirling motion) attract attention by their brilliant displays, others attract it by their deadly toxins. They are the apparent cause of the water that "turned to blood" as reported in Exodus of the *Bible* – red tide organisms known today for the resulting unpleasant odors of dying fish and in some cases very strange effects on humans. Some wear plates of armor and others do not. Their two flagella lie in grooves, one around the middle of the cell like a sash and the other extending from that line down the "back" and up the "front," resulting in their characteristic twirling motion. It is not surprising that they avoid peatlands because most of them prefer alkaline conditions (Hingley 1993).

**Ciliophora (Ciliates)**

These organisms use a series of fine cilia instead of flagella to achieve movement. Some of these, despite their cilia, attach themselves to *Sphagnum* leaves (Hingley 1993). The cilia can serve more than one function. Whereas the primary one is to direct food into the cell, many also use them for locomotion.

Numbers of ciliates among *Sphagnum* water range 0-4.2 x 10^6 cells L^-1 (Gilbert & Mitchell 2006). Many of these organisms may simply use the bryophytes as a substrate. Such is probably the case for the stalked *Vorticella* (Figure 21, Figure 22). Nevertheless, detrital matter that accumulates and algae and bacteria that take up residence among the leaves most likely provide food for ciliates, whether confined by an attachment or free-moving.

Some ciliates occur only among *Sphagnum* (Figure 23), including *Bryometopus* (Figure 24) and *Climacostomum* (Figure 25), the latter often with symbionts (Figure 26) (Gilbert & Mitchell 2006). Other taxa that Mieczan (2006) found to be very constant in Polish peatlands include *Askenasia* sp., *Chlamydionella* spp., *Enchelyomorpha vermicularis* (70%), *Gastronanta* spp. (89%), *Paramecium putrinum*, and *Trochilia minuta*.

![Figure 21. Upper: A member of the genus *Vorticella* that was living on the leaves of the leafy liverwort *Jungermannia cordifolia*. Lower: This same *Vorticella* is shown here with its stalk extended. Photos courtesy of Javier Martinez Abaigar.](image)
Figure 22. *Vorticella*, a stalked ciliate that inhabits bryophyte leaves and other aquatic substrates. Photo by Jason Oyadomari, with permission.

Figure 23. *Sphagnum obtusum* showing the wet capillary spaces among the leaves that support ciliate protozoan communities on these drooping branches. Photo by Michael Lüth, with permission.

The ciliates have a distinct zonation within the peatland, and different communities, fewer in number of individuals and species, occur at the depth of the non-green *Sphagnum* parts (Hingley 1993). Those with *symbiotic* algal partners require light and are thus restricted to areas near the surface where the *Sphagnum* likewise is green. However, some symbiotic ciliates are also able to ingest food and can thus also live farther down the stems.

Figure 24. A ciliate, possibly *Bryometopus*, a bryobiont of *Sphagnum*, showing photosynthetic symbionts. Photo by Yuuji Tsukii, with permission.

Figure 25. *Climacostomum virens* with no symbionts. Photo by Yuuji Tsukii, with permission.

Figure 26. *Climacostomum virens* with dense symbionts. Photos by Yuuji Tsukii, with permission.

Like many other protozoa, the ciliates can survive drought by encysting. *Paramecium aurelia* (see Figure 27-Figure 28 for genus) can survive more than seven years with no test to protect it (Hingley 1993).
Figure 27. *Paramecium*, the slipper animal, is a ciliate that is larger than most protozoa. Photo by Jason Oyadomari, with permission.

Figure 28. *Paramecium* showing two of its round contractile vacuoles that permit it to regulate its water content. Photo by Jason Oyadomari, with permission.

The *Sphagnum*-dwelling ciliate *Podophyra* sp. (Figure 29) has tentacles that are necessary in its capture of prey. These have a knob at the end that excretes substances that narcotize the prey (Samworth). The interesting part of this trapping mechanism is that the cytoplasm is sucked down these tentacle arms to the body and the prey, such as the ciliate *Colpidium* (Figure 30), remains alive during the journey! The prey organism is finally absorbed into the body of the *Podophyra*. But stranger still it is that the prey organism may be released, still alive, after the *Podophyra* has finished feeding!

Figure 29. *Podophyra*, a ciliate found in Perrault Fen, Houghton County, Michigan, USA. Photo by Jason Oyadomari, with permission.

Figure 30. *Colpidium campylum*. Photo by Yuuji Tsukii, with permission.

**Symbionts**

Many of the ciliates have their own symbiotic residents. Those ciliates living near the surface of bryophyte communities where there is ample light often incorporate photosynthetic algae inside their cells (Figure 31), benefitting from the oxygen and photosynthate, and contributing CO₂ to the algae (Hingley 1993). The algae can also transfer organic nitrogen, phosphorus, and sulfur and excrete glycerol, glucose, alanine, organic acids, and carbohydrate released as maltose (Arnold 1991; Dorling et al. 1997). In return, the symbiotic algae can gain inorganic forms of nitrogen, phosphorus, and sulfur and may gain vitamins, while enjoying the safety of a moist cell. Wang (2005) reported that protozoa with algae seemed to be favored by higher oxygen concentrations with concomitant higher concentrations of CO₂. This higher CO₂ undoubtedly aided the algae in their photosynthesis inside the diffusion barrier of the protozoan cell.

Figure 31. *Colpoda* with Chlorophyta symbionts. Photo by Yuuji Tsukii, with permission.

When the alga is to be used as a symbiont, it is protected within a vacuole by a double membrane. Somehow the host cell knows not to digest these, whereas those doomed as food are located in vacuoles that merge with lysosomes and are digested (Karakashian & Rudzinska 1981). In *Hydra*, it is the maltose that apparently signals the host not to digest its symbiont (McAulay & Smith 1982 in Arnold 1991), and this may also be the means of recognition in the protozoa. Anderson (1983) suggests that the protozoan may still later digest some of the symbionts, making these photosynthetic...
organisms into an internal garden to be harvested as needed.

As in Frontalis, the alga may survive with or without symbionts (Figure 32). The common Paramecium bursaria is likely to be home for numerous cells of Chlorella (Figure 33), but it can also have the alga Scenedesmus as a partner (Arnold 1991). Among the ciliate symbiotic hosts, Cyclidium sphagnetorum (see Figure 34) is one of the common ciliate species among peatland bryophytes (Grolière 1977). Others include Frontonia vernalis (Figure 35), Platyphora similis (Figure 36), and Prorodon viridis (Figure 37). Additional species are listed in Table 2.

Figure 32. Frontonia, a peatland-dwelling ciliate. Upper: Cell shape and nucleus. Lower: Frontonia vernalis cell with Chlorella symbionts and desmids (food items?) in the cell. Photos by Yuuji Tsukii, with permission.

Figure 33. Paramecium bursaria (left), a common ciliate that can inhabit bryophytes, showing its Chlorella symbionts. Photo by Yuuji Tsukii, with permission.

Figure 34. Cyclidium, a genus that often has algal symbionts. Photo by Yuuji Tsukii, with permission.

Figure 35. Frontonia, a peatland-dwelling ciliate with Chlorella symbionts and desmids in the cell. Photo by Yuuji Tsukii, with permission.

Figure 36. Platyphora similis, a ciliate known from Sphagnum in Poland (Mieczan 2006). It appears to have both small algal symbionts and larger ingested algae or Cyanobacteria. Photo by Yuuji Tsukii, with permission.

One possible additional advantage to having symbionts, aside from the added energy availability, is that it permits these ciliates to live where the oxygen supply is low, deriving their oxygen from their symbionts (Lawton 1998). This strategy provides them the opportunity to avoid the more oxygen-dependent larger metazoans that might otherwise have them for dinner. In the words of Lawton, it provides "enemy-free space."
Figure 37. *Prorodon viridis*, a ciliate that inhabits *Sphagnum* in peatlands of Poland (Mieczan 2006). It is packed with algal symbionts with a colorless nucleus in the center. Photo by Yuuji Tsukii, with permission.

*Coleps hirtus* (Figure 38-Figure 40) is a facultative host to the *Chlorella* symbiont (Auer et al. 2004), but it grows faster when it is in the light and endowed with endosymbionts (Stabell et al. 2002). Even when it has endosymbionts, it will ingest organic matter, including smaller protozoa and algae (Figure 41-Figure 42; Auer et al. 2004). The alga maintains a coordinated growth rate with the host by its rate of leakage of products to the host.

Figure 38. *Coleps hirtus*, a peatland inhabitant found by Mieczan (2006) in Poland. Cells have internal symbiotic algae. Photo by Yuuji Tsukii, with permission.

Figure 39. *Coleps hirtus* test, showing spines, with diatom. Photo by Yuuji Tsukii, with permission.

Figure 40. *Coleps hirtus* with internal symbiotic algae. Photo by Yuuji Tsukii, with permission.

Figure 41. *Coleps* ingesting the green alga *Chlorogonium*. Photo by Yuuji Tsukii, with permission.

Figure 42. *Coleps* feeding on the diatom *Diatoma*. Photos by Yuuji Tsukii, with permission.
Table 2. Species and genera of Zoomastigophora, flagellate Chlorophyta, Euglenophyta, Pyrrophyta, armored flagellates, Ciliophora, Heliozoa, Cryptophyta, and Ochrophyta I have located in the literature and from observations of protozoologists as those known from bryophytes. Those reported by Hingley are known from peatlands. *Indicates closely associated with *Sphagnum*. Additional photographs are in Chapter 2-2 of this volume.

### Zoomastigophora
- **Distigma proeetus**
  - By **Hingley** 1993
- **Bryophyllum armatum**
  - By **Hingley** 1993

### Flagellate Chlorophyta
- **Carteria globosa**
  - By **Hingley** 1993
- **Carteria sphagnicola**
  - By **Compère** 1966
- **Chilomonas**
  - By **Henebry & Cairns** 1984
- **Chlamydomonas acidophila**
  - By **Hingley** 1993
- **Chlamydomonas sphagnicola**
  - By **Hingley** 1993
- **Gonium pectorale**
  - By **Henebry & Cairns** 1984
- **Monas**
  - By **Henebry & Cairns** 1984
- **Monasiga**
  - By **Henebry & Cairns** 1984
- **Platydorina**
  - By **Hingley** 1993
- **Polytoma uvelia**
  - By **Hingley** 1993
- **Spermatozopsis**
  - By **Hingley** 1993

### Euglenophyta
- **Astasia**
  - By **Hingley** 1993
- **Distigma**
  - By **Hingley** 1993
- **Euglena acus**
  - By **Hingley** 1993
- **Euglena deses**
  - By **Hingley** 1993
- **Euglena mutabilis**
  - By **Hingley** 1993
- **Euglena oxyuris**
  - By **Hingley** 1993
- **Euglena pisciformis**
  - By **Hingley** 1993
- **Euglena sanguinea**
  - By **Hingley** 1993
- **Euglena spirigryra**
  - By **Hingley** 1993
- **Euglena triperis**
  - By **Hingley** 1993
- **Euglena viridis**
  - By **Hingley** 1993
- **Lepocinclis**
  - By **Hingley** 1993
- **Phacus longicaudatus**
  - By **Hingley** 1993
- **Trachelomonas aculeata**
  - By **Hingley** 1993
- **Trachelomonas bulla**
  - By **Hingley** 1993
- **Trachelomonas hispida**
  - By **Hingley** 1993

### Pyrrophyta & Armored Flagellates
- **Amphidinium**
  - By **Hingley** 1993
- **Ceratium hirundinella**
  - By **Hingley** 1993
- **Cystodinium conchaeforme**
  - By **Hingley** 1993
- **Dinococcales**
  - By **Hingley** 1993
- **Ceratium hirundinella**
  - By **Hingley** 1993
- **Amphidinium**
  - By **Hingley** 1993
- **Gymnodinium caudatum**
  - By **Hingley** 1993
- **Gyrodinium**
  - By **Hingley** 1993
- **Hemidinium ochraceum**
  - By **Hingley** 1993
- **Katodinium stigmatica**
  - By **Hingley** 1993
- **Katodinium vorticella**
  - By **Hingley** 1993
- **Peridinium cinctum**
  - By **Hingley** 1993
- **Peridinium inconspicuum**
  - By **Hingley** 1993
- **Peridinium limbatum**
  - By **Hingley** 1993
- **Peridinium umbonatum**
  - By **Hingley** 1993
- **Peridinium volzi**
  - By **Hingley** 1993
- **Peridinium willei**
  - By **Hingley** 1993
- **Sphaerodinium**
  - By **Hingley** 1993
- **Woloszynskia**
  - By **Hingley** 1993

### Ciliophora
- **Amphileptus pleurosigma**
  - By **Bourland pers. obs.**
- **Askenasia**
  - By **Mieczan** 2006
- **Blepharisma lateritium**
  - By **Hingley** 1993
- **Blepharisma steini**
  - By **Hingley** 1993
- **Blepharisma musculus**
  - By **Hingley** 1993
- **Blepharisma sphagni**
  - By **Hingley** 1993
- **Bryometopus pseudochilodon**
  - By **Hingley** 1993
- **Bryometopus sphagni**
  - By **Hingley** 1993

**Addition:**
- **Gyrodinium**
  - By **Hingley** 1993
- **Cristodinium conchaeforme**
  - By **Hingley** 1993
- **Trachelomonas bulla**
  - By **Hingley** 1993
- **Phacus longicaudatus**
  - By **Hingley** 1993
- **Euglena viridis**
  - By **Hingley** 1993
- **Euglena triperis**
  - By **Hingley** 1993
- **Lepocinclis**
  - By **Hingley** 1993
- **Phacus longicaudatus**
  - By **Hingley** 1993
- **Trachelomonas aculeata**
  - By **Hingley** 1993
- **Trachelomonas bulla**
  - By **Hingley** 1993
- **Trachelomonas hispida**
  - By **Hingley** 1993

**Notes:**
- Additional photographs are in Chapter 2-2 of this volume.
- Additional information on species and genera of Zoomastigophora, flagellate Chlorophyta, Euglenophyta, Pyrrophyta, armored flagellates, Ciliophora, Heliozoa, Cryptophyta, and Ochrophyta I have located in the literature and from observations of protozoologists as those known from bryophytes. Those reported by Hingley are known from peatlands. *Indicates closely associated with *Sphagnum*. Additional photographs are in Chapter 2-2 of this volume.
In addition to the taxa listed here, Kreutz and Foissner (2006) have listed many additional taxa from *Sphagnum* ponds in Germany. Many of these are figured with wonderful color images, but pool species are not distinguished from those actually on mosses in or adjoining pools.

**Summary**

There is a rich diversity of protozoans among the bryophytes, much of which has never been explored. Ciliates and testate amoebae (rhizopods with houses) predominate in both peatlands and forests, but some flagellates and other minor groups occur as well. Bryophytes are especially suitable habitats for these organisms that can encyst when dry. And both depend largely on wind for dispersal, with protozoa often dispersing with fragments of their hosts.

*Aufwuchs,* or *periphyton,* are those organisms that live on aquatic substrata, including bryophytes, without being parasites. Epiphyte is a broader term that includes terrestrial associates as well. Identification is difficult and often requires culturing. But more than 2000 organisms per cm$^2$ make the effort worthwhile.

Rainfall can dislocate the protozoa, especially those with heavy testae, and modify their production. Not surprisingly, numbers are highly correlated with moisture.

Some taxa, known as bryobionts, occur only on mosses (e.g. *Cyclidium sphagnetorum*). The naked taxa are mostly bacterivores. In *Sphagnum* the numbers of protozoa are so high (up to 220,000 per gram) that they are important in the microbial loop. In addition to bryobionts, bryophiles are usually found among bryophytes, bryoxenes live elsewhere but regularly spend part of the life cycle among bryophytes, and occasionalis are typical elsewhere, but occasionally are found among bryophytes.

The *Zoomastigophora* (flagellates) include *Chlamydomonas, Euglena,* and *Phacus* among the bryophyte inhabitants. These organisms can swim around in the hooded tips of *Sphagnum* leaves and may inhabit the hyaline cells. The low pH may contribute to the formation of the palmelloid stage in their life cycle, protecting them from rotifer predation. Among the *Ciliophora* (ciliates), *Stentor* and *Vorticella* may attach...
themselves by bryophyte leaves. Other members swim about in the surface water film. Some of these have chlorophyll-bearing symbionts and thus must live near the surface; the symbionts leak maltose and provide oxygen while gaining CO₂.

Acknowledgments

Edward Mitchell provided me with a large number of papers and photographs and William Bourland provided me with wonderful photographs of taxa on my special needs list. Yuuji Tsukii and Jason Oyadomari permitted me to use any of their numerous images. Edward Mitchell and Paul Davison were invaluable in helping me with areas where I was often not personally familiar with the subject.

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Other Ciliophora Known from Bryophytes

Figure 1. *Amphileptus pleurosigma*, a free-swimming, predatory ciliate. Photo by William Bourland, with permission.

Figure 2. *Chilodontopsis depressa*, an algivorous ciliate (Risse-Buhl & Küsel 2008). Photo by William Bourland, with permission.

Figure 3. *Cinetochilum margaritaceum*, a bryophyte-inhabiting ciliate that Mieczan (2007) found in peatland ponds of Poland with pH of 5.0. Photo by William Bourland, with permission.

Figure 4. *Cinetochilum margaritaceum* stained to show organelles. Photos by William Bourland, with permission.

Figure 5. *Didinium nasutum*, a bryophyte-dwelling ciliate that feeds on *Paramecium*. This species is capable of encysting to avoid unfavorable conditions. Photo by William Bourland, with permission.
Figure 6. *Oxytricha fallax*, a ciliate, has a complex grouping of cilia that are used for sweeping food into the gullet. It lives among bryophytes, as well as other habitats. Lower organism has been stained. Photos by William Bourland, with permission.

Figure 7. *Stentor multiformis*, a ciliate that occurs in peatlands (Mieczan 2006) and can attach to moss leaves. Photo by William Bourland, with permission.

Figure 8. *Stentor* showing green algal symbiont. Photo by Wim van Egmond, with permission.

Figure 9. *Colpoda steinii*, a constant member of *Sphagnum* communities in two Polish peatlands (Mieczan 2006). Photo by Yuuji Tsukii, with permission.

Figure 10. Two *Holophyra* species, ciliates that can inhabit *Sphagnum* in peatlands (Mieczan 2006). Photos by Yuuji Tsukii, with permission.
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Figure 11. *Monodinium*, a ciliate that sometimes occurs on *Sphagnum* in peatlands (Mieczan 2006), showing ring of cilia. Photo by Yuuji Tsukii, with permission.

Figure 12. *Monodinium* dividing. Photo by Yuuji Tsukii.

Figure 13. *Paramecium bursaria*, a common species that can occur on *Sphagnum* in peatlands in Poland (Mieczan 2006). This one has algal symbionts. Photo by Yuuji Tsukii, with permission.

Figure 14. *Spathidium muscicola*, a ciliate that can live among mosses. Photo by Yuuji Tsukii, with permission.

Figure 15. *Steinia sphagnicola*. Normal cell. Photo by Yuuji Tsukii, with permission.

Figure 16. *Steinia sphagnicola* cell dividing. Photo by Yuuji Tsukii, with permission.

Figure 17. **Upper:** *Urotricha farcta*. **Lower:** *Urotricha platystoma*. This genus occurs on mosses in Polish peatlands (Mieczan 2006). Photo by Yuuji Tsukii, with permission.
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Michael Lüth kindly sent me the names of several Ciliophora that commonly occur on bryophytes. These include Phacodinium metchnikoffii (Figure 19-Figure 20), Bryophyllum tegularum and B. loxophylliforme (Figure 21).

Figure 18. Strombidium viride, a ciliate that occurs occasionally on mosses in peatlands in Poland (Mieczan 2006). Photo by Yuuji Tsukii, with permission.

Figure 18. Strombidium viride, a ciliate that occurs occasionally on mosses in peatlands in Poland (Mieczan 2006). Photo by Yuuji Tsukii, with permission.

Figure 18. Strombidium viride, a ciliate that occurs occasionally on mosses in peatlands in Poland (Mieczan 2006). Photo by Yuuji Tsukii, with permission.

Figure 19. Phacodinium metchnikoffii, a common species on wet moss. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 19. Phacodinium metchnikoffii, a common species on wet moss. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 19. Phacodinium metchnikoffii, a common species on wet moss. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 20. Phacodinium metchnikoffii showing ribs. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 20. Phacodinium metchnikoffii showing ribs. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 20. Phacodinium metchnikoffii showing ribs. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 21. Bryophyllum loxophylliforme, a common species on wet moss. Bryophyllum tegularum likewise is common there. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 21. Bryophyllum loxophylliforme, a common species on wet moss. Bryophyllum tegularum likewise is common there. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 21. Bryophyllum loxophylliforme, a common species on wet moss. Bryophyllum tegularum likewise is common there. Photo by Michael Plewka <www.plingfactory.de>, with permission.

Figure 22. Actinophrys sol, a moss dweller, showing radiating pseudopodia. Photo by Yuuji Tsukii, with permission.

Figure 22. Actinophrys sol, a moss dweller, showing radiating pseudopodia. Photo by Yuuji Tsukii, with permission.

Figure 22. Actinophrys sol, a moss dweller, showing radiating pseudopodia. Photo by Yuuji Tsukii, with permission.

Heliozoa

The heliozoans look like a sunburst with their sticky, wirelike pseudopods. About 20 species live among Sphagnum in pools with pH ranging 5-5.6 (Hingley 1993). The sticky pseudopods, known as axopods, are used to ensnare food such as algae and smaller protozoa, and to protect the organisms. They also facilitate a slow movement, since these organisms lack cilia or flagella. The beautiful and delicate moss dwellers include Actinophrys sol (Figure 23) and Actinosphaerium eichhorni (Figure 24-Figure 25).
Summary

Although they are more difficult to detect, the **Ciliophora** are quite common among bryophytes. They are best detected by culturing, and then the many species seen in this chapter become active. **Heliozoa** are not common among bryophytes, and only the few species shown here are familiar ones in a bryophyte habitat.

Acknowledgments

This chapter would not have existed without my new, but never seen, friends, William Bourland and Yuuji Tsukii. William Bourland provided me with a set of his pictures of bryophyte inhabitants. Yuuji Tsukii gave me unlimited permission to use his many, many images on the Protist Information Server website. Michael Lüth reported his observations on Protozoa on bryophytes.

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PROTOZOA: RHIZOPOD DIVERSITY

Rhizopoda (Amoebas)

The Rhizopoda are a phylum of protozoa with a name that literally means "root feet" (Figure 1). They include both naked and testate amoebae. Testate amoebae are encased in "houses" of their own making (Figure 2) by way of organic secretions (Hoogenraad & Groot 1953; Wilmshurst 1998). Imagine a tiny pile of sand grains moving across a liverwort leaf.

Despite being only one-celled, testate species construct houses made of various materials such as small sand grains cemented by their own secretions, and even diatoms (Figure 4) may be included among the sand grains. Some even manufacture silica plates that they meticulously arrange into housing. Others may include such items as mineral particles, pollen grains, and the recycled plates and remains of their microscopic food organisms. Such testate rhizopods include Diffugia (Figure 5-Figure 6), Arcella vulgaris (Figure 8-Figure 9), and Centropyxis (Figure 11) among the most common moss-dwellers (Bartos 1949a).
Figure 3. *Sanionia uncinata*, home to testate amoebae in the Antarctic. Photo by Michael Lüth, with permission.

Figure 4. SEM photo of *Amphitrema wrightianum* showing diatoms used in making the test. Photo by Edward Mitchell, with permission.

Figure 5. *Difflugia bacillifera* test with incorporated diatoms. Photo by Edward Mitchell, with permission.

Figure 6. *Difflugia bacillifera* test with incorporated diatoms. Photo by Yuji Tsukii, with permission.

Figure 7. Empty shell of *Arcella vulgaris*, a testate amoeba that forms donut shapes on moss leaves. Photo courtesy of Javier Martínez Abaigar, with permission.

Figure 8. *Arcella vulgaris*, a testate amoeba that forms donut shapes on moss leaves. Photo courtesy of Javier Martínez Abaigar, with permission.
Species Diversity

The diversity of testate amoebae among mosses is quite remarkable. Those dwelling in peatlands are so species-rich and numerous that I have devoted an entire subchapter to them. But terrestrial bryophytes have rhizopods as well.
Török (1993) examined six species of terrestrial mosses in Hungary to compare their rhizopod fauna species diversity. He found 46 testate species, six of which were new for Hungary. The dominant taxa are reviewed in Table 1. The Hungarian diversity exceeded that reported for Arctic mosses (Beyens et al. 1986b). Török found Plagiopyxis labiata on most of the mosses in the study as well as finding them on Sphagnum. Some differences in protozoan species composition seemed evident among moss species. For example, Phryganna acropodia, a soil species, had its highest moss occurrence in Brachythecium velutinum (Figure 14). Trinema penardi, a common Sphagnum inhabitant, was a characteristic species to be found in Cirriphyllum tommasinii (Figure 15). The rhizopod genera with the most species among these six mosses were Centropyxis (Figure 11-Figure 12) and Euglypha (Figure 18). The six mosses are listed with their diversity and numbers in Table 2.

Table 1. Eudominant (X) and dominant (x) rhizopods on six bryophyte species in Hungary (Török 1993).

<table>
<thead>
<tr>
<th>Plagiomnium undulatum</th>
<th>Plagiothecium platyphyllum</th>
<th>Leptodictyum riparium</th>
<th>Cirriphyllum tenuinerve</th>
<th>Brachythecium velutinum</th>
<th>Atrichum undulatum</th>
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<tr>
<td>Tracheleuglypha dentata</td>
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<td>Trinema enchelys</td>
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<tr>
<td>Diffugia lucida</td>
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<td>Euglypha rotunda</td>
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<td>Trinema penardi</td>
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<td>Trinema complanatum</td>
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<td>Diffugiella oviformis</td>
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<td>Centropyxis aerophila</td>
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<td>var. sphagnicola</td>
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Table 2. Total Shannon diversity and species numbers in each of the collections of mosses from Hungary (Török 1993).

<table>
<thead>
<tr>
<th>Moss Species</th>
<th>Diversity</th>
<th># Spp</th>
<th># Indivs</th>
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<tr>
<td>Plagiomnium undulatum</td>
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<td>Plagiothecium platyphyllum</td>
<td>3.65</td>
<td>26</td>
<td>471</td>
</tr>
<tr>
<td>Amblystegium riparium</td>
<td>2.60</td>
<td>14</td>
<td>375</td>
</tr>
<tr>
<td>Cirriphyllum tenuinerve</td>
<td>2.98</td>
<td>21</td>
<td>485</td>
</tr>
<tr>
<td>Brachythecium velutinum</td>
<td>3.52</td>
<td>27</td>
<td>844</td>
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<tr>
<td>Atrichum undulatum</td>
<td>2.80</td>
<td>14</td>
<td>285</td>
</tr>
</tbody>
</table>

In the southeastern Alps in Italy 25 species occurred on the forest moss Hylocomium splendens (Figure 16) in the altitudinal range from 1000-2200 m asl (Mitchell et al. 2004). The most frequent taxa on H. splendens included Assulina muscorum (Figure 17), Centropyxis aerophila (Figure 18), Corythion dubium (Figure 19), Euglypha ciliata (Figure 20), Euglypha laevis, Nebela tincta (Figure 21), Phryganna acropodia, and Trinema enchelys (Figure 22), all with a frequency greater than 10 among 21 samples. Densities per gram of a single species were as high as 12,666 (Corythion dubium, Figure 19). It is interesting that every one of these species is also among the common peatland taxa elsewhere (Table 3); they are all cosmopolitan, a phenomenon suggested by Vinecke et al. (2004) and discussed in a later subchapter. Nebela collaris (sensu lato) is not only common on the leaf surfaces of...
Sphagnum, but can occur within the hyaline (colorless) cells as well (Gilbert et al. 2003).

Figure 16. Hylocomium splendens, a host for many protozoa. Photo by Michael Lüth, with permission.

Figure 17. Assulina muscorum with pseudopodia showing. Photo by Yuuji Tsukii, with permission.

Figure 18. Centropyxis aerophila test. Photo by Yuuji Tsukii, with permission.

Figure 19. Test of Corythion dubium. Photo by Edward Mitchell, with permission.

Figure 20. Euglypha ciliata showing cell contents. Photo by Yuuji Tsukii, with permission.

Figure 21. Nebela tincta showing ingested diatom. Photo by Yuuji Tsukii, with permission.
Table 3. Comparison of similarities in common testate amoebae communities occurring in several locations around the Northern Hemisphere. Note that the list for Bulgaria includes only the most common; others indicate presence. Photos of most follow the table.

<table>
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Figure 22. Test of Trinema enchelys. Photo by William Bourland, with permission.

Mieczan (2006) found that the testate species Difflugia oblonga (Figure 23), Euglypha sp. (Figure 24), and Nebela longeniformis comprised more than 25% of the total numbers in the two Polish peatlands he studied.

In contrast to studies on moist peatland bryophytes (e.g. Table 3), Nguyen et al. (2004) found only 9 rhizopod species in 30 samples of the xerophytic moss Syntrichia ruralis (Figure 25). Mitchell et al. (2004) attributed this depauperate number to the dry conditions and restriction of samples to the photosynthetic tips of the moss.

Figure 23. Difflugia oblonga, a testate amoeba that was common in the Polish peatlands studied by Mieczan (2006). Photo by Yuuji Tsukii, with permission.
Other studies on species richness generally include mosses as a group, rather than examining individual species, with rhizopod richness ranging 9-53 species (Beyens et al. 1986a, b; 1990; Beyens & Chardez 1994; Todorov & Golemansky 1996; Van Kerckvoorde et al. 2000). Additional bryophyte inhabitants from around the world are shown in Figure 26 - Figure 59. A complete list of bryophyte-inhabiting rhizopods is in Table 4.
Table 4. The following taxa are those I have found in the literature and by corresponding with protozoologists as known rhizopods inhabiting bryophytes. Peatland taxa that are I have not found listed for other bryophytes are in the Peatland Rhizopod subchapter. This list is undoubtedly incomplete. *Indicates those not mentioned elsewhere in this chapter and that are found on *Barbula indica* (Figure 30), as listed by Nguyen-Viet *et al.* 2007.

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Figure 30. *Barbula indica*, home of several testate protozoans listed in Table 4. Photo by Li Zhang, with permission.

Figure 31. *Assulina muscorum* test. Photo by Yuuji Tsukii, with permission.

Figure 32. *Assulina muscorum* test. Photo by Edward Mitchell, with permission.

Figure 33. *Assulina seminulum* test. Photo by Yuuji Tsukii, with permission.

Figure 34. SEM photo of *Assulina seminulum* test. Photo by Edward Mitchell, with permission.

Figure 35. *Bullinularia indica* test. Photo by Edward Mitchell, with permission.
Figure 36. *Centropyxis aculeata* test showing spines. Photo by Yuuji Tsukii, with permission.

Figure 37. *Centropyxis aerophila*, a terrestrial protozoan. Photo by Yuuji Tsukii, with permission.

Figure 38. *Corythion dubium* test. Photo by Yuuji Tsukii, with permission.

Figure 39. *Corythion dubium* test showing opening. **Upper:** Photo by Yuuji Tsukii. **Lower:** SEM photo by Edward Mitchell, both with permission.

Figure 40. *Cryptodifflugia ovaliformis* growing on filamentous alga. Photo by Yuuji Tsukii, with permission.

Figure 41. *Cryptodifflugia ovaliformis* test and protoplast. Photo by Yuuji Tsukii, with permission.

Figure 42. Encysted *Difflugia leidyi*. Photo by Edward Mitchell, with permission.

Figure 43. *Euglypha ciliata* live cell. Photo by Yuuji Tsukii, with permission.
Figure 44. *Euglypha ciliata* test. Photo by Edward Mitchell, with permission.

Figure 45. *Euglypha compressa* opening in test. Photo by Edward Mitchell, with permission.

Figure 46. *Euglypha rotunda* test. Photo by Yuuji Tsukii, with permission.

Figure 47. *Euglypha strigosa* duplicating cell. Photo by William Bourland, with permission.

Figure 48. *Euglypha strigosa* single cell with test. Photo by William Bourland, with permission.

Figure 49. *Heleopera petricola* with diatom. Photo by Yuuji Tsukii, with permission.

Figure 50. *Heleopera sphagni* living cell. Photo by Yuuji Tsukii, with permission.
Figure 51. Live cell of *Heleopera sylvatica* showing pseudopodia. Photo by Yuuji Tsukii, with permission.

Figure 52. Test of *Heleopera sylvatica* with protoplast. Photo by Yuuji Tsukii, with permission.

Figure 53. *Hyalosphenia elegans* test with remains of protoplast. Photo by Yuuji Tsukii, with permission.

Figure 54. *Hyalosphenia papilio* test with protoplast and chloroplasts. Photo by Yuuji Tsukii, with permission.

Figure 55. *Nebela flabellulum* living cell and test. Photo by Yuuji Tsukii, with permission.

Figure 56. *Nebela (Physochila) griseola*. Photo by Edward Mitchell, with permission.

Figure 57. *Nebela militaris* test. Photo by Yuuji Tsukii, with permission.

Figure 58. *Nebela tincta* test and protoplasm. Photo by Yuuji Tsukii, with permission.
Testate amoebae that live on bryophytes are mostly cosmopolitan taxa (see discussion of the Baas Becking hypothesis in Chapter 2-5). Even more remarkable than the Northern Hemisphere similarities seen in Table 3 is that the Antarctic displays similar communities. In the Antarctic, where mosses are the dominant flora, testacean protozoa are particularly rich in species. Vincke et al. (2004) found 83 taxa, representing 21 genera, among the mosses on Île de la Possession of the sub-Antarctic. Smith (1974) found them in carpets of the moss Sanionia uncinata (Figure 3) in the severe climate of the South Orkney Islands and near Rothera Station, Adelaide Island, both in the Antarctic. On Île de la Possession of the sub-Antarctic, the bryophyte communities were dominated by Euglypha laevis, E. rotunda (Figure 60), Trinema enchelys (Figure 61), and T. lineare (Figure 62, Figure 63), (Vincke et al. 2004). These four taxa are among those listed in Table 3 as common in the Northern Hemisphere.

Upon analysis, three communities of testate amoebae emerged for Île de la Possession: the Corythion dubium (Figure 39) community occurred in drier and slightly acidic terrestrial moss communities; the Arcella arenaria (Figure 29) and the Difflugiella crenulata communities were both in wetter, circumneutral habitats, with the former occurring in standing water and the latter community typically on submerged mosses of running water. In those habitats, the bryophyte species was important in describing the testate protozoan community. Among these dominant organisms, only Difflugiella crenulata is absent from the Northern Hemisphere taxa listed in Table 3. A word of caution, though: the taxa are difficult to distinguish and one name may have been applied to several taxa, or several names from different regions may actually apply to the same taxon. Morphologies can differ between regions, making the same species appear different (Bobrov et al. 1995). And within a region, cryptic species ("hidden" species that look the same but are reproductively isolated and genetically distinct) can exist.

Many of the known bryophyte inhabitants are never reported as such in the literature. In gathering information for this chapter, I have been able to add several taxa to the published literature I uncovered. Some, like Euglypha bryophila (Figure 64), are suggested by their names. Others, like Tracheleuglypha dentata (Figure 65), have come to me among the images of bryophyte-inhabiting protozoans sent by protozoologists. William Bourland has provided me with images of several moss inhabitants that I
have not found in the literature: *Cyphoderia trochus* (Figure 66); *Quadrulella symmetrica* (Figure 67). I also found many among the Perrault Fen, Michigan, USA images of Jason Oyadomari. Many more taxa are probably lurking among the non-Sphagnum taxa.

Figure 64. *Euglypha bryophila*, a bryophyte inhabitant with a name that means moss-loving. Photo by Yuuji Tsukii, with permission.

Figure 65. *Tracheleuglypha dentata* test with scales. Photo by Edward Mitchell, with permission.

Figure 66. *Cyphoderia trochus*, another member of the Euglyphidae. Photo by William Bourland, with permission.

Figure 67. *Quadrulella symmetrica*, a testate rhizopod that can be found among bryophytes. Photo by William Bourland, with permission.

**Summary**

The rhizopods (amoebae) can be naked or testate (living in a self-made house), with testae made of sand, diatoms, pollen, or mineral particles put together with secretions. Testate species are cosmopolitan and are particularly common on bryophytes, especially in peatlands. These common species even extend to the Antarctic. *Euglypha laevis*, *E. rotunda*, *Trinema lineare*, and *T. enchelys* are among the dominant taxa in both hemispheres. More taxa may be in common but are currently understood as multiple species. Many others undoubtedly remain to be discovered, especially among the non-Sphagnum bryophytes.

**Acknowledgments**

The protozoologists have been especially helpful in preparing this subchapter. Edward Mitchell and Paul Davison helped me find appropriate people to contact to get good images, and Edward Mitchell contributed several of the excellent SEM images used here. William Bourland provided a CD that filled some of my hard-to-find image needs. Images from Jason Oyadomari helped me to expand the list of known bryophyte inhabitants. The images by Yuuji Tsukii, provided through the Protist Information Server website, were invaluable.

**Literature Cited**


Tolonen, K., Huttunen, P., and Jungner, H. 1985. Regeneration of two coastal raised bogs in eastern North America: Stratigraphy, radiocarbon dates and rhizopod analysis from


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CHAPTER 2-4
PROTOZOA: RHIZOPOD ECOLOGY

Figure 1. Test of Centropyxis ecornis with desmids that are common cohabitants in peatlands. Photo by Yuuji Tsukii, Protist Information Server, with permission.

Geographic Distribution

Testate amoeba communities not only are diverse in themselves, but they typically occur with a diversity of algae and other micro-organisms (Figure 1). Moss-dwelling testate amoebae have been reported from the Antarctic (e.g. Richters 1904, 1908a, b; Sudzuki 1964; Smith 1973a, b, c, 1974a, b, 1986; Beyens et al. 1988; Balik 1994), to The Czech Republic (Balik 2001), to the Canadian Arctic (Beyens et al. 1986a, b), to name only a few. Beyens and Chardez (1994) thought that the amoebae formed specific assemblages related to the moss habitats. Working in the Mt. Kurikoma district of Japan, Chiba and Kato (1969) likewise suggested that the testacean community structure is related to the bryophyte habitat. Bartos (1949) reported on the moss-dwelling Rhizopoda of Switzerland. Most of his samples were from aerial mosses, but the Rhizopoda belonged to damp moss associations. The largest numbers of individuals belonged to the testate amoeba genus Centropyxis, including C. aerophila (Figure 3), C. eurystoma, C. kahli, and C. ecornis (Figure 4), in all the mosses. Smith (1992) reported Arcella arenaria (Figure 2). Centropyxis aerophila (Figure 3), Corythion dubium (Figure 5), Difflugia lucida, Diplochlamys timida, Heleopera petricola (Figure 6), and Trigonopyxis arcula (Figure 7) from Antarctica, where numbers were generally low compared to Northern Hemisphere studies. Only Bryum exhibited larger populations, those of Arcella arenaria. Centropyxis aerophila seems to prefer more calcareous situations (Coûteaux 1969), although its distribution in South Georgia (Antarctica) occurs at pH 4.5-5.6 (Smith & Headland 1983). This species is variable, whether due to geography or ecology (Chardez 1979).

Figure 2. Arcella arenaria. Photo by Yuuji Tsukii, Protist Information Server, with permission.
Chapter 2-4: Protozoa: Rhizopod Ecology

As for most of the invertebrates, the highest numbers seem to occur in peatlands. Gilbert et al. (2003) reported 29,582 ± 9650 active individuals per liter of Nebela vas and 2263 ± 1620 for the encysted ones at Pradeaux peatland (Puy de Dôme, France), with the greatest abundance at the end of June (almost 40,000), dropping to the lowest number in July (less than 15,000).

Communities

Although most of the information regarding rhizopod communities is for peatlands (Subchapter 2-5), a few studies have discussed communities in other types of bryophytes. Beyens et al. (1990) compared communities from the coastal lowlands on Devon Island, NWT, Canadian Arctic. These encompassed 57 taxa on mosses, soils, and lichens. The dry, acidic moss habitats were characterized by Assulina muscorum – Corythion dubium assemblages. In wet, neutral pH habitats, Paraquadrula irregularis was dominant. Sedge moss meadows had a soil fauna association of Plagiopyxis callida – Plagiopyxis declivis. Centropyxis minuta was mostly on coarsely textured soils in this study, but is known from mosses elsewhere.

Mazei and Belykova (2011) found 29 rhizopod species/forms associated with mosses at the water edge in seven streams of the Sura River basin (Middle Volga region, Russia). The dominant species are Centropyxis aerophila, Centropyxis cassis, Corythion dubium, and Centropyxis aerophila, Centropyxis cassis, Corythion dubium.
Euglypha ciliata glabra, Tracheleuglypha dentata, Trinema complanatum, Trinema enchelys, and Trinema lineare. The species richness in these communities varies from 2 to 11 per sample, with an abundance of 100 to 4000 individuals per gram dry moss. Mazei and Belykova suggested that the character of the community could be influenced by forest cover, water hardness, "biogenic elements," stream size, and environmental contamination.

Davis (1981) reported that the testate rhizopods were the dominant form of non-photosynthetic life among mosses in the maritime Antarctic. Smith (1986) reported ten species on the moss Sanionia uncinata: Assulina muscorum, Corythium dubium, Diffugia lucida, Nebela lageniformis, Nebela wailesi, Phryganella acropodia, Trigomopyxis arcula, and a species of Diffugia, possibly D. mica. The most abundant of these were Diffugia lucida and Assulina muscorum. The species richness was low, similar to that found in other southern latitudes.

**Moisture Relationships**

Moisture plays an important role in survivorship. Like many other bryophyte inhabitants, the testate amoebae among the bryophytes survive the wet-dry changes so common among the bryophytes (Chardez 1990). When conditions are dry, many rhizopod amoebae can encyst (Sacchi 1888 a, b; Heal 1962), thus escaping the need for water during long periods of drought (Hingley 1993). Some have survived 5-8 years in dry moss (Hingley 1993).

*Chlamydomyxa montana* is one such encysting protozoan. In its amoeboid state it feeds on diatoms, but it is photosynthetic in bright light in its encysted state (Pearlmutter & Timpano 1984). Cysts of this unusual amoeba occur on the branches of *Sphagnum* (Lankester 1896). These cause the moss to be ruddy brown, with a glistening surface due to olive-brown disk-like or ovoid cysts about 1-2 mm in diameter. When these are awakened, a network of threads appears, signifying the amoeboid stage.

In Germany, the death rate of testaceans in the river exceeded that in mats of the terrestrial *Plagiomnium cuspidatum* (Figure 8) (3%/day) (Schönborn 1977). This is perhaps due to the greater resistance to desiccation among the terrestrial taxa and represents a time of optimal conditions. With *Euglypha ciliata* (Figure 9, Figure 10) (429,000 individuals/m²; 15.5 mg/m²) and *Assulina muscorum* (Figure 11) (406,000 individuals/m²; 2.9 mg/m²) dominating, the production rate on the mosses is 40,600 individuals m⁻² day⁻¹ and a biomass of 0.3 mg m⁻² day⁻¹. In drier times, generation time increases as amoebae go dormant, causing fewer generations to be produced and reducing the productivity. Soil organisms spend only half the time for one generation compared to those living on the bryophytes. Not only is the moss subject to more frequent drying, but the number of *Aufwuchs* on the mosses is lower, thus providing less food.

Rhizopod communities are determined by the moisture and temperature conditions available to them (Chiba & Kato 1969). This affects not only the clumps of moss they inhabit, but also their vertical distribution within the clump. For example, in the Canadian Arctic, *Trinema lineare* (Figure 12) occurs deep in the moss mat where conditions are more humid (Beyens et al. 1986b).
Bartos (1949) found that in those mosses that were often dry, Centropyxis labiata occurred, with C. platystoma and C. constricta (Figure 13) in somewhat damper ones. The very dry mosses housed Trigonopyxis arcula (Figure 14) and Bullinularia indica (Figure 15). Several species occurred in all moss probes: *Trinema enchelys* (Figure 16), *Nebela collaris* (Figure 17), *Euglypha ciliata* (Figure 10), and *Assulina muscorum* (Figure 11).
Figure 17. *Nebela collaris*, a common species among mosses. Photo by Yuuji Tsukii, Protist Information Server, with permission.

**Case Building**

The large, shell-forming *Arcella* is a common genus among bryophytes, particularly *Sphagnum* (Hoogenraad & De Groot 1979; Chardez & Beyens 1987). *Arcella* builds a case that is completely organic (Meisterfeld & Mitchell 2008; Figure 18) and resembles a tiny doughnut in bottom view (Figure 19). *Arcella crenulata* and *A. mitrata* (Figure 20) tend to occur together on *Sphagnum* that is constantly wet, low in nutrients, and in a pH range of 4-6. Others such as *A. arenaria* (Figure 19), *A. catinus* (Figure 21), *A. artocrea* (Figure 22, Figure 23), and *A. microstoma* "prefer" *Sphagnum*, but also occur elsewhere.

Figure 18. SEM image of test of *Arcella hemisphaerica* showing organic construction. Photo by Ralf Meisterfeld, with permission.

Figure 20. Living *Arcella mitrata*. Photo by Yuuji Tsukii, Protist Information Server, with permission.

**Food**

The Rhizopoda have long been considered to be bacterivores, but it appears that this conclusion may be somewhat short-sighted. Although most are heterotrophic, a few are mixotrophic, housing photosynthetic algae as symbionts (Gilbert *et al.* 2000). The ability of some taxa to ingest a wide size range (0.2-1000 µm) of organisms and particulate organic matter (POM) offers a potential competitive advantage.

Figure 19. Test of *Arcella arenaria*. Photo by Yuuji Tsukii, Protist Information Server, with permission.

Wilmshurst (1998) found protozoa so common in New Zealand *Sphagnum* peatlands that she estimated that more than 50,000 protozoans could "eke out a living" in a gram of fresh moss. The amoebae survive by consuming particulate organic matter, algae that grow epiphytically on the mosses, bacteria, fungi, plant cells, and even smaller amoebae (Richardson 1981; Gilbert *et al.* 2000). Although bacterivorous taxa are the most frequent, some taxa eat algae and other protozoa almost as large as they are.

Deriu *et al.* (1995) challenged earlier studies that suggested that *Sphagnum* served as a reservoir of mycobacteria as a food source, citing the medicinal properties of *Sphagnum* as evidence of the near absence of mycobacteria. Nevertheless, it is likely that bacteria serve
as the primary food source. Mieczan (2006) found that among the Sphagnum in Poleski National Park in Poland the bacterivorous protozoa had the greatest numbers, whereas those that ate algae were least common.

![Figure 22. Test of Arcella artocrea. Photo by Edward Mitchell, with permission.](image)

Figure 22. Test of *Arcella artocrea*. Photo by Edward Mitchell, with permission.

Figure 24. *Amphitrema flavum*, a protozoan that incorporates green algal symbionts. Photo by Edward Mitchell, with permission.

Figure 25. *Diffugia oblonga* with green algae, possibly living as symbionts. Photo by Yuuji Tsukii, Protist Information Server, with permission.

**Symbionts**

Despite their habitation within a case or test, some of the Testacea also have **symbionts**. Among those inhabiting bryophytes, symbiotic taxa include *Amphitrema flavum* (Figure 24), *Diffugia oblonga* (Figure 25), *Hyalosphenia papilio* (Figure 26), and *Heleopera sphagni* (Figure 27) (Burkholder 1996; Charrière *et al.* 2006; Meisterfeld & Mitchell 2008). Their dependency on light forces them to live in the upper few cm where the algae live both independently and within the rhizopod, and are able to photosynthesize. A more detailed discussion of algal symbionts is in the subchapter on Protozoa Diversity (Chapter 2-1).

![Figure 23. Test of Arcella artocrea. Photo by Yuuji Tsukii, Protist Information Server, with permission.](image)

Figure 23. Test of *Arcella artocrea*. Photo by Yuuji Tsukii, Protist Information Server, with permission.

![Figure 26. Hyalosphenia papilio densely impregnated with symbiotic algae. Photo by Yuuji Tsukii, Protist Information Server, with permission.](image)

Figure 26. *Hyalosphenia papilio* densely impregnated with symbiotic algae. Photo by Yuuji Tsukii, Protist Information Server, with permission.

**Bryophyte Chemistry**

Moss chemistry appears to play an important role in at least some cases in determining species richness. Testate amoebae occupying *Hylocomium splendens* (Figure 28) in the Italian Alps were distributed largely in accordance with differences in C, P, Ca, Mg, Al, Fe, and Na of the moss tissues (Mitchell *et al.* 2004). The researchers suggested that the chemistry affected the prey organisms, thus affecting their consumers, the amoebae. Surprisingly, there was no relationship to the important nutrients N and K. Both Mitchell *et al.* (2004) and Bonnet (1973b) concluded that distribution of testate amoebae among wefts of *H. splendens* was independent of soil type.

![Figure 28.](image)
Pollution – Heavy Metals

Rhizopods, as well as bryophytes, can serve as indicators of pollution damage to a community. In a study of the moss *Barbula indica* in Viet Nam, both richness and abundance of rhizopods were reduced by lead (Nguyen-Viet *et al.* 2007). Shannon diversity was negatively correlated with cadmium. Although several species of rhizopods were negatively correlated with lead, cadmium, zinc, and nickel, lead was the only pollutant that caused a significant change at the community level. Other effects will be discussed in the sub-chapter on Peatland Rhizopods.

In addition to the taxa mentioned above, Mieczan (2006) also found *Codonella cratera* (Figure 29) in two Polish peatlands. There is surely a wealth of species waiting to be discovered in the little-explored bryophyte microcosm. Corbet (1973) managed a 38-page article on the testate species of *Sphagnum* at a single location, Malham Tarn, Yorkshire. Other bryophytes have received much less attention.

Summary

*Centropyxis* and *Arcella* are among the most common of the testate amoebae among epiphytic bryophytes. Communities vary seasonally as moisture changes. Moisture is also the greatest determinant of the choice of bryophyte and vertical location within it, but for some pH also plays a role. Construction of cases may help them to survive brief dry periods, but most encyst until favorable moisture returns. Terrestrial taxa are more resistant to desiccation than are aquatic ones. Generation time is longer on mosses because of the time spent encysted.

Many of the rhizopods are bacterivores, but they also consume fungi, algae, plant cells, and smaller amoebae. Chemistry may affect the available food organisms, but N & K do not seem important. Several of the rhizopods harbor *Chlorella* as symbionts. Their need for light causes these taxa to live in the upper few cm of the bryophyte layer.

Rhizopods often have a negative correlation with pollutants, especially some of the heavy metals.

Acknowledgments

Yuji Tsukii was most helpful in giving me permission to use his images from the Protist Information Server. Edward Mitchell helped me to find literature and provided me with a number of images I couldn't find elsewhere.

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Beyens, L., Chardez, D., Baere, D. De, Bock, P. De, and Jaques, E.  1990.  Ecology of terrestrial testate amoebae assemblages...
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CHAPTER 2-5
PROTOZOA: PEATLAND RHIZOPODS

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CHAPTER 2-5
PROTOZOA: PEATLAND RHIZOPODS

Peatlands Taxa: Sphagnum

Protozoa, and especially Rhizopoda, are apparently most abundant in peatlands (Figure 1) and were among the earliest of the moss fauna to be examined (Jung 1936). But few other bryophyte protozoans have been studied in detail. Among the abundant sphagnicolous taxa (growing in Sphagnum moss) are Nebela (Figure 2), Hyalosphenia (Figure 3), Diffugia pyriformis (Figure 4), and D. globularis (Bovee 1979; Gerson 1982). Table 1 summarizes the species I have found in the literature.

Figure 1. A peatland with *Sphagnum magellanicum* that serves as habitat for protozoa. Photo by Michael Lüth, with permission.

Figure 2. *Nebela collaris*, a sphagnicole. Photo by Yuuji Tsukii, with permission.

Figure 3. *Hyalosphenia papilio*, a sphagnicole. Photo by Yuuji Tsukii, identified by Matthieu Mulot, with permission.

Mitchell *et al.* (2000b) compared testate (with a house) amoebae in peatlands of Switzerland, the Netherlands, Great Britain, Sweden, and Finland. They found that the plant species differed more than the species of amoebae. The high number of rhizopod species among *Sphagnum*, compared to that of other mosses or tracheophytes, supported the usefulness of rhizopods as indicators of both past and present conditions. Furthermore, the mosses were...
less affected by the chemistry of the ground water than were such taxa as *Carex* and *Eriophorum*. But when Booth and Zygmunt (2005) compared the testate amoeba communities of the Great Lakes in North America with those of the Rocky Mountains of North America, the communities differed, perhaps due to differences in climate and the trophic state of the peatlands. Even so, these two regions had many species in common, and these species occupied similar moisture positions in both regions. In the Rocky Mountains, USA, distribution of these testate amoebae in *Sphagnum*-dominated peatlands is dictated primarily by surface moisture (Zygmunt et al. 2003). Communities in the western Great Lakes region are similarly distributed, with 50% of the species also occurring in the Rocky Mountain peatlands, and similar communities exist for Yellowstone National Park.

Testate amoebae abound in peatlands all over the world. Because of their abundance there, testate amoebae have been widely studied in peatlands all over the world (e.g. Leidy 1879; Harnish 1924, 1925, 1927, 1948, 1950, 1951; Hoogenraad 1934, 1935; Jackzo 1941; van Oye 1941, 1951; Conra, 1943; Heinis 1945; Hoogenraad & de Groot 1946; Paulson 1953; Rose 1953; Hoppman 1954; Chacharonis 1956; Varga 1956; Bonnet 1958; Thomas 1959; Heal 1961, 1964; Schönborn 1962, 1963, 1965; Martin 1963; Buttler et al. 1966a; b; Tolonen 1966, 1994; Coûteau 1969; Bovee 1979; Seis 1971; Corbet, 1973; Laminger 1975; Vucetich 1975; Grospietsch 1976; Ruitenburg & Davids 1977; Meisterfeld 1978, 1979a, b; Beyens & Chardez 1984; Tolonen et al. 1985, 1992, 1994; Warner 1987; Hendon & Charman 1997; Gilbert et al. 1998a, b, 2003; Woodland et al. 1998; Bobrov et al. 1999; Strüder-Kypke & Schönborn 1999; Mitchell et al. 1999, 2000a, b; Charman et al. 2000; Booth 2002; Langdon et al. 2003; Laggoun-Défarge et al. 2008).

Bobrov et al. (1999) studied their ecology in peatlands of Russia. Bousquet (1950) studied them in southwestern France, Mieczan (2006) in Poland, and Wilmshurst (1998) in New Zealand. Robson et al. (2001) reported on *Sphagnum* bog microfauna in Tierra del Fuego, South America, demonstrating several of the same familiar genera as those in Switzerland (Bartos 1949a). Among those Northern Hemisphere taxa also identified in Tierra del Fuego were *Assulina* (Figure 5), *Corythion* (Figure 6), *Euglypha* (Figure 7), and *Heleopera* (Figure 8). Just as peatland plants are more cosmopolitan than other plants, these rhizopod assemblages seem to be more affected by ecology than by geography. This is reflected in the small-scale vertical gradients seen among the amoebae, rotifers, and other invertebrates. As noted above, it appears that the number of species of these rhizopods is generally much greater among *Sphagnum* (Figure 1) than among other mosses or tracheophytes (Mitchell et al. 2000b). Nevertheless, Tolonen et al. (1992) found little difference in rhizopod taxa between *Sphagnum* communities and those of bryalean mosses in Finnish mires. Unfortunately, few studies have compared fauna on these two groups of bryophytes at the same location.
Table 1. Species of testate amoebae known from peatlands. *Indicates species closely associated with *Sphagnum*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Authors</th>
<th>Associates with Sphagnum</th>
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*Nebela tenella* Mazei & Tsyganov 2007/08
*Nebela tincta* Gilbert *et al*. 2003
*Nebela tubulosa* Hingley 1993
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*Phryganella acropodia* Hingley 1993
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*Pyxidicula cymbalum* Hingley 1993
*Sphenoderia dentata* Hingley 1993
*Sphenoderia fissioninis* Hingley 1993
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*Sphenoderia macroelepis* Hingley 1993
*Trigonopyx arcula* Hingley 1993
*Trinema enchelys* Hingley 1993
The nature of peatlands may account for their prominent testate amoeba fauna (Booth & Zygmunt 2005). *Sphagnum* itself is particularly rich in species (Hingley 1993; Mazei et al. 2007). The amoebae are able to live in the thin film of water in the concavity of *Sphagnum* leaves (Figure 9; Corbet 1973). Mazei et al. (2007) found 59 species of testate amoebae among the *Sphagnum* plants of a bog in Volga Highland in Russia. Among these, 24 were common and the minimal richness was three species in a sample. Interestingly, the highest densities of organisms occurred in the driest bog habitats, but predictably, the diversity was lowest (3 species), with *Arcella arenaria* (Figure 10) the most common. At medium levels of humidity, the number of species was greater (13-16), with *Nebela tenella* (Figure 11) and *Hyalosphenia elegans* (Figure 12) being the most common. Low oxygen concentrations reduced densities by 50-65%. When oxygen was not limiting, however, both abundance and species richness increased with depth. At high humidity, the dominant taxa were *Hyalosphenia papilio* (Figure 13) and *Heleopera sphagni* (Figure 14). But not all of these testae were occupied by live amoebae. The number of living individuals ranged 35-75% of the testae found.

Lamentowicz and Mitchell (2005) found 52 taxa of testate amoebae in *Sphagnum* peatlands of northwestern Poland. In a later study, in Poland's largest peatland complex, Lamentowicz et al. (2007) found 32 taxa of testate amoebae. In most of the ten sites in this complex, species composition was dominated by *Hyalosphenia papilio* (Figure 13), *Cyclopyxis arcelloides* (see Figure 15), and *Hyalosphenia elegans* (Figure 12); *Amphitrema flavum* (Figure 16, Figure 17) was among the most numerous.
Lamentowicz and Mitchell (2005) identified three groups of testate taxa, based on depth to water table (DWT) and pH: high DWT & low pH, low DWT & low pH, and high pH & mid-range DWT. Species tolerance increases with dryness, with a pattern that reflects that of *Sphagnum*. That is, changes in the water table depth have more effect on those species in wet habitats than on those in drier microhabitats. This appears to indicate that those in dry microhabitats are specialists for drought.

Corbet (1973) found several species that are apparently confined to the *Sphagnum* habitat: *Amphitrema flavum* (Figure 16-Figure 17), *A. wrightianum* (Figure 18-Figure 19), *A. stenostoma* (Figure 20), *Hyalosphenia elegans* (Figure 12), and *H. papilio* (Figure 13). *Cryptodifflugia ovalis* (Figure 21) and *Amphitrema flavum* (Figure 16) can live within the hyaline cells of *Sphagnum* leaves, entering through the pore and experiencing constant moisture.
Figure 19. *Amphitrema wrightianum* using fluorescence to show ingested chloroplasts. Photo by Edward Mitchell, with permission.

Figure 20. *Amphitrema stenostoma* test with sand grains and living protoplast with included chloroplasts. Photo by Yuuji Tsukii, Protist Information Server, with permission.

Figure 21. *Cryptodifflugia ovalis* showing living cell and extruded protoplasm. Photo by William Bourland, with permission.

Those species that characterize *Sphagnum* hummocks (Figure 22) in the western Carpathians [*Nebela militaris* (Figure 23), *N. tincta* (Figure 24), *Assulina muscorum* (Figure 25), *Heleopera petricola* (Figure 26)] seem intolerant of the mineral-rich fens (Opravilová & Hájek 2006). Only *Corythion dubium* (Figure 27) and *Nebela bohemica* occupy both. The Euglyphidae were dominant in all these habitats and were nearly the exclusive testate inhabitants of the moderately rich fens. Hyalospheniidae, on the other hand, characterized the extremely acid habitats, particularly in *Sphagnum* hummocks. The overall vegetation was the best predictor of the testate protozoan composition, and the composition of the bryophyte assemblage was the second most important predictor.
Mazei and Tsyganov (2007/08) reported on a number of taxa in the Sphagnum peatlands of Russia. In a single bog, they found 63 taxa comprising 21 genera. They found two different communities, one that lived in the Sphagnum "quagmire" and one that lived in the bottom sediments of the drainage. The detritivores from the bottom sediments included *Arcella gibbosa*, *A. vulgaris*, *A. hemisphaerica*, *A. discoides*, *A. intermedia*, *A. mitrata*, *Centropyxis aculeata sphagnicola*, *Cyclopyxis kahli*, *Diffugia glans*, *Lesquereusia spiralis*, *Netzelia tuberculata*, and *Phryganella hemisphaerica*. Those species typical of Sphagnum were *Archerella flavum*, *Euglypha cristata*, *Difflugia juzephiniensis*, *Cryptodifflugia compressa*, *Nebela militaris*, and *Sphenoderia fissirostris*. Those inhabiting both the Sphagnum mats and the quagmire included *Assulina seminulum*, *A. muscorum*, *Bullinularia indica*, *Centropyxis aculeata*, *Diffugia globulosa*, *D. parva*, *Euglypha ciliata*, *Hyalosphenia elegans*, *Nebela tenella*, and *N. tincta*. Other species are not so specific and occur in both of the major bog communities: *Arcella arenaria*, *Euglypha laevis*, and *Trigonopyxis arcula*.

But even within the Sphagnum quagmire, Mazei and Tsyganov (2007/08) found three types of testate amoebae communities. The *xerophilous* (dry-loving) community could be found in hummocks made of *Polytrichum strictum*, *Sphagnum papillosum*, and *S. angustifolium*. These dry hummocks house a community characterized by *Assulina muscorum*, *A. seminulum*, and *Cryptodifflugia compressa*. The lawns of *Sphagnum palustre* and *S. magellanicum* make a wet community characterized by *Heleopera sphagni*, *Hyalosphenia papilio*, *H. elegans*, and *Nebela tenella*. Submerged *Sphagnum riparium* is characterized by an association of *Cyclopyxis eurystoma*, *Heleopera sphagni*, *Hyalosphenia papilio*, and *Phryganella hemisphaerica*. Available moisture, determined by depth from the water table, separated the communities. The greatest homogeneity occurs in the moist areas in the middle of the quagmire, whereas dry habitats have the greatest diversity. On the other hand, a greater proportion of amoebae were alive in the moist areas (36-45%) compared to 22-27% of those in dry habitats.

**Medium and Rich Fens**

Bryophytes of rich fens (Figure 28) differ greatly from those of Sphagnum bogs and poor fens, and so do the protozoa. To utilize fully the testate protozoa to reconstruct peatland history, as discussed later in this chapter, it is important to understand these faunal differences. Opravilová and Hájek (2006) studied the spring fens of the Western Carpathians in the Czech Republic and Slovakia to fill in this rather large gap in our knowledge. They found that two species [*Paraquadrula irregularis* (Figure 29, Figure 30) and *Centropyxis discoides* (see Figure 31)] were essentially restricted to fens, while seven rhizopod species characterized the bryophytes there. In moderately rich Sphagnum fens, *Arcella discoides* (Figure 32) was characteristic. In poor fens, testate protozoan species of bryophyte lawns were closely tied to moisture and overlapped widely with those of poor fen sediments and moderately rich fens: *Nebela collaris* (Figure 33), *Phryganella acropodia*, *Sphenoderia fissirostris*.
The protozoan species of *Sphagnum* fens in the Czech Republic and Slovakia are very similar to those known elsewhere, with *Amphitrema flavum* (Figure 34), *A. wrightianum* (Figure 34), and *Hyalosphenia papilio* (Figure 35), being optimal in wet microhabitats, but also tolerating higher mineral concentrations (Meisterfeld 1979b; Charman & Warner 1992; Tolonen *et al.* 1992; Booth 2001; Schnitchen *et al.* 2003; Booth & Zygmunt 2005; Lamentowicz & Mitchell 2005; Opravilová & Hájek 2006). In the drier poor fens, the dominant species are *Assulina muscorum* (Figure 25), *A. seminulum* (Figure 36), *Arcella catinus* (Figure 37), *Nebela militaris* (Figure 23), *N. bohemica*, *Trigonopyxis arcula* (Figure 38), and *Corythion dubium* (Figure 39). *Corythion dubium* also occurs in moderately rich fens (Beyens *et al.* 1986; Tolonen *et al.* 1994; Bobrov *et al.* 1999; Mitchell *et al.* 2000b; Opravilová & Zahrádková 2003; Vincke *et al.* 2004).
Among the "brown mosses" (Figure 40, Figure 41, ) of calcareous fens, *Centropyxis cassis, Cyclopyxis kahlii, Cyphoderia ampulla* (Figure 42), *Diffugia glans, Quadrulella symmetrica* (Figure 43), and *Trinema enchelys* (Figure 44) often predominate (Mattheusson et al. 2005; Opravilová & Hájek 2006). There is indeed a gradient of species from poor to rich fens, with moisture being an important variable in the poor fens and bogs (Opravilová & Hájek 2006; Hájek et al. 2011). Interestingly, the sediments of poor acidic fens support a species composition similar to that of bryophyte tufts of mineral rich fens (Opravilová & Hájek 2006).
Recent, moist stages of succession in the Jura Mountains of Switzerland were dominated by *Hyalosphenia papilio*, with *Archerella flavum* indicating wet, acidic conditions at one site (Laggoun-Défarge et al. 2008). Drier acid conditions supported a greater abundance of *Nebela tincta* and *Assulina muscorum*. *Corythion dubium* also indicated dry, acid conditions.

### Habitat Needs

Mieczan (2007) examined the habitat preferences of eleven testate amoebae in Eastern Poland peatlands. He found that low pH (4.5) favored the amoebae (see also Warner & Chmielewski 1992; Tolonen et al. 1994; Charman & Warner 1997; Mitchell et al. 1999; Bobrov et al. 2002; Booth 2002; Lamentowicz & Mitchell 2005). These acidophilic taxa were dominated by ubiquitous and common taxa, with *Arcella vulgaris, Assulina muscorum, Euglypha* sp., and *Hyalosphenia* sp. having a distinct preference for low pH. The distribution pattern seemed to be controlled by moisture (no surprise there), whereas the total numbers and biomass had a positive correlation with pH and total organic carbon content of the water. Heal (1964) found that pH was a major factor accounting for differences between bog and fen communities in Great Britain. In addition to moisture and pH, the trophic status and concentration of mineral nutrients, including calcium, can play a role in determining numbers (Tolonen et al. 1992).

In the Western Carpathians along the border between the Czech Republic and Slovakia, Hájková et al. (2011) attempted to ascertain the factors that determined which micro-organisms comprised communities at two sites within mineral-rich *Sphagnum*-fens and four within mineral-poor *Sphagnum*-fens. They found that community composition correlated with water pH, conductivity, calcium concentration, and *Sphagnum* dominance. The types of mosses often played a major role, with a significant positive correlation between testate amoebae and *Sphagnum* (*S. fallax, S. flexuosum, S. palustris, S. papillosum*). On the other hand, there was a significant negative correlation with "crawling dense tufts" of bryophytes (*Cratoneuron filicinum, Palustriella commutata, P. decipiens*). There was no correlation with crawling loose tufts (*Brachythecium rivulare, Calliergonella cuspidata, Plagiomnium ellipticum, P. elatum*) or erect species (*Bryum pseudotriquetrum, Fissidens adianthoides, Philonotis caespitosa*). These community distinctions suggest that growth form was an important factor. Growth form often determines water-holding ability, a strong factor in distribution of testate amoebae.

### Food

Although many of the protozoa associated with bryophytes are detritus/bacterial feeders, some common species prefer a different diet. In one *Sphagnum* peatland 17.4% of *Nebela collaris* sensu lato most frequently preyed upon micro-algae (45%, with diatoms comprising 33% of total prey), spores and fungal mycelia (36%), and large ciliates, rotifers, and small testate amoebae in smaller numbers (Gilbert et al. 2003). However, 71% of the food content could not be identified because it was partially decomposed. It appears that when the mosses are

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**Successional Stages**

Differences occur not only between peatlands, but also in different stages of the same peatland, an important factor in permitting us to reconstruct the past history of peatlands. Mazei and Bubnova (2007) demonstrated 42 species in the initial stage of a transitional bog. Early stages were characterized by widespread species such as *Assulina muscorum, Arcella arenaria, Phryganella hemisphaerica,* and *Euglypha laevis,* whereas the sphagnobionts such as *Nebela, Hyalosphenia,* and *Heleopera* were absent. Vertical differences had not developed because the species that characterize the different depths had not yet become established.

Kishaba and Mitchell (2005) carried out a 40-year study on the *Sphagnum*-inhabiting rhizopods to determine successional trends in the Swiss Jura Mountains. They took their first samples in 1961 following peat cutting and lateral drainage that resulted in an increase in tree cover, especially at the edges. By the second sampling date in 2001, three species had increased significantly in mean relative abundance: *Nebela tincta* s. l. (+97%), *Bullinularia indica* (+810%), and *Cyclopyxis eurystoma* (+100%; absent in 1961), while two species decreased significantly: *Assulina muscorum* (-63%) and *Euglypha compressa* (-93%). Furthermore, testate amoebae communities differed among hummocks, lawns, and hollows. Nevertheless, there were no significant changes in the overall community structure between the two sampling dates.
sufficiently wet, most of the food organisms are immobile, senescent, or dead. However, as the water film on the moss becomes thin, it constrains the ciliates and micro-Metazoa, causing them to be a more easily consumed part of the diet.

Vertical Distribution

Peatlands have both horizontal and vertical differences in moisture, light availability, nutrient availability, and pH (Figure 45). The testate rhizopods are distributed both vertically and horizontally with respect to these differences (Meisterfeld 1977).

![Figure 45. Sphagnum teres, demonstrating the zonation from light to dark within the peat. Photo by Michael Lüth, with permission.](image)

Perhaps because of the multiple factors involved in vertical and horizontal distribution, distinct patterns are difficult to discern. Mazei and Tsyganov (2007/8) considered the aggregations of species to blend into each other in patches of varying sizes. For *Assulina muscorum* and *A. seminulum*, patch size seemed to correlate with shell size. As sample size increases, heterogeneity increases. Communities can be distinct on as small as a 1-cm patch, but more typically the minimum size does not exceed several cm. In their study in the Middle Volga region of Russia, Mazei and Tsyganov found that associated with the upper parts of *Sphagnum* the typical species were *Assulina flavum*, *A. muscorum*, *A. seminulum*, *Heleopera sphagni*, and *Hyalosphenia papilio*. Among these, *Assulina flavum*, *Heleopera sphagni*, and *Hyalosphenia papilio* were mixotrophs, requiring light for their algal symbionts (see sub-chapter 2-4), whereas *Hyalosphenia elegans* lacked symbionts and lived in a deeper community. The upper 0-3 cm layer typically had low rhizopod species richness but the highest abundance in the peatlands. And among those tests the proportion of living organisms was highest (75%). Species of *Amphitrema* likewise occur in the upper layer because of the need for light by their symbionts (Gilbert & Mitchell 2006).

When conditions are somewhat drier, the vertical structure of the communities is more pronounced (Mazei & Tsyganov 2007/08). Low moisture typically resulted in empty tests, especially in *Assulina* species. Survival of the rhizopod species is facilitated by the *r*-strategies of reproduction in which these small organisms are able to increase rapidly in response to the return of favorable conditions.

One additional factor that may play a role in distribution for some species is available nitrogen (Mitchell & Gilbert 2004). In cutover peatlands fertilized with N for three years, richness of the peatland was high (22 taxa of testate amoebae), but diversity of individual samples was low (6.6), attesting to the diversity of the habitat. Species richness increased with depth, but there was little response to differences in N levels in the tested range of additions of 0, 1, 3, or 10g N m\(^{-2}\) yr\(^{-1}\) for three years. Only *Bullinularia indica* was significantly more abundant in N-fertilized plots. Although the vertical distributions differed among species, there seemed to be no relationship to either shell type or metabolism type. In the top segment (0–1 cm), *Assulina muscorum* was most abundant. At 3–5 cm *Heleopera rosea*, *Nebela militaris*, and *Phryganella acropodia* were most abundant.

It is not surprising that the taxa with zoochlorellae occur in the green portions of *Sphagnum*. In Obersee near Lunz, Austria, the dominant taxa hosting zoochlorellae are *Amphitrema flavum*, *Heleopera sphagni*, *Hyalosphenia papilio* (Laminger 1975). *Centropyxis aculeata* likewise lives there, but without zoochlorellae. Activity among the rhizopods extended down to 18 cm, with some of the less mobile testate species extending to a depth of 45 cm. Some of the species that lived down to depths of 12 cm were species that also inhabited forest mosses (*Euglypha laevis*, *Trinema enchelys*, and *T. lineare*). At 18 cm, several sediment species of *Diffugia* occurred (*D. amphora*, *D. corona*, *D. acuminata*, *D. lebes*). Furthermore, the populations of *Centropyxis aculeata* exhibited characteristics of sediment-inhabiting taxa, i.e. tests covered with mineral particles and no spines.

Horizontal Differences

Not only do the testate amoebae have a vertical zonation in peatlands, but their horizontal distribution varies as well, reflecting habitat patchiness (Meisterfeld 1977; Mitchell et al. 2000a; Mazei and Tsyganov 2007/8). In the Swiss Jura Mountains, spatial structure accounted for 36% of the observed variation. Imbedded in the horizontal variability, Mitchell et al. found that microtopography played an important role, indicating that in just 0.25 m\(^2\) conditions are not uniform and present a different picture from that seen on a macroscale. In this case, the horizontal scale responds to differences in distance from the water table, whereas vertically within a *Sphagnum* mat, light, moisture, and detrital accumulation all differ. The horizontal scale also differs in pH and ion concentrations, both of which are lower on hummocks than in hollows. These differences in turn cause differences in the bacteria, fungi, algae, and other protozoa available for food. And hummock *Sphagnum* species are usually different from hollow species, having different morphologies that provide different sorts of spaces and different abilities to retain water and detritus.

Seasonal Differences

Communities of protozoa can differ among seasons, just as moisture and other conditions change in their habitat. As a result, species richness will fluctuate, as will abundance. In a *Sphagnum* bog in the Middle Volga region of Russia, species richness increases as the vegetation increases during May to September (Mazei &
Tsyganov 2007/2008). At the same time, evenness and species diversity have little variation. Species abundance changes are less well defined seasonally, most likely being more responsive to available moisture that is not directly tied to season.

Spring brings melting snow in most peatlands (Figure 46), with dormant protozoa awakening as the environment becomes more hospitable. In spring, dominant hygrophilous (water-loving) species in the Middle Volga region included Heloëpera sphagni, Hyalosphenia papilio, and Nebela tincta (Mazei Tsyganov 2007/08). This dominance is replaced in summer and autumn by Hyalosphenia elegans and Nebela tenella. The xerophilous (dry-loving) community is slightly different and the diversity is somewhat greater. In spring, Assulina muscorum, Heleopera sphagni, and Nebela tincta dominate, being replaced in summer by a community of Assulina seminulum, Euglypha ciliata, Hyalosphenia elegans, and Nebela tenella. Yet another community appears in autumn, dominated by Assulina seminulum, Cryptodifflugia compressa, and Trigonopyxis arcula.

Figure 46. As the snow recedes, the Sphagnum habitat will witness the awakening of water-loving protozoa that have remained dormant throughout the winter. Photo courtesy of Andres Filipe Baron Lopez in Alaska.

Heal (1964) found slightly different species in his study of six fen and bog sites in Great Britain, but the patterns were similar. Three species – Amphitrema flavum, Hyalosphenia papilio, and Nebela tincta sensu lato – had peak numbers from May until October. They then either encysted or died. For Hyalosphenia papilio, light is a controlling factor because this protozoan typically contains photosynthetic zoochlorellae (Figure 47). Although many of these rhizopods can reproduce every eight days by cell division, field evidence suggests that they have fewer than ten generations per year. This low number of generations limits their ability to respond to improved environmental conditions. These three species thus accounted for a biomass of 1.0 g m\(^{-2}\) and 30.2 x 10\(^6\) individuals m\(^{-2}\) in Great Britain. Nevertheless, Heal found 98 species and varieties in these six sites with a distribution similar to that found in northern fens and bogs.

One mechanism that maintains closely related species in different niches is their seasonal requirements. For example, Hyalosphenia papilio is dominant in spring, H. elegans in summer-autumn. Nebela tincta occurs in spring, N. tenella in summer. Assulina muscorum appears in spring, A. seminulum in summer.

Figure 47. This protozoan, possibly Bryometopus, contains zoochlorellae. Photo by Yuuji Tsukii, with permission.

Pollution

Pollution can alter the peatland rhizopod communities. Mitchell et al. (2003) found that CO\(_2\) enrichment caused a change in structure, but not in total biomass. Heterotrophic bacterial biomass increased by 48%, whereas that of the testate amoebae decreased by 13%. They suggested that the increase in CO\(_2\) may have caused an increase in Sphagnum exudates that in turn stimulated an increase in bacterial biomass.

Ozone Loss and UV-B Radiation

One of the effects of pollution with refrigerants has been the destruction of ozone in the upper atmosphere. This loss of ozone itself is not dangerous; it is not an oxygen source for life on Earth. But it is a critical shield of the UV rays from the sun, high energy wavelengths that are lethal to many forms of life. This is especially realized in polar regions.

Searles et al. (1999) examined the effects of this "ozone hole" in regions of Tierra del Fuego, southern Argentina, and Chile. Their study was experimental. They chose areas with an ozone hole and used plastic film filters to reduce the UV-B reaching the habitat, in this case a Sphagnum bog. The growth and pigment concentrations of Sphagnum (S. magellanicum) were virtually unaffected during the three months of the experiment. The surprise was that both testate amoebae and rotifers in this Sphagnum habitat became more numerous under the near-ambient UV-B radiation (i.e., under the reduced ozone filter of the ozone hole) than they were under reduced UV-B radiation resulting from the plastic filter (Figure 48). The protozoa were dominated by Assulina muscorum with some individuals of A. seminulum, Nebela, Heloëpera, and Euglypha species.

Protozoan communities are also sensitive to other pollutants (Nguyen-Viet et al. 2008). As in testate amoebae on Barbula indica in Viet Nam, the testate amoebae on Sphagnum fallax declined in species richness, total density, and total biomass and community structure was altered with added lead (Nguyen-Viet et al. 2007, 2008). NO\(_2\) also caused a decline in diversity, but not in density in the more heavily polluted city center of Besançon, France (34.8 ± 9.5 μg m\(^{-3}\) compared to the peripheral area (14.6 ± 4.7 μg m\(^{-3}\)) (Nguyen-Viet et al. 2004). Paraquadrula irregularis differed dramatically, being present in all peripheral samples and completely
absent in the city; no other species differed significantly between the two areas.

![Figure 48. Effects of UV-B radiation on protozoa and rotifers living among Sphagnum magellanicum in the Antarctic ozone hole. Vertical lines represent standard error of differences between treatments. Redrawn from Searles et al. 1999.](image)

Reconstruction of Past Climate

Diatoms and siliceous protozoan plates and scales are common in peat preparations (Douglas & Smol 2001). However, these are seldom used in peatland reconstruction because it is nearly impossible to identify the species from these fossils. Fortunately, rhizopod tests are often present in the same samples and require the same preservation techniques as the diatoms and scales. Since the species are generally identified by their shells, there has been considerable recent interest in using these testate shells for determining the past history of the peatlands.

Both the mosses and the amoebae are well conserved over time, *Sphagnum* because of its resistance to decay, and for testate amoebae it is the unique test (housing) that likewise resists decay (Meisterfeld & Heisterbaum 1986; Coûteaux 1992). Both can be identified thousands of years later.

Even fossil evidence supports the richness of the *Sphagnum* fauna (Douglas & Smol 1988). Fortunately, the species are cosmopolitan (Smith & Wilkinson 2007) and community structure varies little with geography (Mitchell et al. 2000b; Booth & Zygmun 2005), differing much less between geographic areas than does the tracheophyte community (Mitchell et al. 2000b). Even if species have diverged into sister species and become endemic (Mitchell & Meisterfeld 2005), it will often be possible to use these species complexes as indicators. On the other hand, we may be plagued by species that have diverged physiologically without changing morphologically, thus permitting them to live under different conditions but without being recognizable as different taxa.

As already implied, the testate amoebae have a distribution pattern that mimics that of *Sphagnum* (Lamentowicz & Mitchell 2005). Wet habitat species of both are more sensitive to changes in the water table depth than are those of dry habitats such as hummocks. Species of dry habitats are more tolerant of desiccation. Consequently, the testate amoeba shells from the past permit us to reconstruct the past history of peatlands (van Geel 1976; Beyens & Chardez 1987; Warner 1991; Wilmshurst 1998; Bobrov et al. 1999; Charman et al. 1999; McGlone & Wilmshurst 1999a, b; Foisnser 1999; Mauquoy & Barber 2002; Schnitchen et al. 2003; Zygmun 2003; Booth et al. 2004; Gilbert & Mitchell 2006; Payne et al. 2006; Payne & Mitchell 2007; Mitchell et al. 2008). Payne et al. (2008) demonstrated that even such diverse regions as Turkey, North America, and Europe have similar testate communities. Because of the unique assemblages of testate amoebae associated with moisture conditions of the peat mosses worldwide and the effects of climate change on them, the testate amoebae are useful for reconstructing past climate.

Surface moisture of bogs (with only precipitation as a water source), in particular, is controlled by climate. Reconstruction of the testate amoeba history permits reconstruction of the historic surface moisture, and that permits reconstruction of past rainfall. The amoebae are so fine tuned to the water table that they can help a researcher to predict the water table within less than 2 cm (Payne & Mitchell 2007). For example, Hughes et al. (2006) used testate amoebae to identify fourteen distinct phases of near-surface water tables in a coastal plateau bog in eastern Newfoundland, with corresponding time periods beginning 8270, 7500, 6800, 5700, 5200, 4900, 4400, 4000, 3100, 2500, 2050, 1700, 600, and 200 calibrated years BP. The final drainage of glacial Lake Agassiz accounts for the first major phase of pool development at 8400 calibrated years BP, followed by the Ungava lakes ca 7500-6900 calibrated years BP. From 7500 BP to the present the reconstructed bog surface water and the stacked ice rafted debris of the North Atlantic Ocean correlate well. At the same time, long-term changes in air masses may have been a contributing factor. Records of "cosmogenic isotope flux," when compared to the bog surface wetness reconstruction, suggest that reduced solar radiation presents a consistent link with increased bog surface wetness during the Holocene.

But the models are not always so accurate. Payne et al. (2006) were only able to estimate within 9.7 cm of water table depth, and that was after exclusion of selected data. They attributed the less than ideal fit to inaccuracies in water-table measurements, very large environmental gradients, and recent climatic change in the study area. Their pH estimates were only off by 0.2, which is within the error range of many pH measuring techniques.

Using weighted averaging to model species abundance as measures of water table depth and soil moisture, Bobrov et al. (1999) calculated optima and tolerance of species niches. They found that each group of taxa tends to have a gradient of hydrological preference. For example, a wet to dry gradient is exhibited among species of the *Trigonopyxis arcula* group: *T. arcula* var. major > *T. arcula* > *T. minuta*. Likewise, the Assulina-alkanovia group exhibits wet to dry as *A. seminulum* > *A. muscorum* > *Hyalosphenia elegans* and the *Trinema lineare* group appears as *T. lineare* var. truncatum/*T. lineare* > *T. trincatum*.
**lineare** var. **terricola**. Interestingly, these species gradients also follow a large to small size gradient, indicating that small taxa survive better than large ones under dry conditions. It appears that having spines is a disadvantage in dry habitats. Within the genera **Euglypha** and **Placocista**, the spined forms (Figure 49) are typical of wetter habitats than are those with shorter spines or no spines. These relationships suggest that the most effective use of these rhizopods for reconstruction of the past water regime is to use the lowest possible level of identification, i.e. species and varieties.

One interesting question that arises is whether these spined taxa are really different species and varieties, i.e., genetically different, or if they represent ecotypes – morphological representations of the microenvironment where they occur. For example, Laminger (1975) found that **Centropyxis aculeata** from greater depths lacked spines and their tests were covered with mineral particles. To test the possibility of ecological morphs, Booth (2001) examined four of the most common taxa in two Lake Superior coastal wetlands: **Arcella** spp., **Assulina** spp., **Centropyxis cassis** type, and the **Nebela tincta-parvula-collaris** group. Using 74 microsites, Booth compared testate amoeba assemblages based on percent moisture, depth to water table, pH, porosity, depth of living moss, and associated bryophyte and tracheophyte species. He used such parameters as test length and aperture diameter for amoebae from at least ten microsites. In general, there was little correlation between morphological variation and microenvironmental parameters. However, in the **Nebela tincta-parvula-collaris** group, the test size correlated significantly with pH ($r^2 = 0.68$). Booth concluded that these testate rhizopods are sensitive indicators of water-level and pH changes.

In **Sphagnum** peatlands of the Rocky Mountains, USA, surface moisture determines the distribution of fossil rhizopods (Zygmunt et al. 2003). As suggested by the ecological studies of Lamentowicz and Mitchell (2005) and others (Booth & Zygmunt 2005), Booth and Jackson (2001) could track the history of an ombrotrophic peatland in northeastern Lower Michigan, USA, through 2800 years of changes using the moisture preferences of these organisms. Such fossils as these testae of rhizopods permit us to determine past changes in water table depth (Warner 1991; Woodland 1998; Woodland et al. 1998). Booth and Zygmunt (2005) further argued that the widespread geographic nature of the rhizopod relationships makes interpretation of their community structure widely applicable.

Charman and Warner (1997) used 60 samples from 14 peatlands in Newfoundland, Canada, and found 40 species that occurred in more than six samples. They used these to model the relationships between the species and the water table depth. Species with narrow tolerances provided the best indicators. These include **Amphitrema stenostoma**, **Arcella discoides**, **Cryptodifflugia sacculus**, **Diffugia bacillifera**, **Nebela carinata**, **Nebela griseola**, **Nebela marginata**, **Quadrulella symmetrica**, and **Sphenoderia lenta**. Charman and Warner recommend that for most accurate results modern constructs from wide regions should be used to interpret the data from peatland cores that represent palaeoecological time series.

Fortunately, most of the testate amoeba taxa are cosmopolitan, permitting the studies from the Northern Hemisphere to be used in less-studied areas such as New Zealand (Charman 1997; Wilmshurst 1998). In fact, Charman (1997) modelled the hydrologic relationships of protozoa and **Sphagnum** in peatlands of New Zealand and suggested that "palaeohydrology could be accurately inferred from fossil faunas."

Schoning et al. (2005) used peatland amoebae to reconstruct 125 years of peatland amoebae in Sweden. Unlike the cases in other areas in Europe, the changes in water table correlated primarily with changes in mean annual temperature, whereas in most other studies, precipitation was also an important factor. They caution that spatial differences must be considered in these historic interpretations and thus more study is needed on these influences.
In a Michigan, USA, study, Booth (2002) found that most of the eleven peatlands he studied had similar testate assemblages. As in most other studies, depth to water table was the best predictor of the testozoan assemblages. Nevertheless, within a given peatland, community variability was correlated with environmental heterogeneity, adding support to the suggestion of Schoning et al. (2005) regarding spatial considerations. But the testate amoebae in bog/fen habitats also had distinct differences in species between May and late summer/early autumn. Testate amoebae in the swamp community, on the other hand, had no clear difference in community structure between dates. They attributed these differences to the more constant water table and moisture conditions in the swamp.

Warner et al. (2007) add further support to the importance of considering seasons, particularly for living rhizopods. In southern Ontario, Canada, the usual factors of soil water content and water table influenced the distribution of amoeboid species and these differ with seasons. But the big differences were in the open bog/fen community, whereas in the swamp community there was no clear seasonal difference between May and August or October.

The historical record will not take us back forever. In their study on bogs in Ontario and Minnesota, Warner and Charman (1994) found that cores spanning the entire Holocene era only exhibited rhizopods present in the last 6500 years. They indicated that the fauna changed from the early rich fens with sedges and brown mosses. At those early stages, the protozoan communities were dominated by Cyclopyxis and Centropyxis. By 5000 BP, the habitat had become Sphagnum-dominated and the predominant protozoan taxa had shifted to Amphitrema flavum, Assulina muscorum, Heleolopa sphagni, and Hyalosphenia subflava. As the habitat became drier, taxa again shifted to Nebela griseola, N. militaris, and Trigonopyxis arcula.

Geographic Differences

Despite a considerable number of studies indicating usefulness of these organisms, use of testate amoebae to determine past habitats can at times be misleading. Harnish examined mires in Central Europe (1927 in Paulson 1952-53) and in Lapland, North Sweden (1938 in Paulson 1952-53), and found that the communities were not similar. Rather, associations from Central Europe did not exist in raised bogs in Lapland. In fact, the Amphitrema association existed in Lapland, but in different habitats, not raised bogs, whereas in Central Europe it was confined to raised bogs. The Hyalosphenia type was also absent in the Lapland raised bogs.

Problems in Using Rhizopods

There are caveats in using fossilized amoeba tests to assess past communities of testate rhizopods. Not all tests are equally preserved (Mitchell et al. 2007). The Euglypha, which includes the common Euglypha species (Figure 51), are an idiosome group that secretes its own test and its biosilica plates (Beyens & Meisterfeld 2001). This biological test decays more readily than the testae of the other groups (Mitchell et al. 2007). In Sphagnum peatlands, this differential decay seems to make little difference in the estimations of water table depth. However, in minerotrophic peatlands, with large numbers of this Euglyphida group, the loss of these tests leads to an underestimation of the water table depth. Data on more alkaline fens are lacking, and the community structure there is not well known. If this idiosome group is not dominant there, reconstruction may be more accurate.

Swindles and Roe (2007) likewise found that under conditions of low pH, such as found in peatlands, the degree of dissolution was highly variable, but it did not seem to relate to xenosomic (using "foreign" materials) vs. idiosomic tests. Euglypha (Figure 51) is particularly susceptible, whereas Assulina muscorum (Figure 50), Amphitrema flavum (Figure 34), and Trigonopyxis arcula (Figure 52) are affected little by acidity. Payne (2007) found similar results by subjecting rhizopod tests to weak acid, nutrient enrichment, and desiccation over 28-months, and used shorter-term experiments with stronger acids in peatlands. He determined that during dry periods the record may be altered by differential preservations of the tests, as demonstrated by significant effects of long-term desiccation and short-term acid treatment at two different concentrations. This consequence could lead to over-estimating water table depths.

![Figure 51. SEM detail of biosilica plates of Euglypha penardi, a protozoan for which the test is especially susceptible to dissolution. Photo by Edward Mitchell, with permission.](image)

Human Influence on Development

In New Zealand, it appears development of Sphagnum bogs has been dependent on human activity such as clearing or modifying the vegetation, resulting in Sphagnum dominance (Wilmshurst 1998). In other places, clearing of a peatland means that without human intervention it is gone forever. After such loss, it is often desirable to reconstruct the peatland. Testate amoebae have been used to define the past nature of the peatland for reconstruction purposes (Charman 1997; Charman & Gilbert 1997).

In a Polish peatland, a rapid shift in peat accumulation and lower pH occurred ~110-150 years ago, with a shift to
a *Sphagnum*-dominated poor fen (Lamentowicz *et al*. 2007). The protozoa supported this history. Researchers interpreted this to be a result of forest clearance in surrounding areas. Whereas peatlands are often destroyed by human activity, in some cases those activities make conditions more favorable to peatland development. In this case, *Sphagnum* peatland replaced a species-rich poor fen.

Figure 52. *Trigonopyxis arcula* test showing opening for pseudopod. This test is more stable than that of *Euglypha*. Photo by Yuji Tsukii, with permission.

Laggoun-Défarge *et al*. (2008) found testate amoebae can be used to reflect disturbances that result from peat harvesting. Where better carbohydrate preservation was present, along with more heterogeneous peat composition, the testate amoebae exhibited a higher diversity, thus serving as a biological indicator of conditions.

**Use in Peatland Regeneration**

Regeneration of peatlands can use remains of testate amoebae to determine the species to re-introduce or to follow the progress in a less labor-intensive fashion by monitoring the amoebae. In the Jura Mountains, Switzerland, Laggoun-Défarge *et al*. (2008) examined a peatland that had been mined for heating fuel until World War II and found that amoeba communities changed as peatlands changed during regeneration. The *Sphagnum* habitat shifted from moderately acidic, wet conditions to more acidic, drier conditions. During these changes, biomass and mean size of amoebae declined while remaining higher at the undamaged site. At the same time, species richness and diversity increased while density declined. As reported by Mitchell *et al*. (2004), changes in the amoeba community lagged behind that of the returning *Sphagnum* community. Moreover, during the forty years of 1961-2001, overall amoeba richness (33) remained unchanged, but richness per sample decreased from 11.9 to 9.6 (Kishaba & Mitchell 2005). Relative abundance changed, with three species increasing significantly [*Bullinularia indica* (Figure 53) (+810%), *Cyclopyxis euryystoma* (+100%, 0 in 1961), *Nebela tincta* (Figure 54) (+97%)] and two species declining [*Assulina muscorum* (Figure 50) (-63%), *Euglypha compressa* (Figure 55) (-93%)]. The researchers concluded the expected changes in richness were complete before the 1961-2001 study began.

Jauhianinen (2002) demonstrated in an ombrotrophic bog that the testacean shells were present throughout the vertical profile, whereas in the minerotrophic fen they were numerous only at the surface. As in other studies, moisture conditions were important, but peat composition and minerals also played important roles. Following restoration, species that indicated dry conditions disappeared, whereas the moisture gradient seemed to result in less defined community differences. In fact, the minerals seemed to have a greater effect.

Figure 53. *Bullinularia indica*. Photo by Edward Mitchell, with permission.

Figure 54. *Nebela tincta* test with living amoeba. Photo by Edward Mitchell, with permission.

Figure 55. Opening of test of *Euglypha compressa*. Photo by Edward Mitchell, with permission.
Lamentowicz et al. (2008) demonstrated that the testate amoebae record in a Baltic coast peatland in Northern Poland correlated well with the stable isotope data in the same core. The large number of testate protozoans known from peatlands, their relatively cosmopolitan distribution, and the understanding we have of the water table requirements for many of these species provide us with a useful tool for understanding the past history of many peatlands.

**Summary**

Peatlands support an abundant bryophyte fauna, with Amphilcrema, Assulina, Corythion, Diffugia, Euglypha, Heleopera, Hyalosphenia, and Nebela typically being the most common genera. *Sphagnum* sports more species than those found among other mosses or tracheophytes. These taxa are widespread and thus are very reliable indicators of moisture conditions in the peatlands and are less affected by water chemistry than are the tracheophyces.

Diversity is lowest in the driest peatland habitats, but the number of individuals is highest. Abundance increases with depth if oxygen is not limiting. Dry habitat species are more tolerant of changes in water depth than are wet habitat species. Rich fen amoeba species differ from those of acid bogs, but Euglyphidae are prominent in all these habitats. *Paragadula irregularis* and *Centropyxis discoides* are restricted to fens, with *Arcella discoides* indicative of rich fens. Detritus forms a major portion of the protozoan diet in the peatlands.

Vertical zonation presents the symbiotic taxa in the light zone at the top of the moss, with those requiring more moisture occurring at the greatest depths. Shell size, pH, moisture, light, nutrients, and available food all contribute to the distribution. Horizontal variation results from differences in bryophyte species and microtopography, resulting in differences in distance from water table and in pH. Seasonal differences reflect some of these same changes in moisture and food availability and are effective in separating niches of closely related species.

CO₂ enrichment may cause a reduction in testate amoebae while at the same time increasing bacterial biomass. Loss of the ozone filter and consequent increase in UV-B radiation may actually favor some testate amoebae in *Sphagnum* peatlands.

Amoebae form more constant associations in peatlands than do the plants. And testate species, with few exceptions, are well preserved even after death. Therefore, they can serve as appropriate markers of past climates as well as indicators of predisturbance conditions, although tests of some species, especially Euglyphidae, decompose more easily than others and can skew the results. The best indicators are those with narrow tolerance ranges, especially for moisture.

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CHAPTER 2-6
PROTOZOA ECOLOGY

Figure 1. The ciliate protozoan *Blepharisma americana* inhabits the lobules of the liverwort *Pleurozia purpurea*. Photo by Sebastian Hess, with permission.

**General Ecology**

Protozoa can probably be found on almost any bryophyte if one just looks carefully (Figure 1). Larger protozoa tend to occur in bog habitats (Chardez 1967; Bovee 1979). As drier habitats are examined, the species are smaller and smaller. *Difflugia* (Figure 2) species are typical of aquatic mosses; *Cyclopyxis* species occur on terrestrial mosses. *Centropyxis* species distribution depends on the habitat, with *C. aculeata* (Figure 3, Figure 4) in wet locations and *C. platystoma* in dry ones. *Corythion dubium* (Figure 5), *Assulina muscorum* (Figure 6), and *Trinema lineare* (Figure 7) occur generally on forest mosses (Chardez 1957; Bovee 1979; Beyens *et al.* 1986), although *A. muscorum* also is known from the cells of living *Sphagnum recurvum* (Figure 8) (BioImages 1998). *Corythion pulchellum* (Figure 9) and *Trinema complanatum* (Figure 10) occur only on forest mosses (Chardez 1960; Bovee 1979). *Nebela collaris* (Figure 11), *Centropyxis aculeata*, and *Hyalosphenia papilio* (Figure 12) occur on *Sphagnum* and other bog mosses, but not on forest mosses (Chardez 1960; Chiba & Kato 1969; Bovee 1979).

Figure 2. *Difflugia bacillifera* with diatoms in the test. Note the small desmid beside it. Photo by Yuuji Tsukii, with permission.
Figure 3. *Centropyxis aculeata*, a testate amoeba that commonly occurs on bryophyte leaves. Photo courtesy of Javier Martinez Abaigar, with permission.

Figure 4. *Centropyxis aculeata* test. Photo by William Bourland, with permission.

Figure 5. *Corythion dubium* test. Photo by Yuuji Tsukii, with permission.

Figure 6. *Assulina muscorum*. Photo by Yuuji Tsukii, with permission.

Figure 7. Test of *Trinema lineare*. Photo by Edward Mitchell, with permission.

Figure 8. *Sphagnum recurvum var. tenue*, a peatmoss that supports living protozoa in its hyaline cells. Photo by Jan-Peter Frahm, with permission.

Figure 9. *Corythion pulchellum*. Photo by Yuuji Tsukii, with permission.
Protozoa are generally the most numerous invertebrates among the *Sphagnum* plants (Figure 8;antham & Porter 1945). In a Canadian study, flagellates were the most numerous, but testate amoebae are often the most numerous.

**Epiphytes**

Despite the dryness of aerial habitats, protozoa are common among epiphytic bryophytes, drying and encysting as the bryophytes dry, then reviving, eating, and reproducing when the bryophytes are moist. This habitat may hold many species as yet undiscovered because it is a habitat less frequently studied by protozoologists. Nevertheless, a number of taxa are known from this unique habitat (Golemansky 1967; Casale 1967; Bonnet 1973a, b).

**Antarctic**

The role of protozoa is particularly important in the Antarctic. On Elephant Island of the South Shetland Islands in the Antarctic, moss carpets and turf form a major part of the habitat available to protozoa (Smith 1972). Mastigophoran (flagellate) moss inhabitants include 15 species. The Mastigophora are not unique to this habitat. Those that were in most of the moss samples also were in samples of grass/soil, clay, or guano. Furthermore, none of the species that was abundant in the other habitats was absent among bryophytes except *Tetramitus rostratus*, which was abundant only on guano. The Rhizopoda, including the testate amoebae, seemingly avoided the guana on Elephant Island, whereas 16 species occurred in the bryophyte habitats (Smith 1972). Several of those Rhizopoda present in the grass/soil habitat were not found among the moss samples. Fourteen species of Ciliata occurred among mosses.

The small number of Elephant Island moss samples (4 in *Polytrichum–Chorisodontium* turf & 5 in *Brachythecium–Calliergon–Drepanocladus* carpet) precludes comparison of moss preferences (Smith 1972). The most abundant ciliate, *Urotricha agilis* (see Figure 14), was abundant in both turf and carpet. In samples of turf, mean numbers per gram of fresh weight ranged 170-4,500. In carpet they ranged 250 to 7,700. On Signey Island species numbers were higher in moss turf (40), whereas on Elephant Island they were higher in moss carpet (37) than in turf.
Nutrient Cycling

Protozoa are common predators on bacteria and fungi (Hausmann et al. 2003), having the role of nutrient cyclers (Mitchell et al. 2008). In the Pradeaux peatland in France, the testate Nebela tincta (Figure 13) consumed mostly micro-algae, especially diatoms, associated with mosses (Gilbert et al. 2003). In summer they also consumed large ciliates, rotifers, and other small testate species. Micro-organisms collect between leaves and along stems of Sphagnum. When the system is wet, prey organisms are mostly immobile and often dead, but when conditions are drier and the water film is thin, testate fauna are able to ingest more mobile organisms than usual because these prey are slowed down by lack of sufficient free water for rapid swimming. Although we know little about their role among bryophytes, it is likely that at least in peatlands the role of moss-dwelling protozoans in nutrient cycling is significant (Gilbert et al. 1998a, b; Mitchell et al. 2008).

Habitat Effects

Moss Effects on Soil Habitat

The presence of mosses also affects the micro-organisms found in the underlying soil. Miroshnichenko and coworkers (1975) found that the greatest numbers of micro-organisms were under mosses (compared to other soil substrata) in a community in Russia, and Smith and Headland (1983) found similar results for testate rhizopods on the sub-Antarctic island of South Georgia. Smith (1974a, 1986) found protozoa living among the bryophytes in the South Orkney Islands and Adelaide Island of the Antarctic. Ingole and Parulekar (1990) found that the faunal density, including protozoa, was high in moss-associated sediments. These micro-organisms may account for the ability of some macrofauna to remain within the moss mat throughout a major part of their development by serving as a food source (Smith 1974a, 1986).

Epizoites

Some of the fauna, such as Pyxidium tardigradum (Figure 17), an epizoite, are hitch-hikers. This protozoan is recorded as a symphoriont (organism carried by and often dispersed by its host) on two species of tardigrades (Figure 15) [Hypsibius oberhaeuseri (Figure 16) and Milnesium tardigradum] that live among mosses (Land 1964; Morgan 1976). It can be so common on them (up to 35, but more typically 1-3) as to have negative effects on the tardigrade host that must expend extra energy to carry them around (Vicente et al. 2008). For this reason, Vicente et al. (2008) suggest that it should perhaps be considered a parasite.

Soil Crusts

Protozoan communities associated with cryptogamic soil crusts (Figure 18) have hardly been studied. In a study of only five crusts in southeastern Utah, Bamforth (2008) found 28 species of amoebae, 45 ciliates, and 19 testate amoebae. The number of amoebae ranged 680–2500, ciliates 20–460, and testate amoebae 2400–2500 per gram dry mass of crust. As crusts succeeded from Microcoleus (Cyanobacteria) to lichens to bryophytes, numbers of
protozoa increased, perhaps reflecting longer periods of internal moisture in the crusts. Predominant taxa are somewhat different from cosmopolitan ones we have seen elsewhere, comprised mostly of *Acanthamoeba* (Figure 19), *Hartmanella* (Figure 20), *Vahlkampfidae* (Figure 21), two species of *Colpoda* (Figure 22), several other colpodids, *Polyhymenophora* sp., and species of *Cryptodifflugia* (Figure 23) and *Difflugiella*.

Figure 18. Soil crust with the moss *Syntrichia ruralis*. Photo by Michael Lüth, with permission.

Figure 19. *Acanthamoeba* showing ingested carmine particles. Photo by Akira Kihara, with permission.

Figure 20. *Hartmanella*. Photo by Yuuji Tsukii, with permission.

Figure 21. *Vahlkampfia*. Photo by Yuuji Tsukii, with permission.

Figure 22. *Colpoda aspera*. Photos by William Bourland, with permission.
Nitrogen distribution affects the vertical distribution of at least some testate amoebae in *Sphagnum* communities, but nitrogen availability does not seem important for most testate amoebae in the upper centimeters of *Sphagnum* mats in the Swiss Jura Mountains (Mitchell & Gilbert 2004). There were 22 testate taxa among these mosses, although mean diversity of a typical sample was only 6.6. The species richness increased with depth. The moss-dwelling *Assulina muscorum* (Figure 25) was most abundant in the top 0-1 cm; *Phryganella acropodia*, *Heleopera rosea* (see Figure 26), and *Nebela militaris* (Figure 27) were the most abundant taxa at 3-5 cm depth. In this case, species richness increased with depth in the mat. *Only Bullinularia indica* (Figure 28) appeared to be more abundant in plots fertilized with nitrogen.

**Vertical Zonation**

Bryophyte suitability as a protozoan habitat differs in both time and space. Bryophytes offer a vertical series of habitats (Figure 24) that differ in temperature, moisture, and light, and presumably food quality and quantity. Horizontally, the substrate or height above the water table can differ, causing species differences. Hence, the micro-organisms distribute themselves in different communities both seasonally and spatially, particularly in the *Sphagnum* peatlands (Schönborn 1963; Heal 1964; Meisterfeld 1977; Mazei and Tsyganov 2007).

**Spaces:** Several studies indicate that the sizes of spaces within the bryophyte habitat influence the sizes of organisms and influence the available food (Dalenius 1962; Corbet 1973; Bovee 1979; Robson *et al.* 2001). Capillary spaces among branches and leaves hold water. Gilbert *et al.* (2003) suggested that as the *Sphagnum* becomes drier, ciliate protozoa are easier to catch for food because the thin film of water slows them down. As the moss becomes too dry, rather than migrating to lower, moister areas, many of these taxa, like several invertebrate groups, can encyst, permitting them to survive desiccation (Heal 1962; Gerson 1982). And when the moss resumes activity under the stimulation of rain (or fog), the rhizopods do likewise.

**Nitrogen:** Nitrogen from guana seemingly deterred all the testate amoebae on Elephant Island (Smith 1972).
**Temperature:** The Antarctic fauna is dominated by moss-dwelling micro-organisms, including protozoa, rotifers, nematodes, and tardigrades (Schwarz *et al.* 1993). Here, temperature may play a role as important as that of moisture. This need for adequate heat results in a vertical zonation of the fauna. For example, at the Canada Glacier, in southern Victoria Land, the majority of moss-dwelling organisms were in the top 5 mm in the post-melt samples, rather than in the pre-melt samples. However, while temperatures differed, so did the available moisture, making it difficult to determine controlling factors.

**Light:** As one might expect, light determines the absence of protozoa with chlorophyllous symbionts in the lower strata (Chacharonis 1956). Only those surface species contain chlorophyll, either as symbiotic algae or that of their own possession. However, some with chlorophyllous symbionts may occur as deep as 6-10 cm in *Sphagnum* mats (Richardson 1981). Of the 27 species lacking symbionts in a *Sphagnum* mat, all but two exhibited maximum abundance below 6 cm. But even within the first 5 cm, vertical zonation exists. Mitchell and Gilbert (2004) demonstrated a significant difference in number of species between the first 3 cm and the 3-5 cm depth in *Polytrichum strictum* (Figure 29) of a Swiss peatland (Figure 30).

**Community Differences:** As for a number of other moss habitats, the *Sphagnum* peat mat provides vertical differences in microhabitat that are further expressed as vertical community differences (Meisterfeld 1977; Strüder-Kypke 1999; Mitchell *et al.* 2000). Strüder-Kypke found that even in the upper 30 cm of the mat, two very different protistan communities are dictated by the strong vertical zonation. Both light and nutrients differ, causing the upper region to support a denser colonization, mostly of autotrophic cryptomonads and vagile ciliates (able to move about or disperse in a given environment). On the other hand, deeper samples exhibited heterotrophic flagellates and sessile peritrich ciliates.

Presence of testate amoebae at greater depths within the moss mat does not always indicate a retreat to a location of greater moisture. Schönborn (1977) demonstrated that 15% of the shells can be transported to lower depths by 550 mm rainfall, but 400 mm generally does not seem to cause a noticeable downward loss.

**Zoophagy by Liverworts?**

Carnivorous plants are well known among the flowering plants, but the ability of bryophytes to attract and trap organisms has been questionable. Who would guess that these seemingly primitive organisms can attract their own prey? But one interpretation is that the leafy liverwort genera *Colura* (Figure 31, Figure 32) and *Pleurozia* (Figure 33) have lobules (water sacs) that do just that (Hess *et al.* 2005). And this is not an isolated example. In the Aberdare Mountains, Kenya, Chuah-Petiot and Pócs (2003) found many protozoa inhabiting the lobules of the epiphytic *Colura kilimanjarica* (Figure 31, Figure 32).
Figure 31. **Upper:** The leafy liverwort, *Colura*. **Lower:** This lobule of *Colura* houses the ciliate protozoan *Blepharisma americana*. Photos by Jan-Peter Frahm, with permission.

Figure 32. **Upper:** SEM of lobule of *Colura*. **Lower:** Living lobule. These lobules of *Colura* are inhabited by the reddish ciliate protozoan *Blepharisma americana*. Photos by Jan-Peter Frahm, with permission.

Figure 33. **Upper:** SEM of lobule of *Colura*. **Lower:** Living lobule. These lobules of *Colura* are inhabited by the reddish ciliate protozoan *Blepharisma americana*. Photos by Jan-Peter Frahm, with permission.

Lobules are usually considered to be water storage organs. However, in these genera, they might also serve as traps. Goebel (1888, 1893, 1915) did not consider it likely that these were real traps. He argued that insectivorous plants have attractants in order to lure their prey into their traps. Although the lobule resembles the trap of the bladderwort, *Utricularia*, Goebel argued that that does not mean it is used the same way. He furthermore argued that the benefit gained by the excrement from animals (and dead animals?) would be less than that gained from the water. Since having the animals does not preclude also providing a water reservoir, it would seem that zoophagy would simply be an added benefit. Schiffner (1906) even reported chironomid larvae in the lobules, suggesting an even larger source of fecal matter. But the openings in *Pleurozia* are small, only about 300 µm, and closed by a round "lid" of hyaline cells (Hess et al. 2005). What causes these organisms to enter in the first place?

Figure 34. *Pleurozia purpurea*, a leafy liverwort with lobules that can house a variety of invertebrates, including the ciliate *Blepharisma americana*. Photo by Sebastian Hess, with permission.
Figure 35. **Upper:** Lobule of *Pleurozia purpurea* showing lid. Photo by Sebastian Hess, with permission. **Lower:** Lobule redrawn from Hess *et al.* (2005). This lobule of *Pleurozia purpurea* serves as home and apparently ultimately as a trap for a wide range of protozoa and invertebrates.

Barthlott *et al.* (2000), using feeding experiments with the ciliate protozoan *Blepharisma americana* (Figure 1, Figure 36-Figure 38), demonstrated that *Colura* does indeed catch protozoa with its lobules. Hess and coworkers (2005) set out to determine if *Pleurozia purpurea* (Figure 33-Figure 35) is likewise carnivorous.

Figure 36. The ciliate *Blepharisma americana* that inhabits "zoophagous" liverworts. Photo by Yuuji Tsukii, with permission.

Again using *Blepharisma americana*, a cohabitant of *Sphagnum* mats with *Pleurozia purpurea*, Hess *et al.* (2005) performed dozens of experiments in Petri dishes to see if the dispersion of the protozoan remained random. Indeed, the protozoa gradually accumulated around the *Pleurozia*! Within only 30 minutes, 86% of the lobules contained the protozoa. After several hours, up to 16 protozoans were trapped, and further observation failed to reveal any that escaped.

The mode of attraction is only speculation. Barthlott *et al.* (2000) found that older parts of *Colura* were more effective at attracting *Blepharisma americana* (Figure 37, Figure 38) than were younger parts, suggesting that concentrations of bacteria may have been a factor. In fact, in experiments on *Colura*, Barthlott *et al.* (2000) found that *B. americana* moves over the bryophyte surface "like a vacuum cleaner," devouring the bacteria.

Figure 37. A stained *Blepharisma americana*. Photo by Yuuji Tsukii, with permission.

The shade provided by the plants could also contribute to the higher concentrations of protozoa near the branches of *Pleurozia purpurea* (Hess *et al.* 2005), but if so, the liverwort would probably be less effective as a refuge in the field where other mosses were also present.

Hess and coworkers (2005) claim that the large number of organisms in the lobules in such a short time is too great to be attributed to chance. However, they fail to provide any statistical evidence or probability to support this claim, for example, alternative liverworts or mosses. They furthermore state that the organisms die there, but they provide no data on the deaths of the organisms. They do point out that there is no direct evidence that any nutrients provided by the organisms are used by the liverworts, but there is likewise no evidence to the contrary. In any case, the liverworts could benefit from the cleaning of bacteria that block light and compete for nutrients.

Figure 38. SEM photo of *Blepharisma* demonstrating small cell on top and large, cannibalistic cell below. Under starvation conditions, larger individuals become cannibalistic. Photo by Pauline Gould, with permission.
Zoophagy is the process of eating animals (phag = eat, devour; Hanson 1962; Lincoln et al. 1998). There is a fine distinction in what constitutes just eating compared to true carnivory, wherein living organisms are killed (or not) and digested. In this case, it seems that the animals may be trapped, but there is no real proof that they are consumed by the plant. Does admitting the animals into the trap (lobule) then make the liverworts zoophagous? Hess et al. (2005) argue that animals die in the traps and subsequently release their cell contents, bursting in the case of Blepharisma americana. These dead animals are then decomposed by bacteria. Surely some of the nutrients released are absorbed by the liverworts. Is this not a process parallel to that of the pitcher plant Sarracenia purpurea? Many so-called carnivorous plants, like S. purpurea, seem to lack enzymes to digest all or some of the parts of their prey and depend on resident bacteria to accomplish the task. With this broad definition of carnivory, could we not call the liverworts carnivorous? I think I want more data on whether this is a chance event or true trapping before I make that claim. Such experiments would need controls of leafy liverworts with no "traps" to see if the protozoa simply accumulate wherever there is shelter. On the other hand, I wonder how many leafy liverworts with locules provide preferred housing for protozoa.

Dispersal

For any organism to succeed, it must have a means of dispersal. Protozoans can't go very far on their own. They are too small to crawl far on pseudopods or paddle their way with a flagellum or cilia, the common means of transportation for the majority of protozoan moss dwellers. But they can travel reasonable distances as passengers on the mosses, riding on fragments that establish a new home where they land.

Sudzuki (1972) conducted experiments using electric fans to determine the success of wind as a dispersal agent, using mosses as one of the sources of invertebrate fauna. He found that the smaller organisms – micro-organisms, including protozoa, were easily dispersed by light breezes as well as wind. Larger organisms such as gastrotrichs, flatworms, rotifers, nematodes, oligochaetes, tardigrades, crustaceans, and arachnomorphs, on the other hand, rarely were dispersed at wind velocities of less than 2 m per second [tornadoes are generally 27-130 m per second (Allaby 1997)]. In the field, colonization progressed from flagellates to ciliates to rhizopods, suggesting that passive dispersal was not the only factor controlling their colonization rates.

Once an organism becomes airborne, turbulent air may take them 3,000 to even 17,000 m on thermal drafts, with winds carrying them much higher and farther (Maguire 1963). Puschkarew (1913) found that protozoan cysts average about 2.5 per cubic meter, making these organisms readily available for dispersal and colonization on suitable bryophytes.

Smith (1974b) likewise considered that the mosses themselves served as dispersal agents for the protozoa. In particular, moss invasions of volcanic tephra on Deception Island in the Antarctic greatly increased the protozoan fauna. Not only do the mosses provide a great increase in suitable niches, but since they were most likely colonized by protozoa in their former locations, fragments arriving on the island could easily carry communities of fauna as passengers.

Rain can carry many algae and protozoa (Maguire 1963). Rain-borne organisms seem to originate predominantly from splash, typically from plants and soil, and do not travel far vertically, so that mechanism is most likely only suitable for local habitat travel.

In streams, the water movement itself serves as an effective dispersal agent, and aerial dispersal from waterfalls and rapids can carry algae and other Aufwuchs to new locations.

Raccoons are very effective in carrying whole communities of organisms, particularly protozoa, and can accomplish distances of at least 60 meters (Maguire 1963). Both terrestrial and aquatic birds contribute to dispersal, and other mammals contribute, but their relative role is not known.

Several scientists have discussed the dispersal of micro-organisms by insects (Maguire 1963; Parsons et al. 1966). Such mechanisms could easily contribute to the colonization of bryophytes by their micro-inhabitants. The many aquatic insect inhabitants will be discussed in an upcoming chapter. Consider the activity of insects among bryophytes, especially in streams, and their subsequent relocation due to swimming or stream drift. The Aufwuchs could easily be carried from one location to another by these mobile inhabitants (Figure 39). Emerging insects may also swipe micro-organisms trapped by the surface tension and carry them to resting locations, including bryophytes, on land.

Figure 39. Dragonfly Aeshna grandis female ovipositing and exposing herself to possible transport of protozoa. Photo by David Kitching, with permission.

Although few studies seem to have directly addressed the dispersal of micro-organisms by insects to bryophytes, we can infer at least some possibilities from more general studies on dispersal by insects. Maguire (1963) examined the distance both horizontally and vertically to which organisms were dispersed from a pond in Texas and another in Colorado. Dragonflies (Figure 39) and wasps, in particular, carried several species of protozoa and one species of rotifer. Parsons et al. (1966) found amoeboid and other protozoan cysts on adult Odonata, suggesting the possibility of a relatively long dispersal range. Odonata in
a short-term experiment dispersed up to 860 m to the farthest pond in the experiment (Conrad et al. 1999). Michiels and Dhondt (1991) estimated that 80% of adult dragonfly *Sympetrum danae* had migrated 1.75 km or more to their study site. But more importantly, evidence suggests they can migrate 3500 km or more across the Indian Ocean (Anderson 2009). This and other long-distance migrations provide a potential yearly means of dispersal for the micro-organisms.

**Cosmopolitan**

'Everything is everywhere, but, the environment selects' (in Wit & Bouvier 2006; O'Malley 2008). This statement, often called the Baas Becking Principle, has been applied to microscopic organisms that are globally distributed by high dispersal, and that lack biogeographic patterns (Fontaneto et al. 2008). But Wit and Bouvier made it clear that the original hypothesis "did not disregard the biogeography of free-living microorganisms." Finlay et al. (1996) extend the concept to suggest global species diversity is inversely related to body size. Therefore, the huge number of protist individuals makes global dispersal inevitable through normal events such as ocean circulations, groundwater connections, dam, fur, dust storms, etc. (Weinbauer & Rassoulzadegan 2003). This argument is supported by the fact that the estimated number of free-living ciliates is about 3000, whereas there are about 10,000 species of birds and 120,000 species of Lepidoptera (butterflies and moths) (Lawton 1998).

The concept of global distribution describes well the major protozoa associated with bryophytes. This concept does not preclude, however, the presence of cryptic species that differ in less recognizable traits (Richards et al. 2005; Fontaneto & Hortal 2008; Fontaneto et al. 2008; Kooistra et al. 2008), and in recent detailed studies distinct genetic species have been found in disparate parts of the world (Telford et al. 2006; Fontaneto et al. 2008; Kooistra et al. 2008).

One consideration to support "everything is everywhere" is the small number of species of protozoa relative to 750,000 species of insects and 280,000 species of other animals (Papke & Ward 2004). Morphological data support the concept that dispersal is worldwide, suggesting there would be fewer than 5000 morphological protozoan species. Could this also be the explanation for the small number of bryophytes relative to other plants? In both cases, molecular evidence is starting to suggest that there may be cryptic species with genetic differences that are not expressed morphologically (Logares 2006), revealing distributions that are much more restricted.

Bryophyte protozoan communities are remarkably similar no matter where the bryophytes occur and consist primarily of cosmopolitan species. Davidova (2008) compared the testacean communities of epiphytic bryophytes to those of soil bryophytes in Strandzha Natural Park, South-Eastern Bulgaria, and found them to be quite similar in their taxonomic richness, species diversity, and community structure. The most common taxa in both habitats were *Centropyxis aerophila* var. *sphagnicola*, *C. aerophila* (Figure 40), *Phryganella hemisphaerica*, *Euglypha rotunda* (Figure 41), *Corythion dubium* (Figure 5), *Trinema enchelys* (Figure 42), and *T. lineare* (Figure 7). Among these, only *Phryganella hemisphaerica* is missing from the sites in Switzerland, Alaska, Sweden, Finland, Netherlands, Britain, Bulgaria, and North America as summarized in Table 1 of Chapter 2-2. The epiphytic community had 34 taxa in 13 genera, whereas the soil mosses had 31 taxa in 13 genera.

![Centropyxis aerophila test.](image1)

Figure 40. *Centropyxis aerophila* test. Photo by Yuuji Tsukii, with permission.

![Euglypha rotunda.](image2)

Figure 41. *Euglypha rotunda*. Photo by Yuuji Tsukii, with permission.

![Trinema enchelys.](image3)

Figure 42. *Trinema enchelys*. Photo by Yuuji Tsukii, with permission.

The moss-dweller *Nebela* (*Apodera*) *vas* (Figure 43) has been touted to refute the Baas Becking Principle (Mitchell & Meisterfeld 2005; Smith & Wilkinson 2007). In 89 collections, representing 25 publications, mosses represented 59% of its habitat, with *Sphagnum* being the most common (Smith & Wilkinson 2007). Its distribution
is throughout the equatorial region at high altitudes, southern cool-temperate, and sub-Antarctic zones, but it is conspicuously absent in the Holarctic northern hemisphere. Its absence from hundreds of samples from seemingly suitable habitats in the northern hemisphere support the contention that its absence is not a fluke of sampling (Mitchell & Meisterfeld 2005) This distribution is definitely not cosmopolitan, despite its wide pH range (3.8-6.5) (Smith & Wilkinson 2007). Although it has a rather defined climatic range (temperate to sub-Antarctic), its absence in this climate throughout most of the more frequently studied northern hemisphere cannot support the concept of "everything is everywhere." Evidence such as this has been used to argue that micro-organisms are dispersed following the same principles as macro-organisms (BioMed Central 2007). Genetic differences that are not detectable from morphology suggest that global diversity of micro-organisms may be greater than has been suspected (BioMed Central 2007; Fontaneto et al. 2008). Such evidence suggests that care is needed in assigning names to microbial/protozoan collections.

**Communities as Biological Monitors**

Ciliates living among bryophytes in Czechoslovakia are sensitive to air pollution, giving us another way to assess the effects of air pollutants (Tirjakova & Matis 2003). Testate amoebae, including *Assulina* (Figure 25), *Assulina* (Figure 25), *Corythion* (Figure 5, Figure 9), *Euglypha* (Figure 41), and *Heleopera* (Figure 26), as well as *Euglena* (Figure 44) and Cyanobacteria, in a *Sphagnum* bog of Tierra del Fuego, South America, were sensitive to UV-B radiation (Robson et al. 2001). But surprisingly the testate amoebae and rotifers were significantly more abundant and had greater species diversity under current levels of UV-B radiation than those that received reduced UV-B. The fungal component likewise had significantly greater abundance and species diversity under the current dosage than under the reduced dosage.

![Figure 44. Euglena mutabilis](image)

Because pollution affects the entire community, moss-dwelling protozoans can often be a more efficient means of assessing pollution damage than other biological components. In a study in France, Nguyen-Viet et al. (2007a, b) assessed the response of the protozoan community under simulated lead pollution. Using Pb²⁺ concentrations ranging from 0 to 2500 µg L⁻¹, they found that biomass decreased significantly for bacteria, microalgae, testate amoebae, and ciliates at 625 and 2500 µL⁻¹ Pb²⁺ after six weeks. The microbial biomass decreased as the densities of testate and ciliate protozoa decreased, but the relative biomass of bacteria to that of the protozoa remained constant. The correlation between the two groups increased as the lead concentration increased. Hence, the protozoa provided an effective and relatively inexpensive means of assessing the community response.

Enhanced CO₂ had the opposite effect on the community relationships (Mitchell et al. 2003). Biomass of the testate amoebae decreased by 13% while the heterotrophic bacteria increased by 48% when the CO₂ was increased to 560 ppm, compared to those at an ambient CO₂ concentration of 360 ppm. Mitchell et al. (2003) suggest that the increase in bacterial biomass may be a response to increased exudation from *Sphagnum* under the higher CO₂ regimen.

As discussed in an earlier sub-chapter, the testate amoebae can serve as indicators of drainage in *Sphagnum* mires, as noted by Warner and Climbie (1992) in northern Ontario, Canada. As the water level falls, some species increase while others decrease.
**Collecting and Sorting**

There are lots of references for collecting, preserving, and enumerating aquatic and soil taxa of protozoa, but few on methods for bryophyte fauna. However, many methods for soil will apply equally well to the bryophyte fauna. A thorough coverage of methods is in Adl et al. (2008), with methods for peatland microfauna in Gilbert and Mitchell (2006). A special method for holographic viewing of live testate amoebae is presented by Charrière et al. (2006).

**Collecting**

Collecting protozoa that live among mosses is simple and requires no special equipment. In thick cushions or mats of bryophytes, extraction can be achieved with a stainless steel corer. In some circumstances, a knife can be used to cut a core and the core then placed into a cylindrical plastic container (Lamentowicz & Mitchell 2005). Stream bryophytes should be collected in a way that avoids as much loss downstream as possible. This can be achieved by shielding the bryophyte from most of the flow and especially shielding it as it breaks through the surface. One's hands are often sufficient to achieve this, but a container might be used over the bryophyte, enclosing as much of its depth as possible while dislodging it from the substrate. For non-quantitative collections in almost any habitat, a hand-grab is usually sufficient. For diversity studies, it is important to get the moss down to its substrate because zoonation often occurs.

**Storage & Preservation**

Bryophytes and adhering water/moisture can be kept in jars or polyethylene bags until they are returned to the lab. If the weather is warm, it is desirable to place the containers in a cooler with ice. Oxygen is a problem, so open containers or vials with loose lids will help. For aquatic collections, some free water might be needed, making it necessary to confine the water by such means as a wad of paper towel or cloth above the water level to avoid splashes out of the jar. Parafilm may suffice for short time periods, or two, separated layers of screen or mesh.

The most rewarding experience is to observe the protozoa live as they swim about in the water film, gyrate from a stalk, or engulf a food item. Some species will remain alive only a few hours after collection (Samworth 1995). If the organisms are to be kept for a few days, place them in a refrigerator (not freezer) or incubator that is set in the range of 5-15°C (Glime pers. obs.). The container should be covered to reduce evaporation, but not sealed. Jars with lids should have the lid on loosely to permit air exchange. If the jar is opened and a foul odor escapes, there has not been enough air exchange, and many of the organisms will be dead – and perhaps subsequently eaten by the more hardy ones.

**Preservation**

If the sample is to be kept for long in the field before returning to the lab, and the weather is hot, it might be necessary to preserve the organisms. This is fine for testate amoebae, but may make counting and identification of other protozoans difficult or impossible.

Preservation of bryophyte protozoan samples is like that of other protozoa, using 2% glutaraldehyde (final solution) (Mitchell et al. 2003), formaldehyde (Fisher et al. 1998; Gilbert et al. 1998a, b), or glycerol (Hendon & Charman 1997b), but the water content of the bryophyte must be considered in calculating the dilution. For example, saturated Sphagnum typically has 95% water content (Gilbert & Mitchell 2006).

**Long-term Storage of Cysts**

One choice for long-term storage is to let the mosses and their fauna dry slowly in air for several days. This can be done in open paper bags, a method typically used for drying bryophytes, or in open jars. Cool drying is preferable for many species, but survivorship will vary depending on the climate of origin and should be tested against fresh samples if the samples will be used for quantitative or diversity work.

Once the samples are dry and the protozoa have encysted, they can be sealed in containers and stored at 4°C. Again, the effects of storage should be tested for any quantitative or diversity work. Tropical taxa may require a warmer storage temperature (Acosta-Mercado & Lynn 2003). This method will only work for species that readily encyst and for testate rhizopods.

**Extraction**

Organisms can be extracted from the bryophyte-water matrix with a teat pipette (i.e. volume is unimportant) and placed as a drop on a glass microscope slide. Bryophyte inhabitants can be squeezed into a sample bottle with little danger to them, but this may have disastrous results for larger fauna that may be of interest. Protozoa can be concentrated in a centrifuge or by running the water through a fine nylon mesh (Samworth 1995), but smaller organisms will be lost and adhering organisms will remain behind on the bryophyte.

Gilbert et al. (2003) reduced the negative effects of squeezing by pressing a sieve (1.5 mm mesh) on the moss surface and sucking the water up with a syringe. They were unable to solve the problem of adhering organisms, including some microbial groups. Others are missed because they live inside Sphagnum cells. This method creates minimal destruction of the Sphagnum mat, even through repeated sampling, except for the trampling by the people doing the sampling.

In their book on Sphagnum ponds, Kreutz and Foissner (2006) suggest a slide on slide method (Figure 45). Mosses can be washed in a small amount of suitable water, preferably rainwater or other water that won't kill the fauna. In most cases, lots of detrital matter will come off the mosses, along with many members of the fauna. Dense material will collect on the bottom of the container and can be drawn into a pipette/dropper (ca 2 mL). Material can be transferred onto a glass slide to cover most of the slide. A second slide is then used at an angle to push the flocculent detrital matter to the end of the slide. When the edge of the top slide reaches near the end of the bottom slide, the top slide is lowered onto the bottom one and used as a coverslip. A smaller version of this method (i.e. a smaller sample of water and detritus) can be done in the same way with a drop of the water and detritus in the middle. This case, a coverslip of the desired size can be used in the same manner as the top slide described above. Note that both
methods will be biased toward mobile organisms. Tardigrades, rotifers, sessile protozoans, and other attached organisms will be poorly represented, if at all, by this method (and most others!). To see these, branches of moss need to be examined under the microscope.

Figure 45. Slide on slide method of concentrating and extracting micro-organisms. Drawing by Janice Glime based on images in Kreutz and Foissner 2006.

**Testate Amoebae**

The non-flooded Petri dish method (below) can be used to culture testate amoebae as well, but a longer time may be needed to wake up the cysts (Adl et al. 2008).

One method to extract testate organisms is to dry the bryophytes at 65°C, then sieve and back-sieve them with a sieve that retains all particles in the range of 10-300 µm. The standard method seems to be that of Hendon & Charman (1997b). A standard length of moss is cut and boiled for 10 minutes to loosen the amoebae. The boiled samples are filtered first at 300 µm, then back filtered through 20 µm. The organisms retained by the 20 µm filter are stored in 5 ml vials with glycerol.

Another method for extracting testate species is to put single shoots of bryophyte samples in a vial and shake them with a vortex mixer (Nguyen-Viet et al. 2004). This solution with moss is then sieved through a 300 µm sieve to remove large constituents. The filtrate can then be concentrated with a centrifuge at 3000 rpm for 4-6 minutes. The tests can be stored in glycerol.

**Non-testate Taxa**

The non-testate taxa are somewhat more difficult to work with because they are best seen while active. One alternative is to culture them, using the non-flooded Petri dish protocol described by Adl et al. (2008):

1. Place bryophyte sample in a 5- or 10-cm Petri dish. Several Petri plates can be set up initially and drained on different days to avoid depleting nutrients with the wash.
2. To culture, moisten sample with distilled water or wheat grass medium.
   a. To make wheat grass medium, combine 1 g wheat grass powder and 1 L distilled or deionized water in a 2-L Erlenmeyer flask.
   b. Boil at a gentle rolling boil for 2 minutes, then let settle and cool for 1 hour.
   c. Filter into a new flask through several layers of cheesecloth to remove the grass residue.
   d. Adjust the pH to appropriate level (based on sample pH) with a phosphate buffer.
   e. Autoclave in screw top bottles for 20 minutes.
   f. Bacteria growth can be reduced by diluting to 1/10 or 1/100 strength.
3. Alternatively, a culture can be made from a dilute solution of detritus from the moss.
4. Incubate at 15°C in the dark or at ambient field temperature. Be sure plates do not desiccate.
5. Observe every few days for signs of activity, up to about 30 days. Some testate amoebae will take several weeks or even months to leave the encysted stage and become active.
6. To observe, moisten the culture plate with a squeeze bottle of distilled or deionized water.
7. Tilt the plate until there is enough to drain the water into a new plate.
8. Observe the drained water in the new plate with a dissecting microscope and oblique transmitted illumination; capture organisms with micro-dissecting tools or a micropipette, then observe with an inverted microscope with phase contrast if possible (see observation section below). Most will require 100-400X to be seen well.
9. Note that the often abundant cercomonads form thin filopodia that explore tiny pores (<1 µm diameter). These adhere to flat surfaces and are not easily seen or dislodged. They may require staining (see below).
10. The original plate can be returned to the incubator.

**Observation**

Live observations can be done with a small branch, a leaf, or just a drop of adhering water on a glass slide with a compound microscope. A few larger protozoa might be observed with a dissecting microscope. A cavity slide will avoid crushing as the slide dries. Further confinement can
be achieved with this type of slide by putting a drop of water on the cover slip, then inverting it over the cavity, making a hanging drop slide. Alternatively, putting Vaseline at the corners of a cover slip on a standard flat slide will keep the cover slip from crushing them. More water can be added at the edge of the cover slip and will be drawn under by capillary action.

Ciliates and flagellates can be slowed down by a viscous substance such as methyl cellulose. Observing them in the interstitial water of intact bryophytes also tends to slow them down. Note that these organisms are mostly transparent and viewing may be improved by using darkfield and/or closing down the diaphragm of the microscope. An inverted microscope has the advantage of giving you a better view of those protozoa that settle on the bottom, especially testate amoebae.

Start your observations with a low magnification and move up after you have found a quiet one you want to observe, preferably surrounded by a bryophyte leaf or other confinement.

For testate amoebae, observation of dead material is not a problem, albeit not so interesting. The test is well-preserved and can be observed and identified at the convenience of the observer.

**Staining**

Staining can make the organisms easier to see (Figure 46), and vital stains may help to provide behavioral information. For example, neutral red can be used to follow digestion (Howey 2000). Newly formed vacuoles will stain bright red. As digestion proceeds, the vacuole will become yellowish, indicating a change in pH toward alkaline. Powdered carmine can also be used to indicate the location of the vacuole. Subsequent observation with Nomarski differential interference contrast can provide clear visibility. The observer should experiment with brightfield, darkfield, India ink in the solution, oblique illumination, phase contrast, or whatever types of optical contrast may be available. Unfortunately, all stains appear eventually to be toxic, so the viewing time is limited (Howey 2000; Table 1). WARNING: Read the labels carefully; many stains are also highly toxic to humans!

### Table 1. Concentrations needed to stain *Paramecium* and toxicity after one hour. Table from Howey 2000.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Min Conc to Stain</th>
<th>Toxicity - % dead in hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>bismarck brown</td>
<td>1:150,000</td>
<td>0</td>
</tr>
<tr>
<td>methylene blue</td>
<td>1:100,000</td>
<td>5</td>
</tr>
<tr>
<td>methylene green</td>
<td>1:37,500</td>
<td>5</td>
</tr>
<tr>
<td>neutral red</td>
<td>1:150,000</td>
<td>3</td>
</tr>
<tr>
<td>toluidine blue</td>
<td>1:105,000</td>
<td>5</td>
</tr>
<tr>
<td>basic fuchsin</td>
<td>1:25,000</td>
<td>30</td>
</tr>
<tr>
<td>safranin</td>
<td>1:9,000</td>
<td>30</td>
</tr>
<tr>
<td>aniline yellow</td>
<td>1:5,500</td>
<td>0</td>
</tr>
<tr>
<td>methyl violet</td>
<td>1:500,000</td>
<td>20</td>
</tr>
<tr>
<td>Janus green B</td>
<td>1:180,000</td>
<td>40</td>
</tr>
<tr>
<td>Nile blue</td>
<td>1:30,000</td>
<td>0</td>
</tr>
<tr>
<td>Rhodamine</td>
<td>1:20,000</td>
<td>0</td>
</tr>
</tbody>
</table>

**Identification**

There are some specialty keys available, and lots of pictures on the internet. However, internet pictures and keys should be used with caution and the source of information evaluated because these are unrefereed and often contain errors. A good general reference for identification is the publication by Lee *et al.* (2002), “The Illustrated Guide to the Protozoa.” Its nomenclature is in places outdated, so usage should be checked in Adl *et al.* (2005). A more recent aid is a book by Kreutz and Foissner (2006). This book has wonderful color pictures, but there is no designation to tell which were on bryophytes and which were in open water.

**Quantification**

Adl *et al.* (2008) advised that taxa must be counted within one or two days of collection because temperature and moisture changes will shift the bacterial communities and this will, in turn, cause a change in community structure of the protozoa.

To quantify the sample size, the bryophyte can be weighed after drying. However, some amoebae will become glued to the bryophyte by the attending algae and detrital matter, thus contributing to the weight. Biovolumes can be estimated by using the geometrical shapes and an appropriate formula for that shape, then multiplying by the number obtained (Mitchell 2004).

Adl *et al.* (2008) provided a method to estimate protozoa per gram of dry soil. It could be modified for bryophyte purposes. For any quantification, the method must be consistent among those communities being used for comparison. One can use stem length, wet weight, or dry weight, but these have different biases for different bryophytes and those must be dealt with. Furthermore, different methods may favor the observations of some protozoan taxa. For example, larger organism are more easily seen, testate organisms are more likely to fall from the moss upon shaking, sessile organisms will most likely not fall at all.

Charman (1997) suggested a method for quantifying the testate amoebae and warned of its shortfalls. You may be familiar with methods of determining pollen density by including a known number of *Lycopodium* spores in the sample (for example, 200) and using the ratio of those...
observed on the slide to those put in the sample. Unfortunately, in the testate samples extracted from mosses, the number of tests estimated was reduced by up to 80% and the number of taxa was reduced by 60%, probably due to differences in weight, making this a less than desirable method. Using KOH to digest the organic matter did not destroy the tests, and permitted extraction of more tests, but they were damaged and more difficult to identify. Charman concluded that a water-based preparation with sieving was the best method.

Various combinations of filtration, vortex, and centrifuge can be used to get the best results for particular circumstances. Different mesh sizes can be used with back filtration to classify the organisms into size groups (Kishaba & Mitchell 2005). The organisms collected between 15 and 350 μm are a typical size group of Testacea examined (e.g. Warner & Charman 1994; Booth & Zygmunt 2005).

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**Summary**

Larger protozoa tend to occur in moist or bog habitats, whereas drier habitats have smaller ones. Some even occur within the hyaline cells of Sphagnum. Some protozoa are exclusive to Sphagnum; others occur only on forest mosses. Those on epiphytic bryophytes are able to dry with the mosses and encyst during periods of drought. Moisture also contributes to the vertical zonation of protozoa in peatlands. Soil crusts can have some of the highest numbers of species. Moisture is the major determining factor on species distribution and survivorship, with terrestrial species able to withstand drying more than wet habitat species can. Over 400,000 individuals can occur in one square meter of terrestrial mosses. Studies in the Antarctic suggest that temperature and moss growth form play roles in the number of species.

Drying slows the mobile organisms and permits larger protozoa to capture them. Their consumption of micro-organisms places the moss-dwelling protozoa in the role of nutrient cycling. The bryophytes further contribute to ecosystem processing by affecting the moisture and temperature, hence altering the protozoan fauna, in the underlying soil.

Some protozoa are hitch-hikers on other bryophyte inhabitants, such as those that ride around on tardigrades. Others have green algae as symbionts and are thus restricted to photic zones on the bryophytes, whereas those without these symbionts typically occur below 6 cm depth. Yet others (Pleurozia, Colura) seem to trap protozoan prey in leaf lobules. In fact, it appears that the leafy liverwort Pleurozia purpurea may actually attract Blepharisma americana.

Dispersal is likely to be as passengers on bryophyte fragments. A successional pattern from flagellates to ciliates to rhizopods suggests that other factors determine colonization rates. Some colonization comes from dormant cysts awaiting suitable conditions. Dispersal of cysts and living organisms can be facilitated by splashing raindrops. Some may even be facilitated by insects, birds, raccoons, and other mammals.

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The small size of protozoans and other micro-organisms led to the assumption of cosmopolitan distribution, a concept known as the Baas Becking Principle, or "everything is everywhere." However, recent studies on distribution and genetic differences have brought this principle into question.

Bryophyte-inhabiting protozoa are sufficiently sensitive to some types of air pollution that they can be used as monitors, but not all are sensitive to the same things, so community structure is likely to change.

Collecting is relatively simple, but quantification is tricky. Testate species can be separated by physical means, but other taxa often require culturing to awaken cysts. Some may be amenable to staining to further clarify identification.

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**Acknowledgments**

Paul Davison has been wonderful in helping me with the methods portion of this chapter, including the suggestion to include it. Edward Mitchell provided me with a large number of papers and photographs. Both of these researchers were invaluable in helping me with areas where I was often not personally familiar with the subject.

**Literature Cited**


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