

Whither the Field Mycologist?

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n the past century, we have gone from the age of physics to the age of chemistry (which began just after World War II) to our current scientific epoch: the age of biology. Some would argue that, more specifically, we're in the age of biotechnology. Or molecular biology. It's no surprise that molecular biology and genetics are now incredibly important to current biological research. Likewise, our understanding of the living world around us is accelerating at a pace that makes many of us, even those with Ph.D.s in biology, dizzy. In this, the bicentennial of the birth of Charles Darwin, and the 150th anniversary of his landmark On the Origin of Species (1859), which many consider to be THE most important published work ever in biology, it's interesting to consider how much he got RIGHT about how organisms evolve-and without any knowledge of the genetic basis of inheritance.

Darwin's tremendous success, of course, was due to his incredible patience and eye for detail. He devoted decades to the microscopic study and illustration of morphological forms of thousands of barnacles, as one example, before publishing seminal volumes on their taxonomy. How would modern research tools and techniques – many of them DNA sequence-based – have shaped Darwin's work? Is there even a place nowadays in the world of taxonomy for a modern-day Darwin? If you attend a single mushroom foray this year, you will no doubt hear this issue being lamented.

Although fungi have been studied for centuries, mycology has never gotten the acclaim of other branches of biology; botany, zoology, and medicine—all have much more storied pasts. Furthermore, fungi were only the fifth kingdom to be recognized. No doubt many readers remember when fungi were still thought simply to be evolutionarily lower forms of plants—it wasn't all that long ago. Many thousands of species of fungi have been described and named, but most mycologists agree that the known species account for somewhere around five to ten percent but maybe only about one percent—of the total species of fungi on the planet.

"Classical" taxonomy of fungi (as well as of plants and animals) has always relied on those characteristics of a group of organisms that could be seen with the naked eye and, later (thanks to Antonie van Leeuwenhoek and others), the microscope. Every one of you reading this, no matter the proficiency of your skills, notes characteristics about macrofungal sporophores ("mushrooms") on a regular basis that tell you: first off, is it fungal or not; then maybe it's the presence of gills or pores; color of the spore print etc.; on down the line. These are morphological characteristics. They can be macromorphological-those seen with the naked eye-or they may be microscopic. Some of the characteristics you may note about mushrooms may be chemical, such as when you break open a bolete and watch for the tissues to turn blue; or perhaps it's a reaction the mushroom tissues may have when treated with some simple compounds like Melzer's Reagent, potassium hydroxide, or iron sulfate. But keep in mind that the *phenotypic* (or physical) makeup of an organism is the manifestation of the organism's genotypic (or genetic) genetic makeup.

The morphology of sexual reproductive structures, sexual compatibility, and the ability to produce viable progeny are characteristics that taxonomists have long employed for many organisms, especially plants. This can be tricky with fungi as many types are not even known to reproduce sexually, and sexual reproductive structures may not be known either. Fungi are enigmatic in many other ways: phenotypically, too. For these reasons and more, the tools to use for investigating the genotypic characteristics of fungi have been attractive to researchers since they became available only a few short decades ago.

So, is the "classic" or morphological fungal taxonomist or field mycologist going by the wayside? Is DNAsequence-based taxonomy going to rule the day? To hear the talk of mushroom foray-goers and some taxonomists, you would think that this is already the case (Korf, 2005). It's justifiable, I suppose, that attendees of mycological society lectures roll their eyes when they hear "new" names placed on fungal species they've long grown accustomed to calling something else. And what's with the phylogenetic "trees" that seem to sprout from the pages of all taxonomic research papers these days. (Time was, a tree was something that grew in the forest, had leaves and played host to numerous fungal symbionts and pathogens!) Are we going to have to get used to a perpetuation of name changes and arguments over who is related to whom, evolutionarily? (Never mind that those arguments were ongoing long before DNA was discovered!) So . . . whither the field mycologist?

To find out, I needed to seek the opinions of well-regarded mycologists on both sides of the aisle - from a field mycologist as well as a molecular mycologist. The opportunity presented itself last summer at the Annual Meeting of the Mycological Society of America. During a lecture by Nathan Wilson (of MushroomObserver.org) I looked around at the audience filling the huge lecture hall. There were mycologists of every variety: molecular taxonomists, yeast biologists, medical and field mycologists. After the lecture ended and during the discussion of many questions that followed, I made my way to the back of the auditorium and positioned myself near the exit. Following thunderous applause, the audience filed out and began a slow stampede towards the pastry table that had been assembled in the lobby, in time for afternoon coffee break. I nabbed two esteemed mycologists and, knowing no academic will pass on a freebie, offered to treat them to a proper coffee and gourmet pastries at a nearby coffee shop.

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I sat down, took out my notepad, and explained to the two scholars my purpose for the interview. Professor Ivanna Forré, Curator of Mycology Collections at an esteemed museum in the Midwest, who also serves as Adjunct Professor at an equally well-known university in Chicago, and Doctor Gene Jøkke, the endowed Chair of the Molecular and Cell Biology Department of a worldrenowned university in New England that everybody knows about, both welcomed the opportunity. I put the same questions to both academics: what does the future hold for fungal taxonomy research? Will molecular biology mean the end of the field mycologist?

FUNGI: So, Professor Forré, how do you think molecular biology will shape the future of mycology?

Forré: Please, you can call me Ivanna. Well, I think it's myopic to put too much faith in taxonomic studies based solely on DNA sequences. Field mycology seeks to explore and document biodiversity. DNA phylogenies are based on a few individuals and often sequences simply are compared to collections of other sequences. I really don't see how much can be learned from this approach. Collections of DNA sequences are incapable of handling unique aspects of biodiversity studies such as diverse and large collections of specimens, and taxonomic uncertainties and revisions.

Jøkke: Let me first say that unless you are from Scandinavia, you cannot possibly pronounce my name correctly, but it is close enough to simply say it "jockey" like the man that rides on the racehorse. Now, to rebut Professor Forré. First, let me say that the many collections of DNA sequence data held at universities and elsewhere are already an extraordinary sampling of biological diversity and this is unmatched by any museums or natural history data. As one example, in GenBank there are nearly 200,000 species represented which is five to ten percent of all known species – not just fungi – and this includes some 900 complete genomes.

Forré: I'm glad you mentioned GenBank, Professor Jøkke, because, as you know, GenBank does not require vouchering of specimens, DNA extracts, or other molecular data, whereas the study of natural history always anchors information with specimens. Without vouchers, revisionary work — which is part of the tradition of natural history and is always ongoing in the field of taxonomy — cannot be conducted. As a result, GenBank contains a considerable amount of unidentified or misidentified sequences and this is one of the biggest criticisms of the use of genetic data versus field data.

Jøkke: Yes, my colleague is correct, technically. It is widely known that collections of DNA sequence data do contain some bad sequences, for example, some primer sequences or contaminants occasionally get deposited. I think for GenBank, since this was the example used, it is on the order of one-tenth of one percent. But ALL significant natural history museums likewise contain unidentified and misidentified specimens. Indeed, as you point out, systematic vouchering of specimens strengthens the reliability of biological collections, and it is for this reason that for more than a decade GenBank has included a data field for the specimen voucher. Presently, more than 600,000 sequences contain some kind of voucher information. Now, it is also true that museums have long been repositories for specimens and vouchers, but that does not mean that all specimens are actually available to researchers. I can think of collections that were or have been kept off limits to the

scientific community. For example, many specimens of the famous Burgess Shale where kept private for years while select information was slowly leaked out. Even the great Charles Darwin sat on his data on evolution for decades before finally making it known to the world.

Forré: Even so, you cannot possibly argue that collections of DNA sequence data are as likely to reach amateurs in the way that natural history has. Take our field, mycology. Although I doubt that you've attended many mushroom forays, but if you had, you wouldn't see a lot of folks standing around tables, peering down at long strings of Gs, Cs, As, and Ts! Nobody's running electrophoresis gels during the lunch breaks! (We all chuckled.) Field mycology generally does not require expensive equipment and is exciting and rewarding to thousands of amateur mycologists in North America. In comparison, DNA sequence information is costly, offers little aesthetic reward or recreational value, and requires specialist knowledge. Mycological clubs have an opportunity to make real contributions to the field through forays where they collect specimens, get them identified and vouchered, and keep good records over decades. Several of the legendary mycologists of North America, who have made monumental contributions to our knowledge and named countless species, were amateurs. I don't see a future where amateurs can make any sort of contribution to a molecular data driven field. And thus a lot of ecological information will cease being collected.

Jøkke: It is certainly true that much of the DNA sequence information, until now, has been provided by professionals. Who knows, that might change someday with the falling cost of genome sequencing. However, natural history collections are not so different, since most specimens have been identified by professionals, not by amateurs, and this is true in mycology. Professor Forré may be taking some liberties when she romanticizes about the availability of amateurs to contribute to field mycology when she suggests that it doesn't require expensive equipment. Her multimillion dollar grants belie this notion. The truth is that most natural history endeavors have been anything but inexpensive. Field surveys that have taken her all over the world have advanced the field, surely, but at no small expense. Funding her museum or the Kew Gardens or even a university herbarium requires that institutes seek out all sorts of private monetary support because it is costly.

Forré: Well . . .

Jøkke: And another thing: just because you have a museum full of specimens — actual skin and teeth and bones, doesn't mean you have enough information to make conclusive statements. Keep in mind that we didn't know who was humans' closest relative, gorilla or chimpanzee, until DNA techniques came along. Or breeds of dog – if we had only fossils to go on, surely morphological taxonomists would conclude that a Chihuahua and a Newfoundland were different species! Likewise, DNA sequence data has revealed species hidden right under our noses. For example nobody knew that there were two species of elephant on the African savannahs until someone starting playing with the DNA. So molecular data certainly has a place in biodiversity studies and in the conservation of flora and fauna.

Forré: Well, I just think that the data coming from field mycology work has many important facets, such as specimen locality data, images of morphology, and ecological notes. Specimen collections will continue serving as the primary record of natural history and biodiversity, while molecular data can never truly be anything more than a supplement to real field mycology work.

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Jøkke: Professor Forré, I share more of your opinions than you can imagine. But the heart of the matter is that you seem to fear that molecular data will replace specimen collections. And I don't think so. We scientists are all about seeking the truth. You ignore the fact that natural history is not fundamentally about specimens, but about natural facts. Mushrooms are among these, sure, but so are rocks, bones, shells, and other items that end up in data collections. And now DNA sequences. Your fears are nothing new . . . this is the same concern that numerous naturalists have voiced since the beginning of the 20th century, which is that the lab-based science would take over field-based science. That field mycology and morphological taxonomy would be supplanted by molecular taxonomy. I assure you, DNA sequence technology is just one more tool we can use - in conjunction with all the other tools in our mycological toolbox-to get at the facts. A classification system, that is, the species names and their arrangement into higher, named categories, is most useful to the amateur, professional, or applied scientist — someone like a plant pathologist, for example — if that system is based on the best estimates of evolutionary relatedness. Mycologists were in the business of changing species names long before DNA came onto the scene and will likely continue to do so. And I would be willing to bet that molecular data agrees with and strengthens the comparative morphological data from the classical taxonomists about 95% of the time anyway.

FUNGI: Does anyone have anything else to add?

Jøkke: I would simply reiterate that DNA sequence technology is just one more tool we can use to better understand fungi and their evolution. I would argue that the controversy between field mycology and molecular mycology is becoming increasingly irrelevant in the 21st century as the boundaries between specimen collections and molecular data collections are becoming increasingly blurred. Going back to GenBank, many DNA sequences are now linked to specimens in natural history museums. The convergence of specimen and sequence collections has proceeded smoothly precisely because both function in the same tradition of field research.

FUNGI: Well it certainly seems like there is less of a divide between your two camps than I originally thought. It's also made me realize that identification is a different exercise than classification. Regardless, I think we can all agree that the fungi are amazingly fascinating and beautiful organisms.

Forré and Jøkke (*in unison*): Agreed! [And at that same time, they both reached for the last piece of coffee cake, whereupon their hands met . . . then their eyes locked on the other's. A sudden feeling on third-wheeledness came over me.]

Jøkke: I wonder if the Professor would like to join me for some lunch where we might discuss some future research collaborations?

Forré (pushing herself away from the table): I think THAT is a splendid idea!

FUNGI: Yeah, you two go enjoy your lunch . . . I need to stay here and organize my notes . . . get ready for some more lectures or something . . . don't mind me . . .

Forré and Jøkke (walking away and chatting): . . . and next, I think we should turn our attention to the northward spread of *Amanita thiersii* . . .

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No doubt some mycological "curmudgeons" (to use the words of Korf, 2005) will continue to dismiss phylogenetic studies based on DNA sequences. Others seem to be prepared to say good riddance to the "classical" fungal taxonomy based on morphological characters (Kuo, 2007), finding it suitable for the purposes of mushroom identification, but that's about it. Kuo argues that comparative morphology is pretty much "unscientific" and of little use in making evolutionary (thus, taxonomic) statements about fungi. Some would point out that there is importance to being able to identify fungal species without having to be burdened with making sure that the name you use is evolutionarily based (Leonard, 2008). Laurie Leonard would be quick (and was so in his reply to Kuo) to point out that when he has mere minutes to determine whether or not a mushroom was involved in a poisoning case, the last thing on his mind are phylogenetic inferences. Efforts to elucidate the toxic properties of some mushrooms, even common ones responsible for many deaths (e.g. Russula subnigricans), has been hampered by "incorrect classification" (Matsuura et al., 2009).

That many outside the academic community are resistant to give much credence to phylogenetic conclusions (I'm not referring to Leonard, by the way) is not surprising when half the US population doesn't even believe in the very obvious facts about evolution. To me, this is very disturbing, but outside the scope of this discussion.

Many (myself included) will continue to struggle with new names placed on species that have long been familiar to us. I, for one, can appreciate recent attempts to elucidate a more sound phylogeny of fungi. And this was the crux of Kuo's paper (2007), concluding that if we are to ever attain a better understanding of the fungi around us we will need to take into account morphological characteristics and DNA sequences, sure, but also ecological information (habitat, meteorological and temporal information, insect associates, etc.). And when making mushroom collections (attention foray leaders!), the importance of annotated vouchers cannot be over emphasized (a dried *Lactarius* without notes on latex color and color changes is not much help, even if one can get DNA from it!). And this is where mycological clubs and amateurs can play a significant role. Who better to document ecological information than those who observe and collect mushroom species on a regular basis! Routine foraying by expert "amateurs" (for lack of a better descriptor) does unveil new species (Minnis et al., 2006) and new regional, country or continental records (Bunyard et al., 2008; Thorn et al., 1990). You never know when well-taken records of mushroom clubs can lead to landmark discoveries about the planet's health (Gange et al., 2007). Sometimes a chemical difference between two well-known groups within a "species" is shown, in the light of DNA sequence analysis, to have been useful all along to discriminate between two closely

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related species. Such was the case with different populations of *Sarcodon imbricatus* that had long been collected by those making natural dyes from the mushroom fruitbodies (Johannesson et al., 1999). Conversely, different morphological characters between "species" wellknown to us all, can at times be misleading (as in the case of *Morchella* spp.) (Bunyard et al., 1994).

The science of mycology is going to continue to rely on field collections and field collectors. The field mycologist—be she professional or be he an enthusiastic amateur—will remain important to the science. And you can help! Get out there and collect specimens; attend forays; take part in field studies. In fact, field collections are now being made with greater urgency as we witness our planet's ever-diminishing wild habitats and corresponding biological diversity.

Acknowledgments

I would like to express my sincere thanks to George Barron (University of Guelph), Gary Lincoff (NY Botanical Garden), Nicholas Money (University of Miami, Ohio), Moselio Schaechter (Emeritus, Tufts University), R. Greg Thorn (University of Western Ontario), Else Vellinga (University of California-Berkeley), and Michael Wood (MykoWeb) for their helpful comments during preparation of this manuscript.

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