

## **Protozoa of Microbiotic Crusts in the Grand Canyon**

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### **Abstract**

Thirty-five microbiotic crusts - Cyanobacteria, lichens, and bryophytes - were collected from desert soils of the Grand Canyon between Lees Ferry (mile 0) and Whitmore Wash (mile 187), in May 2000, and twenty were examined for protozoa. Fifty species of ciliates, 27 of amoebae, and 14 of testacea were found. In all three groups, r-selected species, responding rapidly to wetting and exploiting resources, predominated. The few nematodes present were principally stylet-bearing tylenchids, which feed on fungal hyphae and moss roots. The protozoan population is adapted to the short periods when crusts are moist.

### **Introduction**

Microbiotic crusts - Cyanobacteria, lichens, bryophytes, and combinations of these photosynthetic bionts - are now recognized to be important flora in arid ecosystems because these communities fix nitrogen into soils (to aid vascular plant growth) and hold soils in place. The National Park Service recognizes this latter function by restricting park visitors to trails. At the Visitors Center in Moab, UT, a display case shows crusts and explains their value, and the front panel of the brochure to Canyonlands National Park shows crusts prominently in the foreground of the photo and describes their importance.

A number of studies have elucidated the floristics, structure, photosynthesis and nitrogen fixing activities of crust bionts, and the effects of disturbance on crusts, but essentially nothing is known of the water film fauna, mostly protozoa (Evans & Johanson, 1999), which are involved in recycling of phosphorus and other nutrients within crusts, as in litter and soil communities.

The National Park Service permitting me to collect microbiotic crusts on my May 2000 raft trip through the Grand Canyon provided an opportunity to study protozoa in crust communities, and help meet this deficiency in crust biology. This report updates, corrects a few misidentifications of crust bionts, and expands my preliminary report (sent April 20, 2001).

#### **Materials and Methods**

The floor of the Grand Canyon is a northern extension of the Sonoran Desert of southern Arizona. Lateral, on either side of the bands of riparian and *Acacia/Mesquite* vegetation along the Colorado River and its tributaries, is the desert flora of various cacti, *Ephedra*, *Encelia*, *Agave*, and *Larrea*, growing on slopes and terraces, to about 1200 m above sea level above the Canyon floor, and entirely dependent on sporadic rainfall for its water supply. Grand Canyon soil pH ranges from 7.2 to 8.4 (Bamforth, 1984).

Crusts were collected between May 22 and 28, 2000, during day and overnight visits of the Tulane University Colloquium 412 float trip (with the Hatch Company) on the Colorado River from Lees Ferry (mile 0) to Whitmore Wash (mile 187). At all but two locations, crusts were collected in the Canyon, at least 30 meters distal from the *Acacia/Mesquite* border, and 10-30 meters above river level. At Deer Creek, crusts were collected from the terrace, 0.5 km upstream from the waterfall, and

20 meters from the creek. In Havasu Canyon, crusts were collected near Beaver Falls, along a path 30 meters from the stream. Thirty-five crusts were collected; twenty were analyzed for protozoa and their collection sites and photosynthetic biont descriptions are presented in Table 1.

Crusts were collected by slipping the bottom of a 60 mm plastic petri dish below the crust, raising the dish to enclose it, and then placing the top on and sealing with tape, for transport to the laboratory at Tulane University. Samples of non-Bryophyte crusts were sent to Dr. Jayne Belnap, at USGS, Moab, UT, to ascertain identification of the cyanobacteria *Microcoleus* and *Scytonema*.

Crusts were wetted and studied with a dissecting microscope to determine biont structure (Cyanobacteria, lichen, bryophyte). Initial protozoan extractions were obtained by placing portions of crusts in (a) small (3 cm diameter) dishes, (b) in 1.5 mm diameter polypropylene rings imbedded in non-nutrient agar in petri dishes, (c) on a substrate of sterile sand in petri dishes, and adding sterile de-ionized water to induce protozoan excystment (packed petri dish method, Foissner, 1987). Small aliquots were drawn and examined at 1, 2, 4, 7 10 and 15 days intervals to find protozoa. Additional amoebae were found by (a) placing two streaks of bacteria on non-nutrient agar plates and overlaying streaks of crust material, and (b) cutting 1 cm diameter wells (with a sterilized cork borer) in bacterized agar plates and placing bits of crusts in the wells, and examining the agar surfaces on following days. Soil flagellates are difficult to study by these methods. Serious study requires photography and "non-interpretive" methods for proper identification, and extensive literature study, due to extensive taxonomic revisions (Larsen & Patterson, 1990). Hence flagellates are only briefly considered in this report.

The frequency of taxa at the twenty sites were determined, and colpodid/polyhymenophoran (C/P) ratios (Luftenegger et al, 1986) were determined for ciliates as a measure of ciliate populations on the r/K continuum. r/K designations for ciliates are taken from Luftenegger et al (1986), amoebae from Bamforth (2000), and testacea from Wodarz et al (1992).

## Results

The photosynthetic bionts of the twenty examined crusts are listed in Table 1. Ninety-one species of ciliates, naked amoebae, and testacea were found (Tables 2-4).. Flagellates appeared in all crusts and included the usually reported genera: *Spumella* (*Monas*), *Oikomonas*, *Cercomonas*, and species of *Bodo*. The less frequently reported genera *Goniomonas* and *Cryptalax* were also identified.

Fifty species of ciliates, 46 identified to at least genus level, were found (Table 2), and can be grouped into four categories on the basis of frequency at sites: (1) everywhere (all sites;); (2) common, at 55-90% of the sites; (3) moderate, at 30-45% of sites, and (4) rare, often only at one site. *Copoda inflata* and *C. maupasi* were found everywhere, and eight other species were common, occurring at about two-thirds of the sites. All but one of the ten ciliates in these two groups were colpodids, the exception being *Urosoma acuminata*, a polyhymenophoran. Ciliates averaged 15 species per site (Table 5), of which usually about seven were colpodids and four were polyhymenophrans. The average C/P ratio was 2.36, reflecting the dominance of colpodid species. Most ciliates were bacterivores, exceptions being many omnivorous Polyhymenophora (Table 2).

Twenty seven species of naked amoebae were found (Table 3). Three taxa were widespread: *Acanthamoeba*, Vahlkampfiidae, and *Mayorella microerucia*. *Acanthamoeba* is listed by genus only because species can only be reliably identified by isoenzyme methods (Stodhard et al, 1998). Vahlkampfiid amoebae are listed under the family name because Brown and DeJonckheere (1999) have shown that species of the genus *Vahlkampfia* should be distributed among several of the family's genera, hence the application of the name Vahlkampfiidae to describe limax amoebae with eruptive pseudopodia in this report. Although most amoebae were found by the agar plate methods, several appeared frequently in the non-flooded dish cultures, and vahlkampfiid amoebae appeared early and frequently, often on days 4 or 7. With the exception of *Saccamoeba*, an algivore as well as a bacterivore, and *Thecamoeba*, an omnivore, the amoebae were all bacterivores.

Fourteen species of testacea, mostly small acrostomes were found (Table 4). The three largest species, *Arcella arenaria*, *Centropyxis aerophila*, and *Trinema enchelys*, were found only in bryophyte crusts, and most of the other species appeared more frequently in bryophyte crusts than in cyanobacterial and lichen. There were no such associations among ciliates and amoebae with types of crusts.

Protozoa furnished almost all of the water film fauna. A single monogont rotifer, *Trichodera* sp., was found in a lichen crust, and a single bdelloid rotifer in the cyanobacterial-bryophyte crust. A rhabditid (bacterial feeding) nematode was found in another lichen crust, but all the other few nematodes were species of Tylenchidae, with piercing stylets adapting them to sucking fluids from fungal hyphae and bryophyte roots (Yeates et al, 1993).

## Discussion

Protozoa cannot be extracted from soil with the same efficiency as metazoa (nematodes, annelids, and arthropods), hence may lead to some uncertainties to representativeness of species found. Thus, in interpreting biodiversity and ecological functioning, abundance and species richness may appear to be determined by stochastic processes of metapopulation dynamics, except in species-poor communities, where species richness within a trophic level is likely to reduce functional gaps, and differences in species richness are likely to be more pronounced on larger spatial scales (Eckshmidt and Griffiths, 1998). For example, Foissner (1996) investigated 59 moss and soil samples over a distance of 60,000 km (Signy Island to Victoria Land) and used species richness to describe the patchy species pauperization of soil ciliates dominated by r-selected colpodids in western Antarctica..

In this study, some crusts were spaced only a few meters apart, but other crusts were separated by distances of 18-65 km in an elongated area extending over 300 km (from Lees Ferry to Whitmore Wash) (Table 1).

Photosynthesis, nitrogen fixation, and respiration commence in crusts a few hours after wetting (Evans and Johanson, 1999). The rapid respiration response reflects in part the bacterial-protozoan activities in mineralization and nutrient recycling. But crusts usually desiccate in a few days, requiring rapid response dynamics from small flagellates and amoebae, particularly *Acanthamoeba* and Vahlkampfs, and r-selected colopodid ciliates. Individuals of the genus *Colpoda* can excyst after 4-6 hours of wetting (personal observation), and because the members reproduce in the cyst, they can spend all of their active time feeding. The diversity of colpodid species exploit a variety of biotic (bacterial and fungal) and physical niches: *Colpoda inflata* moves along particle surfaces like a vacuum cleaner,

while more flexible *Colpoda maupasi* can squeeze into small spaces, and thinner *Cryptolophosis* enters narrower pores and channels in search of bacteria.. *Platyophora vorax* eats flagellates and algae (Foissner, 1987), but I saw two individuals with ingested *Microcoleus* filaments! Disturbance affects community structure in a systematic way because tolerance range and functional contributions are correlated (Eck Schmidt and Griffiths, 1998). The similarity of crust ecologies in the Grand Canyon determines the basic species composition of their protozoan communities. Despite the immigration by the daily winds blowing upstream in the Canyon, only certain protozoan species are able to colonize and integrate into the microbiotic crusts.

These desert crusts are similar to lichens in temperate forests and grasslands (Wenzel, 1953; Gellert, 1956; Foissner, 1986) in the prominence of colpodid ciliates. The desert crust mosses differ from their counterparts in temperate forests (Wenzel, 1953; Gellert, 1956) in possessing fewer polyhymenopharan species and intermediate taxa, especially predatory species of *Dileptus* and *Spathidium*. The predominance of r-selected species is also apparent among the small, bacterivorous testacea, which appeared more frequently among the mosses, but which differed from temperate moss populations in the absence of larger species of *Euglypha*, and large-size genera such as *Centropyxis*, *Heleopera*, *Nebela*, and *Hyalosphenia*.

The Tylenchid nematodes, that comprised almost all the metazoa, are described as algal-lichen (both algal or fungal component)-moss feeders (Yeates et al, 1993), and their occurrence parallels parasitic nematode- plant roots associations.

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Table 1. Collecting sites and the twenty microbiotic crusts examined in this report.

| River Location |       | Site Description      | Elev.<br>m | Photosynthetic Biont                                                                                                                                                                        |
|----------------|-------|-----------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mile           | km    |                       |            |                                                                                                                                                                                             |
| 8              | 13    | Badger Creek          | 937        | Bryophyte                                                                                                                                                                                   |
| 47             | 78    | Saddle Canyon         | 856        | Bryophyte<br>Bryophyte                                                                                                                                                                      |
| 61.5           | 102.5 | Little Colorado River | 826        | Lichen ( <i>Microcoleus</i> , <i>Scytonema</i> )                                                                                                                                            |
| 72             | 120   | Unkar                 | 795        | Lichen (green algae)<br>Lichen (green algae)                                                                                                                                                |
| 104            | 173   | Beach ("Lunch" stop)  | 680        | Bryophyte                                                                                                                                                                                   |
| 118            | 196   | Camp site             | 640        | Bryophyte<br>Bryophyte<br><i>Microcoleus/Scytonema/Bryophyte</i><br><i>Microcoleus&gt;Scytonema/Bryophyte</i><br><i>Scytonema&gt;Microcoleus</i>                                            |
| 136            | 226   | Deer Creek            | 649        | Lichen (green algae)/Bryophyte                                                                                                                                                              |
| 156            | 260   | Havasui Canyon        | 565        | Lichen/ <i>Microcoleus/Scytonema/Bryophyte</i><br>Lichen/ <i>Scytonema</i><br>Lichen/ <i>Microcoleus</i>                                                                                    |
| 187            | 311   | Whitmore Wash         | 505        | Lichen (green algae)/ <i>Scytonema</i><br>Lichen (green algae)/ <i>Microcoleus/Scytonema</i><br>Lichen(green algae)/ <i>Microcoleus/Scytonema</i><br>Lichen (green algae)/ <i>Scytonema</i> |

Note: Canyon data extracted from "Powell Centennial Grand Canyon River Guide" (Westwater Books, 1969).

**Table 2.** Ciliates in microbiotic crusts: common = 55-90%, moderate 30-45%, rare =  $\leq$  20%.  
C = Colpodid; P = Polyhymenophora; all other taxa unlabeled

| Species                           | Group | Frequency  |
|-----------------------------------|-------|------------|
| <i>Amphisiella tericola</i>       | P     | rare       |
| <i>Balantidioides muscicola</i>   | P     | moderate   |
| <i>Bardiella pulchra</i>          | C     | common     |
| <i>Blepharisma hyalinum</i>       | P     | rare       |
| <i>Bresslaia vorax</i>            | C     | rare       |
| <i>Bryometopus pseudochilodon</i> | C     | rare       |
| <i>Colpoda aspera</i>             | C     | common     |
| <i>Colpoda cucullus</i>           | C     | moderate   |
| <i>Colpoda inflata</i>            | C     | everywhere |
| <i>Colpoda maupasi</i>            | C     | everywhere |
| <i>Colpoda steini</i>             | C     | common     |
| <i>Cyclidium muscicola</i>        | C     | moderate   |
| <i>Cyrtolophosis elongata</i>     | C     | common     |
| <i>Cyrtolophosis mucicola</i>     | C     | common     |
| <i>Drepanomonas sphagni</i>       |       | rare       |
| <i>Enchelidium</i> sp.            |       | moderate   |
| <i>Enchelyodon</i> sp.            |       | rare       |
| <i>Euplotes muscicola</i>         | P     | rare       |
| <i>Gastrostyla steini</i>         | P     | rare       |
| <i>Gonostomum affine</i>          | P     | moderate   |
| <i>Hemisincirra gellerti</i>      | P     | rare       |
| <i>Histriculus muscorum</i>       | P     | rare       |
| <i>Holosticha sigmoidea</i>       | P     | moderate   |
| <i>Holosticha</i> sp.             | P     | rare       |

**Table 2** (continued)

| <u>Species</u>                        | <u>Group</u> | <u>Frequency</u> |
|---------------------------------------|--------------|------------------|
| <i>Lagynophrya geleii</i>             |              | moderate         |
| <i>Leptopharynx costatus</i>          |              | rare             |
| <i>Litonotus</i> sp.                  |              | rare             |
| <i>Microdiaphanosoma arcuata</i>      | C            | rare             |
| <i>Nassula picta</i>                  |              | rare             |
| <i>Niviella plana</i>                 | C            | rare             |
| <i>Oxytricha setigera</i>             | P            | rare             |
| <i>Plagiocampa rouxi</i>              |              | rare             |
| <i>Paruroleptus muscorum</i>          | P            | rare             |
| <i>Platyophrya vorax</i>              | C            | common           |
| <i>Protospathidium</i> sp.            |              | rare             |
| <i>Pseudochilodontopsis mutabilis</i> |              | moderate         |
| <i>Pseudocyrtolophosis terricola</i>  | C            | moderate         |
| <i>Pseudoplatyophrya nana</i>         | C            | common           |
| <i>Spathidium</i> sp.                 |              | rare             |
| <i>Sphaerophrya</i> sp.               |              | rare             |
| <i>Steinia</i> sp.                    | P            | rare             |
| <i>Strongylidium wilberti</i>         | P            | rare             |
| <i>Urosoma acuminata</i>              | P            | common           |
| <i>Urosoma macrostyla</i>             | P            | rare             |
| <i>Urosomoida agilis</i>              | P            | moderate         |
| <i>Vorticella astyliformis</i>        |              | rare             |
| 4 unidentified ciliates               |              | rare             |

Table 3, Amoebae in microbiotic crusts. Frequency designations same as in Table 2.

| Species                            | Frequency |
|------------------------------------|-----------|
| <i>Acanthamoeba</i> spp.           | common    |
| <i>Amoeba diminiutiva</i>          | rare      |
| <i>Amoeba</i> sp.                  | rare      |
| <i>Cashia limacoides</i>           | moderate  |
| <i>Dermamoeba minima</i>           | rare      |
| <i>Echinamoeba exundans</i>        | moderate  |
| <i>Filamoeba nolandi</i>           | rare      |
| <i>Gephramoeba delicatula</i>      | rare      |
| <i>Gocevia</i> sp.                 | rare      |
| <i>Hartmanella cantabrigiensis</i> | moderate  |
| <i>Hartmanella vermiformis</i>     | rare      |
| HELIOZOAN                          | RARE      |
| <i>Korotnevella</i> sp. *          | moderate  |
| <i>Leptomyxa</i> sp.               | rare      |
| <i>Mayorella microeruca</i>        | common    |
| <i>Mayorella riparia</i>           | rare      |
| <i>Mayorella</i> sp.               | rare      |
| <i>Nuclearia</i> sp.               | rare      |
| <i>Penardia</i> sp.                | rare      |
| <i>Platyamoeba placida</i>         | moderate  |
| <i>Platyamoeba stenopodia</i>      | moderate  |
| <i>Rosculus ithacus</i>            | moderate  |

|                            |                               |          |
|----------------------------|-------------------------------|----------|
| <b>Table 3 (continued)</b> | <i>Saccamoeba stagnicola</i>  | moderate |
|                            | <i>Stachyamoeba lipophora</i> | rare     |
|                            | <i>Thecamoeba striata</i>     | rare     |
|                            | VAHLKAMPFIDAE                 | common   |
|                            | <i>Vanella simplex</i>        | rare     |

\* *Korotneveella* is the preferred name for *Dactylamoeba* (O'Kelly et al, 2001).

**Table 4.** Testacea in microbiotic crusts. All occurred singly in crusts.

|                                  |                                           |
|----------------------------------|-------------------------------------------|
| <i>Arcella arenaria</i>          | <i>Microcorycia flava</i>                 |
| <i>Centropyxis aerophila</i>     | <i>Phryganella acropodia</i>              |
| <i>Cucurbitella minutissima</i>  | <i>Pseudodifflugia fascicularis</i>       |
| <i>Difflugia lucida</i>          | <i>Pseudodifflugia gracilis</i>           |
| <i>Difflugia oviformis</i>       | <i>Pseudodifflugia gracilis terricola</i> |
| <i>Difflugia oviformis fusca</i> | <i>Pseudodifflugia senartensis</i>        |
| <i>Euglypha rotunda</i>          | <i>Trinema enchelys</i>                   |

**Table 5.** Species summaries for ciliates, amoebae, and testacea.

| Group            | Total | Range   | Average |
|------------------|-------|---------|---------|
| Ciliates         | 50    | 9 - 28  | 15      |
| Amoebae          | 27    | 3 - 11  | 6       |
| Testacea         | 14    | 0 - 4   | 1       |
| Total - 3 groups | 91    | 15 - 33 | 23      |