Mushroom Citizen Science in USA: From Species Lists to Mycofloras 2.0[1]

Bill Sheehan, Ph.D. *bill@productpolicy.org*

The tagline of the North American Mycoflora Project – "without a sequenced specimen, it's a rumor" – reflects the importance of two activities that are central to 21st century mycology: *vouchering* specimens in established fungaria, and obtaining genetic information by *sequencing* DNA. The traditional practice of amateur mushrooming is to collect specimens, try to identify them, create species lists … and then discard the specimens. That's similar to the model of amateur birders who create unvouchered species lists at Christmas bird counts. Amateur birding is often held up as the quintessential citizen science activity, but mushroomers have an advantage in that specimens can be readily collected and preserved.

Since I have scientific training (in insect ecology) and an interest in citizen science, I wondered what mushroom clubs were doing to voucher and sequence mushrooms. On behalf of the North American Mycological Association's Mycoflora Committee, I queried clubs through a survey and direct contact. What I found was a delightfully chaotic variety of projects, notable for the diversity of protocols employed as well as the lack of coordination between them. The projects demonstrate considerable potential while highlighting significant challenges. The purpose of this paper is to describe club vouchering and sequencing projects, assess challenges, and report on a proposed project, called Mycoflora 2.0, to make vouchering and sequencing simple and inexpensive for clubs and organizations that want to do it.

North American Mycoflora Project

A stimulus for sequencing activity and a renewed push for vouchering appears to be the North American Mycoflora Project. The Project is a collaboration between professional and amateur mycologists that aims to create online compendia for macrofungi in defined geographic areas, similar to floras for plants and lichens. Macrofungi are fungi that are easily visible without a microscope—essentially mushrooms, polypores, truffles, corticoid fungi, and Ascomycota with large fruiting bodies (Bruns and Beug, 2012). The goals envisioned by Vellinga (2013) for a North American Mycoflora include "keys, descriptions, accompanied by photos, notes of the fresh specimens, data on habitat, location and date, and with DNA sequence data [and] available on the web, portable, free, and easy to update."

An early advocate for a North American mycoflora was mycologist Tom Bruns of the University of California at Berkeley (Bruns, 2011; see also Matheny and Vellinga, 2009). Bruns and colleagues secured National Science Foundation funding for a networking project that had enough funds left over for a meeting in 2012 at Yale University. The meeting included 75 leading professional and amateur mycologists from the Mycological Society of America (MSA; professionals) and the North American Mycological Association (NAMA; amateurs). Meeting presentations are documented at www.namycoflora.org and several articles summarized the event (Bruns, 2012; Bruns and Beug, 2012; Vellinga, 2013).

Unfortunately the Mycoflora Project has not gained traction among professional mycologists since the 2012 meeting. Much of this has to do with funding, but over-committed professional mycologists is another hindrance. The 2012 meeting stimulated considerable interest within mushroom clubs in vouchering and sequencing. Whether motivated by accurate identification of edible, medicinal or psychedelic mushrooms, by the possibility of finding new species, or by fascination with fungal diversity, many mushroom enthusiasts seem eager to make their endeavors more scientific. Some clubs hosted talks and workshops (like the New Jersey Mycological Association's 2014 DNA Workshop) that have not yet resulted in active projects. Other clubs launched a range of projects, many described here.

Methods

There are 80 mycological clubs affiliated with NAMA; approximately 1,500 members (of the approximate 10,000 total club membership) are NAMA members, not including several large Canadian clubs, according to NAMA president David Rust. Data for this study were gathered initially by outreach to several NAMA clubs known to voucher or sequence specimens. In December 2016 a link to an online survey ("Mycoflora Survey") was sent to 76 club presidents by David Rust. The survey asked about club capacity, foray frequency, vouchering and sequencing activity, and whether clubs post photos and documentation to the Internet. Survey responses were amplified by direct contact via phone or email with club members responsible for vouchering or sequencing.

Thirty-eight of the 76 clubs contacted responded to the Mycoflora Survey (50% response rate), and four additional clubs were researched for a total of 42 clubs. Thirty-seven of those clubs are in the US, the other five in Canada. Membership in clubs surveyed ranges from 10 to more than 1,200 members. Dues range from $0 to $35 USD per year for individuals. The number of specimens identified on all forays in the past year varied from a few dozen to many hundreds. Interestingly, several active clubs (e.g., Long Island, Santa Cruz, Asheville) report lifetime species lists of around 1,000 species identified (this was not a survey question).

The survey sought to determine whether and how clubs post information to the Internet. Posting photo-rich observations to sites such as Mushroom Observer, iNaturalist and Flickr is important to scientific documentation. But high-quality citizen science needs to make information accessible to scientists around the world. Professional mycologists seeking

[1] A version of this article with hyperlinks can be found at www.fungimag.com.

vouchered specimens in North America increasingly search on MycoPortal, which recently completed digitizing almost 2 million records of macrofungi. The fungi are stored in "established" fungaria—i.e., institutions with curation staff. (Most fungaria in the USA are still called "herbaria," a throwback to the time when fungi were considered plants.) The core website repository for DNA sequences is GenBank. Few clubs are currently posting on, or linking to, either MycoPortal or GenBank.

This report makes a distinction between two models of mushroom citizen science to emphasize potential for club initiative. "Professional-led" projects are projects designed and led by full-time mycologists who typically have academic positions, access to university herbaria for vouchering and funding for DNA sequencing. We use the term "professional" here broadly to include recognized taxonomic experts who do mycology full time but are not paid to do it, as well as external institutions like the National Park Service. For our purposes, the point is that direction and resources come from outside a mushroom club. "Club-led" projects, by contrast, are initiated, organized and stewarded by amateur club members – albeit usually in partnership with academic mycologists who provide technical expertise and guidance when asked. The selected projects detailed below are not comprehensive; rather they are intended to highlight different approaches to common challenges.

Professional-led Projects

Originated in 1996, "bioblitzes" occur all over the world. In the USA they were organized by the National Park Service and the National Geographic Society between 2007 and 2016. They are biological surveys by groups of scientists, naturalists and volunteers that "attempt to record all living species within a designated area over a continuous time period, usually 24 hours" (Wikipedia). Fungi have been a part of many bioblitzes; those focused specifically on fungi are called "mycoblitzes." When vouchering or sequencing has been done, it is usually by professional mycologists or fungarium professionals.

- **Great Smoky Mountains National Park:** A large and long-running mycoblitz was organized in 2004 by Ron Petersen from the University of Tennessee to sample agarics in the Great Smoky Mountains National Park. Part of a larger bioblitz, the project received significant funding from the National Science Foundation. The project included a 3-day event in conjunction with an annual meeting of the Mycological Society of America in Asheville, NC.
- **Point Reyes National Seashore and Yosemite Mycoblitzes:** Five mycoblitzes occurred at the Point Reyes National Seashore between 2005 and 2007. The events were coordinated by Tom Bruns of the University of California, Berkeley; Darvin DeShazer, founder of the Sonoma County Mycological Association; and David Rust, co-founder of the Bay Area Mycological Society (BAMS) and current NAMA president. BAMS sponsored a "box party" of volunteers in 2007 to process collected specimens for the UC Berkeley Fungarium (UC). Challenges highlighted by the project include the fact that while some specimens were accessioned, others were apparently not and remain in boxes, according to Rust. The Point Reyes project has continued in the form of an annual Fungus Fair co-hosted by the National Seashore and the Bay Area Mycological Society. UC Berkeley mycologists look through the collections and cherry pick unusual or new species on collection day, which they may then voucher or sequence in their lab (David Rust, personal communication). Bruns, Else Vellinga and BAMS also organized a mycoblitz (fungal survey) in Yosemite National Park; many of those specimens were sequenced as well.

Some other mycoblitzes with vouchering include:

- Colorado Mycological Society, Rocky Mountain National Park and Denver Botanic Gardens Sam Mitchel Fungarium of Fungi (DBG) held mycoblitzes in 2008 and 2009, as well as one in 2012 cosponsored by the National Geographic Society.
- The Metchosin Biodiversity Project has held four mycoblitzes on the south coast of Victoria Island, British Columbia, the latest in 2016.
- The National Park Service and National Geographic have sponsored several bioblitzes that included mycoblitzes, including in 2013 at the Jean Lafitte National Historical Park and Preserve in Louisiana, and in 2016 at Shenandoah National Park in conjunction with the NAMA national foray in Front Royal, Virginia.

Taxonomist-led Projects

Another model occurs when a club helps an expert taxonomist collect species in a particular taxon, which the expert then vouchers and sequences. Two examples involve experts who are professional-level but self-trained in mycology. Rod Tulloss is the leading *Amanita* expert in North America; he is a retired engineer who runs the site www.amanitaceae.org. Another example is Henry Beker who is studying the genus *Hebeloma*. Having completed a monograph on European Hebolomas he is now working on a North American monograph and is seeking help from mushroom clubs in finding specimens, as described in a recent NAMA newsletter article (Beker,

Survey Results: Clubs Vouchering and Sequencing

Vouchering	
Club members voucher at home	8
Club members voucher at established fungaria	12
% of 38 clubs with some vouchering	53%
Sequencing	
Clubs conducting club-led barcoding	8
Clubs engaged with professional-led barcoding	12
Clubs with members interested in sequencing	10
% of 38 clubs sequencing or interested	63%

2016). I recently posted a *Hebeloma* observation to Mushroom Observer and within an hour received an email from Dr. Beker offering to arrange a FedEx pick-up "so that you incur no cost." A third example is Michael Kuo's Morel Data Collection Project which ran between 2001 and 2010. Collections were in part crowdsourced from visitors to Kuo's MushroomExpert.com website. They were sequenced and vouchered at the Field Museum in Chicago; 518 observations with photos are posted on Kuo's site. These are only three of many examples of professional or semi-professional taxonomists engaging amateur mushroomers to collect specific taxa.

Club-led Voucher Projects

Mycoflora Survey (Vouchering): Twenty (53%) of 38 clubs voucher at least some specimens, and, of those, 12 clubs (32%) voucher or plan to voucher specimens in established fungaria. It is likely that more amateur mushroom experts save specimens at home, and clubs that voucher may have been more likely than non-vouchering clubs to fill out the survey. There is little pattern as to how clubs post observations to the Internet. Nine clubs post to Mushroom Observer (MO), including one to both MO and iNaturalist and one to MO and MycoPortal (MP). Five clubs post to MP and one to MP and Flickr. Twelve clubs post only to a club website, two post to Facebook, and 11 do not post any observations.

North American Mycological Association (NAMA) Voucher Program: NAMA national forays (annual forays open to all NAMA members) have been vouchered for 20 years (since 1997), overseen by Patrick Leacock from the Field Museum of Natural History in Chicago. NAMA forays move to a different location every year but all vouchered specimens are accessioned at the Field Museum. The Voucher Collection Program has databased 6,640 records, but not all records have vouchers. Some of the records have been posted to MycoPortal. All of the vouchers "should now be in the Museum collections database online," according to Leacock, "but we still have images to load for half of the forays." Martin Livezey used a bulk uploader developed by Raymond Suelzer to get observations on Mushroom Observer, where voucher photos for the last seven annual forays are posted. For example, the 2016 Shenandoah Foray Species List has 339 observations. Challenges include reporting observations separately to both Mushroom Observer and MycoPortal, without links between those sites.

The **Northeast Bolete Consortium** is an informal network of mushroom enthusiasts from several Northeastern clubs focused on boletes. It was formed in 2015 at the suggestion of Roy Halling of the New York Botanical Garden. Eight experienced collectors (Robert Gergulics, Roy Halling, Renée Lebeuf, Scott Pavelle, John Plischke III, Igor Safonov, Walt Sturgeon, David Wasilewski and Bill Yule) contribute bolete observations to Mushroom Observer, which are linked to the group's Project Page (MO Project 199). The page states: "the project is a concerted effort to collect, document, and analyze two confusing sets of boletes: (A) The various species referred to as 'bicolors'; i.e. those with yellow pores and red-and-yellow caps/stems; and (B) The various species referred to as 'red mouths'; i.e., those with red pores that readily stain blue." Consortium members voucher individually. The Western Pennsylvania Mushroom Club and several members also sequenced selected specimens. WPMC member Scott Pavelle created the Bolete Filter as an online "encyclopedia" of North American boletes that uses images from Mushroom Observer and Consortium members.

Gulf States Mycological Society (GSMS): GSMS president David Lewis has been vouchering along the Gulf Coast for four decades, often with the help of Jay Justice. Lewis has 5,300 collections with detailed notes vouchered at the Field Museum in Chicago (also on MycoPortal); 3,544 specimens from the Big Thicket National Preserve and other East Texas sites at the Tracy Herbarium (TAMU) at College Station, TX (not on MycoPortal); and 394 Russulas and *Cantharellus* vouchered at the National Museum of Natural History in Paris, France.

Long Island Mycological Club (LIMC): In addition to being the first club to participate in Henry Beker's *Hebeloma* Project (see above), LIMC has a history of vouchering specimens. The club submitted 75-100 specimens to the New York State Museum (NYS) around 2011, and another 154 specimens to the New York Botanical Garden Herbarium (NY) over the last few years, according to Joel Horman. There is no online record of the NYSM specimens on MycoPortal, indicating that they may not have been accessioned yet – a recurring theme at staff-limited fungaria. NYBG has accessioned the LIMC specimens and posted them to their on-line database (NYBG Virtual Herbarium) though not yet on MycoPortal "for technical reasons," according to Barbara Thiers.

New Jersey Mycological Association (NJMA): NJMA maintains its own fungarium next to Rutgers University's Chrysler Herbarium (CHRB). The club fungarium has approximately 2,900 specimens accessioned, representing more than 1,000 species, most collected before the year 2000. Club president John Burghardt, with help from Igor Safanov, is working to post the records to MycoPortal in 2017. Burghardt also matched fungarium records with the club's New Jersey species list and generated a list of species which have no fungarium specimens – as targets for future collecting.

Illinois Mycological Association (IMA): In addition to being in charge of the NAMA Voucher Program (above), Patrick Leacock is IMA club president. The club has 4,900 databased observations from IMA forays for the past 23 years, representing 650 species. "Some 2,000 specimens (vouchers) were saved, but only about 300 of these have been accessioned into the permanent Field Museum of Natural History (F) collections," according to Leacock. "The rest are in herbarium cabinets with our other local research specimens waiting to be selected for packaging, barcoding, and transferring to the permanent collections."

Club-led Voucher and Sequencing Projects

DNA barcoding, or sequencing, is a powerful tool that probes the essence of a species' identity. It's so important that professional mycologists can barely publish research on fungi

without including reference to DNA sequences. Some amateurs are setting up DIY (do-it-yourself) labs to extract and amplify small DNA fragments from the nuclear ribosomal internal transcribed spacer (ITS) region of the fungal genome, and then analyze results after a commercial lab has converted the amplified extract into a string of letters representing DNA base pairs. Amateur mushroomers Alan Rockefeller, Stephen Russell, Richard Jacob and Christian Schwarz have all given talks to clubs on the brave new world of DNA sequencing. (It's noteworthy that each of these individuals is significantly younger than the median age of NAMA members, if the prevalence of grey-haired baby boomers at NAMA forays is any indication.) Sequencing the ITS locus has been the standard for fungi for the past decade but new technologies are becoming more widespread that can sequence multiple loci at once. Rytas Vilgalys of Duke University says he will soon be able to provide multi-locus "next-gen" sequencing to mushroom clubs, possibly for equal or less cost than the current cost of ITS sequencing.

It should be clearly noted, however, that sequencing is not essential for doing valuable citizen science, nor is it a "slam dunk" for making species determinations. Traditional macroscopic and microscopic descriptions, high quality photographs, and accurate field information are no less important than they have always been to scientists studying fungi. Preserving specimens is more critical for high-quality citizen science than sequencing. When specimens are properly documented, dried and preserved in curated fungaria, scientists can examine them decades from now, and probably sequence them with more powerful technology than is presently available.

In many cases a sequence will not provide a clear identification for many reasons. For example, if the type specimen of the species has not been sequenced – a common situation – it may be impossible to determine a mushroom's identity. Samples may be contaminated, reference data in GenBank may be erroneous, and a host of other problems. On the other

hand, even if a sequence does not yield a positive species determination, valid sequences posted to GenBank are still extremely useful to mycologists working on phylogenies and ecology (Tom Bruns, personal communication).

Mycoflora Survey (Sequencing): Fourteen (37%) of the 38 clubs responding to the survey are engaged in DNA barcoding, either directly as a club-led project (8) or indirectly as part of a professional-led project (6). Of the 24 clubs not engaged in DNA barcoding, 10 reported member interest in sequencing. Hence, 24 (63%) of 38 clubs either have members interested in sequencing or are engaged directly or indirectly (see Table above).

NAMA Voucher Sequencing Program: In 2015, the NAMA Voucher Program (see above) added sequencing. Sampling of new collections for DNA barcoding was conducted using Whatman FTA Plantsaver cards. La Monte Yarroll (Western Pennsylvania Mushroom Club) did the sample collection at the 2015 Black Mountain foray and Stephen Russell (Hoosier Mushroom Society) at the 2016 Shenandoah foray. A first pass was made through the 2015 FTA card samples using Sanger sequencing, but the results still need to be proofread and edited before posting to GenBank, according to Rytas Vilgalys.

Amanita Projects: Amanitas have been an early focus of sequencing, perhaps due to interest and encouragement from Rod Tulloss. The Western Pennsylvania Mushroom Club (see below) decided that half of their sequencing targets would be *Amanita* specimens. In 2016 the NAMA Board authorized a project led by Stephen Russell to sequence all the Amanitaceae specimens vouchered at NAMA annual forays over the past 20 years: 241 specimens of *Amanita* and 3 of *Limacella* vouchered between 1997 and 2015. Extractions and amplifications will be done in the Aime Lab at Purdue University, and sequencing will be done by Genewiz. The budget approved for sequencing is $3340, or about $14 per sample. Metadata will be posted to Russell's MycoMap platform and final sequences will deposited into GenBank and added to the original record reported on Mushroom Observer.

Western Pennsylvania Mushroom Club (WPMC): An excellent model has been created by WPMC under the leadership of club president Richard Jacob. Their website excels in thorough explanation and documentation of each step in collection, description and DNA barcoding specimens. The web page Introduction to DNA Barcoding provides a valuable tutorial on the process as well as links to record-keeping sheets. The club has created detailed binders that can be checked out by members wanting to engage in recording, vouchering and DNA barcoding. Currently, specimens are being sequenced at Duke University for the cost of labor and analyzed by Jacob; sequenced specimens are also stored at the Duke University Herbarium (DUKE). WPMC used the DNA barcoding work and a web-based bolete synoptic key in a project called the Northeast Bolete Consortium (see above). A key challenge to club-led mycoflora projects is illustrated by the fact that WPMC is keeping its extensive specimen documentation in a local database because critical web portals do not communicate with each other.

Southeastern Clubs: My colleague Ton Tran and I tested out the WPMC protocols on 12 haphazardly selected specimens collected during the annual Oconee State Park (SC) Foray in October 2016. The foray includes three southeastern clubs: our Mushroom Club of Georgia; Asheville (NC) Mushroom Club; and South Carolina Upstate Mycological Society. Observations are published as Species List 973 on Mushroom Observer. There is no better way to gain an appreciation of the many moving parts and challenges involved in this work!

Fungus Federation of Santa Cruz (FFSC): The most extensive club-led vouchering and sequencing project to date, along with an elegant website and Youtube videos, is that of the Santa Cruz Mycoflora Project (SCMP; www.scmycoflora.org), created by the Fungus Federation of Santa Cruz (California). Project leader Christian Schwarz views the project as "using citizen science to create a two-way flow of information between amateur naturalists and professional scientists … and to foster communities with a shared love of nature." The goal of SCMP is to create an Internet-based mycoflora of all the macrofungi in Santa Cruz County, based on vouchered and sequenced specimens. They have already identified around 1,000 species, and estimate that there may be 2,000 to 3,000 total species. FFSC allocated $15,000 and PSMS another $2,000 for DNA sequencing in 2014. As of June 2016, 500 vouchered specimens had been processed and sequenced. According to Henry Young, "Not all of them were successful for a variety of issues completely normal to the process, but we had a >90% success rate," with more analysis to be done. Finding fungarium space is a major challenge for many clubs. The Santa Cruz club solved this by devoting considerable volunteer effort, along with intern labor, to creating a fungarium at the natural history museum at the University of California at Santa Cruz.

The SCMP website is the most elegant club mycoflora site I have viewed to date. Webmaster Adam Ryszka created it from scratch with the idea it could be used as a template for other clubs (contact Adam at drcarparts@gmail.com). In the Species Index section on the SCMP website, each genus link leads to a page with links to MycoPortal and Mushroom Observer. The MycoPortal link shows all vouchered material for that genus in official fungaria reported from the county, while the Mushroom Observer link shows all observations for the genus from the county (good photos but few vouchers). For example, the SCMP web page for *Amanita* says that "approximately" 21 *Amanita* species have been recorded from Santa Cruz County. The link to MycoPortal shows 88 specimens in various fungaria (some collected more than a century ago) while the link to Mushroom Observer shows 292 contemporary observations from Santa Cruz County. Such external links are simple devices that any club could readily create on their own websites. The Western Pennsylvania Mushroom Club uses a similar approach in its Mushroom Catalog. WPMC's website also has an innovative section on Species List Visualizations.

But updating static websites is tedious work, according to Schwarz. Stephen Russell's MycoMap aims to automate pulling all internet observations into a common platform for use by the Mycoflora 2.0 Project (see below). This will facilitate the creation of club species

lists and geographic-based mycofloras.

Missouri Mycological Society (MOMS): The MOMS web page, Voucher Specimen List, provides another model for club websites. The page lists links to 155 specimens (different species), each with voucher information including multiple photographs, micrographs, voucher slips, collection information, spore descriptions and, in some cases, reference (though not yet links) to DNA barcode information. The New York Botanical Garden has agreed to take them and make the data available online through its website and MycoPortal. The club allocated $5,000 for training Brad Bomanz to do the DNA sequencing at Missouri Botanical Garden; 155 specimens have been sequenced and funds remain for another 95 specimens. At this writing, sequences for five specimens have been posted to GenBank (e.g., *Sarcoscypha occidentalis*), and Brad is working on posting the remainder to both GenBank and the club web page.

Hoosier Mushroom Society (HMS): The long-term goal of HMS is to create a vouchered and sequenced mycoflora for the State of Indiana. The club currently has more than 1,000 individual specimens vouchered and sequenced. Club president Stephen Russell, who also chairs NAMA's Mycoflora Committee, is developing an ambitious web hub for mushrooms called MycoMap (www.mycomap.com), currently in beta mode. MycoMap aims eventually to be the "catch all mycological database/platform" described by Halme et al. (2012): a one-stop shop for uploading and tracking mushroom specimens, as well as providing integrations with essential datasets including Mushroom Observer, iNaturalist, MycoPortal, MycoBank, Index Fungorum, Discover Life, Encyclopedia of Life, UNITE, and GenBank. MycoMap even has a smartphone app (in development) designed to be used on forays. A novel element is that Russell aims to conduct environmental DNA sampling of soil, wood, and tree roots, with the environmental dataset forming the "backbone" list of species they are looking to find in the state.

Oregon Mycological Society (OMS): OMS's Mycoflora Working Group aims to identify, voucher and sequence at least some specimens found during club forays. The Group is led by Joe Cohen, who is also a developer for Mushroom Observer (MO) and species lists are posted to MO. Of some 1,200 observations, 11 have been sequenced in cooperation with the Forest Service Interagency Special Status/Sensitive Species Program (see MO Species List 965). More than 100 dried specimens are kept at the homes of club members. The Mycoflora Working Group has a budget of $500 per year, most of which is anticipated to be used for sequencing.

Puget Sound Mycological Society (PSMS): PSMS is the largest mushroom club in the US. While the club has not started sequencing, they donated $2,000 to the Santa Cruz Mycoflora Project to help the latter gain experience with sequencing that will be useful to all clubs. The club's identification coordinator, Danny Miller, is currently the main programmer for MatchMaker (see next). Such keys are important components of mycofloras. Daniel Winkler is leading a new project to voucher macrofungi from Bridle Trails State Park (195 ha).

Pacific Northwest Key Council (PNWKC): The Key Council was created in 1974 as an invitation-only group of experienced amateur and retired professional mushroomers whose purpose was to create field keys to the fungi of the Pacific Northwest. Another resource is MatchMaker, a mushroom identification application for the entire Pacific Northwest with both synoptic and pictorial keys. Developed by Ian Gibson of the South Vancouver Island Mycological Society, the application can be downloaded free from the SVIMS website. Several years ago a decision was made to start vouchering and sequencing unusual finds. But that was "more of an aspiration than a reality," according to PNWKC member Joe Cohen. Council president Paul Kroger put it this way in an email to Joe Cohen, illustrating a key challenge for all mushroom clubs:

> "I recall that at certain forays we attempted to voucher all taxa found when we had collecting permits in a National Forest and the local Forest Service desired it and assisted with documenting and disposition of the preserved specimens. The task is a daunting one and involves much labor. The logistics of actually processing all the material within a weekend is a real problem, and involves enormous amounts of follow-up work. There are limited numbers of herbaria able and willing to accept such large collections."

Arizona Mushroom Society (AMS): Vouchering and sequencing at AMS is mainly due to one motivated member, Terri Clements. She is working with the Gilbertson Mycological Herbarium (ARIZ) at the University of Arizona to voucher and sequence 150 specimens currently stored at her home and posted to Mushroom Observer (MO). The club encourages all members to create a MO observation before vouchering and to note the MO number on the voucher. Last year the club paid $212 to get eight *Morchella* specimens sequenced in Spain, and has allocated $250 for sequencing next year. Notes Terri: "Our small first effort resulted in two significant findings which are indicative of what citizen science can bring to the table. The eight specimens turned out to be seven species of *Morchella* that had not yet been documented from Arizona. And one of the specimens is a new species [confirmed by Kerry O'Donnell] that will now get a name."

San Diego Mycological Society (SDMS): SDMS has a web page, Mushroom Barcoding Project. Observations are not posted online; however, a March 2014 presentation said that 70 specimens had been vouchered at the San Diego Natural History Museum and sequenced by the International Barcode of Life project at the University of Guelph in Ontario, Canada.

Canadian Clubs

In the course of researching this article I became aware of an impressive amount of fungal citizen science activity in Canada. It's worthy of a separate article. Making no attempt to be comprehensive, I'll only mention several items of interest here. (Mexico has no amateur clubs and all sequencing is done at universities, according to Alan Rockefeller who collects extensively there.)

- **MycoQuébec's** website is one of the best resources for mushroom identification in North America, with detailed descriptions of 2,958 species found in Québec Province

and 20,365 high quality photographs imported from Flickr. They also have a (static) smartphone app, *La fonge du Québec*. "MycoQuébecis doesn't represent a club in particular, but seeks to involve all mycological clubs and independent amateur mycologists (professionals are welcome too)," according to Renée Lebeuf. Several members have large personal fungaria with hundreds to thousands of specimens and numerous collections have been deposited in Université de Montréal's Centre sur la Biodiversité (many of which appear on MycoPortal).

- British Columbia has several strong clubs. Oluna and Adolf Ceska, members of the **South Vancouver Island Mycological Society**, have collected, recorded (on Mushroom Observer) and vouchered about 3,500 specimens, of which 1,166 species come from a 75 ha area that was intensively sampled over a ten-year period – a model for citizen science projects. Victoria BC is also home of Ian Gibson, the original developer of MatchMaker (see above, PNWKC).
- The **Alberta Mycological Society** has some 600 members in a province larger than California with no professional mycologists. Since 2005 they have been gathering all existing mushroom records, many vouchered, in a central website called Alberta Fungal Database. The club vouchers many specimens on forays (the default fungarium is Department of Agriculture in Ottawa). "Anything that is vouchered is sequenced," according to past-president Martin Osis. The club is currently adding DNA sequence data to its web database.
- **Foray Newfoundland and Labrador** recently uploaded collection data to MycoPortal for 2,100 specimens from five years of forays (2011-2015). MycoPortal noted: "As one of the first amateur mycology groups to send us its data, we are pleased to see your high standards for collecting, identifying, and recording appropriate specimen information" (Kuhn et al., 2016).

Challenges

Club-led projects described above have faced similar challenges that must be addressed if mushroom citizen science is to become widespread. Key operational challenges fall in three categories: documentation and data management, vouchering, and sequencing.

Documentation and Data Management: For specimens to become valuable to science, documentation of morphological and ecological data is essential. Good photographs of fresh specimens and recording features that are lost upon drying (such as taste, odor and color of the sporocarp and spore prints) is important, as are micrographs. DNA sequences can be useful for identification but specimens that are otherwise well documented will still be valuable to future mycologists who can get genetic information from dried specimens.

A critical data management issue that needs to be solved is creating an easy process for sharing multifaceted observations with key Internet websites (modern mycofloras should be Internet-based). The problem is that there are multiple web portals that do not communicate easily or at all with each other. One web portal specializes in US fungaria records (MycoPortal), another keeps DNA sequences (GenBank), and several support uploading photo-rich observations, including Mushroom Observer, iNaturalist, Flickr (preferred by Eastern Canadian clubs), and Discover Life, in addition to club websites. (Facebook and Yahoo groups are used for sharing and identifying fungal finds, but those sites are not designed as databases so they do not function as repositories.) Mushroom Observer is dedicated to fungi so it has the largest community of expert mycologists who can help with identifications, but Mushroom Observer lags behind some other sites, especially iNaturalist, in functionality for taxonomic databasing. What's needed is the capability to upload multiple records to one site; have that information propagated to other key sites via a unique identifier; and have additional information (for example, the addition of a DNA sequence or fungarium accession number) update on all linked sites.

Vouchering: Curating specimens at established fungaria (those accessible to international scientists and likely to be around a long time) has labor, equipment and space costs. These costs are difficult to assess, vary locally and are long-term investments; but they are real. A key issue for amateur mycologists is the long time to accession specimens and get catalog numbers that clubs can link to their observations. Several cases were noted above of club specimens being submitted to fungaria but not accessioned, even after years.

Sequencing: DNA sequencing costs, compared with vouchering costs, are upfront and easier to calculate. Of the three steps involved in DNA sequencing, step 1, extraction and amplification, can either be done locally or out-sourced; step 2, sequencing the extract, is generally out-sourced to a specialized lab with expensive DNA sequencers; and step 3, analysis of results, can be done locally by tech-savvy people using one's own computer and public Internet databases. Extraction and amplification (step 1) can be done in a DIY club lab for about $5 per specimen, if you don't count the cost of labor or initial cost of lab equipment. A commercial lab is needed for the actual sequencing (step 2); that reportedly costs about $5-$8 in California (Alan Rockefeller, personal communication). Commercial labs can also do both steps 1 and 2, for around $30 per specimen currently. Clubs have used MCLAB in California (mclab.com) and ALVALAB in Spain (alvalab.es) which specializes in mycology. The Osmundson lab in Wisconsin is willing to take on sequencing club vouchers at cost. And technology is constantly evolving. As noted above, Duke's Rytas Vilgalys is developing multi-locus next-gen extraction and sequencing that he hopes to offer to clubs for $10 per read. Stephen Russell is working to make his MycoMap platform provide an analysis pipeline (step 3) where users can upload their raw sequence files and get an analytical report that includes phylogenetic trees from reference datasets.

It should be noted that vouchering and sequencing are starting points rather than end points for mushroom citizen science. As vouchering and sequencing become more common practices, the most avid amateurs may be drawn into advanced taxonomic projects like finding and sequencing neotypes for missing type specimens, researching old taxonomic literature, and describing and publishing new species. Others will be

Selected Club Expenditures for DNA Sequencing

Mushroom Club	Amount
Fungus Federation of Santa Cruz (500 specimens)	15,000
Missouri Mycological Society	5,000
NAMA Vouchering & Amanita Project	4,340
Hoosier Mushroom Society (for 2016)	2,000
Puget Sound Mycological Society (to FFSC, one-time)	2,000
Western Pennsylvania Mushroom Club (annual)	1,000
Oregon Mycological Society (for 2017)	500
Arizona Mushroom Society (for 2017)	250
Mycological Association of Washington, DC (for 2017)	200

drawn to answering ecological questions.

Mycoflora 2.0 Project Overview

This review of club activity suggests that interest in citizen science is widespread and the time is ripe for developing simple, standardized protocols and workflows for vouchering and sequencing, and for providing timely feedback to collectors. A group of amateur and professional mycologists is planning a pilot citizen science project to develop such protocols so that any mushroom club—or organization such as Radical Mycology—that wants to do citizen science can do so efficiently and inexpensively. Our plan is to start with seven NAMA clubs and academic partners in different regions of the United States in the fall of 2017, with the goal of making processes created available to all serious amateur mycologists. Collection and sampling strategies will be developed in consultation with expert mycologists so that vouchering and sequencing effort is focused on filling data gaps. (For updates visit the Mycoflora 2.0 Facebook group.)

Data management will be based on a unique identifier (url or barcode) for every specimen which will link all components of each observation from all websites, including MycoPortal and GenBank. Several clubs have access to "established" fungaria in their states or regions; more will need to be founded and protocols for submitting specimens developed. During the pilot phase, clubs will upload documentation and photographs to any platform that databases individual observations (including Mushroom Observer and iNaturalist). Experts will review observations and help determine which should be vouchered and sequenced. DNA "smash cards" will be sent to Duke University for sequencing (see below).

Observations will be pulled into MycoMap and fungarium information will be linked to MycoPortal (pending application program interface, or API, access). MycoMap software will facilitate creation of club checklists of documented specimens, lists that can also be displayed on a club's website. The software will also facilitate creation of mycofloras of defined areas (e.g., county or state) by incorporating all species records posted anywhere online, including historical records from MycoPortal. MycoMap will generate maps and display data on phenology and distribution. Observations can be filtered by reliability and completeness of information. DiscoverLife will archive all images from resulting species lists and mycofloras.

Sequencing will be done, initially at least, at Duke University, in Rytas Vilgaly's lab. At the outset the single ITS locus will be sequenced, but eventually Vilgalys plans to make multi-locus "next-gen" sequencing technology available to the project. Whatman FTA Plantsaver cards (a.k.a. "smash" cards) will be provided to participating clubs or individuals, who will only need to take tiny tissue samples from specimens

to be vouchered, smash the card with a hammer, and mail the cards to the central location. Our goal is to enlist academic mycologists to help raise funds so that sequencing, analysis and posting results on GenBank is done for all clubs at little or no cost, thereby valuing and integrating the contributions of amateurs into professional mycology (see next section). We are currently looking into funding from the National Science Foundation (for a Research Coordination Networks grant or writing in citizen science outreach components to new research grants) as well as to clubs, organizations and other sources.

Roles of Amateur and Professional Mycologists

Engaging amateur mycologists in the Mycoflora 2.0 Project is critical because there's simply no way that academically trained professional mycologists alone can create a North American mycoflora. For one thing there are simply not enough of them. There's been a significant waning of funding support for taxonomic research over the last several decades (Wheeler et al., 2004), resulting in a steep decline of professional taxonomists (Pearson et al., 2011). Mycology is no exception. And compared with botanists there are considerably fewer professional mycologists, and far more species of fungi than plants. Combining both factors, Bruns and Beug (2012) calculated a ratio of organisms to scientists that is conservatively 30 times worse for fungi than for plants. As academically trained professional taxonomists become rarer, the importance of self-supported expert amateurs increases. Vellinga (2013) noted that in Europe "in the last 10 years the number of species described by non-professional mycologists is greater than the number of species described by people who have mycology as their profession."

The role of academic mycologists also needs to change. Pearson et al. (2011) note that the decline of all fields of taxonomy means that surviving taxonomists need to work more closely with expert amateurs. Virtually all taxonomic fields started out dominated by amateurs but "over time theoretical and institutional developments lead reliably or even inevitably to the exclusion of expert amateurs" (Pearson et al., 2011). As taxonomy transitions "out of the museum and into cyberspace" opportunities are opened up for citizen scientists to engage and make substantial contributions. Besides providing technical support, academic mycologists could solicit funding from their traditional research sources (primarily National Science Foundation in the US) for DNA sequencing and fungarium space for specimens collected by citizen scientists. Other actions suggested by Pearson et al. (2011) include: (1) "writing grant proposals that are more likely to be funded by a wider range of private and government agencies through emphasizing support of youth education and cost-effective 'pro-ams' (professional-amateurs, i.e., "serious and committed citizen scientists who function at the level of some professionals but are not paid for their work"); (2) recruiting and mentoring amateur enthusiasts; and (3) sponsoring workshops and symposia in which professionals and pro-ams can interact and socialize with one another." For more on the economic value of 'pro-ams' see Leadbeater and Miller (2004).

It seems clear from activities described here that many amateur mushroomers are ready to make mushroom collecting a more scientific hobby. Harnessing the enthusiasm of amateurs through mushroom clubs and other organizations to do high-quality citizen science can not only help professional scientists, it can inspire members of the broader public to understand and want to conserve nature. With appropriate coordination, development of protocols, cyberinfrastructure, and some external funding, amateur mycology could become a poster child for high-quality citizen science. The results will benefit both amateur and professional mycology, while advancing taxonomic, ecological and conservation science.

Acknowledgements

I wish to thank the many people who shared detailed information on their clubs and programs, most of whom are mentioned in the club descriptions. Thanks also to the 38 NAMA club leaders who responded to our survey. Special shout out to those who commented more broadly on the manuscript, especially Stephen Russell, Richard Jacob, David Rust, Christian Schwarz, Joe Cohen and Else Vellinga. Finally, thanks to John Pickering for encouraging the pilot project in the first place; and to Rytas Vilgalys and Tom Bruns who are providing guidance on development of Mycoflora 2.0.

Literature Cited

Beker, H., 2016. The *Hebeloma* Project Progresses: Citizen Science at Work. *The Mycophile* 56(5): 1-3.

Bruns, T.D. 2011. President's corner: Working toward a North American mycobiota for macrofungi – what's stopping us? *Inoculum* 62(4): 1-3.

Bruns, T.D. 2012. The North American Mycoflora project–the first steps on a long journey. *New Phytologist*, 196(4): 972-974. http://onlinelibrary.wiley.com/doi/10.1111/nph.12027/full

Bruns, T.D., and M.W. Beug. 2012. Working toward a North American Mycoflora for Macrofungi. *McIlvainea* 21. http://www.namyco.org/working_toward_a_north_america.php

Halme, P., J. Heilmann-Clausen, T. Rämä, T. Kosonen, and P. Kunttu. 2012. Monitoring fungal biodiversity–towards an integrated approach. *Fungal Ecology* 5(6): 750-758.

Kuhn, A., R. Baldree, T. Iturriaga and A. Miller. 2016. Foray Newfoundland and Labrador collections on MyCoPortal. *Omphalina* 7(7): 12-13. http://www.microfungi.org/files/5114/7777/4138/O-VII-7.pdf

Leadbeater, C., and P. Miller. 2004. The Pro-Am revolution: How enthusiasts are changing our society and economy. *Demos*, 70 pp. https://www.demos.co.uk/files/proamrevolutionfinal.pdf

Matheny, P.B., and E.C. Vellinga. 2009. A North American flora for mushroom-forming fungi. *Inoculum* 60(5): 28–29.

Pearson, D.L., A.L. Hamilton, and T.L. Erwin. 2011. Recovery plan for the endangered taxonomy profession. *BioScience* 61(1): 58-63. http://bioscience.oxfordjournals.org/content/61/1/58.full.pdf+html

Vellinga, E.C. 2013. The North American Mycoflora Project: without a sequenced specimen it is a rumor. *McIlvainea* 22: 2-10. http://www.namyco.org/mycoflora_project.php

Wheeler, Q.D., P.H. Raven, and E.O. Wilson. 2004. Taxonomy: impediment or expedient? *Science* 303(5656): 285.