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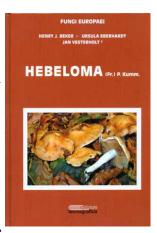
September-October 2016

www.namyco.org

The *Hebeloma* Project Progresses: Citizen Science at Work

By Henry Beker

For the last two decades Ursula Eberhardt, Jan Vesterholt and I have been studying the genus *Hebeloma*. Our European monograph is complete (published earlier this year as *Fungi Europaei* Volume 14: *Hebeloma*)¹. We have already begun to extend this work to the rest of the world. The next major area we wish to address is North America. As well as understanding the North American taxonomy we also hope to address the species overlap between North America and Europe. In order to make this study meaningful we need collections from throughout North America, where we anticipate discovering some new species. Ideally we need good collections, carefully dried and with good pictures; also good macroscopic descriptions particularly of any characters that may disappear with drying such as odor. We can attend to the microscopic descriptions. We have developed a recording sheet for the macroscopic description (See p. 3). We are thankful to all who have already submitted collections but need help to assemble a more



representative sample, across the whole continent. This will be Citizen Science at its best. Our goal is a future monograph on the *Hebeloma* of North America, although this is probably several years away. However, we will of course send information regarding our determinations to contributors of material, and all such contributions will be fully acknowledged. In due course we will establish a website so that all contributors will be able to see their collections on a map of North America.

This genus has long been regarded as difficult and consequently *Hebeloma* are rarely recorded. Within Europe there are some 300 published names and in North America there are over 200 additional published names. In Europe the list of published names boiled down to 54 species, and, during the course of our studies, 30 species new to science were discovered. In order to unravel the taxonomy and phylogeny of this difficult group, we developed a methodology combining molecular analysis with the functionality provided by a powerful database, allowing the comparison of hundreds of morphological characters (macroscopic and microscopic) and molecular characters from several loci.

Our database has details of more than 5000 collections, of which over 4000 are European and already almost 700 are from North America. The database also contains details of all the European holotypes, isotypes, lectotypes, epitypes and neotypes that we have been able to locate. We have also now started work on the North American types. Our monograph, which was published earlier this year, describes in detail the 84 species of *Hebeloma* that we currently recognise within Europe, provides keys based on morphological characters and also extensive molecular data as well as more than 500 pages of colour photographs. It also includes a commentary on all the existing European names, on their synonymies and their various interpretations. We are sure that there are still more new species to be described from Europe (as well as new species from North America) and we hope that our monograph will act as a catalyst to enable this discovery.

1. See book review of *Hebeloma* by Andrus Voitk in *Omphalina*, Vol VII, No. 6, August 26, 2016, pp. 16-17.

UPCOMING FORAYS & OTHER EVENTS

The events page of *The Mycophile* publicizes forays and events of NAMA affiliated clubs which may be of interest to our members. If you would like to list your club's next big event, contact Dianna Smith, Editor: mycophile@namyco.org.

Include date, location, brief description, link for information, and host organization name. To post your event on the NAMA website, contact the webmaster: webmaster@namyco.org.

<u>September 8-11</u>: NAMA Shenandoah Foray located in the unique environment of the bio-regions of the Blue Ridge Mountains and the Shenandoah Valley of Virginia. Walt Sturgeon will be chief mycologist. The foray will be stationed at the Northern Virginia 4-H Center in Front Royal. http://www.nova4h.com/#landing. Registration closed May 15, 2016.

<u>September 22-25</u>: Annual COMA Clark Rogerson Foray located in Copake NY in the Berkshires at the intersection of NY, CT and MA. Check for updates and registration online at <u>www.comafungi.org</u>.

<u>September 24:</u> Western Pennsylvania Mushroom Club's 16th Annual Gary Lincoff Mushroom Foray with Gary Lincoff, Nicolas Money and Chef George Harris. Go to http://www.wpamushroomclub.org/ to register.

October 17-22: 17th International Fungi & Fibre Symposium, Madeira Park, British Columbia. http://fungiandfibre2016.org.

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(Hebeloma Project: Continued from p. 1)

Joel Horman of the Long Island Mycological Club has kindly agreed to act as receiver for North American collections which he will then package together to send on to us. We have set up a FedEx system so that the sender should incur no cost and as little inconvenience as we can manage. Just contact Joel at jlhorman@optonline.net and he will provide delivery instructions. Please include a copy of the filled out form printed on the next page with your specimens.

We appreciate any help we can get with this project.

Henry Beker (henry@hjbeker.com) & Ursula Eberhardt (ursula.eberhardt@smns-bw.de)

<u>Hebeloma Recording Sheet</u>

Place Name County/Region GPS Altitude Date	Ш	
Trace Name County/Region 015 Attitude Date	\neg	
Habitat Descriptor Habitat Qualifier Special Habitat Special Soil Conditions Growth Habit		
Associated Organism Substrate Descriptor Substrate Qualifier Smell Taste (if recorded		
<u>Pileus</u>		
Shape? Unicolour or 2-colour? Margin?	\neg	
Spotted? Colour in Centre? Hygrophanous?		
With remains of Universal Veil? Colour at Margin? Rugulose?		
Lamellae		
Tears? Attachment?		
White Fimbriate Edge? Depth of Lamella?		
<u>Stipe</u>		
Basal Shape? Discolouring? Mycelial Cords?		
Interior? Cortina?		
Floccosity? With remains of Universal Veil?		
Basidiome Dimensions		
Pileus Width Length of Stipe Stipe Width Mid Stipe Width Base L: # of Full Length		
Lamellae		

Leucoagaricus jubilaei: First N.A. Record

By Joel Horman, Editor of the *L.I. Sporeprint*, originally published in Vol 4, No. 2, Summer 2016

Leucocoprinus and Leucoagaricus are strikingly similar genera, indeed sister taxa (Vellinga, 2010). There has been much contention over the years about principal distinguishing differences, sometimes said to be "the fragile coprinoid basidiomata, the plicate-sulcate-striate pileal margin, the relatively large cheilocystidia, and the abundance of pseudoparaphyses (pavement cells) in the hymenium of Leucocoprinus (Kumar & Manimohan, 2004). Despite these and other differences, a number of species have been placed in both genera, and Mycobank considers Leucoagaricus jubilaei and Leucocoprinus jubilaei to be synonymous.

It is a European taxon, with no previous records evident on the Mycoportal website, and previous literature reports its presence in northern France, Spain, Bulgaria, Turkey and into India. Some of these reports are recent, within the past decade.

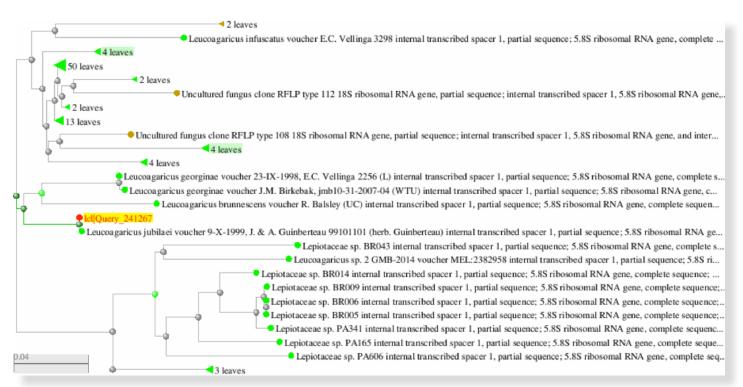
Our collection of two caps was discovered by my wife, Peggy, among wood chips in our garden on October 1, 2015. At first, I thought this might be *Leucoagaricus brunnesens* Peck (originally *Lepiota*) but many of the characters did not fit, and the identity could not easily be resolved. Therefore, a small sample of one cap was sent for DNA analysis to AlvaLabs in Spain, a commercial lab that specializes in sequencing fungi for professional researchers but also for "amateur researchers, both independent and members of Mycological Societies from different countries." While the reader has already been informed of the identity of the mushroom, this result came as a complete shock to us, so much so that doubt was not easily eradicated.



Leucoagaricus jubilaei

To get technical for a moment, the DNA segments sequenced were Internal Transcribed Spacer 1, 5.8S ribosomal RNA gene, and ITS 2, which produced a nucleotide sequence of 753 letters. This sequence was entered into the NCBI GenBank database, which search produced a 99% similarity match to a sequence of *Leucoagaricus jubilaei*, a French sample dated 1999 which had been analyzed by Else Vellinga at the University of California Berkeley. Else Vellinga is known worldwide as the foremost expert in Lepiotaceous fungi. She kindly responded to my inquiry stating that "your specimen is indeed so close to the European *jubilaei* that it is really the same species!...it is exciting though that this species would be present in North America as well!"

GenBank also generates a phylogenetic tree showing the degree of relationships between the specimen submitted and other closely related taxa, an abbreviated version of which is shown below:



The yellow marks our specimen, (Query 241267) nestled next to Else Vellinga's sequence of the French collection of *La. jubilaei*. Note that on the branch immediately above lies *Leucoagaricus brunnescens*, closely related enough to be considered a sister species, and with a common ancestor, forming a clade.

Else was also kind enough to provide me with Josserand's original description and other documentation which enabled me to go beyond the DNA evidence to realms I am more at home with: macro and microscopic character descriptions. (I am also grateful to LIMC's science advisor, Benjamin Wolfe, Dept of Biology, Tufts University, for providing instructions on GenBank submissions and other support.) There were two characters that gave me pause because of an imperfect match: firstly, the size. Our specimens were fairly robust, with caps measuring up to 8 cm., while Josserand's measured a maximum of only 5 cm. But we all know that size is a variable character, and not infrequently encounter specimens which are "off the charts". The other character is the "plicate-striate" cap margin, but this is not emphasized by Josserand and others, with Kumar's key making a point of indicating that the pileus of *L. jubilaei* is non-striate.

While the molecular evidence is paramount in clinching this identification, this is an abstraction for most amateur mycologists, so we need to provide a more concrete picture, and a physical description follows:

Cap shallowly depressed, (in mature stage) 6.5—8.5 cm., ivory to cream colored, with numerous small. pointed, brownish- purple scales, the disc darker. Stipe enlarged toward the base, with fixed median annulus, about 5 cm. long, 1.5 cm. at base, 1 cm at narrowest point. Cream colored at apex, brownish below. Stipe, flesh and gills all turning reddish brown on handling and injury. Gills free, close (~ 90 full gills) with 4 layers of lamellulae. No discernible odor.

Microscopically, spores dextrinoid, elliptic with one flattened side, 6.5- $7.5 \times 4 \,\mu m$, germ pore lacking. Cheilocystidea abundant, crowded, clavate, to $50 \times 20 \,\mu m$. No pleurocystidea. Pellis a trichoderm, hyphae over $100 \,\mu m$ long, up to 23 wide, ends rounded. No clamp connections. Unfortunately, several chemical reactions (gill ammonia reaction, spore to cresyl blue) were not tested as I was initially unaware of them. On drying, the entire specimen became very dark, as is reported for other species in *Leucoagaricus* section Piloselli.

Interestingly, Josserand in 1973 named this species *jubilaei* as the species account was to be offered for publication in the Jubilee volume of his mentor Prof. Robert Kühner, the noted French mycologist, upon his seventieth birthday. Its presence in northeast NA mirrors several closely related species in *Leucoagaricus* sec. piloselli, which Vellinga recently reports as being present in both California and Italy. And it matches an existing pattern of species present both on the East Coast and Europe, e.g., *Tricholoma colossus* and *Pluteus romellii*.

The DNA sequence was duly registered with GenBank, by Pablo Alvorado of AlvaLabs, where it is publicly available to researchers (Accession number KX258658). Our collection was donated to the NYBG Herbarium. *La. jubilaei* will be added to our LI checklist and hopefully will be reencountered by others.

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History of Eastern Mushroom Teaching Kit

By Carol Dreiling Asheville, NC

In the beginning was *The Mushroom Trunk*. I first heard about it, as we called it back then from Theresa Rey-Oursler who worked with Sandy Sheine and Allein Stanley to create the *Mus-room Educational Trunk*. I became interested in using this resource in 2001 in the Children's Room when the Asheville Mushroom Club put on a Mushroom Fair at the North Carolina Arboretum in September. The *Trunk* provided a helpful teaching tool to introduce children to fungi.

List of Original Trunk Items in 2001:

- 1. Packet of lesson plans and hand-outs for K-12 with bibliography
- 2. 8-4x3 colored mushroom drawings by Louise Freedman on hard backing
- 3. 9 microfiche pictures on fungi stages, variety of shapes, mushrooms anatomy and spore bearing surfaces
- 4. 5 spore prints
- 5. 3 'What can you do with mycology?' posters
- 6. 26 slides
- 7. 25 hand lenses
- 8. 13 dried samples
- 9. Poisonous Mushroom poster

As Theresa was starting graduate school, she asked me to be responsible for renting it out to people and clubs all over the country that needed it. It soon became apparent that there was a need for a kit designed for the West that was appropriate to the area served, and to cut down on postage for shipping across the country. Sandy Sheine and Catharine Gunderson, who taught in the Santa Cruz Public Schools, created the *Western Mushroom Teaching Kit*. So now we offered two kits-- the *Western Mushroom Teaching Kit*—Catharine Gunderson's responsibility and the Eastern Mushroom Kit, my responsibility In 2003 Maggie Rogers took took charge of the *Western Mushroom Teaching Kit* and rented it out until 2014.

Contributions over the years presented a longer list of materials to use for teaching:

Susan Hopkins gave us her dyeing skeins and doll to show the different colors of dye derived from fungi.

Nancy Parker donated her book, A New Home for Lil Gnome

Taylor Lockwood gave us his teaching DVDs: Mushroom Identification Trilogy, The Good, the Bad and the Deadly and Treasures from the Kingdom of Fungi

Mary Woehrel contributed Kit Scates's Mushroom Identification Sheets

Roy Watling gave us his book Fungi

Bryce Kendrick sent us book Young Person's Guide to Fungi

Mike Wood contributed his MykoCD

Maggie Rogers gave us fungi stamps

Dean Abel's gave us his slime mold educational material

Walt Sundberg's teaching material included illustrations depicting growth stages of *Amanita* species.

Sandy and Jerry Sheine were instrumental in collecting items for kits and supplying mailing boxes. Jerry created our CDs with photos for digital use.

(Continued from p. 7)

Children learn through their senses. When a child (and also older people!) smells, touches, hears, sees and tastes, they connect with material presented to them. The senses help us to relate to information. Holding a mushroom and smelling it, touching it, seeing it and even tasting it when safe, helps a child to retain what they've learned. The *Mushroom Teaching Kits* provide this hands-learning. Learning on a computer screen does not replace this hands-on learning.

Since 2001 both *Kits* have been used extensively and succesfully throughout the United States and have been enjoyed by a wide range of ages from K-12 to college to adults to senior citizens. They have also inspired many teachers to develop their own versions specific to the local fungal community. The *Eastern Mushroom Teaching Kit* is available if you live east of the Mississippi River. The link to check it out is: Mushroom Teaching Kits - North American Mycological Association

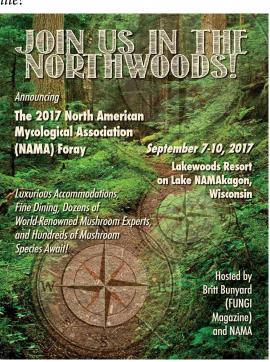
More on the Teaching Guide

Sandy Sheine, NAMA Education Chair, sandysheine@aol.com.

We encourage the NAMA clubs in the Rocky Mountain states, the Western states, in Canada and in Mexico to make your own teaching *Kits* for your local use in schools, nature centers, libraries and homes. Carol Dreiling has set an example of developing an *Eastern Mushroom Teaching Kit*. You can model your *Kit* after Carol's and you can also use the information in the Education section of the NAMA website. This section has extensive information on developing a Basic 45 minute Lesson on Fungi. You will find many other activities suggested such as drawings of How Mushroom Grow, questions to ask in class and on walks in the woods. A full pdf of terra Brie Stewart's excellent 91 page e-book, The Fungus Files: An Educator's Guide to Fungi, Grades K-6 (Second Edition), can be downloaded. You can also browse individual chapters to find the activity that suits your immediate needs. The Education secftion also offers *Guidelines for a Successful Mushroom Fair*, children and adult book lists and *Online Teaching Resources*. Twenty five teaching programs, developed by Michael Beug, are available from NAMA. You will also find directions for making dyes for wool and silk, paper-making with fungi, aansd a brand new page about lichens.

I am happy to answer any questions that you may have. I would also like you to tell me about your programs and to send photos as well. Your *Club Kits* might be featured in the *Mycophile*!

Britt Bunyard's New Poster Advertizing the 2017 North American Mycological Association Foray Save the Date! September 7-10, 2017



Renowned Mycologist Richard Korf Dies at 91

By Krishna Ramanujan

Noted plant pathologist, scholar and mentor Richard P. Korf '46, Ph.D. '50, professor emeritus of mycology, died Aug. 20 at his Ithaca home. He was 91 years old.

Korf had a major impact in the field of mycology, where he specialized in a group of fungi called discomycetes, or cup fungi, which include morel and truffle mushrooms and other cup-shaped fungi. He described or reclassified many hundreds of fungal species.

"He was a world leader in the understanding and taxonomy of discomycetes; there are now three genera and at least 16 species of fungi named after him, including the locally common false morel, *Gyromitra korfii*," said Kathie Hodge, associate professor of mycology in the Plant Pathology and Plant-Microbe Biology Section of the School of Integrative Plant Science.

Another area where Korf had a significant impact was in how fungi are named. He fought for rule changes to make assigning names to species more logical and practical, Hodge said. As an authority in the field, many colleagues relied on his advice on naming newly discovered fungi, she said.

Korf co-founded the journal *Mycotaxon* in 1974 with a friend, Gregoire Hennebert. The journal promotes rapid publication of discoveries in fungal biodiversity by allowing authors to format papers and assign reviewers themselves.

At Cornell, Korf directed Cornell University's Plant Pathology Herbarium, the fifth-largest herbarium of fungi in North America. Korf's contributions include specimens from expeditions to Japan, Bermuda, Macaronesia and Southeast Asia, plus local New York fungi. His personal collection comprises 5,000 specimens of fungi, including 257 types – each the first of its kind to be named.

Along with publishing more than 400 papers on cup fungi and fungal nomenclature, Korf trained and advised 27 graduate students and eight postdoctoral researchers.

"Richard Korf mentored many of the prominent fungal taxonomists in the world today," said Gary Bergstrom, chair of the Plant Pathology and Plant-Microbe Biology Section.

"He had a great store of knowledge and used it generously and gracefully," Hodge said. "The pride he took in his many students was a real gift to receive."

Hailing from Westchester County, New York, and New Fairfield, Connecticut, Korf received his bachelor's degree in botany in 1946 and his doctorate in plant pathology and mycology in 1950. He joined Cornell's faculty in 1951 in the Department of Plant Pathology; he retired in 1992. During his career, Korf traveled and taught in Japan, Canada, Denmark and China, among other places.

Korf was also an active thespian and served as chair of Cornell's Department of Theater Arts 1985-86.

His many honors and awards include an Ainsworth Medal for extraordinary service to international mycology from the International Mycological Congress (2010); selection as a British Mycological Society Centenary Fellow (1996); a Gamma Sigma Delta Cornell Chapter 1992 Distinguished Teaching Award (1993); a State University of New York Chancellor's Award for Excellence in Teaching (1992); and a Distinguished Mycologist Award from the Mycological Society of America (1991).

Korf is survived by his wife, Kumi, and four children.

A memorial service for Korf is being planned for later this year.

This obituary was originally published in the *Cornell Chronicle* on August 25, 2016 by Krishna Ramanujan, Life Sciences Editor of the Cornell News Service.

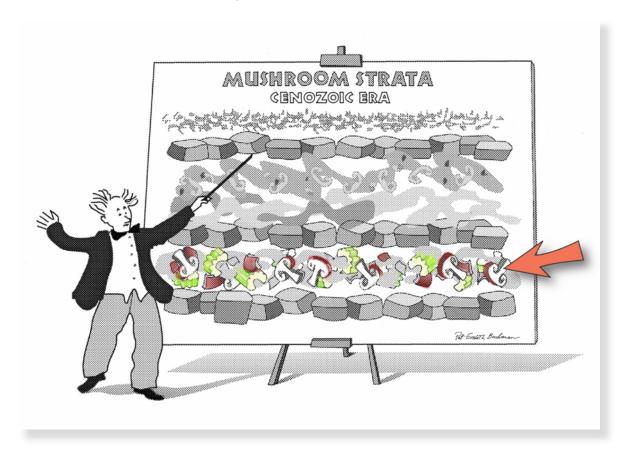


Richard Korf in his laboratory 9, 1985

K. E. Loeffler/Cornell Plant Pathology Herbarium Image Collection

Peregrinations of an Illustrator

By Patricia Buchanan



Back in 1994 I was invited to make pictures for a cookbook called "Recipes from Many Lands", that was to be published by the Eastern Massachusetts Literacy Council. I was free to choose whatever recipes in the book happened to inspire me. One was named "Mushroom Strata" — the mushrooms were nestled between two layers of cubed bread, and other stuff was piled up on top. Naturally a geological diagram came to mind.

When I drew the professor and his representation of the Cenozoic era with its layer of fungi between the cubes, I had no idea that twelve years later I would develop a passion for finding fungi. In 2006, a few months after retiring as a technical illustrator at an R&D firm, I saw a mushroom sitting in my front yard which looked just like a fried egg, sunny side up, sprinkled with paprika. Bending down to get a closer look I dislodged it from the earth and, much to my astonishment, rather than finding gills on the belly side of the cap, I saw an array of tiny pores. Yes it's true — I can only imagine that although I might have noticed mushrooms in the wild, I had never really seen them. Four things became imperative — that I draw this creature with the tiny pores, that it must be identified (*Suillus americanus*) and explained (drawing can be a way of explaining a thing to oneself), and that I find a group of like-minded enthusiasts, which of course I did when I was introduced to the Boston Mycological Club.

At first I began to record my finds in a rather loose, impressionistic way, but it wasn't long before I gravitated toward doing more precise renditions in my sketchbooks. Sometimes I sketch in the field, but more often I will bring a specimen home, where I have the luxury of a dining room table and can spread around my pens, pencils (both graphite and color) and watercolors. By bringing a specimen home, perhaps a young Amanita for example, it sometimes happens that I can record its changing form over the next few hours or even days.



My sketch kit



A time-lapse portrait of an Amanita muscaria var. formosa (guessowii)

Still, every so often I am inspired to do an "illustration", a rendering that provides a setting for the main topic. These bird's nest fungi, *Crucibulum laeve*, were on a twig I found near my house. I added some grass and a leaf and, to help with the scale, a couple of red ants I found running around on my doorstep.



Here in Massachusetts this summer we've had a seemingly endless parade of rainless days, and the mushrooms aren't having any of it, at least not in my front yard. One of the last live appearances I witnessed was of a morel, *Morchella americana*, which had popped up in a friend's yard across town in mid May. It was an exciting find and my friend gave it to me to bring home to sketch. When the present drought ends, I daresay I will be almost as excited to see a little brown mushroom as I was to see that morel in May and I will definitely be sketching it. The name of the mushrooms called for in the Mushroom Strata recipe were left to the imagination.

Pat sketching on Jekyll Island, Georgia



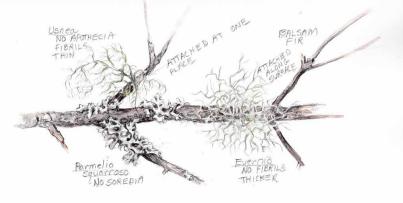
Drawing Mushrooms By Carol Govan

I like to look at the natural world but have a hard time slowing down to see anything unless I draw and/or identify it. Drawing allows me to see details I don't see at first glance. Struggling to find a name reveals information from many sources that encourages me go back and look again. My interests tend to combine art and science, both of which require taking notes about concentrated observations.

I enjoy drawing mushrooms. They have the most unusual sculptural shapes with colors, patterns and textures unlike any others I find in the woods. I even like all the different shades of brown and gray. I am interested in their ecological niche and want to identify them. However, I find fungi hard to name. And of course as soon as I learn a name it changes. Plants are much easier to identify. In any case, I really like to look at fungi and appreciate forays and classes where someone else names them. And, if I am lucky, I can take some home before they get thrown away.



I am not confident enough to pick any to eat. The state forest behind my condo is filled with beautiful mushrooms. I have hundreds of photos in situ (mostly unnamed) and will gather a few to draw at home. Sometimes I meet people from other parts of the world who really know their edible mushrooms. They get upset when they see the ones I am carrying and will point to my collection to say: "poison, poison," I quickly say: "no, no, drawing, drawing, drawing."



After I take my selected mushrooms home I often have many other things to do before I draw them. When I finally get a drawing and a spore print I hate to throw my mushroom away because I have become quite attached. I learned my lesson though after finding a melted glob of dark icky stuff with little maggots wriggling around. A little off-putting for visitors. I have a house full of twigs, dried leaves, flowers and fruits, old dried mushrooms (that didn't decompose), lichens, all which encouraged my granddaughter to bring me a handful of stuff (dirt) she grabbed outside because she knew I would like it. Of course I did.

I have a hard time focusing but see or learn something new each time I go outside and draw, or try to identify something new. These activities help me focus (a little) and encourage me to keep doing what I enjoy doing: slowing down to observe new things outside.



Cortinarius iodes



Russula sp.

Illustrations:

The illustrations are from my sketch books drawn at Eagle Hill, Maine, (where I took wonderful courses about fungi and lichens), behind my house in the Ashland State Park and a after a Boston Mycological Club (BMC) foray.

Please don't hold me to the names. Or maybe you can help me identify what I have mislabeled. I sent Zaac Chavez, our BMC Newsletter editor, a picture from my sketchbook that was labeled *Boletus campestris*. He wrote back and said: "are you sure?" I immediately wrote back and said "Noooooo, I am not sure at all." So he put it in the newsletter as a contest to name.



Entoloma strictius

Hydnellum suaveolens

Carol Govan at an Eagle Hill fungi class lab table concentrating on capturing details of specimens in watercolors.

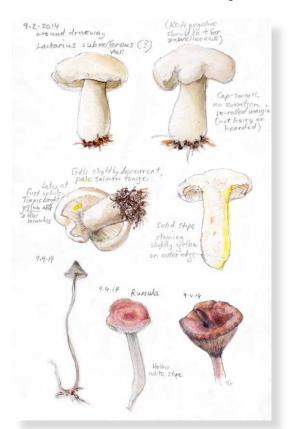


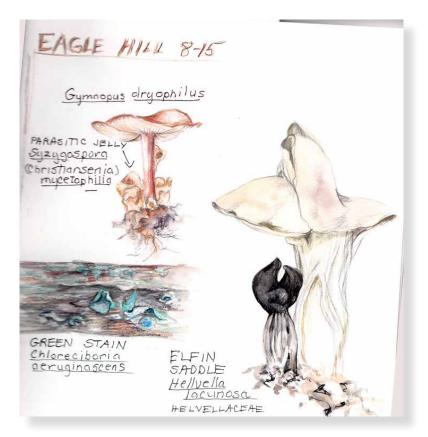
THE MYCOPHILE, SEPTEMBER-OCTOBER 2016





Abandoned Parking Lot





The artists Pat Buchanan and Carol Govan have been going to Eagle Hill Research Center in Steuben, Maine for five years to study mushrooms, lichens, mosses, and slime molds. They have taught several drawing courses together for the Boston Mycological Club and most recently offered two drawing workshops at the 2016 NEMF Sam Ristich Foray in Fitchburg, MA. To see additional drawing from these talented mycophile artists go to http://www.nemf.org/fungi-illustration-new.html.

Letter to the Editor

In her excellent and exhaustive review of the clinical trials of three popular medicinal mushroom supplements,¹ Megan Frost unfortunately misidentified the Royal Sun Agaricus as *Agaricus blazei*, listing *A. subrufescens*, *A. sylvaticus*, *A. rufotegulis* and *A. brasiliensis* as deprecated "regional" synonyms. In so doing she perpetuates a long-standing and persistent nomenclatural error that continues to cause confusion despite the fact that this has been corrected in several important papers. The correct name of this mushroom is *Agaricus subrufescens* Peck. *Agaricus blazei* Murrill is a different species entirely. Please permit me to clear up this issue with some brief comments and a paragraph excerpted from our forthcoming book: Mary L. Woehrel and William H. Light, *Mushrooms of the Georgia Piedmont and Southern Appalachians*, The University of Georgia Press: Athens (in press, publication scheduled for September, 2017).

This mushroom has long been misidentified as *Agaricus blazei* Murrill (1945) stemming from a misidentification by a Belgian mycologist to whom the Brazilian specimens were sent by the Japanese collector who brought samples of this species to Japan.^{2,3} This identification error was later recognized and the species was described as a new species, *Agaricus brasiliensis* Wasser, Didukh & Stamets (2002).⁴ Alas, this turned out to be a homonym of yet a different taxon, *Agaricus brasiliensis* Fries (1830) and is therefore an illegitimate name. Despite this, it is sometimes still erroneously called *A. brasiliensis*. Molecular genetic and breeding studies finally revealed that the North American, Brazilian, European, Hawaiian, and Asian populations are all later synonyms of a North American species, *Agaricus subrufescens* Peck (1893).^{3,5} Nevertheless, this species and the medicinal extracts and pharmaceutical products prepared from it continue to be widely and incorrectly referred to as *A. blazei* (and to a lesser extent, also *A. brasiliensis*) in both the medical research and popular literature and in commercial packaging. Bioactive extracts from this mushroom are still typically referred to as "ABM [for *Agaricus blazei* Murrill] glucans" in the biomedical literature and a steroid recently isolated from this mushroom in Japan (where it is popularly called himematsutake) was recently named "blazein" on the basis of this continuing misidentification.⁶

The synonymy of *A. subrufescens* was clarified and fully discussed by R. W. Kerrigan³—a paper that Frost cited in her article but, whose nomenclatural priority she misinterpreted—and was reviewed by Wisitrassameewong et al. (2012).⁷Unfortunately, these and several other deprecated synonyms and misapplied names—particularly *Agaricus blazei*—continue to be widely used, as in this case.

Given the long-standing, widespread and persistent misidentification and nomenclatural confusion of this important taxon despite its clarification in several important papers, it seemed appropriate to bring this to the attention of your readers.

Very sincerely,

William H. Light, M.A., Ph.D. Naturalist, Author, Educato

- 1. Frost, M. "Three popular medicinal mushroom supplements: a review of human clinical trials." Mycophile 56, no. 4 (2016):5-13.
- 2. Heinemann, R. 1993. "Agarici Austroamericani VIII. Agariceae des régions intertropicales d'Amérique du Sud." Bulletin du Jardin botanique national de Belgique 62 (1993):355–384.
- 3. R.W. Kerrigan. "Agaricus subrufescens, a cultivated edible and medicinal mushroom, and its synonyms." Mycologia 97, no. 1 (2005):12–24.
- 4. In: Wasser, S.P., M.Ya. Didukh, M.A.L.A. de Amazonas, E. Nevo, P. Stamets & A.F. da Eira. "Is a widely cultivated culinary-medicinal Royal Sun *Agaricus* (the Himematsutake Mushroom) indeed *Agaricus blazei* Murrill?" *International Journal of Medicinal Mushrooms* 4, no. 4 (2003):267–290.
- 5. Agaricus subrufescens Ellis & Everhart (1894) is an illegitimate homonym of *A. subrufescens* Peck (1893), and is properly referred to *Tricholoma subrufescens* Saccardo (1895) [Index Fungorum: http://www.indexfungorum.org/Names/HomoSpecies.asp?RecordID=504759].
- 6 Ito, Hiroko, Hitoshi Ito & H. Hibasami. "Blazein of a new steroid isolated from Agaricus blazei Murrill (himematsutake) induces cell death and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells." *Oncology Reports* 20, no. 5 (2009):1359–1361.
- 7. Wisitrassameewong K., S.C. Karunarathna, N. Thongklang, R. Zhao, P. Callac, S. Moukha, S. Férandon, E. Chukeatirote & K.D. Hyd. "Agaricus subrufescens: a review." Saudi Journal of Biological Sciences 19, no. 2 (2012):131–146.

Fungal Sequencing on a Budget for the Advanced Amateur Mycologist

By Alan Rockefeller

Overview

DNA sequencing enables the discovery of new species, the determination of which features delineate species, and the building of phylogenetic trees which show how various organisms are related.

DNA Extraction

The goal of DNA extraction is to get a few nanograms of your organism's DNA into solution so it can be used as a <u>PCR template</u>.

Two good DNA extraction methods are the NaOH extraction and ExtractNAmp.

NaOH extraction (Wang et al. 1993, Osmundson et al. 2013). 200 μ L of 0.5 M NaOH is added to the ground tissue. 5 μ L of the extract is diluted in 495 μ L of 100 mM Tris-HCl, pH 8.0 (pH is adjusted with pH meter and HCl until it is 8.0), and 1 μ L of the dilution is used as template DNA for a 25 μ L PCR reaction.

The amount of mushroom you use is not critical, but smaller pieces often have a higher chance of working since there is less likelihood of contamination. I usually use about 20 milligrams, and if it is a tiny collection and I want to use less I use less DNA extraction solution. Since only 1 uL is needed for the template DNA, in theory you can get a good sequence from a piece of mushroom that is nearly too small to see. If the mushroom has gills, use that part because they have more DNA. If the mushroom is thick, break it in half and use part of the inside of the cap to reduce the chance of contamination. Get the mushroom piece with forceps and heat sterilize them between samples to reduce cross contamination.

Optional: Autoclave or filter sterilize the DNA extraction solutions. Success increases if you wear gloves to reduce cross contamination and DNase.

PCR works at a wide range of DNA concentrations, and a likely cause of PCR failure is the presence of PCR inhibitors. More lengthy DNA extraction protocols can separate the DNA from PCR inhibitors. This is more often required with samples that have a lot of pigments.

Papers on the NaOH extraction:

File: Wang et al NaOH.pdf

File: Osmundson et al. - 2013 - Back to basics an evaluation of NaOH and alternat.pdf

Polymerase Chain Reaction (PCR)

I use a 25 μ L PCR reaction which includes 1 μ L of 10 nM forward primer, 1 μ L of 10 nM reverse primer, 1 μ L DNA template and the <u>PCR master mix</u>. I use the Gene and Cell master mix which is available from <u>ebay</u>.

The current PCR program I am using is an initial denaturation of two minutes at 95 degrees C, followed by 30 cycles of denaturation at 95 degrees for 30 seconds, annealing at 54 degrees for 25 seconds and an extension phase of 70 degrees for 45 seconds. Since the DNA we are amplifying isn't very long, it is probably ok to omit the final extension phase of 6 minutes at 70 degrees. The 54 degree annealing temperature was chosen by looking up the melting point of the ITS4-b primer and subtracting a couple degrees. The 70 degree extension phase was chosen because when I had it set to 72, an external thermocouple that I used to measure the temperature of the PCR block indicated that it was actually going up to 74, which was too hot. Setting it to 70 ensures that it stays within an optimal range for copying DNA.

and for ascomycetes the less specific reverse primer its4 (TCCTCCGCTTATTGATATGC). LSU and EF1-a and various plant primers are also in the CCL freezer. <u>Primer sequences from UC Berkeley</u>.

PCR math: Add 1 or 2 to however many samples you want to run to ensure that you have enough PCR mix for all of your tubes. Multiply the number of samples by 25, assuming that you are doing a 25 uL PCR reaction. This number is your total PCR mix volume. Divide the total volume by 5 to see how much PCR master mix to add, assuming that you are using 5x master mix concentrate. Subtract the amount of concentrate, primer and DNA you will add from the total volume to see how much ultrapure water you should add. PCR math example.

Gel Electrophoresis

Agarose gel electrophoresis

2% agarose, 3 uL Ethidium Bromide or GelRed solution per gel, TAE buffer.

To make a gel, add agarose to room temperature water, then heat in the microwave to dissolve it into solution. It is important to make it completely clear - no grains of agarose should be visible when you swirl the beaker in front of a light. This often involves a couple minutes of boiling with the microwave - run the microwave until it boils, stop it before it boils over, swirl and run it again every few seconds for a couple minutes. Be careful not to fill the beaker too full or it can get superheated and boil over, possibly burning your hand.

Gels can be used more than once, but if you try to use them too many times the DNA stops showing up. I have had success remelting gels or reusing them without recasting however for optimal sensitivity it is best to make new gels each time.

To use the gel, put it into a <u>gel box</u> and pour 1x TAE buffer over it until the buffer barely overflows to cover the gel, making sure to fill all the wells as you pour the buffer. Keep in mind that the DNA will move towards the positive electrode, so make sure you orient the gel correctly. Set a small sheet of parafilm in front of the gel box and pipette 2.5 uL drops of loading buffer for as many wells as you have in your gel. Do this in rows of 8 so it is easy to keep track of which sample is going into each lane. Add 8 uL of your PCR products to the drops on the parafilm and pipette them into the wells on the gel. This process should be done quickly so the drops don't evaporate too much and the DNA does not disperse throughout the gel before you run it. You don't need to hurry, but you also shouldn't stop in the middle and go do something else for awhile.

Optionally, you can use one of your lanes for a <u>DNA ladder</u> to verify that the gel is showing DNA correctly and see the size of your PCR product.

Run the gel at 120 volts DC for 20 minutes. If you leave it for 30 minutes or longer, you will lose your results.

Visualize the gel using a <u>UV transilluminator</u>. Do not look at the UV light for more than a half second - you can use a sheet of glass to stop most of the UV and make it safer to view. Yellow safety goggles can be used to filter out the blue, making the results easier to see and providing additional UV eye protection. It is best to quickly photograph your gel and record your results from the photo to save your eyes and prevent sunburn.

If you get a strong or medium-strong clear band, it is very likely that you will get a clean sequence from the sample. If you get a weak band, smear or no band at all, <u>nested PCR</u> can be used to further amplify the DNA.

TAE buffer is the standard buffer used, but it may not be the best choice - sodium borate can be used instead, allowing higher voltage and therefore faster gels. See <u>Faster even cool DNA gels</u>.

Sequencing

Sanger sequencing

If the gel electrophoresis indicates that the PCR reaction worked, it can be brought in for sequencing. For sequencing I use <u>Genewiz</u>, which has a sample drop off box at 626 Bancroft in Berkeley. If you sign up for a new account, the first two sequences are free. If you have Genewiz do the DNA quantification and PCR cleanup, they charge \$7 per sample or \$5 per sample if you do 96 at a time.

Genewiz sample submission guidelines

MClab does DNA sequencing less expensively

Bioinformatics

Download the <u>sequence</u> and <u>chromatogram</u> from your sequencing facility. <u>BLAST</u> the sequence.

- At the <u>base pairs</u> where you see a difference between your sequence and the closest BLAST match, verify on the chromatogram to see if the differences are real or just sequencer errors. Fix any sequencer errors and save the text file. I use the free software <u>FinchTV</u> to view chromatograms.
- BLAST the fixed sequence and download some of the matches in FASTA format. You can make a tree with all close matches, or just select the sequences that you want to compare your sample to.
- Add your sequence to the <u>FASTA file</u> with a text editor.
- Align your sequence using your favorite <u>sequence alignment</u> program.
- Use the FASTA file to build a phylogenetic tree using Mega 7 for Windows, uGene for Linux or the more difficult but powerful multiplatform command line tool RaxML and the java program FigTree to visualize the tree. The easiest way to make a tree is to use the one-click mode at http://phylogeny.lirmm.fr this website allows you to upload a FASTA file, aligns the sequences and makes a tree using the <a href="mainto-main

How to use Mega on Windows to make phylogenetic trees

Supplies Needed

Gel box with tray and comb \$115 or make your own for \$21

PCR machine \$59

10 uL and 200 uL pipettes 1<u>0 - 50 uL \$27 P200 \$22</u>

PCR master mix \$46 for 200 reactions \$20 for 50 reactions

Electrophoresis DC power supply \$57

Pipette tips (sterile, for p10 and p200 pipettes) \$40

PCR tubes \$20 for 120 tubes

Ethidium Bromide or other DNA stain \$10

100 bp DNA Marker \$10

Agarose \$10

DNA extraction supplies (NaOH and TRIS buffer, plastic pestles) \$10 \$10 \$55 for 100 pestles 500 tubes for \$10

PCR primers \$10

Gel loading dye <u>\$5</u>

TAE buffer concentrate \$10

Tube rack for DNA extracts \$5

Blue LED flashlight for viewing DNA on gel \$3

Pure, DNA-free water \$2 (bottled distilled water works)

Total cost to set up a DNA Lab at home: \$455 (or about \$200 less if you DIY some of the pieces). Recurring costs are about \$5 - \$8 per sequence.

This article was last updated August 8, 2016. We are publishing it with permission of the author and administrator of the Facebook group called 'Fungal Sequencing' https://www.facebook.com/groups/FungalSequencing/.

Keys to Lichens of North America: Revised and Expanded

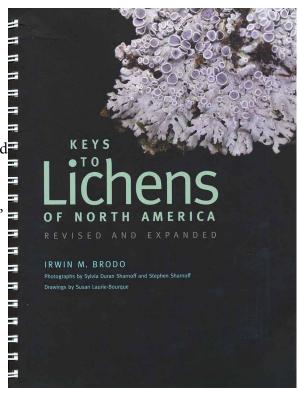
Irwin M. Brodo, 2016

Yale University Press (www.yalebooks.com)

ISBN 978-0-300-19573-6 (spiral bound paper, 424 pages)

\$29.95

I reviewed *Lichens of North America* in the May-June 2002 issue of *The Mycophile* and had this to say: "... this splendid nine-pound compendium (definitely NOT a field guide) will allow anyone in North America, expert or neophyte, to identify our more common foliose, fruticose, squamulose, and crustose lichens (yes, even crusts) ... The writing is clear and easy to understand, even for beginning lichenologists, and the illustrations are absolutely marvelous (there are over 920 color photographs taken mostly in the field) ... It's clearly the best buy in a natural history book that I've seen in a long, long time." Although the list price has increased from the original (subsidized) \$69.95 to \$135, used copies can be found for much less and the book remains highly recommended.



In addition to the wonderful photos, Lichens of North America

(*LNA*) contains extensive keys. According to the preface of this new volume, many users asked that the keys be issued separately to facilitate their use in university courses and workshops, and to protect the main volume from wear and staining during lab use. Rather than simply extract the keys from *LNA*, Brodo chose to update, revise, and expand the keys to reflect all that had happened, nomenclaturally and otherwise, since the original versions were completed in 1998.

The new keys cover 382 genera and 2028 species (up from 1086 in the original book), which is 42% of the 4881 species known to occur in the continental U.S. and Canada. The keys are arranged alphabetically by genus, and species that are illustrated in LNA are indicated by bold font. Although the 2028 species do not include every lichen in North America, they do cover all, or nearly all, of the common or conspicuous species one is likely to find.

Anyone interested in identifying lichens in North America will find this a must-have publication and, if you don't own a copy already, you'll want to track down *LNA* to get the most out of these keys.

Steve Trudell

WEB ARTICLES ON FUNGI & LICHENS:

THE NEXT LEATHER JACKET WILL BE MADE FROM MUSHROOMS http://www.healthlivenews.com/the-next-leather-jacket-will-be-made-from-mushrooms/

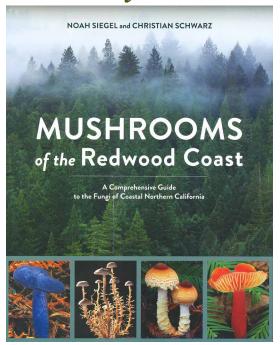
HOW A GUY FROM A MONTANA TRAILER PARK UPTURNED 150 YEARS OF BIOLOGY http://www.theatlantic.com/science/archive/2016/07/how-a-guy-from-a-montana-trailer-park-upturned-150-years-of-biology/491702/

Mushrooms of the Redwood Coast: A Comprehensive Guide to the Fungi of Coastal Northern California

Noah Siegel and Christian Schwarz, 2016 Ten Speed Press (www.tenspeed.com) ISBN 978-1-60774-817-5 (plasticized paper, 602 pages) \$35.00

Brief review: Beautiful book. Lots of species. Reasonable price. Buy it.

Long review: Things are continuing to get better and better for identification-minded California mushroom hunters ... on the book front at least. This new guide from two of North America's younger field mycologists is the third of three recent books dealing with the mushrooms of the Golden State. Preceding it were *Field Guide to Mushrooms of Western North America (MWNA*, with very heavy emphasis on California) by Mike Davis, Bob Sommer, and John Menge (*The Mycophile* March-April 2013) and *California Mushrooms: The Comprehensive Identification Guide* (*CM*) by Dennis Desjardin, Mike Wood, and Fred Stevens (*The Mycophile* September-October 2015).



Content-wise, *Mushrooms of the Redwood Coast (MRC)* is a typical field guide. The front matter includes Introduction, What Are Mushrooms? Ecology of Fungi, Humans and Fungi, The Redwood Coast, Trees of the Redwood Coast, Finding Mushrooms, Collecting Mushrooms, Identifying Mushrooms, Making Spore Prints, Tools of the Trade, Making Collections, Photographing Mushrooms, Collecting for the Table, Taxonomy and Cladistics, How to Read the Species Descriptions, and How to Use the Pictorial Key to the Major Sections, all in 16 tiny-font pages. The Pictorial Key to the Major Sections, which follows, comprises an additional six pages. The species treatments and accompanying section descriptions occupy a full 548 pages. They are followed by back matter that includes Acknowledgments, About the Authors, Future Directions, Glossary, Resources for the Mycophile (clubs and websites), Bibliography (fairly extensive), General Index, and Genus and Species Index.

The 25 categories ("Major Sections") used to group the species treatments are Chanterelles and Gomphoids, *Amanita, Lepiota* and Allies, *Agaricus* and *Melanophyllum*, Dark-Spored Mushrooms, Brown-Spored Decomposers, Mycorrhizal Brown-Spored Mushrooms, *Cortinarius, Entoloma* and Allies, *Pluteus* and Allies, *Russula* and *Lactarius*, Waxy Caps, The White-Spored Multitude, Pleurotoid Mushrooms, Gilled Bolete Relatives, Boletes, Polypores and Allies, Crust Fungi, Tooth Fungi, Coral and Club Fungi, Puffballs / Earthballs / Earthstars / Stinkhorns / Bird's Nests, Truffles, Jelly Fungi, Morels / False Morels / Elfin Saddles, and Cup Fungi. While these will probably work fine for those with enough experience for the genus names to be meaningful, the breakdown might prove difficult for those with less experience, and the use of ecological role could prove troublesome to even those with a fair amount of experience, because whether the species is a saprotroph or a mycorrhizal associate isn't something that is obvious from its outward appearance. Although there is no perfect arrangement, I would have preferred sticking to a strictly macromorphological approach. There are no keys other than the up-front pictorial one, but the authors will be posting keys and other identification resources online at www.redwoodcoastmushrooms.org.

The species treatments contain the usual stuff—photo (usually one, but occasionally more), species name with author, common name(s) if it has one or if the authors decided to coin one, descriptions of cap, gills / pores / spines, stipe, partial veil, flesh, odor, taste, KOH reaction, spore deposit color, key microscopic features

(usually spore size, shape, and ornamentation and cystidia types), ecology, edibility, and comments. Where appropriate, misapplied names, synonyms, and nomenclatural notes follow. The names are as up-to-date as could be given their rate of change and the time it takes to go from manuscript to printed book, and the comments sections typically are very useful in pointing out key ID features and mentioning similar species.

The photographs are generally wonderful (probably due to Siegel's "unrivaled" technique and attention to detail). Taken in the field, most are staged documentary portraits with the mushrooms arranged in aesthetically pleasing fashion. Key identification features are shown well and color rendition, sharpness, and lighting typically are excellent. Often a long-sectioned fruitbody showing the flesh, or dribbles of KOH to demonstrate a color reaction, are included. Most are cropped very closely, including a number where, unfortunately, stipe bases have been cut off. This makes it easier to see detailed features, albeit with less of a sense for the habitat and relative size of the mushrooms. One can't have it all.

Quibbles. The strict one or two species per page layout results in a mix of large and small photos. Many of the large (6×4 inches) ones are striking, and the size makes it easy to see important identification features. However, the small $(2-7/8 \times 1-15/16 \text{ inches})$ photos have much less impact and the features are not as easily seen. A "freeflow" layout, such as was used in CM, would have allowed the photos to be of a more uniform size, still large enough to impress and, more importantly, large enough for the features to be readily appreciated. The text is the smallest I can recall having seen in a book and I couldn't begin to attack it without my reading glasses. Again, one can't have it all—use of a larger font would no doubt have meant reducing the number of species that could

be included. On balance, I support the more-species approach and will just have to make sure the glasses are nearby at all times.

The fact that this is the third of the California mushroom books to come out might lead some to wonder whether Siegel and Schwarz cover any new ground. Indeed they do. Three hundred eighteen of the 768 species they describe and illustrate appear only in MRC (CM and MWNA include 669 and 299 total species, and 207 and 34 "unique" ones, respectively). Taken together, the three books cover an impressive 1046 species, providing California mushroom hunters with a valuable new resource. Although MRC does not often mention the range of the species beyond California, I counted at least 478 species that extend into the Pacific Northwest and so the book will be useful throughout the West Coast, particularly in southern Oregon.

As the final editorial touches were being applied, the publisher asked me to write a blurb for the back cover (apparently I was too wordy and so there wasn't room to include it) — "California's north coast is home to the most awe-inspiring forests on Earth. Those who take time to look down from the towering redwoods will find that, as the motto of the North American Mycological Association says, there is also a 'world of wonder at your feet.' Accomplished field mycologists/ photographers Siegel and Schwarz have made that col-



orful and fascinating world accessible through this most excellent new book that will be indispensable for those seeking to know California's mushrooms." Beautiful book. Lots of species. Reasonable price. Buy it.

to crowded, White or creamy ivory at first, soon with yellow tones. STIPE: 8–15 cm long, 2–3 cm thick at apex, swollen or enlarged toward base. Base distinctly enlarged (but not

or enlarged toward base. Base distinctly enlarged flost not abruptly bulbously and also tapered downward, resulting in a spindle-shaped appearance. Creamy white, but lower stipe covered with many yellow or gray, pointed, curved scales. Often developing reddish status at base. PARTAL VEIL: A creamy white membranous skirt, usually with a ring of yellow universal veil scales adhering to odge. VOLVAS Sometimes very indistinct, otherwise appearing as concentric rings of flat scales, often as a fused "sheet" that detaches from base of stipe and remains in soil. FLESH: Firm to soft, whitish Purising slowly dull reddish, specially around base of stipe. DDOB: Indistinct. TASTE: Indistinct. SPORE DEPOSIT: White. MICROSCOPY: Spores 8.5—9.5 x 6—7 µm, broadly ellipsoid to ellipsoid, amyloid. Basidia not clamped at base.

COMMENTS: The cap color ranging from dark brown to bright yellow, creamy gills, skirtlike partial veil, yellowish universal veil remnants, and reddish staining at the stipe base are distinctive. Few other mushrooms approach this

species in appearance. A. gemmata and its brethren migh

cause confusion, but they are rarely as richly colored and have white (not yellow) universal veil patches.

MISAPPLIED NAMES: Amanita franchetii (Boud.) Fayod and

North American Mycological Association

Steve Bichler 6018 Illinois Lane SE, Unit B Lacey, WA 98513-3617

Change Service Requested



Mushroom of the Issue



Exsudoporus frostii (formerly Boletus frostii) is commonly known as 'Frost's Bolete' or the 'Apple Bolete'. Amateur American mycologist, Charles Christopher Frost, published the first scientific description of this species in 1874. These stunning boletes can be recognized by their dark red sticky caps, red pores, deeply reticulate stipe, and promptly blue-bruising tissue. The fruit bodies grow solitarily, scattered, or in groups on the ground under hardwood trees, especially oak. This fungus fruits in summer to early autumn. I typically find the largest concentration of them in late summer, growing in sandy soil under scrub oak in Sw Michigan.

Although considered edible, some sources do not recommend for consumption because of the risk of confusion with other poisonous redpored, blue-bruising boletes. I myself have eaten these a few times, they are very pleasant with a light citrus flavor. The young fruit bodies often exude amber-colored drops on the pore surface called guttation. You can slightly see some guttation on the specimen to the right, another thing you may notice about the this unique specimen is that it has developed a rose comb (mutation). There is some controversy over whether this phenomenon is caused by pollution or genetics. One thing is for sure, it is always a pleasure to see this very beautiful and photogenic mushroom in the forest. I spend most of my time taking photos of fungi and *Exsudoporus frostii* is one of my favorites to find and photograph.

By Anthony Michael Blowers