



Report on sequencing funded by MSA and done by Fungal Diversity Survey (NAMP) for community scientists

August 2021

The mission of Fungal Diversity Survey (FunDiS; previously North American Mycoflora Project, or NAMP) is to increase scientific knowledge and public awareness of the critical role of fungi in the health of our ecosystems and to better utilize and protect them in a world of rapid climate change and habitat loss. We do this by equipping community scientists, working with professionals, with the reporting tools to document the diversity and distribution of fungi across North America.

FunDiS's sequencing program for community scientists has already generated thousands of barcode sequences that are being uploaded to GenBank, and as a result of this process, has documented numerous new-to-science species. Equally importantly, our sequencing program has given many amateur and paraprofessional mycophiles the opportunity to turn their collecting passion into valuable scientific contributions and has facilitated their collaboration with experts. Through this partnership they are motivated to learn more about fungi, scientific collecting protocols, and the role of fungi in local habitats.

MSA authorized a \$20,000 contribution to FunDiS (as NAMP) in 2017 for sequencing by amateur mycologists. (FunDiS also received \$20,000 from the North American Mycological Association: half directly from NAMA paid over three years and half passed through NAMA from Paul Stamets and Dusty Yao.) FunDiS issued sequencing grants between January 2018 and April 2021, by which time all funds had been allocated through grants. We will receive and process the last specimens over the next year.

FunDiS has already sequenced more than 7,000 specimens collected throughout the United States and Canada, including grant specimens, sequencing for NAMA forays, and sequences paid for by individuals and clubs. In addition, we expect about 1200 samples from 2020 and 2021 grants (listed below) will be sequenced over the next year. Of the already-sequenced specimens, 3,605 were from 200 [local projects registered](#) on the FunDiS website and 1,264 were from specimens collected at NAMA forays. Sequencing of the remaining specimens was paid for by clubs and individuals.

The FunDiS sequencing operation was conducted by more than a dozen volunteers who contributed collectively thousands of hours over the past four years for the benefit of the amateur mycology community; only in the past year did we hire a part-time contractor to coordinate sequencing.

FunDiS sequenced specimens from these NAMA forays:

- **NAMA 2017 Foray** - 152 sequences; all uploaded to GenBank.
- **NAMA 2018 Oregon Foray** - 300+ sequences
- **NAMA 2018 Regional Canton MS Foray** - 135 sequences
- **Amanita specimens** from various forays - 227 successful sequences; all uploaded to GenBank. Project with Rod Tulloss.
- **NAMA 2014 Foray** - 450 sequences (sequenced in 2019; paid for by Puget Sound Mycological Society via Danny Miller)

Sequencing Grants

2018-2019 - 64 registered projects were awarded grants for sequencing of 30 (mostly) or 50 specimens each: **1,960 specimens**.

- Many sequences were generated by graduate student Steve Russell using resources from the Cathie Aime lab at Purdue University. Participants were expected to validate or redetermine the identity of their specimens using blast results posted by Russell on his MycoMap platform, training documents and videos on sequence analysis & interpretation on the NAMP [website](#).
- Additional sequences were generated by the labs of Todd Osmundson at the University of Wisconsin-La Crosse and Rytas Vilgalys at Duke University. Validating identifications and uploading data to GenBank were performed by these labs.

2020 - 27 registered projects were awarded grants for sequencing between 10-50 specimens each: **685 specimens**.

- In 2020 we simplified our processes and switched to a commercial lab, Barcode of Life Data Systems (BOLD) at the University of Guelph in Ontario, Canada (plus two orders went to another commercial lab, Molecular Solutions LLC, run by Matt Gordon in Portland OR). The quality of sequences from BOLD has been excellent. We hired a part time Sequencing Coordinator contractor in Eugene, Oregon to receive samples, fill plates, send them to BOLD, track processing, run GenBank BLAST searches, and communicate with participants.
- Identification of sequences in BOLD has been facilitated by the volunteer efforts of Jean Lodge and Bitty Roy who examine every sequence and specimen record in iNat/MO (>900 so far). They work with the users to get the best possible IDs on each sequence, including contacting experts of particular

genera, when needed. They are working with the sequence coordinator to get all the sequences submitted to GenBank within a year of sequencing.

2021: January-July - The final round of grants (#5) were awarded in April 2021. 24 registered projects were awarded grants for sequencing between 10-50 specimens each: **960 specimens**. Two of these are FunDiS Rare Fungi Challenges, one on the West Coast and one in the North East (Challenges are in both Canada and USA).

- We have been fortunate to have the continuing volunteer services of two “retired” mycologists, D. Jean Lodge and Bitty Roy, to analyze and interpret sequences from BOLD for participants.

Completion of grant sequencing (August 2021-July 2022) - All outstanding grant specimens must be submitted by November 30, 2021. These specimens will work their way through the sequencing, data editing, analysis, and GenBank submission processes in early 2022.

- Historically, about 70% of grant allocations result in specimen submissions. (Most participants were able to follow the documenting, collecting, drying and submission protocols, but others found the process overwhelming and learned that they “just want to collect fungi.”) We expect about **1,200 samples** from the 2020 and 2021 grants will be sequenced in the winter of 2021-2022.
- We hope that Jean Lodge and Bitty Roy continue to analyze and interpret DNA sequences for participants; otherwise interpretation will be up to participants, as it mostly was in FunDiS’s first two years.

GenBank Submission

All 7,000 specimens have either been or will be submitted to GenBank. Of these:

- Those completed by the Osmundson and Vilgalys labs have been submitted to GenBank.
- Sequences from FunDiS registered projects completed in 2018 and 2019 are now in the process of being uploaded to GenBank by Jeff Stallman, a graduate student in the Aime lab at Purdue University and longtime FunDiS volunteer.
- Those completed at BOLD in 2020 are in the process of being uploaded to GenBank and made public on the BOLD platform, after they have been edited, analyzed, and IDs validated or corrected.

Vouchering

FunDiS strongly encourages vouchering of sequenced collections and others deemed significant, and offers resources to make vouchering practical. Some projects routinely deposit their collections at CUP, FLAS, MU, NY, OSC, PUL, WISC and WTU. It

is not possible to determine how many of the sequenced specimens will be vouchered because the participants are responsible for this step and there is a time lag while they obtain final determinations; moreover, some fungaria require that barcodes have been analyzed and data submitted to GenBank before accepting vouchers. Collections of species that are new to science or represent rare collections of known species have usually been shared with expert mycologists who were involved in determinations, and those experts have deposited these duplicates at their institution's fungaria.

The Future

FunDiS is currently looking for funds to continue the sequencing programs for interested amateur mycophiles as well as for specimens from Rare Challenges, Habitat Challenges, the Diversity Database and amateur projects. More importantly, we are looking for funding to hire staff to manage these programs, starting with an Executive Director to oversee FunDiS program management and fundraising. We are currently exploring a range of funding options.

In the meantime, amateurs can get Sanger sequencing done at commercial labs (which charge about twice what FunDiS charged). On [our website](#) we refer interested parties to two well-regarded commercial labs: Molecular Solutions LLC (Portland, Oregon) and ALVALAB (Oviedo, SPAIN).

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