

**Proposal to the National Park Service
as a CESU Agreement**

**Investigations of the Biological Soil Crusts and Associated Fungi
of Grand Canyon-Parashant National Monument**

**A Cooperative Proposal between
Thomas H. Nash III
School of Life Sciences
Arizona State University
(480) 965-7735**

and the

**U.S. National Park Service
Kari Yanskey, NPS
Grand Canyon-Parashant N.M.
(435) 688-3298**

Introduction

Throughout much of the Intermountain West, a cryptogamic or microbiotic crust covers some or all of the interspace between vascular plants (Nash, 1996a). The crust is composed of water-stable surface soil aggregates bound by algae, fungi, lichens, and moss (Johansen, 1993; St. Clair and Johansen, 1993). Lichens and moss are the largest and arguably, the most significant of the soil cryptogams.

Soil cryptogams play several roles in semi-arid regions (West 1990; Metting 1991; Belnap and Lange 2001). Soil cryptogams can stabilize the soil, reducing wind and water erosion (Metting 1991; Johansen 1993; Eldridge and Greene 1994). They contribute nitrogen (Harper and Pendleton 1993; Jeffries et al. 1992; Evans and Ehleringer 1993) and organic carbon (Johansen et al. 1993) to the soil. Some researchers have found an increase in water infiltration into the soil with cryptogamic soil crusts (Brotherson and Rushforth, 1983). Cryptogamic crusts play a significant role in ecosystem structure in the shrub-steppe. Recently, variation in seed banks was related to variation in cryptogamic crusts on the Monument (Boudell et al. 2002). Boudell et al. (2002) found significantly more seeds in the cracks surrounding soil surfaces covered by lichens and moss than beneath the lichens and moss. These intact cryptogamic crusts can enhance seedling establishment of some species in semi-arid ecosystems (Belnap 1994). In contrast, weedy, 'r' selected species are inhibited by intact cryptogamic crusts in arid ecosystems (Zaady et al. 1997). At the Monument, Boudell et al. (2002) observed significant numbers of *Bromus tectorum* (an "r" selected species) seeds in the seedbank of intact soil crusts, but few established plants. Hawke et al. (1998) found a significant and negative correlation between cryptogamic crust cover and the cover of *Bromus tectorum*.

Target Area – Parashant National Monument

The 1,540,264 acres of Parashant National Monument, which is located just north of the Grand Canyon National Park in northwestern Arizona in part of the region known as the Arizona Strip, includes complex and often remote landforms. Because of its highly variable climatic conditions across its major ecological regions and of its heterogeneous landscape, the area supports a diverse flora, with vascular plant vegetation ranging from ponderosa pine forests on Mt. Trumbull to yucca and Mojave desert vegetation at lower elevations, and contains some of the most remote and least surveyed lands in the American Southwest. In addition to their vascular plants, these communities also hold a biologically diverse lichen flora (Sweat et al. 2004) based on an initial survey of 16 sites. No information on the soil crust free-living fungi is currently available.

Research Goal

The goals of this proposal are to (1) expand the sites investigated for lichens by at least 4 and to collect soil crust fungi (free living) at 4 additional sites (soil crust dominated). We will concentrate in the Mr. Trumbull area due to the moister habitats available in this region and include previously unvisited sites near the rim above the Grand Canyon National Park. Soil crust sites will include the Mud Mountain mesa top ca. 1500 m (36°40'41" N, 113°37'52" W), where we found an excellent soil cryptogamic cover in 2003. Other relatively underdisturbed soil sites will be sought.

Cryptogamic soil crusts present also have the potential for high species diversity of lichens and other taxa. Research (e.g. Belnap and Lange, 2001) suggests that fungi play a considerable role in promoting crust development, but the development and ecology of these crusts is currently poorly understood. Although most of the previous studies in this area have used traditional culturing techniques to investigate fungal diversity, using molecular tools will greatly enhance our ability to investigate many aspects of fungal constituents in biological crusts, and there is a high probability that novel fungal lineages will be discovered. The molecular analysis will also provide information about ecological relationships within the soil in the context of a much larger survey across the intermountain West and including the near-by Fort Pearce Area of Critical Environmental Concern (ACEC).

Methods

In mid-May, 2006, a 7-day field excursion to PARA by the Arizona State University lichenological and fungal team (graduate students Mr. Ken Sweat and Mr. Scott Bates and with Dr. Nash) is planned. At each additional lichen site a range of potential lichen substrates will be surveyed, including soils, litter, rocks (including the range of basic and acidic types available), bark (recorded by vascular plant species or genus) and wood. Longitude and latitude for each site will be measured with a GPS device and recorded. Lichen specimens will be prepared according to standard herbarium practices (Nash et al. 1993) and deposited in the Arizona State University lichen herbarium (ASU) for lichens. A representative collection of duplicate specimens will be deposited in the Monument. We will follow Esslinger and Egan (1995 and subsequent web based updates) for nomenclature of the lichens. These are an integral part of the USDA Integrated Taxonomic Information System (ITIS) used in our respective herbarium management systems.

In the case of lichens identification frequently requires microscopic examination, both with stereomicroscopes and compound microscopes, both of which will be provided by Arizona State University. For lichens, knowledge of secondary metabolites occurring in them is frequently essential for accurate determinations. These will be identified with standard thin layer chromatographic procedures (e.g. Culberson 1972).

Completion of the molecular analyses of the fungi associated with the soil crusts is dependent on receiving additional funds for Scott Bates in 2007, and will involve the following techniques:

Fungal Collection of crust samples: In May of 2006 samples will be collected along 4 linear transects distributed across each of the 4 crust sites within Parashant N.M.. At each site, 20 samples will collect, two every 50 meters, for a distance of 500 meters. One sample from each pair is analyzed in the lab, and the other sample is archived. For sampling, the crust is wetted with sterile distilled water to make the top centimeter of the soil soft. Petri plate bottoms are inserted into the surface to excise a circular portion of the crust, with each sample collecting crust to a depth of approximately 1 cm without disturbing the upper layer. Samples are given unique identifier numbers, allowed to dry, sealed and transported to the laboratory, where they are stored dry until analysis in 2007.

Isolation of DNA: Starting in 2007 approximately 1.0 g of crust soil sample is transferred aseptically into vials containing bead solution of commercial Soil DNA Extraction kits (e.g., Ultra Clean Soil DNA isolation kit, Mo Bio Laboratories). DNA is isolated according to the protocol described by the manufacturer. Extracted DNA is eluted with 50 µl of elution buffer and stored at -80°C. The isolated DNA is assessed for quality by standard agarose gel electrophoresis followed by ethidium bromide staining. Concentration of DNA in extracts are quantified from the agarose gels using a Bio-Rad Fluor-S MultiImager system (Bio-Rad Laboratories) using a Bio-Rad EZ molecular weight marker as a standard.

PCR-amplification of 18S rRNA gene: Amplicons of fungal 18S nrDNA are produced using a nested PCR routine. This routine employs the outer fungal specific primers EF4f (5'-GGAAGGG[G/A]TGTATTTATTAG-3') and EF3r (5'-TCCTCTAAATGACCAGTTTG-3') of Smit et al. (1999) to amplify ca. 1500 bp long 18S rRNA gene fragments from the community DNA. PCR product amplified using the EF4f-EF3r primers are then subjected to a second round of PCR using the primers NS2f (5'-GGCTGCTGGCACCAGACTTGC-3') with a 'GC clamp' (Muyzer et al., 1993) and Fung5r (5'-GTAAAAGTCCTGGTTCCC-3') developed by J.D. van Elsas (2000). This second round of PCR produces ca. 230 bp 'GC clamped' amplicons that suitable for use with DGGE. The PCR reactions are performed according to the protocol described by J.D. van Elsas et al. (2000).

Denaturing Gradient Gel Electrophoresis: PCR-amplified 18S rRNA gene alleles are separated by Denaturing Gradient Gel Electrophoresis (DGGE) using a 30 to 60% chemical gradient of denaturants (urea and formamide) on a 6% polyacrylamide gel (Muyzer et al., 1993). About 300 ng of PCR product is loaded on a DGGE gel and electrophoresed for 10 hr at 50 V in a Bio-Rad Dcode universal mutation detection system. The bands are visualized under Bio-Rad Fluor-S MultiImager where banding patterns represent a unique 'finger print' of the fungal community present in the BSC sample. Conspicuous bands representing distinct fungal alleles are excised with sterile scalpels and diffused for 4 days at 4°C in to 10mM Tris buffer.

Sequencing of DGGE eluted alleles: The bands eluted from DGGE gel are PCR amplified with primers NS2f and Fung5r. The PCR products is purified on a 1% agarose gel, where the band corresponding to ca 230 bp are excised and 'cleaned' using commercially available purification kits (e.g., Gel QuikClean kit, Quiagen) according to the protocol described by the manufacturers. The purified DNA is commercially sequenced using the above mentioned primers in the forward and reverse direction.

Sequence analysis: Sequence similarity searches are preformed using GenBank BLASTN (Altschul et al., 1997). Sequences of closely related taxa are retrieved from GenBank and aligned using the Clustal W module in the Mega 3 software package (Kumar et al., 2004). Pair wise evolutionary distances between the sequences can be calculated using Kimura's two-parameter model (Kimura, 1980). Phylogenetic trees are constructed using three tree-making algorithms (e.g., Neighbor joining, Maximum Parsimony, and Unweighted Pair Group Method with Arithmetic Mean) in the MEGA 3 software package (Kumar et al., 2004). Clade stability in the phylogenetic trees is assessed using 1000 replicate data sets in a 'bootstrap' analysis.

DELIVERABLES

For FY 2006

- By 30 Sept, 2006, list of sites sampled and a preliminary list of lichenized fungal species based on visual identifications from all 8 sites will be provided.
- A final report due 31 May, 2007 listing all lichen species found by site. Information on lichens will be available at <http://seinet.asu.edu/collections/selection.jsp> and may be searched at Southwestern Environmental Information Network through the same web site at any point in the future to provide updates as identifications are inevitably revised by subsequent systematic investigations. Hard copies and electronic copies of the final report will be provided to Kari Yanskey. The Principal Investigator will prepare a brief report abstract suitable for public distribution and two hard copies and an electronic version (in PDF file format) of the final report and mail all to the NPS Research Coordinator, CPCESU, NAU, P.O. Box 5765, Flagstaff, AZ 86011-5765

For FY 2007

- to be negotiated pending availability of funds for 2007.

TO BE PROVIDED BY COOPERATOR

- Motorized vehicle travel (ATVs, other vehicles as appropriate), especially for NPS personnel
- Lodging in government bunkhouses or otherwise as appropriate
- Existing maps, imagery, photographs, GIS layers as described above for site selection

P.I. AND CONTRACTOR JUSTIFICATION & INSTITUTIONAL COLLABORATION

Dr. Thomas H. Nash III will (1) be responsible for the verification of lichen names in the NPSpecies Database, (2) participate in the field excursion, and (3) provide full use of the Arizona State University Lichen Herbarium facilities. Dr. Nash was the PI on a National Science Foundation supported greater Sonoran Desert Lichen Flora project, which includes the PARA region. The flora has been (is) published in three volumes (Nash et al. 2002, 2004 & 2006). In addition, he is a past president of the American Bryological and Lichenological Society. He has international collaborations with colleagues in Europe and Australasia and has published approximately 2000 research papers and 5 books.

PROJECT SUMMARY AND SCHEDULE.

This is a two-year project. This research consists of the following tasks:

1. Field work at PARA, starting May 15th, 2006, including all collections in collaboration with PARA staff.
2. Curation of collected material (lichens at Arizona State University) – June and July 2006

3. Identification of curated material (lichens at Arizona State University) – June to December, 2006. Interim report compilation – May, 2007
4. Identification of fungi, additional field work if needed. 2007
5. Final report May, 2008

Annual Project Schedule

Project initiation -- 15 May, 2006

Annual Report – 30 Sept, 2006, list of sites sampled and a preliminary list of lichenized fungal species based on visual identifications from all 8 sites visited in 2006

Investigator's Annual Report – March 31, 2007 for 2006, March 31, 2008 for 2007

Duplicate herbarium vouchers, Database and Maps Provided to Park Management – with final reports

Interim Report – 31-May 2007 for 2006 field work listing all lichen species found by site.

Final Report -- 31 May 2008 for 2007 work and final summary report for project.

BUDGET

Budget 2006

Salaries & Wages

T.H. Nash III, PI \$5506.

Undergraduate (hourly)\$1000.

Fringe Benefits \$1416.

Travel (domestic)\$450.

Materials\$138.

Total direct costs\$8510.

Indirect costs \$1490.

Total project costs\$10,000.

Budget Justification – Grand Canyon Parashant – NPS -2006

A. Salaries

Dr. Nash will devote 0.5 months to the project, traveling to the field with Mr. Ken Sweat and Mr. Scott Bates, two Ph.D. students at ASU, and working with them to certify proper identifications. The undergraduate, working on an hourly basis, will assist with lichen sample preparation, with thin layer chromatography of the specimens to determine their secondary metabolites, and with data entry into the Arizona State University lichen herbarium system (see <http://seinet.asu.edu/collections/selection.jsp>).

B. Fringe Benefits

Dr. Nash's summer salary carries a 25% fringe benefit rate and the undergraduate student is calculated at 4% as per ASU policies.

- C. Travel – Dr. Nash will be reimbursed for driving their personal vehicles to the Parashant research sites from Phoenix according to ASU travel guidelines.
- D. Materials and Supplies – This budget of \$138. will allow purchase of half a box of TLC plates for chemical analyses of lichen secondary products, a necessary step for many lichen identifications.
- E. Indirect Costs – Due to agency limitation (e.g. National Park Service negotiation with Northern Arizona University; C. Redman as the Arizona State University representative), indirect is calculated as 17.5% (see <http://jan.ucc.nau.edu/~envsus-p/new/index.htm>).

ASU contributions to the project:

Scott Bates currently receives summer salary (for 2006 only) for developing the molecular techniques for soil fungi and will travel to the Parashant National Monument this summer. One month of his salary is \$2863. Robin Schroeder is an ASU staff employee, who is assistant curator of the lichen herbarium, and she maintains the ASU databases and other web applications. She will supervise data entry and maintenance of the data generated on this project. One tenth of her f/y salary + benefits is \$6250. Dr. Nash will easily contribute two weeks of his a/y salary to administration of this grant including writing requirements. Based on current salary, this would be equivalent to \$6247. Thus, total ASU contribution is at least \$15,360.

Budget 2007

Estimated at \$11,340. Detailed budget and products to be developed pending results of the 2006 surveys

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Appendix

CESU SUBSTANTIAL INVOLVEMENT DOCUMENTATION (form from the NPS Pacific West Region)

1. Why was this cooperator selected?

The PI, Tomas Nash, is internationally recognized for his lichen expertise, and these researchers are absolutely top caliber. They are more familiar with the local lichen and fungal communities than probably anyone else, period. ASU is preparing a 3-part “Flora of the Sonoran Desert” which includes all of Arizona, and before they started their work here in 2003-2004 the only lichen collections in Arizona north of the Colorado River consisted of only a few spot collections at the Grand Canyon North Rim--NO lichen collections had been made and documented on the BLM Arizona Strip lands or the BLM and NPS lands of the Grand Canyon – Parashant National Monument. Their first surveys in 2003 gave us a preliminary list of 171 species from 57 genera, subsequent studies (2005-2006) are giving us heavy metal depositional information across the Monument for historic air pollution levels and sources and to provide a baseline for future monitoring. This study will give us more information on the crustose lichen species present, continuing the general surveys and looking at the fungal components of the biological soil crusts.

2. Explain the nature of the anticipated substantial involvement.

The Monument botanist (Kari Yanskey) will be fully participating in field work, as well as suggesting direction of inquiry and high-potential survey area possibilities, completing budget, CESU, and other required forms and paperwork, arranging field housing, and interpreting the resulting data for its relevance and usefulness for monument management and planning. She has been and will continue to apply for grants to finance previous, this, and subsequent work, and interacts with other resource specialists, particularly the BLM soil scientist, to identify areas that have the best potential of intact or unique soil crusts, and meet the management needs of both the BLM and the NPS.

3. Why is the substantial involvement considered to be necessary?

There is only a limited amount of money available. The more involvement the NPS/BLM staff have in the planning and implementing of the study, the more time and money the researchers can devote to their specialty—collecting, identifying, analyzing, and interpreting the results. Involvement is also necessary to ensure that the process remains flexible to accommodate the needs of the Monument, other resource staff, management, and the researchers. It allows flexibility in designating survey areas and data collection to take advantage of unique, interesting, or special sites as they are found or information becomes available

4. Explain why the project or activity entails a relationship of assistance rather than a contract.

This is a project of mutual assistance and benefit. We have no information on the lichen and fungal components of the NPS and BLM lands under our management (with the exception of the previous CESU agreements with these same researchers from 2003-2005). NPS directives and guidance state that we shall inventory, and both NPS and BLM directives and guidance state that we shall protect our natural resources. This is impossible to do when we don't know what they are or where they're located. From the University's standpoint, they need the information on the species present to make their flora more complete (see #1 above). The research that the PhD candidate students are doing under the PI not only helps meet our needs for resource information, it allows them to get their Doctorate degrees. The Monument Proclamation states that scientific enquiry is encouraged on the Monument.

5. How was the determination made that the costs proposed are accurate and proper?
Provide a breakdown of costs and rationale for determining they are acceptable.

See 2006 budget, above. The amount requested has been pared down to the absolute minimum, to meet what funding we have available. ASU is contributing over \$15,000 in salary of their researchers to this project. The travel will probably only cover the gasoline—field per diem and any vehicle maintenance required due to the Parashant’s notoriously bad roads will be paid out of their pockets. Supplies covers the TLC plates, but does not cover herbarium supplies, (which will be reimbursed out of the 07 funding). Not only are the costs acceptable, we’re getting a “bargain basement” price for a “top of the line” product!

Approved:

_____/s/ Kari Yanskey_____
Key Official
Kari Yanskey, Botanist,
Grand Canyon – Parashant National Monument

____4/4/03_____
Date

Contracting Officer

Date