

Mushrooming in the Age of DNA: Now Comes the Fun Part

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The first reward of tree study—but one that lasts you to the end of your days—is that as you walk abroad, follow a rushing stream, climb a hill, or sit on a rock to admire the view, the trees stand forth, proclaiming their names to you. Though at first you may fix their identity with more or less conscious effort, the easy-to-know species soon become like the faces of your friends, known without thought, and bringing each a host of associations.

—D. C. Peattie (1948/1991, p. 156)

I LOVE THIS passage, which comes from Peattie’s 1948 *A Natural History of Trees of Eastern and Central North America*. Peattie’s words capture perfectly a sense of familiarity with nature known to many naturalists. Last summer, my wife Kate and I walked through woods in southeastern Kentucky, where entire ecosystems are being laid to waste by a coal mining practice called mountaintop removal (if you have Google Earth, zoom in on the region; you can see the devastation from an altitude of 400 miles). We wanted to see what mushrooms might be threatened by destruction of an ancient Appalachian ecosystem known for its incredible biodiversity, and we spent about half an hour walking, looking for mushrooms, and cataloguing what we could recognize—a cursory, preliminary list. We will be returning with mycologists from Illinois, Ohio, and Tennessee to create a rigorous survey of fungi, but in that 30 minutes we wrote down more than 60 mushroom names, from *Craterellus cornucopioides* to a host of *Amanita* and *Lactarius* species that we did not recognize at sight. It is a wonderful feeling to know the names of mushrooms—and while fungophiles must frequently be content with recognizing a genus rather than a species, the feeling that Peattie describes contributed deeply to our enjoyment of those beautiful Appalachian woods.

But it must be said—not just as an ironic aside, but as a fundamental matter of science—that the trees and mushrooms *do not care* what their names are. The taxonomic system we inher-

ited from Linnaeus reflects *our understanding* of natural organisms, not the organisms themselves. Hopefully, our understanding is “accurate,” meaning that it corresponds with evolution and selection in the natural world—but this happy agreement between nature and taxonomy is fundamentally out of reach, and never a certainty, since taxonomy is composed of *hypotheses*, to be supported (or not) by available evidence. I restate these well known principles here because they are frequently forgotten in biology and *especially* in mycology, where the hypotheses that underlie traditional taxonomy are almost always unstated—probably for good reason, because they are often ludicrous. To demonstrate this, one need only put words to the actual hypothesis that underpins most of the taxonomic keys in mycology:

1	Displaying a morphological feature whose predictive value is as far as we know coincidental, since we have no working theory about how the feature is actually related to natural selection and speciation.	Subgenus <i>Somethings</i>
1	Not displaying this feature.	2

This is the essence of the vast majority of the couplets found in mycological monographs. Cystidia, colors, sporal dimensions, reactions to chemicals and reagents, tubes that are individually discrete . . . the list, of course, goes on and on, because mycologists have rarely bothered even to think about what selective advantage any of the features might provide for an organism; taxo-

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onomic mycology has been predominantly *atheoretical*—which is, frankly, another way of saying it has been largely unscientific. It sounds odd to claim that an effort dependent on highly technical monographs, jargon, and advanced microscopy skills is often “unscientific,” but we should not confuse the trappings of science with science itself, which involves hypothesis and theory. This becomes clear if we consider an alternative to the key couplet above—one in which a morphological feature’s selective relevance is theorized:

1	Rhizomorphs aggregated into a stemlike structure that holds the spore-producing machinery high enough to avoid being covered with drifting sand on exposed beaches and dunes.	<i>Scleroderma septentrionale</i>
1	Rhizomorphs present but not aggregated into a stemlike structure since the organism grows in wind-protected environments.	2

Here we have an actual hypothesis involved with the taxonomy—one that we can test with the experiment of further collection and morphological analysis. If we collect hundreds of *Scleroderma* specimens and find that the ones growing in sand dunes all manifest the pseudostipe, while specimens growing elsewhere do not, we have supported (not proved!) our hypothesis, especially if we can correlate this data with other support (morphological or otherwise). Just to be clear, it is not the inclusion of the hypothesis in the wording of the couplet that is at issue here; it is the presence of a hypothesis at all. It is not as though the many couplets in mycological monographs referring to cystidia, for example, merely leave the authors’ hypotheses about cystidia unstated in order to avoid being wordy and repetitive. The intent of mycological taxonomy, according to Korf (2005) in a controversial paper that I will discuss in more detail below, “was almost always to provide a classification that reflected relationships, deduced from comparative morphology.” This sounds suspiciously like what I have been describing, and while it is certainly a more presentable form of the idea than “If they look different to me they must be different,” there is considerable conceptual overlap between the two ideas.

The use of morphological features like cys-

tidia or the color of the cap can be very useful, of course, when it comes to *identifying* mushrooms—as long as we acknowledge that the presence of the cystidia (for example) is as far as we know coincidental and prepare ourselves for the possibility that our flimsy hypothesis will be stood on its head. But keys that have identification, rather than classification, as their sole *raison d’être* are few and far between in the world of technical mycological publications. Korf’s wonderful “Synoptic Key to the Genera of the Pezizales” (1972) is an excellent example of an identification key without taxonomic pretenses—as is the Volk & Burdsall (2005) key to *Armillaria* (in which the researchers defined the species on the basis of mating studies, then searched for morphological characters that might successfully predict the species) and the den Bakker & Noordeloos (2005) key to *Leccinum* (species defined by DNA and ecology, morphological characters used in part for identification). However, the vast majority of keys in mycology (at least, the ones I am familiar with) are taxonomic, not merely identifying but *defining and arranging* taxa on the basis of untheorized morphological features.

Anyone who studies fungi, even casually, is familiar with the taxonomic frustrations that permeate the discipline. We are told that *Lepiota americana* is really *Macrolepiota americana*, then *Leucoagaricus americanus*—more closely related to *Agaricus campestris* and *Coprinus comatus* than to *Lepiota cristata*. DNA studies like the ones that produced these mind-blowing revisions are in vogue, but while I readily concede that a few DNA studies may be irresponsible and unscientific, most of them are not—or are at the very least no more irresponsible and unscientific than what preceded them. In short, the frustration we feel is the result of having built a huge taxonomic house of cards on the basis of a flimsy theory; it is the inability of “comparative morphology” to predict natural relationships consistently that has created the problem, rather than competing evidence from DNA studies, mating studies, or anything else.

In the words of one mycologist, we are in a period of transition as far as the study of mushrooms is concerned. We know the faults of the old system, but since the new one is still in the forma-

tive stage undoubtedly many of its faults have not yet been discovered.

Some readers will recognize these words, which do not come from a contemporary mycologist but, perhaps ironically, from Alexander Smith's 1949 introduction to his *Mushrooms in Their Natural Habitat*. I quote them here to underscore the fact that Smith—who is generally seen as an iconic representative of micromorphology-based mycological taxonomy in North America in the last century—was immersed at the beginning of his career in a similarly frustrating era of taxonomic instability as *Psalliota*, for example, exploded into *Agaricus*, *Oudemansiella*, and other genera. More importantly, however, Smith's words make it clear he understood, at some level, that taxonomy is *perpetually* unstable because it is an elaborate hierarchy of hypotheses (read: “potential house of cards”) that may prove to be untenable.

There are many complaints that can be lodged about DNA research in mycology. Some of these complaints (“I don't want to have to learn a new name for *Lepiota americana*,” for example, or “In the woods, those molecular biologists wouldn't know a *Leccinum* from a beer bottle”) have more to do with personal misgivings or social concerns, and are probably not worth discussing here. Other complaints—especially the one that faults some DNA papers for proposing sweeping taxonomic changes based on molecular data from something like 10–20 mushrooms—may be more reasonable. But it must be acknowledged that my complaint against comparative morphology—that it (often) lacks any decent theory about natural selection—cannot be maintained against molecular biology. The theory that an organism's DNA expresses its genetic identity is one that no scientist would deny, and the quickest way to demonstrate the massive evidence supporting the theory is to try imagining the kind of evidence that would make it unstable: discovery of a living organism that has no DNA, or an organism whose DNA appears to code for features that are *not* present when the features are present in every other organism whose DNA is so coded. On the basis of current evidence, both scenarios are preposterous.

In my view the most significant finding of mycological DNA studies is, frankly, one that should have been “discovered” long before

DNA entered the picture: that mushrooms have evolved with other organisms within ecosystems, and their classification cannot be adequately accomplished without keeping ecology in the foreground. Results from DNA studies support this idea again and again; if you are interested in seeing a few examples I recommend the recent papers on *Leccinum* by den Bakker and his collaborators (2004, 2005) and a paper by Kretzer and collaborators (1996) on *Suillus* (full citations for these works can be found below).

When DNA studies do not support ecological hypotheses, it is often the case that ecological data is simply missing or too poorly documented to be incorporated into the research. A recent study of the genus *Gymnopilus* (Guzmán-Dávalos et al., 2003) is an excellent example, and also serves to underscore some of the points I have been making about morphology-based taxonomy and its pitfalls. The paper's title, “Traditional infrageneric classification of *Gymnopilus* is not supported by ribosomal DNA sequence data,” pretty much sums up what the researchers discovered when they sequenced DNA from over 50 *Gymnopilus* specimens. The “traditional” way of looking at *Gymnopilus* (Romagnesi, 1942; Singer, 1986; Hesler, 1969) divides the genus into two major groups: the *Annulati* group, which features mushrooms in which there is a “[v]eil forming a membranous to densely fibrillose, persistent annulus” (quoting Hesler, 1969—the major monograph for the genus in North America); and the *Gymnopilus* group (“[v]eil absent, or present and fugacious, not forming a persistent annulus”). The *Gymnopilus* group is further subdivided by Hesler as follows:

1	Spores 3.5–7 μm long; if 6–8 μm long, take next choice.	sect. <i>Microspori</i>
1	Spores (6–) 7–9 μm long.	sect. <i>Gymnopilus</i>

Guzmán-Dávalos (1995) later proposed three, rather than two, subdivisions to the *Gymnopilus* group:

1a	Spores 3.5–6.5 μm long.	sect. <i>Microspori</i>
1b	Spores longer.	2

2a	Spores 6–9 (-10) μm long.	Sect. <i>Gymnopilus</i>
2b	Spores 8–11 (-12) μm long.	Sect. <i>Macrospori</i>

I am quoting the “traditional infrageneric classification of *Gymnopilus*” at length because—with no disrespect intended to those who constructed it—it is *self-evidently* unscientific. Entire taxonomic divisions have been erected on the basis of whether the ring is “persistent” or not, and on differences in spore lengths—without even a passing guess as to how these features might relate to natural selection and evolutionary history in the genus. This is the “comparative morphology” mentioned above by Korf, but the hypothesis-based, deductive reasoning he champions is simply missing here. Not unstated: missing. We did not need a DNA study to tell us that our taxonomic arrangement of *Gymnopilus* was artificial and that the species we named on the basis of that arrangement are questionable; there could easily have been a paper entitled “Traditional infrageneric classification of *Gymnopilus* is not supported by anything approaching scientific hypothesis” long before 2003.

So it should come as no surprise that Guzmán-Dávalos and her collaborators found no DNA support for previous arrangements of the genus. The results supported dividing the 50+ *Gymnopilus* specimens studied into five groups, some of which “have no obvious morphological synmorphies that clearly define them,” and the study concludes that “[p]artial veil characters and basidiospore size are highly homoplastic characters.”

The study does not correlate any ecological data, so we do not know whether the five species groups might be partially or completely understood with reference to the ecology of the mushrooms. I tried to uncover such a possibility by plotting out each species in the study by its DNA-defined group and by its substrate—but while there are hints of a few patterns, they are not well supported because documentation of substrate in *Gymnopilus* literature is almost entirely insufficient. Phrases like “conifer and deciduous logs” or “rotten wood, West Indies” are the rule (my favorite is: “small excavation near a farm house”); more precise information (on “decayed stipe palm,” for example) is the rare exception.

Some headway might be made by a thorough investigation of collectors’ notes in herbaria, but the kind of rigorous ecological data that might reliably support conclusions is not likely to be hidden on collection cards, since collectors have never held ecological data to be very significant.

Mycology, if it is ever going to reach some kind of actual *understanding* of the genus *Gymnopilus*, must return to somewhere in the vicinity of Square One and a Half and begin to compile a robust record of ecological data, accompanied by morphological and molecular data (as well as data from mating studies). Collectors will need to document substrates with precision, as well as forest types, weather patterns, evidence of animal (especially insect) activity—in short, the fullest documentation of ecology possible. This way there is a more legitimate, though still unfocused, hypothesis being tested: that the mushroom has evolved in an ecosystem and that such data will be integral to understanding the mushroom. Subsequent, more specific hypotheses will undoubtedly suggest themselves as the data come in—including hypotheses regarding the morphology of the mushrooms. Perhaps the scales on the cap of one species represent an adaptation to drier ecosystems, handily holding precious moisture on the mushroom rather than letting it slide away. Or perhaps the same scales are the side-effect of an adaptation for a thicker, denser cap surface that protects the vital spore-producing hymenium below it from increased sunlight. Perhaps a species has developed darker pigments to hold heat in low-sunlight forests. Yes, I have sewn all of these examples out of whole cloth on the spur of the moment, and the stitchwork is not even particularly adept. But the concept is clear enough: a robust base of ecological data will allow us to think this way and restore a sound basis in hypothesis to mushroom taxonomy.

Korf’s paper, “Reinventing taxonomy: a curmudgeon’s view of 250 years of fungal taxonomy, the crisis in biodiversity, and the pitfalls of the phylogenetic age,” has become a rallying point for amateurs and professionals who are frustrated with impenetrable, jargon-laden DNA studies and long for the good-old days when *Lepiota americana* was *Lepiota americana*. I disagree with Korf on whether what he calls “comparative morphology” is, in and of itself, sound science—and I think he is probably wrong when he says it is

“clearly impossible to equate DNA sequences with taxonomic insights.” But whether I am right (comparative morphology usually lacks a sound theoretical base; DNA studies have one) or Korf is right (comparative morphology is hypothesis-driven and based on sound theory; DNA studies are not), we are headed, believe it or not, for the same conclusion. In Korf’s words, “[w]e *must* collect, collect, and collect.”

What mycology needs now is data from collections—*especially* ecological data, but also data from morphological studies, DNA studies, and mating studies. Korf documents a sad state of affairs in contemporary academic mycology: grant funding given primarily to DNA studies of a few crusty and poorly documented specimens in herbaria; the “bean-counting mentality” of universities and research institutes that prioritize faddish publications; the inability of fungal taxonomists to find positions . . . all of this in a field that was never highly popular to begin with and has been struggling to keep itself afloat within biology departments for decades. Some of these problems may be inherent to academia (this, anyway, is what my 20 years of experience in academic literary studies, where more or less equivalent problems occur, suggests), but mycology is very fortunate to have a large body of experienced and able collectors and enthusiasts *outside* of academia: amateur mycologists, mushroom hunters, and a large network of mycological societies and mushroom clubs across the continent.

Now comes the fun part. It is up to us, the amateurs, to provide mycology with the mushroom data it needs so badly. If the science must wait for academics to do it, it will never happen. I teach English at a university with over 10,000 students. In my department we have about 300 majors and provide a minimum of two classes for every student on campus; in Biology there are two mycology students and two elective courses in mycology, one of which is taught every other year. In fact Kim and Vince may represent a disproportionately high number when compared to the average number of mycology students at American universities, since the chair of our biology department is a prominent mycologist who attracts students to the field. However, there are two major mycological societies within four hours of our school, both of which have a large membership and hold many meetings and forays

every year. The number of mushrooms collected and scrutinized by these two mushroom clubs every year is astounding.

What a potential resource for the science! But, as anyone who has been to a mushroom club’s foray knows, the resource is only a “potential” one. The mushrooms are not picked with an eye toward documentation of ecological data; they are placed on collection tables after being hastily sorted and identified; the edible mushrooms are removed; someone may make a list of the species names that have been applied to the mushrooms . . . and everything is thrown away on Sunday. This is all very fun—but to be honest none of it, even the occasionally produced species list, is very useful to science. With just a few changes, however, the process could easily provide mycology with lots of invaluable data.

I plan to make my suggestions for mushroom clubs more specific and detailed in further publications and in my lectures, but here I will paint them in broad strokes. At a minimum, three things must happen for mycological society forays to make more scientifically useful contributions: collection of ecological data, documentation of macromorphology, and preservation of specimens.

Documenting the ecology of mushroom collections is fun, and can provide mushroom clubs with new areas to explore when it comes to finding speakers and activities. If the Friday night speaker is a club member with tree expertise or a guest with this expertise from a local university or department of natural resources, speaking about the ecosystem and trees in the Saturday foray location, foray participants are better prepared to write down information about potential tree associations and substrates—perhaps on collection cards that stress the collection of such information rather than simply including a small line for “Habitat.” With an eye toward understanding mushrooms as parts of ecosystems, collection tables might be organized, not by constantly changing and usually outdated taxonomical schemes but by ecosystems, and labeled “Spruce-Fir Zone” or “Cottonwood-Sycamore Lowlands” instead of “Russulaceae” and “Polyporaceae.” Ecological documentation can provide clubs with activities even in the off-season, since research into a collection site’s ecological history (using sources in local libraries, courthouse records, state

archives, and so on) can provide crucial information.

The advent of digital photography has made recording the macromorphology of specimens much easier, and if one or two club members were simply to take photographs, from all angles, of each collection while it is still fresh (obviously a numbering system will be required to keep everything straight), meticulous journal-style descriptions could be bypassed—though such descriptions would be ideal as an accompaniment to the photos. At a minimum, information that may not be discernible from the photos (odors, for example) should be documented on the collection card.

Perhaps most importantly, the collections must be dried and saved. If clubs were to add “Curator” to the list of positions, along with “President,” “Foray Coordinator,” and other traditional positions, that person (or persons) could be responsible for drying the specimens, keeping the numbering system intact, coordinating the digital photos and collection cards, and maintaining the club’s herbarium (which sounds fancy but could easily consist of a few old filing cabinets in a club member’s garage).

Ideally the process I’m recommending would be undertaken for most (even all) of the mushrooms collected on forays—but this goal is probably unrealistic. However, members might discuss at a regular meeting what groups of mushrooms seem to be well represented in the club’s regular foray locations and if, for example, many boletes are regularly collected at the club’s annual foray to Such-and-Such State Park, the club could produce an invaluable resource for mycologists by going through the process I’m suggesting for boletes alone over the course of several annual forays at the location.

Mycologist Tom Volk tells his students that a mushroom’s name is far from being the most interesting thing about it. Picking a bunch of mushrooms from a state park, using 40-year-old monographs to label them, gawking at them for a few days, then throwing them in a dumpster is lots of fun—but it is also quaint, and a little bit like what Civil War re-enactment clubs do: act out something from the past. We might as well wear thick plastic glasses *a la* 1965, wrap our mushrooms in waxed paper pulled from a baking roll and twisted at both ends, drive to the forays

in two-toned sedans with car-top carriers, and call it *Lepiota americana*. Don’t get me wrong; if there were indeed an official “I Want To Be Alexander Smith Retro Foray,” I would be the first to sign up; I am a huge fan of Smith and his contemporaries, and North American mycology is inconceivable without their Herculean efforts and genius.

But it is time to move on, as far as the science of mycology is concerned. We *must* accumulate specimens—and it is time to consider our mushrooms from an ecological perspective. I have been framing my argument, so far, in terms of the pursuit of the advancement of scientific knowledge—but I will close by framing it in other terms: our planet’s biodiversity crisis. Korf writes:

An oft-quoted estimate for fungi is that we have described only 4 to 5% of the world’s species, leaving 95% or more yet to be recorded. The loss of habitats is proceeding so swiftly that the problem is critical. Unless these habitats are sampled now we will have lost forever our chance to document the world’s living biodiversity, to save that in museum specimens and, in the case of fungi, often in culture collections.

Even the fungi from well-studied areas are subject to habitat loss and potential extinction. Many of Smith’s mushroom collections, for example—including type collections for taxa that have never (or rarely) been subsequently collected—come from locations in Emmet and Cheboygan counties, in Michigan, that have long since been clear-cut. In one of these locations the logging company left a scrawny swath of trees standing next to the road in an attempt to hide the acres of damage. Somewhere behind these trees, in what is now a landscape reminiscent of Tolkien’s Mordor, Smith collected in 1963 what he called *Leccinum imitatum*—and apparently never collected it again. Unfortunately, his ecological data consisted of two words (“under aspen”), and we may never know much more about *Leccinum imitatum* than we do now.

There is evidence suggesting that cultivated *Agaricus bisporus*, the common “button mushroom” sold in grocery stores, may have “escaped”

cultivation to threaten native *Agaricus* populations (see Kerrigan et al., 1995)—and while there are as yet no scientific studies to confirm these observations, some highly adaptive mushrooms—including *Amanita thiersii*, *Morchella rubrunnea*, *Stropharia rugosoannulata*, and many stinkhorns such as *Phallus rubicundus*—appear to be increasing their geographic ranges at alarming rates, very possibly assisted by commercial distribution of substrates like wood chips and sod.

Korf's warnings about biodiversity cannot be ignored, and the time is now for mushroom collectors to begin documenting our fungal flora. This is why Kate and I, along with Midwestern mycologists, are returning to southeastern Kentucky. The species list we created as we walked through the woods was fun to produce, and it was nice to be able to name so many mushrooms—but the list must now be replaced by actual documentation of ecology and preservation of specimens before that beautiful, diverse, and ancient ecosystem is destroyed.

ACKNOWLEDGMENTS

I wish to thank Ron Meyers, Nicholas Money, Patrick Leacock, Richard Korf, Andrew Miller, and Carol Schumde (none of whom, of course, necessarily agrees with me) for valuable advice on this manuscript.

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