

**Progress Report: BPS 29697 ACEC Cryptogamic Crust Study - Kari Yanskey, AZ
110 - Science project**

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Objective:

The goal of this research is to survey cryptogamic species (i.e., macrolichens) associated with biological soil crusts (BSCs) at two localities on the Colorado Plateau and to examine the affect that different land management plans in place at these sites might have on the biological diversity encountered there. In addition to traditional survey practices, the study also employs modern molecular techniques, such as polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE), to further quantify this diversity. Specimens and BSC samples collected as part of the survey will also contribute to understanding of cryptogamic diversity within the Grand Canyon-Parashant National Monument (GCPNM) as well as on the Colorado Plateau.

Project status (2007):

Field Work - Three days were spent on the Arizona strip in the spring of 2007 collecting soil samples at the Ft. Pearce ACEC and Gyp Hills (GCPNM) sites for a culture survey of fungi associated with BSCs found on these sites. Additional collections of macrolichens where made at these sites, as well as other localities within GCPNM, in order to further document lichen diversity.

Curation and Identification - Two sets of specimens of nearly every species collected have been curated according to standard lichen herbarium techniques. These specimens have been identified by the investigators and have been used to train a volunteer ASU undergraduate student worker in the science of lichen identification. These specimens are being processed and databased for accession in the ASU lichen herbarium or to be sent to PARA. In addition to previous new records (*see project status 2006 below*), these specimens continue to contribute lichen records that are novel for the state of Arizona and GCPNM (*Placopyrenium insuetum* and *Aspicilia hispida*).

Molecular Study - Community DNA has been extracted from triplicate transects sets (a total of 60 samples) for both the Ft. Pearce ACEC and Gyp Hills (GCPNM) sites following protocol specifically developed for this study. These samples are currently stored at -20° C in the Garcia-Pichel Microbial Lab (ASU). Representative transect community DNA (to date, one each for both sites) have been amplified by PCR using fungal specific primers (NL1f-GC and LS2r). The PCR product has been used in a culture independent DGGE analysis of the fungal community associated with BSCs in these sites (*see results below - figures and tables 1-2*). Sequencing of phylotypic bands cut from the DGGEs is currently underway. This sequence data will assist in surveying the diversity present in these sites from a taxonomic and phylogenetic point of reference. In the process of conducting a portion of this lab work, a volunteer undergraduate student

from Universidad Metropolitana (San Juan, Puerto Rico) was trained in molecular microbial ecology methods.

Statistical analysis of data – We are in the process of conducting a correlation analysis of the transect disturbance data collected at both the Ft. Pearce ACEC and Gyp Hills (GCPNM) site transects. A preliminary survey of the data show that the Ft. Pearce ACEC site has a higher occurrence of anthropogenic disturbance (7% of the transect points) and water related erosion (27% of the transect points) than the Gyp Hills (GCPNM) site (4% and 6% of the transect points, respectively); however, we are not able to state at this time whether these results are significantly correlated. It is probable that other factors (e.g., topography) could contribute to the higher occurrence of water related erosion at the Ft. Pearce ACEC site.

An analysis of representative transect DGGE data for each site has been conducted using the Student's T Test for comparison between sites and a control transect DGGE (*table 3*, Colorado Plateau dark crust/sandy soil site, 'Sunday Churt', near Moab, UT). These analyses show that richness and Shannon index of diversity values from the Ft. Pearce ACEC site are significantly lower than the values obtained from the Gyp Hills (GCPNM) site ($P=0.001$ and 0.014 , respectively). Richness and Shannon index of diversity values from the control site were not significantly different from the Ft. Pearce ACEC site ($P=0.07$ and 0.669 , respectively), but were significantly different from the Gyp Hills (GCPNM) site ($P=0.019$ and 0.001 , respectively). These results suggest that factors other than disturbance (e.g., geography) might also explain the lower diversity encountered at the Ft. Pearce ACEC site.

Publications – Results from this study have already contributed to publications. The *Aspicilia hispida* specimens (*fig. 3*) collected at GCPNM will be published as a new record for Arizona and included in a taxonomic treatment of the genus *Aspicilia* in the forthcoming book, *The Lichen Flora of the Greater Sonoran Desert Region: Vol. III* (T.H. Nash III et al.). This record and that of *Placopyrenium insuetum* will be included in a publication that is in preparation (*Additions to the lichens of Grand Canyon-Parashant National Monument*). It is hoped that this publication will be submitted for publication by the spring of 2008. Result from the study of fungi (lichenized and non-lichenized and including the molecular study) will be included in the PhD. dissertation of one of the investigators (STB). Publications resulting from that work will acknowledge this study, Grand Canyon-Parashant National Monument (GCPNM), Kari Yanskey, and the Bureau of Land Management.

Project status (2006):

Field Work - Four days were spent on the Arizona strip in the summer 2006 collecting data at the Ft. Pearce ACEC and Gyp Hills (PARA) sites. Eighty-four BSC samples were collected along eight 500 meter transects (four at each site). Data was also gathered assessing site disturbance and BSC cover. In addition to these data, numerous collections of macrolichens were made at these sites, as well as several other localities within Parashant NM, in order to document this diversity.

Curation and Identification - Two sets of specimens of each species collected are currently being curated according to standard lichen herbarium techniques. One set will be sent to PARA and the other will be housed at the ASU lichen herbarium. These specimens have contributed lichen records that are novel for Parashant (*Diploschistes diacapsis*) as well as new to the state of Arizona (*Gypsoplaca macrophylla*).

Molecular Study - Protocols related to the molecular techniques to be used in the study have been refined and preliminary DGGE data has been obtained using Colorado Plateau BSC samples. Statistical analyses of the field data is currently underway in order to address questions pertaining to the relationship between cryptogamic diversity at the sites associated with BSCs and current land management practices.

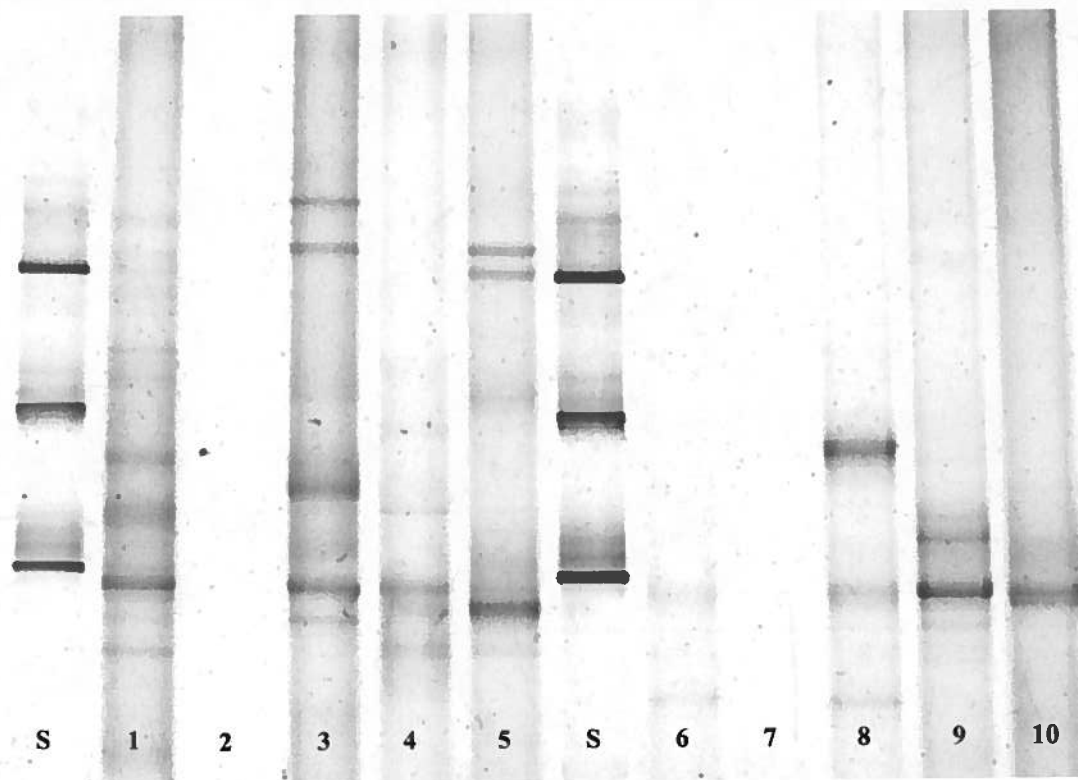


Figure 1. Comparison of Colorado Plateau fungal communities within BSC samples taken along a 500 m transect at the 'Ft. Pearce ACEC' site (Mohave County, Arizona - DN011) using denaturing gradient gel electrophoresis (DGGE). Lane numbers are denoted at the bottom of the image (S = standard).

Table 1. Denaturing Gradient Gel Electrophoresis (DN011, see fig. 1) fungal community diversity analysis of BSC samples from a gypsic soil site ('Ft. Pearce') transect on the Colorado Plateau. ('S-W Index' refers to the Shannon index of diversity; richness values of '0' represent transect samples where fungal community DNA was not obtained in the fungal specific PCR reaction).

<i>Lane #</i>	1	2	3	4	5	6	7	8	9	10
Richness	7	0	5	3	4	2	0	3	3	2
S-W Index	1.923	NA	1.591	1.092	1.342	0.665	NA	1.074	1.045	0.689

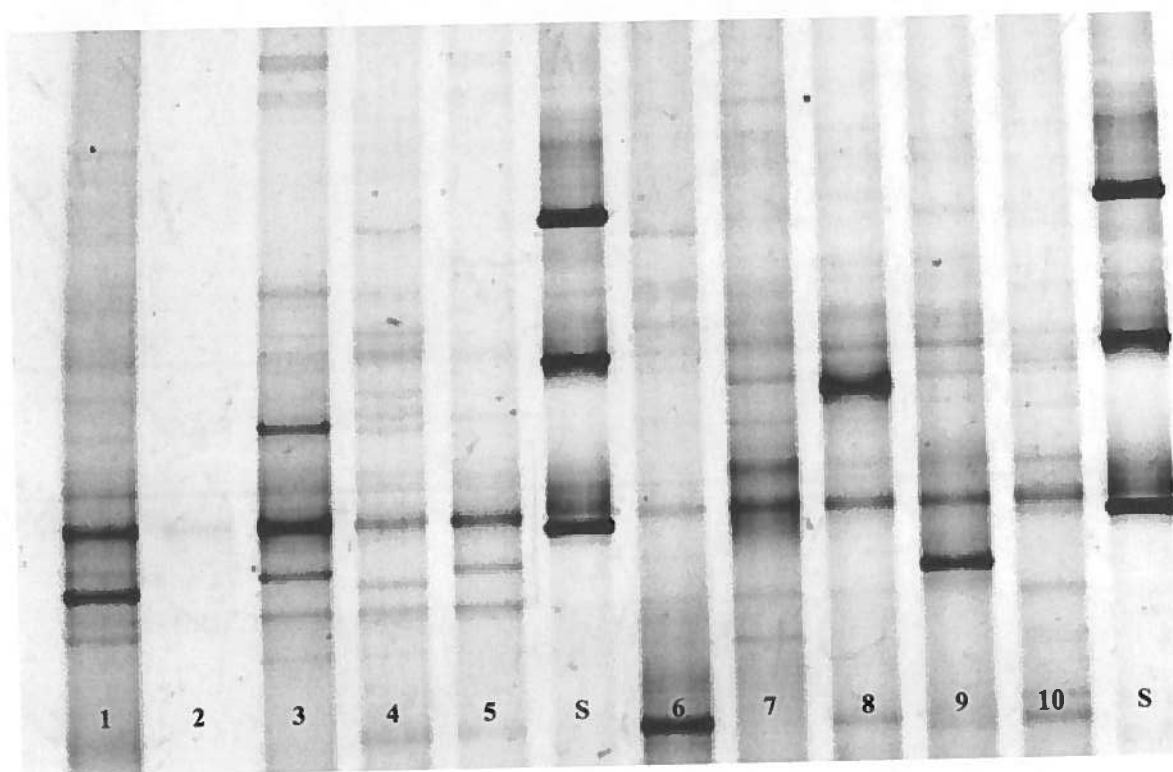


Figure 2. Comparison of Colorado Plateau fungal communities within BSC samples taken along a 500 m transect at the 'Parashant, Gyp Hills' site (Mohave County, Arizona - DN016) using denaturing gradient gel electrophoresis (DGGE). Lane numbers are denoted at the bottom of the image (S = standard).

Table 2. Denaturing Gradient Gel Electrophoresis (DN016, see fig. 2) fungal community diversity analysis of BSC samples from a gypsic soil site ('Parashant, Gyp Hills') transect on the Colorado Plateau. ('S-W Index' refers to the Shannon index of diversity; richness values of '0' represent transect samples where fungal community DNA was not obtained in the fungal specific PCR reaction).

<i>Lane #</i>	1	2	3	4	5	6	7	8	9	10
Richness	12	0	11	11	6	6	9	7	9	7
S-W Index	2.406	NA	2.339	1.195	1.705	1.697	1.315	1.835	1.390	1.876

Table 3. Denaturing Gradient Gel Electrophoresis (DN005) fungal community analysis of BSC samples from a dark crust site ('Sunday Churt') transect on the Colorado Plateau. ('S-W Index' refers to the Shannon index of diversity; richness values of '0' represent transect samples where fungal community DNA was not obtained in the fungal specific PCR reaction).

<i>Lane #</i>	1	2	3	4	5	6	7	8	9	10
Richness	8	4	4	5	5	3	2	5	7	3
S-W Index	1.576	1.083	1.204	1.329	1.503	0.818	0.551	1.095	0.937	0.917



Figure 3. *Aspicilia hispida* Mereschk. (STB001547). This species, collected near poverty mountain (Parashant-Grand Canyon NM, 36° 26' 15.50 N, 113° 32' 40.9" W), is a new record for the state of Arizona. *A. hispida* exhibits a unique growth form for lichenized fungi and is commonly called a 'vagrant lichen'. Lichens typically have thalli that are attached to a substrate (such as bark or rock); however, the thallus of a vagrant lichen is not attached to a substrate, thus it is free to be blown about the soil surface.