

Names, Names, Names: When Nomenclature Meets Molecules

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IN EASTERN North America, the Appalachian Mountains have their southern origin in northern Georgia, and extend to the northeast to Maine, a distance of over 3200 kilometers. Although not as spectacular as other ranges (i.e. Alps, Himalaya, Andes, Rockies, etc.), their height (up to 2250 m) combined with their longitudinal range provide a host of ecological niches. Glaciation of the northern portion of the range 10- to 20,000 years ago produced climatic conditions which forced the forest flora to colonize farther south into more hospitable climatic refugia, taking its fungi with it and eventually to recolonize northward once the glaciers receded. The conifers of the Canadian Shield still can be found at high elevation as far south as Tennessee (N 37°). Subsequent temperate conditions fostered survival of a rich flora and in many places in the southern Appalachians, one can count 15–25 species of trees within a radius of 30 m, leading to the common denotation on herbarium labels as “mixed woods.” With such a spectrum of forest types (supported by underlying geological formations and moderate to abundant rainfall), the mushroom mycota is also rich. A list of agaric species from the Great Smoky Mountains National Park (GSMNP), straddling the border between Tennessee and North Carolina, counts over 1400, not including other basidiomycotina and ascomycotina.

In surveying the list of mushroom species in GSMNP, it is impressive to see how many of the names of our mushrooms originated in Europe, including Scandinavia. A comparison of illustrations in popular mushroom books from both sides of the Atlantic often show rather similar mushrooms, even when comparing microscopic features. It has been easy to use names from the literature to apply to our local mushrooms. A case

in point: for years it was assumed that *Amanita caesarea* (Caesar’s mushroom; Fig. 1A) occurred in the Smokies. Confronted with our mushroom in 1968, Marinus Donk and Roger Heim, with deep experience in Old World tropics (Indonesia and New Caledonia), told us that our species was, in fact, *A. hemibapha* (Fig. 2A), with which they were familiar. Creating further confusion: Vassilieva described *A. caesarioides* (Fig. 2B) from far eastern Russia. Finally, we have come to call our version of Caesar’s mushroom *A. jacksonii* (Fig. 1B).

But if such confusion is possible over such a sensational mushroom, what other surprises could lurk over other, more arcane worldwide mimics?

While herbarium specimens can be (and have been, hopefully with notes and photos) preserved for careful examination, in the past few years mycologists have been presented with a “new” tool with which to examine fungi. Reading of the DNA code furnishes new information which can be applied to identification and taxonomy. Of the several genes being investigated, the sequence of a length of DNA called the internally transcribed spacer (ITS; in fact, two spacers which separate three functional genes) has been shown to be useful as more or less parallel to the traditional rank of species (as opposed to genus or variety). As ITS sequences of several collections of a single “species” are gathered, the sequences can be compared and can show relationships of these collections through DNA (closer similarity of sequences implies closer evolutionary/genetic relationships). When comparative analyses produce a related cluster of sequences, that cluster is called a clade, and when such clades are considered sufficiently separate (that is, when the molecules comprising the DNA differ at a sufficient number of sites) they can be termed as “phylogenetic species.” A “phylogenetic reconstruction” (phylogeny for short) is merely a graphic representation of the relationships among clades based on DNA sequences.

As other workers generate ITS DNA se-

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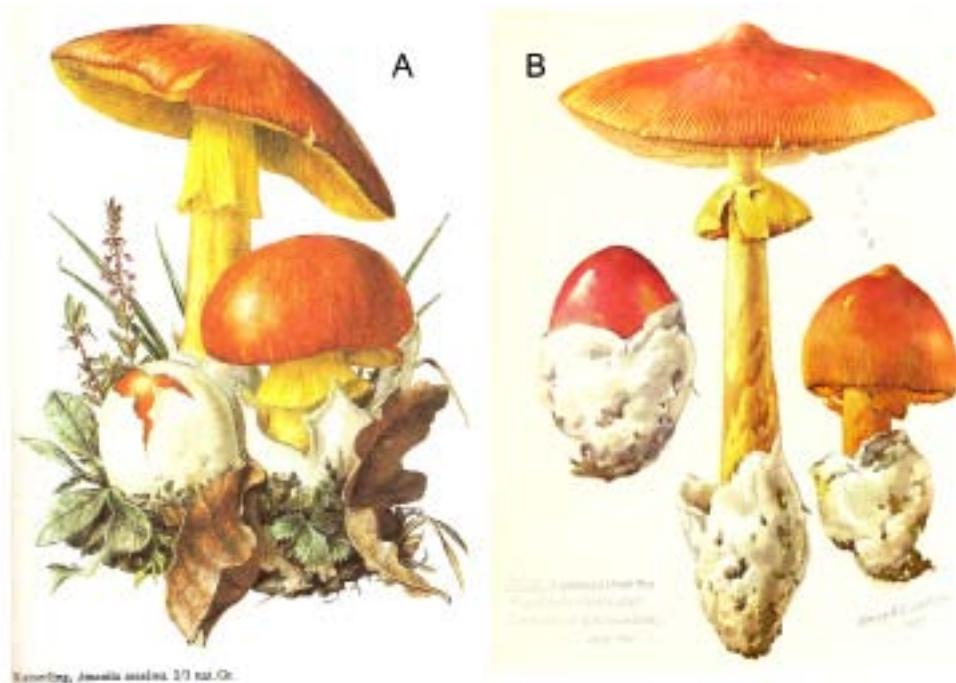


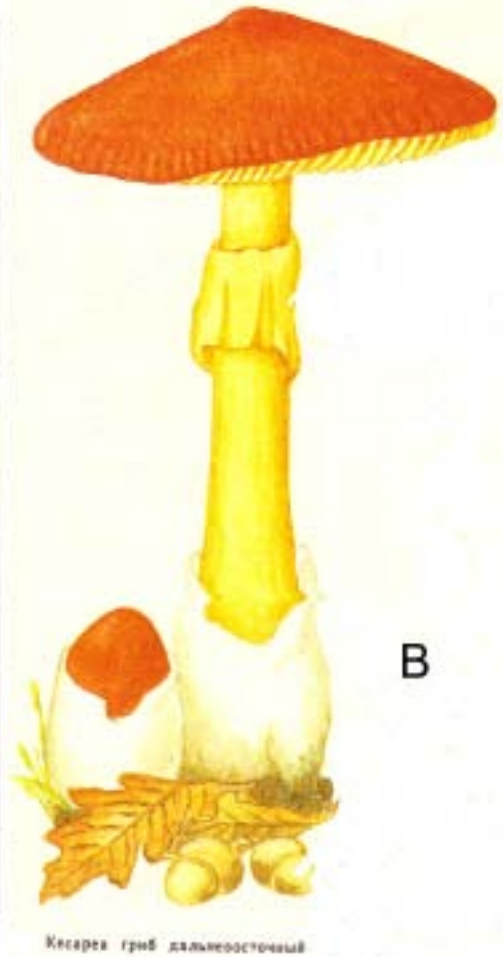
Fig. 1A. *Amanita caesarea*. Michael et al., 1987. 1B. *Amanita jacksonii*. Cazort, 1979.

quences, these sequences are deposited into DNA databases—one such is “Genbank,” another is “Unite.” Sequences from these databases can be added to those generated for specific projects in order to build a richer, deeper data set for analysis. But such sequences are “naked”—not accompanied by morphological or biological data. Moreover, they may originate from rather intriguing geographical sources. A basic and substantive question is: Are collections of the same putative species from widely separated geographical areas really the same species or do they represent different species? Another way to ask this question is: Are collections from the Appalachian Mountains with European names really the European species or are they separate and distinct species requiring new names? In order to begin to answer this question, the author (RHP) and Dr. Andy Methven collected in Sweden during September 2008, focusing on collections representing species with putative intercontinental distributions. The area was Fries’s home territory, so it serves as the “type” location for numerous mushroom species

Some data on intercontinental distributions already exist. Figure 3 is extracted from a larger

phylogeny intended to show the relationships of a new genus, *Cruentomycena*, to other members of its clade based on ITS sequence data. Note, however, that as a side product there is a distinct geographic signal for *Panellus stypticus*, a very common, small, pleurotoid fungus found throughout North America. The clades of *P. stypticus* collections sort into nicely distributed geographic areas: Southern Hemisphere (all South American), eastern North America, Asia/western North America, and Europe. Since *P. stypticus* is a cold-tolerant fungus, the Eurasia + western North American distributions may be linked by the Bering land bridge, which existed before and just after glaciation, when ocean levels were lowered and the bridge was exposed.

We also know that all the collections we have from the world over are sexually compatible and therefore potentially belong to the same biological unit. But a strange distinction is that only fruitbodies from eastern North America are bioluminescent – those from all other regions are not so, even though they can be made so by interbreeding in the lab with appropriate strains. Our graduate student, Jiankang Jin (2000) was able to tease out microscopic differences as well. In short, there are mor-



Кесарея гриб дальневосточный

Fig. 2A. *Amanita hemibapha*. Photo courtesy of Dr. Masato Ohtani. 2B. *Amanita caesarioides*. Vassilieva, 1978.

phological differences and DNA differences, but only a single potentially interbreeding unit. DNA clearly points to distinct intercontinental entities.

But which of these several strains is the real *Panellus stypticus*? Nomenclature to the rescue! This name (as *Agaricus stypticus*) was originated by Jean Baptiste François Bulliard, who lived and collected around Paris, France, in the late 18th century. Although the name was used as the cornerstone species of Petter Adolph Karsten's genus *Panellus*, the origin of the species name is clear. For the careful worker today, only a DNA sequence from a northern French collection can legitimately be used to characterize the species and by DNA, the fungus from the Appalachians belongs to a different clade and (if bioluminescence is thrown in) perhaps a different taxon (i.e. variety, species) than the European.

Figure 4 shows a phylogeny simplified from a recent Japanese paper using data from the ribosomal large subunit gene (Shimono et al., 2007), intending to show the relationships of numerous collections of the milk mushroom, *Lactarius volemus*, common in eastern North America and Europe. The authors report that there are several strains of the species in Japan and China (Groups A → C), but ask which of them is the true *L. volemus*? LSU-sequence data are commonly accepted as a coarser resolution than ITS, with clades often representing species complexes rather than individual species.

Toward the bottom of the phylogeny appears a sequence AF 506413 (bold face). Further investigation reveals that this sequence is from Sweden, deposited in Genbank by Karl-Henrik

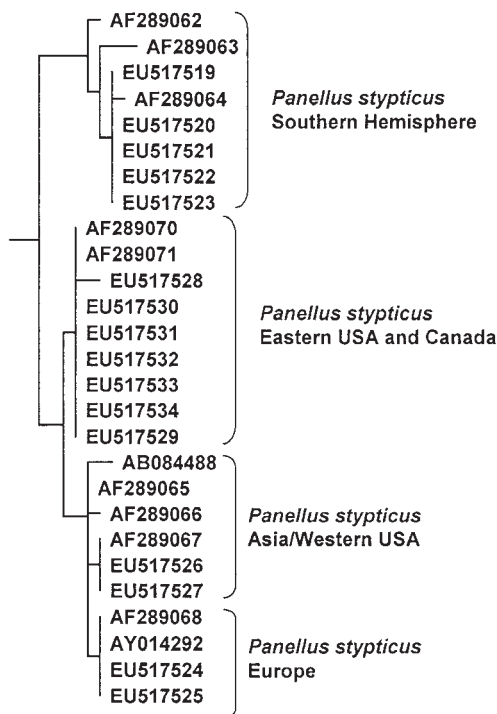


Fig. 3. Extracted phylogeny of the *Panellus stypticus* complex based on ITS sequences (from Petersen, et al., 2008). Sequences labeled with alphabetical prefixes are accession numbers in GenBank.

Larsson from Göteborg. In fact, Karl-Henrik has additional sequences of the species, all identical. The sequence just below this is AF 042574, which was deposited in Genbank by Rytas Vilgalys from North Carolina, USA. The name (as *Agaricus volemus*) was originated by Elias Magnus Fries in 1821. An “authentic” sequence, therefore, ought to come from southern Sweden (AF 506413 certainly qualifies). And if this is so, then not only are all the Asian strains doubtfully the same species as the Swedish, but our Appalachian strain is surely not the same species as the Swedish either. Ribosomal ITS sequences from Europe and from the southern Appalachians differ by 5.07%, dissimilar enough to suggest different species. Only careful re-examination of specimens, however, will tell us if there are any morphological differences, and at this time, we cannot test the biological species concept because spores of this fungus will not germinate on artificial medium in the lab.

Two additional examples can be offered here.

One of the more deceptively easy taxonomic identifications in the agarics has been *Megacolhybia* (*Tricholomopsis* for some folks) “*platyphylla*” (within quotation marks because the same name has been used haphazardly throughout the Northern Hemisphere). Once numerous collections are compared using DNA sequence data, however, it becomes obvious that not only are there several taxa (of species rank?) involved, but they are geographically limited (Hughes et al., 2007). Figure 5 is greatly simplified but by using DNA it is easy to separate the collections from the Old World (far eastern + western Europe) from those of the New World. In fact, there are two species even within temperate Asia (we have called them *M. clitocyboidea* and *M. marginata*). The true *M. platyphylla*, described originally from Germany, seems restricted to Europe and Scandinavia, while there are several species in the New World.

If only collections from the New World are shown (Fig. 6), even here several taxa can be recognized. The common eastern North American species, known traditionally as *M. platyphylla*, has been

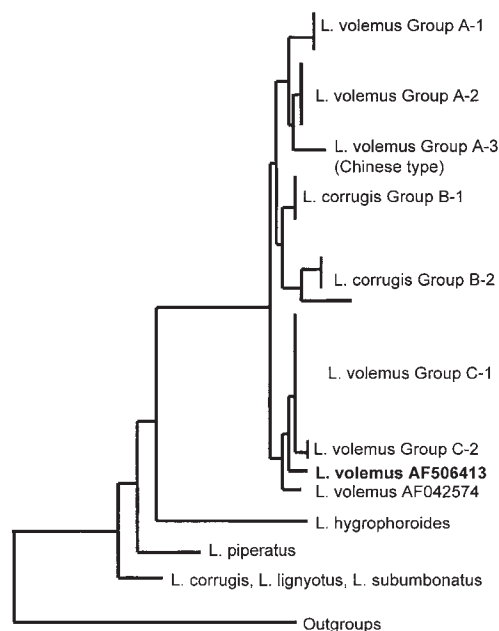
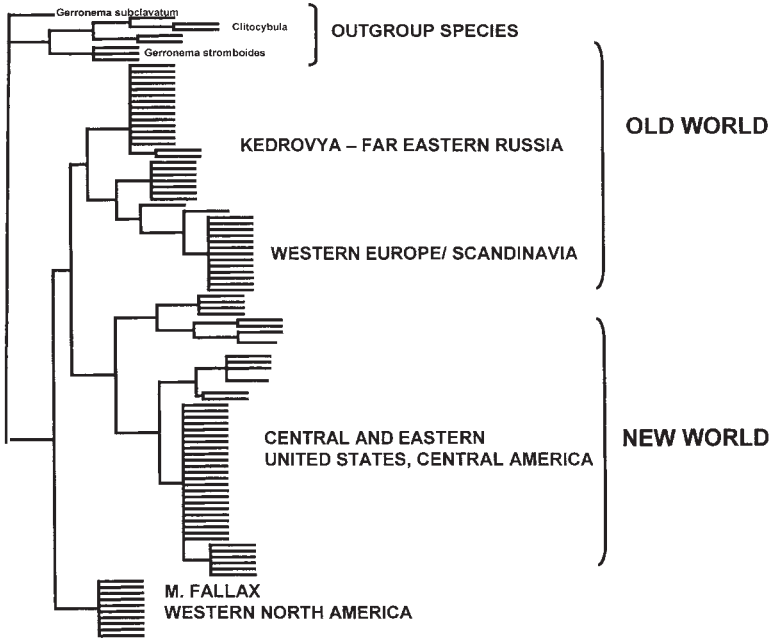


Fig. 4. Phylogeny of the *Lactarius volemus* complex in Asia, with two extra-Asian collections, based on nLSU sequences. Groups A, B and C all represent Asian collections.



renamed *M. rodmani*, and a species described from the Pacific Northwest by Alex Smith (as *Tricholomopsis*) has been transferred as *M. fallax* (Fig. 5, bottom).

Parenthetically, more can be squeezed from the careful alignment of sequences. In *Megacollybia*, it can be conjectured that the North American glacial refugia included Central America and perhaps the Gulf Coast and lower Mississippi River delta (Fig. 7). While the stock of American *Megacollybia* was compressed into these climatically livable areas, glaciers slowly receded northward and the various strains of *Megacollybia* were

Fig. 5 (above). Simplified phylogeny of *Megacollybia* based on ITS sequences, showing distinct Old and New World clades. Fig. 6 (below). Phylogeny of DNA sequences from New World collections of *Megacollybia*.

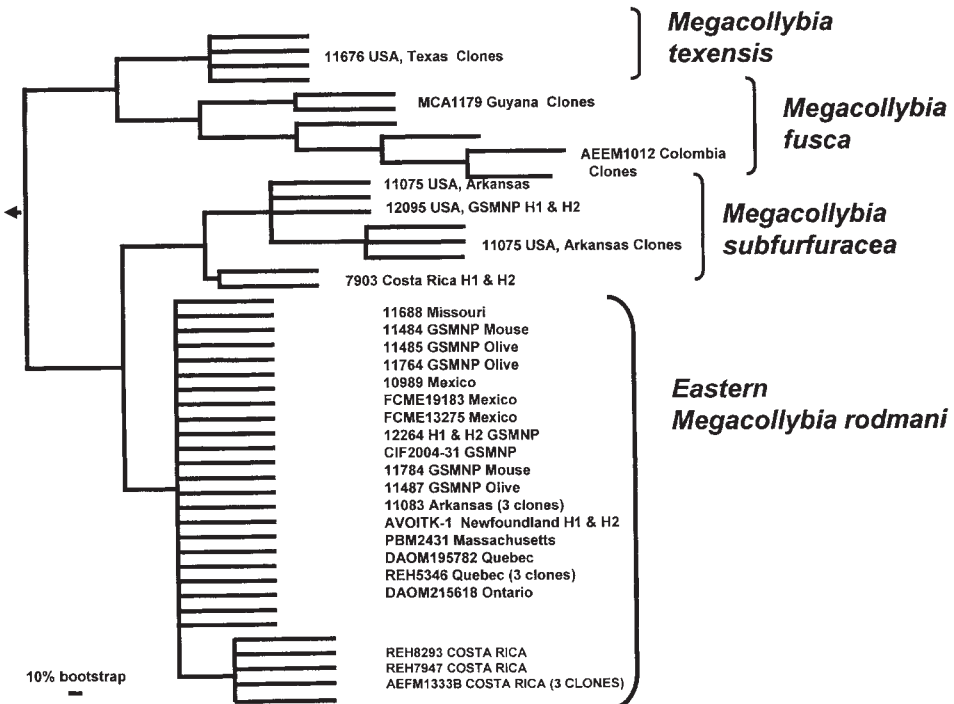




Fig. 7. Proposed refugia and migration avenues for *Megacollybia* strains, producing several DNA species.

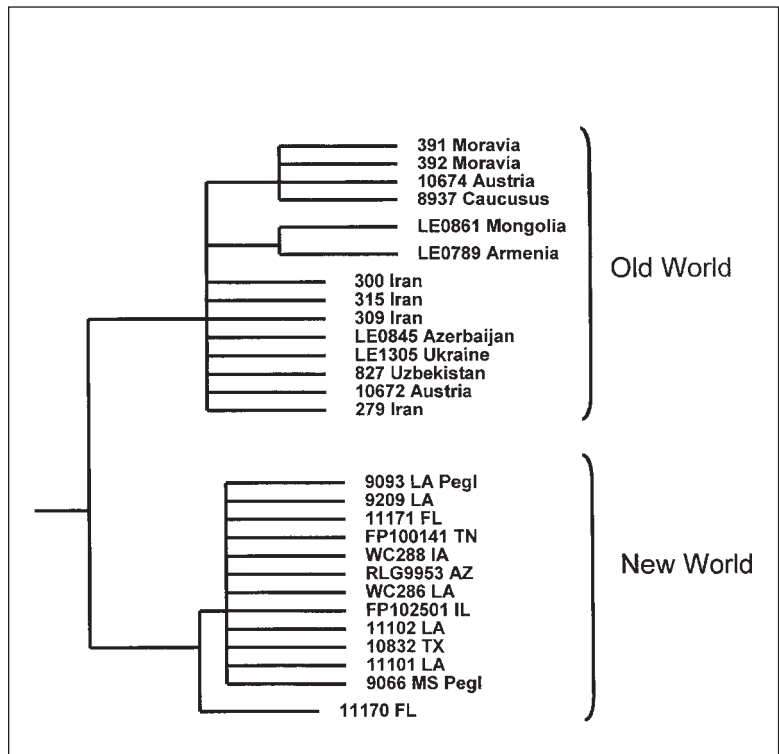
able to migrate also, diverging into at least four groups which are now separable on the phylogeny; *M. rodmani* in the east, *M. texensis* and *M. subfurfuracea* in the central United States, and *M. fusca* in South America.

Finally, Figure 8 is an extraction from a much larger phylogeny of *Lentinus* and *Panus*. It shows worldwide collections of only *Lentinus tigrinus* based on ITS sequences. It is clear that, while all cultured collections were sexually compatible, they sorted into two distinct distribution clades (Grand, 2004). The upper clade shows collections from eastern Europe, several locations within Russia and Ukraine and northern Middle East. The lower clade is all United States. To be sure of our identifications, identification of some collections was checked by David Pegler (“Peg”), who authored the comprehensive monograph

of the group (Pegler, 1983).

There are numerous other examples in which phylogeographic signal indicates differences in mushrooms called by the same name across wide expanses of geography (See Taylor *et al.* 2006; Taylor 2008). Most of the old names we use in the Appalachians can be traced to a few European authors: Fries, Bulliard, Persoon, Scopoli, Schaeffer, etc. Each had his favorite collecting grounds, and it might be possible to recollect numerous species with matching names if such comparisons are intended.

These days, there is a movement to assign a DNA “barcode” to each unit of living organisms, including fungi. It is assumed that each organism will have a unique barcode, but reality, including the examples above, shows otherwise. *Panellus stypticus*, *Lactarius volemus*, *Megacollybia platyphylla*, *Lentinus tigrinus* and others all have multiple “barcodes” depending on their geographical origin. The correct barcode (note the omission of quotation marks) must be assigned to the organism as it occurs *in the place where it was named*, not merely anywhere it is found. To envision a project to untangle this molecular/geographical knot renews



interest in specimen collecting, travel and scientific cooperation.

To think that all these problems can be solved in one career is “wishful thinking.” The breadth of experience and time for careful work is too limited. But to ignore the problem or to make no effort to stabilize the names we use is to cultivate the seeds of confusion even more than now. Many of the “problem” species are easily recognized (others are not so) and serious amateurs can play an important role in bringing order to their taxonomy. Euro-Scandian collectors live on “sacred” ground, and their goodwill, wisdom and hospitality are especially welcome in these efforts. But European mycologists are discovering names by Peck, Smith, Stuntz, Hesler and other Americans (to say nothing of Asian workers), so a good-hearted quid pro quo seems probable to develop. Keep tuned.

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