

**Environment and Natural Resources Trust Fund**

# 2023 Request for Proposal

## **General Information**

**Proposal ID:** 2023-173

**Proposal Title:** Survey, Protection and Application of Rare Minnesota Fungi

## **Project Manager Information**

**Name:** Christine Salomon

**Organization:** U of MN - College of Pharmacy

**Office Telephone:** (612) 626-3698

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## **Project Basic Information**

**Project Summary:** Survey, characterization and assessment of rare and endangered fungal species found in old growth forests and protected habitats in Scientific and Natural Areas (SNAs) throughout Minnesota

**Funds Requested:** $647,000

**Proposed Project Completion:** June 30, 2026

**LCCMR Funding Category:** Foundational Natural Resource Data and Information (A)

## **Project Location**

**What is the best scale for describing where your work will take place?** Statewide

**What is the best scale to describe the area impacted by your work?** Statewide

**When will the work impact occur?** During the Project

## **Narrative**

**Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Minnesota’s 169 Scientific and Natural Areas (SNAs) provide habitats for many rare species and often contain relict populations of plants and old growth forests that now survive just in these protected locations. Although these locations are well known for protecting flora and fauna, they also protect an important relict fungal resource that we know very little about. Fungi are some of the most important components of healthy ecosystems, serving as decomposers, nutrient recyclers, and fundamental keystone species, but are generally not included in comprehensive biological surveys. There are significant gaps in knowledge about the occurrence, frequency and identity of Minnesota fungi.
Fungi are a valuable group of organisms with immense potential for many biotechnological applications. This not only includes their important uses as a source of antibiotics, anti-cancer and immunosuppressive agents but also for bioremediation, biofuel production, plastic degradation, enzyme production and even nontoxic dyes. Our preliminary studies have identified rare and possibly new species that only exist in protected old growth hardwood and conifer forests. The rare and often endangered fungi that exist in Minnesota as well as those that are found more commonly but have not been previously studied need to be evaluated.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

We propose to survey, culture, and characterize fungi from Scientific and Natural Areas (SNAs) throughout Minnesota. In collaboration with the MN DNR, we will select SNAs with a special focus on sites that have old growth forests and unusual habitats that likely harbor unique fungi. Emphasis will be placed on lignicolous (fungi that grow on wood) and other culturable fungi. Samples will be cultured using a variety of culture media following previous methods that have been found to be successful. Pure cultures will be obtained and DNA extracted and sequenced to identify the fungi. For taxa that do not match known species, we will sequence additional genes and carry out phylogenetic analyses needed for describing the new species.
To assess the potential applications of the fungal species that can be grown in culture, we will prepare extracts and test them for biological activities, including anti-bacterial, anti-fungal, anti-cancer, immuno-modulatory, anti-parasitic and anti-viral activities. The combination of biological data will be added to the database for each species which will provide a chemo-taxonomic “fingerprint” and allow for rapid comparison with other closely related species.

**What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state’s natural resources?**

The goal of this project is to provide a survey of fungi (especially rare and unusual species) from Scientific Natural Areas (SNAs) throughout the state. This survey will include in-depth analysis of each specimen, including DNA sequencing and taxonomy, culturability, and biological activity to assess potential applications. These data will be shared with the DNR and added to the Minnesota biological Atlas to develop a more complete assessment of fungi throughout Minnesota. The development of a culture system for most species will provide an enduring resource for further research, and provides a source for reintroduction of rare and endangered species.

## **Activities and Milestones**

### **Activity 1: Survey and investigation of fungi from Scientific and Natural Areas throughout Minnesota**

**Activity Budget:** $318,250

**Activity Description:**In collaboration with the MN Department of Natural Resources, we will select Scientific and Natural Areas with a special focus on sites that have old growth forests and unusual habitats that likely harbor unique fungi. We anticipate visiting at least 8 sites each year for 3 years for a minimum total of 24 SNAs. Since fungi have different development stages and fruit at different times, sites will be visited multiple times each year (spring, summer, fall). Although the number of collections will vary from site to site depending on the abundance of fungi, we anticipate at least 15 to 20 unique fungal collections will be obtained from each site for further investigation. Emphasis will be placed on lignicolous (wood associated) and other culturable fungi. Samples will be cultured using a variety of culture media following previous methods that have been found to be successful. Pure cultures will be obtained and DNA extracted and sequenced to identify the fungi. For taxa that do not match known species, we will sequence additional genes and carry out phylogenetic analyses needed for describing the new species.

**Activity Milestones:**

|  |  |
| --- | --- |
| **Description** | **Completion Date** |
| Survey and collect fungi from 24 Scientific and Natural Areas throughout Minnesota | May 31, 2026 |
| DNA sequencing and taxonomic analysis of collected fungi | June 30, 2026 |
| Cultivation of collected fungi | June 30, 2026 |
| Development of online database of collected fungi | June 30, 2026 |

### **Activity 2: Biological activity assessment of cultured fungi**

**Activity Budget:** $328,750

**Activity Description:**We estimate collecting 15-20 unique specimens per collection site (SNA), and a minimum of 8 sites per year, resulting in ~120 specimens in activity 1. Assuming that approximately 75% of these fungi can be cultivated in laboratory conditions, we anticipate that 90 samples x 3 culture conditions will result in a minimum of 270 samples each year. For samples that consist of significant quantities of mushroom fruiting body material, a portion of these will also be extracted. Each of the cultures and mushroom materials will be extracted with solvents to produce a crude extract. These extracts will be analyzed by high performance liquid chromatography (HPLC) to develop a chromatographic signature for each sample. The extracts will then be tested against a panel of 8 human microbial pathogens (bacteria and fungi), cancer cell lines (breast and colon), viruses (SARS-COV2 and other RNA viruses), and parasites (cryptosporidium and toxoplasmosis). Extracts will also be tested for their ability to modulate the immune response in human cells. Samples will be prioritized for potency versus toxicity as well as for those that appear to have new chemical compounds that have not been previously described.

**Activity Milestones:**

|  |  |
| --- | --- |
| **Description** | **Completion Date** |
| Cultivate each sample under 3 conditions and make crude extracts | June 30, 2026 |
| Test each sample in all bioassays (antimicrobial, anti-cancer, anti-viral, anti-parasitic, immuno-modulatory) | June 30, 2026 |
| Compile data in fungal database | June 30, 2026 |

## **Project Partners and Collaborators**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Organization** | **Role** | **Receiving Funds** |
| Robert Blanchette | University of Minnesota, Department of Plant Pathology | Dr. Blanchette will be responsible for the identification, collection, cultivation and DNA sequencing of fungi in selected Scientific and Natural Areas. His group will also produce, store and maintain voucher specimens for all samples. | Yes |

## **Long-Term Implementation and Funding**

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?**This project will provide foundational data on both cultured and uncultured fungi in protected Scientific Natural Areas with an emphasis on rare old growth forests. We expect these results to lead to a better understanding about the occurrence and frequency of rare and unusual fungi in these unique habitats. Ultimately, we hope to increase both public and scientific awareness of these ecosystems to inspire continued research and protection. Additional research could be funded by the NSF, USFW and/or through a "phase II" request from the LCCMR.

## **Other ENRTF Appropriations Awarded in the Last Six Years**

|  |  |  |
| --- | --- | --- |
| **Name** | **Appropriation** | **Amount Awarded** |
| Biological Control of White Nose Syndrome in Bats - Phase II | M.L. 2016, Chp. 186, Sec. 2, Subd. 06d | $452,000 |

## **Project Manager and Organization Qualifications**

**Project Manager Name:** Christine Salomon

**Job Title:** Associate Professor

**Provide description of the project manager’s qualifications to manage the proposed project.**Dr. Salomon (PI) is an Associate Professor at the Center for Drug Design and a faculty member in the Biotechnology Institute at the University of Minnesota. Dr. Salomon earned her Ph.D. at the Scripps Institution of Oceanography, UCSD, in the area of natural products chemistry from invertebrates and microbes. She continued her training in the Department of Microbiology at the University of Minnesota where she worked on understanding how soil microbes biosynthesize chemical compounds. Dr. Salomon’s current research program is focused on the discovery and utilization of novel microbes that can be used for biological control of agricultural and wildlife pathogens and production of unique compounds for biomedical and biotechnological applications. She has successfully secured both internal (Academic Health Center, Biotechnology Institute) and external (US Department of Agriculture and US Fish and Wildlife) support for her research program. Dr. Salomon was the Project Manager on a previous funded ENRTF project "Biological Control of White Nose Syndrome in Bats – Phase II", ML 2016 Chp 186, sec 2.

Dr. Robert Blanchette (Co-PI) is a professor in the Department of Plant Pathology. He has been involved with research and teaching of forest and landscape trees at the University for over 30 years. He currently teaches undergraduate and graduate classes at the University of Minnesota on forest and shade tree diseases. Research is in the area of forest pathology and wood microbiology with investigations underway on the biology and ecology of tree pathogens, tree defense mechanisms and managing tree diseases using integrated control procedures. Dr. Blanchette has served as project leader on several past projects including 2015-084 Preventing a new disease of pines in Minnesota, 2013-19B Finding Disease Resistant Elm Trees in Minnesota and 2016-131-D Winning the Dutch elm disease battle Phase II.

**Organization:** U of MN - College of Pharmacy

**Organization Description:**Dr. Salomon is in the Center for Drug Design at the University of Minnesota and a member of the Biotechnology Institute. These centers play a central role in providing training and coordinating research in biological, chemical and engineering sciences at the University of Minnesota. The University of Minnesota Biotechnology Institute was established to catalyze the development of a biotechnology industry in Minnesota. It also serves as an important resource for industry by providing connections with academic research partners.

The Department of Plant Pathology at the University of Minnesota is one of the oldest plant pathology departments in the country. Since 1907 the Department has had a strong impact on plant health, agricultural development, and ecosystem vitality on a local, national, and international scale. It is involved with solving today's complex plant health problems through cutting-edge research. The department provides sound plant health advice to stakeholders throughout Minnesota and around the globe and is educating the next generation of plant health professionals and change-makers through a modern and broad plant pathology curriculum.

## **Budget Summary**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Category / Name** | **Subcategory or Type** | **Description** | **Purpose** | **Gen. Ineli gible** | **% Bene fits** | **# FTE** | **Class ified Staff?** | **$ Amount** |
| **Personnel** |  |  |  |  |  |  |  |  |
| Principle Investigator |  | Project mananager and fungal chemistry and bioactivity testing lead. |  |  | 25.1% | 0.15 |  | $28,153 |
| Research associate |  | Responsible for fungal cultivations, extract production, compound purification and biological testing |  |  | 25.1% | 3 |  | $212,505 |
| Research Associate |  | Fungal collections, cultivations, sequencing and analysis of pure cultures |  |  | 25.1% | 0.75 |  | $84,700 |
| graduate student |  | Fungal collections, cultivations, sequencing and taxonomic analysis, database building |  |  | 44.2% | 3 |  | $160,582 |
| undergraduate research assistant |  | media and sample prep, sample management, fungal cultivations, general lab support |  |  | 0% | 0.9 |  | $20,629 |
| Co-Principal Investigator |  | Project manager for fungal isolations, cultivation and taxonomic analysis |  |  | 0% | 0.03 |  | - |
| junior scientist |  | General lab support, media preparation, strain cultivations, bioassays, database assistance |  |  | 22.3% | 0.9 |  | $24,193 |
| undergraduate research assistant |  | general lab support, ordering, glassware washing, solution, reagent and media preparations |  |  | 0% | 0.9 |  | $20,629 |
|  |  |  |  |  |  |  | **Sub Total** | **$551,391** |
| **Contracts and Services** |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | **Sub Total** | **-** |
| **Equipment, Tools, and Supplies** |  |  |  |  |  |  |  |  |
|  | Tools and Supplies | Field collections and microbiology supplies: media, reagents, petri dishes, tubes, gloves, field sampling materials | For making collections of fungi from the field and cultivating fungi in the lab |  |  |  |  | $22,090 |
|  | Tools and Supplies | Molecular biology/sequencing costs: (DNA isolation kits, PCR supplies, enzymes, reagents, sequencing costs) x 300 samples/year | Sequencing of DNA from pure cultures and fruiting bodies |  |  |  |  | $15,000 |
|  | Tools and Supplies | Chemical supplies (solvents, chromatography materials, reagents, tubes, glassware, pipettes) | Supplies for conducting chemical extractions for biological assays and fractionation of extracts for additional testing |  |  |  |  | $22,090 |
|  | Tools and Supplies | Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, media, 96 well plates, reagents, gloves), estimated 1000 samples per year | Supplies for conducting biological assays: antimicrobial, anti-cancer, anti-viral, anti-parasitic and immuno-modulation assays |  |  |  |  | $15,000 |
|  |  |  |  |  |  |  | **Sub Total** | **$74,180** |
| **Capital Expenditures** |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | **Sub Total** | **-** |
| **Acquisitions and Stewardship** |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | **Sub Total** | **-** |
| **Travel In Minnesota** |  |  |  |  |  |  |  |  |
|  | Miles/ Meals/ Lodging | estimated 6 trips per year with 2-3 scientists, 1-2 days, distance from 60-450 miles round trip x 3 years, lodging for longer trips. | In-state round trip travel for sampling from SNAs throughout the state Spring, Summer and Fall each year for 3 years (estimated 15 trips per year) |  |  |  |  | $15,250 |
|  |  |  |  |  |  |  | **Sub Total** | **$15,250** |
| **Travel Outside Minnesota** |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | **Sub Total** | **-** |
| **Printing and Publication** |  |  |  |  |  |  |  |  |
|  | Publication | Scientific manuscript publication costs (open access) -estimated 2-3 publications over 3 years | Publication of scientific data and results obtained during this project, including descriptions of new species |  |  |  |  | $3,089 |
|  |  |  |  |  |  |  | **Sub Total** | **$3,089** |
| **Other Expenses** |  |  |  |  |  |  |  |  |
|  |  | Repair of equipment and instrumentation (e.g. vacuum pumps, water baths, incubators, shakers, etc.) and calibration of instruments (pipettes, balances) estimated at $1000 per year for 3 years | Funds for inevitable breakage, repair of glassware and instrumentation and calibration of instrumentation |  |  |  |  | $3,090 |
|  |  |  |  |  |  |  | **Sub Total** | **$3,090** |
|  |  |  |  |  |  |  | **Grand Total** | **$647,000** |

### **Classified Staff or Generally Ineligible Expenses**

|  |  |  |  |
| --- | --- | --- | --- |
| **Category/Name** | **Subcategory or Type** | **Description** | **Justification Ineligible Expense or Classified Staff Request** |

### **Non ENRTF Funds**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Category** | **Specific Source** | **Use** | **Status** | **Amount** |
| **State** |  |  |  |  |
|  |  |  | **State Sub Total** | **-** |
| **Non-State** |  |  |  |  |
|  |  |  | **Non State Sub Total** | **-** |
|  |  |  | **Funds Total** | **-** |

## **Attachments**

### **Required Attachments**

#### ***Visual Component***

File: [8e3ce1b5-a91.pdf](https://lccmrprojectmgmt.leg.mn/media/map/8e3ce1b5-a91.pdf)

#### ***Alternate Text for Visual Component***

A map of Minnesota showing the location of protected Scientific and Natural Areas (SNAs) with proposed focus habitats containing old growth hardwood and conifer forests and environments likely to harbor unusual or rare fungi. There are 2 photographs of rare (endangered) fungi that we identified in an SNA in 2021....

## **Administrative Use**

**Does your project include restoration or acquisition of land rights?**
 No

**Does your project have potential for royalties, copyrights, patents, or sale of products and assets?**
 Yes

**Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?**
 Yes

**Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF? If so, describe here:**
 Yes, Although we do not currently anticipate any immediate products or assets from this project in this three year time-frame, it is possible that we will discover fungi with useful activities that we may want to develop further.

**Does your project include original, hypothesis-driven research?**
 Yes

**Does the organization have a fiscal agent for this project?**
 Yes, Sponsored Projects Administration