

Copyright 2011 by Rodham E. Tulloss, P. O. Box 57, Roosevelt, NJ 08555-0057.

Cover images left to right (in 3 rows): *Amanita cinereopannosa*, *A. daucipes*, *A. morrisii*, *A. pseudovolvata*, *A. borealisorora*, *A. cinereopannosa*, *A. sinicoflava*, *A. amerimuscaria* (yellow variant) [= *A. muscaria* var. *guessowii*], *A. magnivelaris* (photo: Yves Lamoureux), *A. whetstoneae*, *A. jacksonii*. All photographs not otherwise credited are by R. E. Tulloss.

About *Amanitaceae*

The family *Amanitaceae* R. Heim ex Pouzar is typified by the genus *Amanita* Pers. and presently comprises two genera: *Amanita* and *Limacella* Earle. In most of the world, the species in these two genera are gilled mushrooms with central stipes. It is very likely that the genus *Amanita* will be found to contain 900 to 1,000 taxa (over 745 are presently listed on the new www.amanitaceae.org website). So far as is known, the genus *Limacella* is much smaller, apparently an evolutionary relict group, with about 60 - 100 species to be expected (51 are now listed on www.amanitaceae.org, with some synonymy among the names expected).

The small number of taxa not having an agaricoid habit (that is, not having the form of a gilled mushroom with a central stem) occur in arid, often sandy areas where rain is seasonal and not well retained in the ecosystem—(a) countries surrounding the Mediterranean (one species) and (b) southwestern Australia (about a half-dozen species). These exceptions were formerly treated in two genera—*Amarrendia* Bougher & T. Lebel and *Torrendia* Bres.—that are now considered synonyms of *Amanita*.

Recent (including some unpublished) molecular studies concur with the morphological view that *Limacella* is a distinct and older (more basal) genus than *Amanita*, and the two share a common ancestor.

Agaricoid forms

All agaricoid taxa in the *Amanitaceae* have two, defining, microscopic characters in common:

- a cross-section of a gill will reveal that the gill's tissue (lamella trama) has an interior structure that is some variation of a constant theme—if you imagine a line running down the center of the cross-section from the connection of the gill to the cap to the gill's free edge, (1) the two halves of the gill divided by that line are approximate mirror images of each other and (2) the tissues on both sides of the center line are composed of cells that individually and in groups are clearly curving away from the center line. This anatomical structure is called a bilateral, divergent lamella trama.
- a thin, vertical slice of the stem tissue will always reveal vertically aligned inflated cells that are shaped like clubs or baseball bats, these may be in short chains in some taxa, but are more commonly solitary and arising from the end of a simple hypha. This sort of inflated cell has been given a technical name "acrophysalide." Stem tissue with such a structure is called longitudinally acrophysalidic. It is unknown outside of the family *Amanitaceae*. It is persistent—even after an amanita has been chopped and thoroughly cooked and been in a poisoning victims stomach, the fact that the tissue is longitudinally acrophysalidic can be determined with a microscope.

Sequestrate forms

What about the taxa formerly placed in the genera *Amarrendia* and *Torrendia*?

Both of these genera included sequestrate species—species that had lost the ability to auto-eject spores from their basidia—they sequester their spores. These species retain basidia; and some of the cellular structure of gills is also preserved; but true gills no longer exist. The term that is used for the tissue in which spores develop in common puffballs and truffle-like basidiomycetes—"gleba"—is used for the spore bearing tissue in the truffle-like amanitas formerly in *Amarrendia*. The term "lamella trama" is not applicable.

On the other hand, the former members of the genus *Torrendia* all have a stem. The stem was little affected by the evolutionary changes that produced the secotioid ("puffball on a stick") form of the species of *Torrendia*. One of the consequences is that the stem of an *Amanita* formerly placed in *Torrendia* is longitudinally acrophysalidic.

The taxa formerly placed in *Torrendia* comprise the epigeous (above ground) sequestrate forms in *Amanita*, and the taxa formerly placed in *Amarrendia* comprise the hypogeous (underground), truffle-like species of *Amanita*. A number of hypogeous amanitas have retained an internal element called a columella which is the remnant of a stem much altered by evolution, but still including the typical longitudinally acrophysalidic tissue.

Hence a formal definition of the family *Amanitaceae* can be reduced to this: The *Amanitaceae* include all and only those species of the Agaricales that have a stipe or columella that comprises longitudinally acrophysalidic tissue.

To find an alphabetical listing of all species of the *Amanitaceae* that are listed on www.amanitaceae.org, go to www.amanitaceae.org/?family+Amanitaceae

NOTES

About *Limacella*

Species of the genus *Limacella* Earle are strongly differentiated from the genus *Amanita* by their mode of basidiome (fruiting body) development (ontogeny). Whereas the species of *Amanita* share the unique form of ontogeny that is called “schizohymenial” (see “About *Amanita*”), basidiome development in *Limacella* is generally like that of all other terrestrial (ground-growing) Agaricales in exhibiting the following stages:

- growth of a minute, vertically oriented, rudimentary stipe (stem)
- initiation of pileus (cap) growth at the top of the rudimentary stipe [In cross-section, the rudimentary pileus expands at first by extending its edge outward, then downward, and then into a self-enclosing spiral.]
- initiation of lamella (gill) growth on the underside of the developing cap (the inside of the cap-edge spiral).

In other words, for the species that have been investigated, the lamellae of a *Limacella* grow into empty space from the undersurface of the developing pileus. As a result, unlike the species of *Amanita*, limacellas have a fertile edge on their lamellae—basidia appear on the faces and on the edge of a *Limacella* lamella. A fertile gill edge of a specimen demonstrably belonging to the *Amanitaceae*, is demonstration that the organism in question produced this gill on a fruiting body that did not arise through schizohymenial ontogeny—the gill edge did not have to be separated mechanically from a partial veil or a stipe of an *Amanita*. Hence, when the gill edge of a member of the *Amanitaceae* is fertile, that specimen is a *Limacella*, not an *Amanita*.

In *Limacella*, the analog of the universal veil of *Amanita* is a glutinous (slimy) matrix supported by tightly packed, vertically oriented hyphae (sometimes with distinctive tip cells) that arise not from a pileipellis (cuticle or cap skin), but from a dense layer in the uppermost part of the pileus context (cap flesh). Indeed, as in most taxa of *Amanita* [sect. *Lepidella*] subsect. *Vittadiniae* there is no pileipellis present in *Limacella*. In the literature, a reference to a pileipellis in *Limacella* is a reference to the vertical hyphae and associated slime that are the analog of the universal veil in *Amanita*. At least for the present, we will call this structure the volva or universal veil or universal veil analog in our descriptions of species of *Limacella*.

What do we know about the volva in *Limacella*? Apparently, it develops in at least two stages.

In the first stage, very narrow hyphae grow vertically from the outer surface (eventually, the upper surface) of the developing pileus. These hyphae soon begin to gelatinize and collapse creating a slimy covering for the immature cap. A second set of hyphae (of larger diameter than the first group) then develop from the tissue just below the bases of the first set of vertically aligned hyphae. The second set of hyphae is also vertically aligned and very tightly packed and carry the previously created slime upward on their closely packed tips. The tip (terminal) cells of these hyphae take on shapes that fall approximately into three groups that (given present knowledge) are considered as a possible foundation for the hypothetical division of the genus *Limacella* into three groups which are called “sections” on the amanitaceae.org site (until we learn a reason to change this rank).

With regard to the “slimy partial veil” seen in some species of *Limacella*, this structure is actually a remnant of the development of the universal veil. Since the young cap has its edges curled under (putting part of the pileus surface in close proximity to the surface of the developing stipe), some hyphae from the pileus surface may form wispy (spiderweb-like or cortina-like) connections between the cap and the stipe. These hyphae gelatinize and/or are covered with gluten slipping down from the higher parts of the immature pileus; and then they create a slimy “partial-veil-like” structure (that will include some hyphae). When the cap unfurls and breaks the tenuous connection with the stipe, small tufts of broken hyphae covered with slime may be left encircling a narrow region on the upper stipe. The resulting ring of material looks like a partial veil in *Amanita* and may be protective of the maturing lamellae for a short time, but its origin and development differ from the origin and development of the partial veil in the schizohymenial genus *Amanita*.

A membranous partial veil (i.e., ring, skirt, or annulus) is present in some species of *Limacella*. **...more to be developed...**

The state of understanding of *Limacella* is behind that of the genus *Amanita*. The user of the amanitaceae.org site that looks at the technical tabs of taxa in *Limacella* will see that there is very little uniformity in the collection of data by past authors and revisers of taxa. The last attempt at revision of *Limacella* for North America was published in 1945—sixty-five years before this sentence was drafted. As a consequence, revisions of all type collections and many more recent, well-documented collections will have to be made to gain a worldwide grasp of the diversity, taxonomy, and systematics of this genus.

Part of the reason for the lack of understanding is lack of subject matter experts using modern methodologies. Lack of expertise may be due, at least in part, to the very small number of collections that exist to support research. The amanita-

ceae.org site lists about 50 taxa or probable taxa in *Lepidella*, and there are a few pairs of "taxa or probable taxa" that probably consist of material of a single taxon.

There is a very significant role to be played by disciplined collectors who collect carefully, annotate thoroughly, and photograph well. So few collections of *Limacella* are reported each year (e.g., in journals or newsletters or on mushroomob-server.org or in blogs of mushrooming groups, that it is a shame not to have more of them documented more thoroughly and dried well for deposit in working herbaria that are accessible to specialists. The way to make progress certainly must involve soliciting quality collections from as broad an audience as possible.

The type species of *Limacella* is *Agaricus delicatus* Fr. : Fr. (1821).

To begin exploring the taxa of *Limacella*, we suggest using the alphabetic directory to be found at www.amanitaceae.org/?genus+Limacella

About Amanita

As discussed in the previous section, the definition of the genus *Amanita* has been slightly complicated from a morphological point of view by the inclusion of at least seven species in the genus that are not agaricoid (do not have the form of a typical gilled mushroom with a central stem and have lost the ability to mechanically discharge spores). Having accommodated these species in a morphological definition of the *Amanitaceae*, our task of defining the genus *Amanita* is made easier.

The genus *Amanita* includes all and only those members of the *Amanitaceae* that produce a fruiting body (basidiome) satisfying exactly one of the following conditions:

- It is hypogeous (it has lost the ability to mechanically discharge its spores and grows under ground).
- It is secotioid (it has lost the ability to mechanically discharge its spores and grows above ground).
- It is agaricoid and exhibits the mode of basidiome development (ontogeny) that is called schizohymenial.

In most of the world this reduces the practical matter of identification of agaricoid specimens of *Amanita* to the tasks of finding in that specimen the same evidence that has been very clearly required since the publication of the thesis of Dr. Cornelis Bas in 1969. The specimen that is a member of the *Amanitaceae* is an *Amanita* if and only if you can demonstrate that the specimen

- has longitudinally acrophysalidic stipe tissue
- is not a species of *Limacella*.

If an unopened button of the species is available, and you find that all the developing elements (cap, stem, gills, volva) of a mature mushroom are visible as distinct, shadowy regions in a cross-section of the button and that these developing elements are interconnected by tissue so that there is no open space within the button, then you have demonstrated that the probable ontogeny of the button is schizohymenial—literally that the faces of adjacent gills must be split apart from each other as the development of the mushroom continues.

If an agaric exhibits schizohymenial development it can only be an *Amanita*—this ontogeny is restricted entirely to the genus *Amanita*. Hence, you don't have a *Limacella*.

If the collector of your specimen found no buttons are found them but did not retain them for your edification, then you should consider the following section (“About *Limacella*”) in which distinctive morphological features of *Limacella* are described; and you must show that the material you have in hand lacks those distinctive characters.

Here is a simple method of separating dried specimens of *Limacella* and *Amanita* with microscopic examination of the gill edge. Check whether the edge of a gill is fertile (has spore-bearing basidia growing from it) or sterile (doesn't have basidia growing from it).

In the *Amanitaceae*, the fertile condition occurs only in *Limacella*. The sterile condition is found only in *Amanita*.

The sterile condition can be recognized as follows: In *Amanita*, the gill edge is comprised of a “cable-like” grouping of hyphae running the length of the gill edge and giving rise to balloon-like cells of various shapes (singly or in short chains) which separate, collapse, gelatinize, and/or break, facilitating the the separation of the gill edge from the stem or from the partial veil (ring, annulus, skirt) as the elements of the expanding *Amanita* basidiome are separating.

The reader may think, “Surely, I recognize an *Amanita* when I see one.” In response it must be said that, in many cases (especially with regard to taxa similar to locally familiar taxa), the reader probably does know his/her amanitas by sight. On the other hand, it still happens that mycological taxonomists name species in the genus *Amanita* that are not amanitas. Wouldn't you like to avoid that happening to you?

The type species of *Amanita* is *A. muscaria* (L.: Fr.) Lam. [≡*Agaricus muscarius* L. (1753)].

To start exploring *Amanita* with an alphabetized directory of the taxa listed on the amanitaceae.org site, go to www.amanitaceae.org/?genus+Amanita.

Amanita is divided into two subgenera depending on the reaction of spores to an iodine solution (e.g., Melzer's Reagent). A darkening reaction of a spore's wall in this solution is called an amyloid reaction and lack of such a reaction classifies a spore as inamyloid.

Species having spores producing the amyloid reaction are classified in *Amanita* subgen. *Lepidella*. The type species of this subgenus is *A. vittadinii* (Moretti) Vitt. The directory for this subgenus on the amanitaceae.org site is found at www.amanitaceae.org/?subgenus=Lepidella

The species with inamyloid spores are placed in *Amanita* subgen. *Amanita*. The type species for subgenus *Amanita* is the same species that is the type for genus as a whole—*A. muscaria*. For an alphabetized directory of the taxa of subgenus *Amanita*, go to

www.amanitaceae.org/?subgenus+Amanita

The subgenera are further divided into sections. There are seven sections currently recognized in *Amanita*. In this workshop/seminar, sectional names follow the usage of Corner and Bas (1962) and Bas (1969) as emended in Yang (1997). A description of the sections of the genus can be found here:

www.amanitaceae.org/?sections+of+Amanita

sections of *Amanita*

Because so many of the species of *Amanita* are not formally described (perhaps as many as half of world taxa in the genus), RET has urged persons just beginning to learn *Amanita* to start with an understanding of the sectional level of the genus and to learn select, "iconic" taxa in their home area that can represent the sections present in that area.

In this workshop, sectional names will follow the usage of Corner and Bas (1962) and Bas (1969) as emended in Yang (1997).

In *Amanita* subgenus *Amanita* (spores inamyloid):

Amanita* sect. *Amanita

In this section, the basidiome (fruiting body) develops eccentrically upward (off-center, toward the top) in the primordium (the button stage of development). As a result, whether or not there is a saccate (sack-like) volva (and this occurs in very few species), there is very likely to be a bulb at the stipe base—at least in young specimens. The known toxins of this section are related to muscimol and ibotenic acid. These chemicals cause the "Pantherine Syndrome" in humans and some other mammals. The type species of this section is *A. muscaria*.

Amanita* sect. *Caesareae

In this section, the basidiome develops approximately centrally in the primordium. The stipe is totally elongating (does not have a bulb at the base). All species have a partial veil (annulus or ring); all species have a saccate volva; and all species bear clamps at the bases of their basidia. Most of the *Amanita* species that have a hypogeous (truffle-like) or secotioid ("puffball-on-a-stick") habit, are placed in section *Caesareae*. While no statement can be made covering all the taxa of this section (many of which are not formally described), a number of the species are eaten and, in some areas of the world, are market commodities. The type species of this section is *A. caesarea*.

Amanita* sect. *Vaginatae

In this section, development of the basidiome and elongation of the stipe are as in section *Caesareae* (above). However, none of the species of section *Vaginatae* have a partial veil on the stipe. The universal veil (volva) is usually saccate, but it may have a very weak internal structure that may cause it to break up in a variety of ways in different species. Clamps are usually not reported at the bases of basidia in this section. While no statement can be made covering all the taxa of this section (many of which are not formally described), a number of the species are eaten and, in some areas of the world, are market commodities. The type species of this section is *A. vaginata*, which unfortunately is interpreted in different ways by different authors.

In *Amanita* subgenus *Lepidella* (spores amyloid):

Amanita* sect. *Lepidella

In this section, the margin of the pileus is appendiculate (decorated with more or less floccose or powdery, hanging material) at least at first; and the base of the stipe is not encased in a saccate volva (although there may be a membranous, thin limb (flap) of universal veil attached at the top of the stipe's bulb in a few species). This is the only section of *Amanita* known to include a few species (about 40) that sometimes or always live without a mycorrhizal partner (symbiotic relationship with a plant). All toxic species known from this section contain an amino-acid toxin (allenic norleucine) that has significant destructive impact on both the liver and kidneys of humans. The type species of this section is *A. vittadinii*.

It was a brilliant monograph on this section (by Dr. Cornelis Bas, 1969) that played a major transformative role in the study of *Amanita*.

Amanita* sect. *Amidella

Species of this rather small section have pilei with an appendiculate margin as described for the taxa of section *Lepidella*; however, the appendiculate material is more scanty in section *Amidella* and disappears much more quickly as the basidiome of a member of section *Amidella* matures. In many of the group of section *Amidella* taxa most similar to *A. volvata*,

the stipe is totally elongating and its base is enclosed in a very thick, multilayer, saccate universal veil. The shape of the volva can range from nearly globose to test-tube-like to very large and baggy. Basidiomes of this same group of taxa often stain pinkish on bruising (at least when very young and fresh) and take on a brownish red (brick red) color with time. Only one of the known species in sect. *Amidella* (*A. peckiana*) has a partial veil (annulus or ring) and this is found only in the early stages of expansion of the basidiome. Three taxa of section *Amidella* are reported to lack all brownish staining reactions; two of these are considered edible (*A. ovoidea* and *A. neoovoidea*); and one is dangerously toxic (*A. proxima*)—causing symptoms similar to those produced by the species of section *Lepidella* that contain allenic norleucine. The type of this section is *A. volvata*.

Amanita* sect. *Phalloideae

In this rather small section, the species have a pileus margin that is not appendiculate even in very young specimens. All species of this section also have a stipe that always has a bulbous base and always has a persistent partial veil (annulus or ring). The universal veil is always membranous; and is present on the stipe's bulb as either a limbate (flap-like) or saccate volva. Section *Phalloideae* infamously includes the taxa that are the most common causes of death by mushroom poisoning in the world. The primary causes of the deaths are the chemicals known as amatoxins. The oldest (basal) taxa of this section lack amatoxins and are edible, market commodities in eastern and southern Asia. The type species of this section is *A. phalloides*.

Amanita* sect. *Validae

In this section, the pileus margin is never appendiculate; and the stipe always bears a persistent partial veil. The stipe is always bulbous at its base—although the breadth of the bulb may diminish with age. The universal veil is always friable (fragile, breakable, crumbly) in whole or in part. The known toxin(s) of the section are hemolytic—they cause the destruction of red blood cells, which results in gastrointestinal distress. These toxins are destroyed by heat during cooking. As a result, several of the rubescent taxa (for example) of section *Validae* in Europe, Africa, and the Americas are market commodities and are eaten after cooking by indigenous peoples in the areas where they are found. The type species of this section is *A. excelsa*.

Morphological study of *Amanita* (Fungi: *Agaricales*)—notes on methodology

DRAFT

RODHAM E. TULLOSS^{1A} AND ZHU LIANG YANG^B

^A<ret@njcc.com> P. O. Box 57, Roosevelt, New Jersey 08555-0057, U.S.A

^B<fungi@mail.kib.ac.cn> Kunming Institute of Botany, Academia Sinica, Kunming 650204, China

*This paper is dedicated to Dr. Cornelis Bas, Leiden,
to whom we wish to express our profound gratitude for his work, his mentoring, his wisdom,
his subtle criticism, his generosity, his humor, his encouragement, and his discipline.*

Abstract—The similar methodologies of the authors are presented in detail. An appeal is made for broader use of such methods in order to advance taxonomy and systematics of *Amanita*. Detailed forms to support uniform specimen annotation are provided.

Key words—basic components of tissues, biometrics, macromorphology, micromorphology, spores

Amanita is a fascinating genus consisting of many edible, as well as poisonous, even lethal, mushrooms. Species of this genus are valuable and important to human beings due to their mycorrhizal relationship with vascular plants and consequent important role in ecosystems (Yang 2000a) and their commercial value as foodstuffs in many cultures (e.g., Buyck 1994; Montoya Esquivel 1997; Tulloss and Bhandary 1992).

Despite the many contributions to the knowledge of *Amanita* (e.g.: Coker 1917; Gilbert 1940-41; Corner and Bas 1962; Bas 1969; Fraiture 1993; Jenkins 1977; Miller 1992a & 1992b; Neville and Poumarat 1996 & 2004; Pegler and Shah-Smith 1997 [as well as Pegler's regional floristic studies]; Reid 1980; Tulloss *et al.* 1992; Tulloss 1994b; Tulloss *et al.* 2001; Wood 1997; Yang 1997), many species of the genus are still not well known, even in Europe after about 300 years of mycological research (Bas 2000). The taxonomy is in an unsettled state for most sections of the genus.

One reason for this unsatisfactory state of affairs is that only infrequently are herbarium collections accompanied by any detailed field annotation or illustrations of fresh material. Another reason is that many collections were not well-dried or are not well-preserved resulting in destruction of microscopic characters that now seem critical to the taxonomy of the genus. Still another reason is that many of these valuable characters persist in being ignored by taxonomists. It was not until the sixties of the Twentieth Century that a beginning was made with systematic and detailed analysis of many microscopic structures (Bas 1969; Bas 2000). Unfortunately, after more than thirty years, few workers have sufficiently adopted the methods that Bas put forth; and the study of *Amanita* is still held back by descriptions of new species that are written in the style of earlier times—omitting characters that would permit a reader to make relevant comparisons with other taxa. It is like creating islands in a lake— islands that are difficult to access—when accessible peninsulas could have been created instead.



Fig.1. Basket used in Tlaxcala, Mexico, showing cardboard carton dividers separating *Amanita* specimens and other useful and beautiful objects. Photo: R. E. Tulloss.

When compounded by the great diversity of the genus, these difficulties make for a further problem: The taxonomy of the genus is only in good order in the one section monographed by Bas—*Amanita* sect. *Lepidella* Corner & Bas. How then, is a scholar (without decades of study) to determine if a collection represents a known taxon or a novel one? To what taxa must a collection be compared to confirm novelty—what are its probable phenetic close relations? The sometimes haphazard selection of taxa compared to proposed novelties in recent literature (e.g., not even restricted to a single section of the genus) serves to underline this point.

Considering all these points, it seemed important to us to publish some notes on study methods in an “international” language. We hope this will result in better communication among *Amanita* researchers, better understanding of the genus, and increased value of future studies of *Amanita* throughout the world. Some of our observations are specific to *Amanita*, but others may be generally applicable in the *Agaricales*.

1. Research Associate (hons.), New York Botanical Garden, Bronx, New York, USA.

Document road map

This paper covers the entire process of dealing with collections of *Amanita* from gathering in the field to annotation when fresh and review of microscopic characters. Some readers may find the level of detail excessive in the first section, but the state of many collections in herbaria suggest to us that inclusion of this information will be of value.

The text is divided into three major sections entitled: “In the field,” “In the laboratory—macroscopic characters,” and “In the laboratory—microscopic characters.” Below this level, sections are marked by numbered headings and, as needed, are further organized in subordinate outline form.

In the field

1. Basket design

For carrying *Amanita* collections in the field, use a very deep basket that is cross-laced with strings so that many rectangular “compartments” are outlined by the strings. The first author got this idea from Dr. David T. Jenkins, University of Alabama, Birmingham. *Amanita* specimens are wrapped in (e.g.) wax paper and arranged with stipes vertical (as they were in the soil) supported by the web of strings (strings in two layers are needed—say, one-third of the way up from the basket bottom and two-thirds of the way up). By storing the specimens in this way, the stipes don’t coil up due to geotropism in the stipe apical region—as they would have a tendency to do in many species of *Amanita* were the specimens laid on their sides. One can arrive home with a photogenic specimen...and one that is easier to measure than it might have been otherwise.

When expecting to collect with a borrowed basket, bring a set of slotted cardboard strips or cardboard dividers from a carton of bottles or cans. The dividers can be expanded and inserted in the borrowed basket. They can be folded to fit baskets of various dimensions. It seems that dividers from cartons of U.S. tomato ketchup bottles are very well-proportioned as far as *Amanita* collecting goes. If such dividers are lacking, a pocket knife is useful to cut up cardboard to make similar dividers on the spot (Fig. 1).

2. Collecting

Remove the basidiome from the soil carefully. Having the whole mushroom is often valuable in determining a collection. A bulb or lack of a bulb and the form and nature of any universal veil remains on the stipe base may be lost or badly damaged due to careless collecting. Some species of *Amanita* section *Lepidella* are deceptively deeply rooting. Some of the species of other sections can have half or more of the stipe below the surface of the ground. It is best to assume deep insertion in the substrate and excavate each specimen carefully. For digging one can use a large-bladed knife or a narrow garden trowel. An metal tent peg can be used to good advantage for the same purpose.

3. Field Annotation

“Field notes” is a poor term for what is intended to be entered on our note forms; some of these notes are best made after returning from the field (see the following section). Copies of forms mentioned in this paper appear as appendices.

In the field, it is valuable to note collector's names, collection number, locality, date, quantity and distribution of basidiomes, soil type, and habitat. It is important to take the time to note habitat information carefully. Trees in the area of collection (not just the closest tree) are important to know about. Scrub trees in undergrowth are also noteworthy (e.g., *Quercus* seedlings in a forest of *Pinus*). The absence of trees is also very important to note. There are some amanitas (notably in subsection *Vittadiniae* Bas) that apparently are not symbiotic with woody plants.

If a color book can be carried into the field, colors of just-collected material are worth noting in terms of a color code. Otherwise, a best estimate of color should be made in common terms. Careful annotation of color using a color book can be done on return from the field, but beware of colors that change between collection and the laboratory. The first author has experience with two cases in which color change was associated by slight change in spore size and shape when specimens aged in the field or after collecting (e.g., Tulloss and Borgen 1996).

4. Photography

If a camera with macro or other close-up lens has been taken into the field, one should make color slides of the whole basidiome as well as of unique features such as the universal veil material on the pileus, anastomosing lamellae, and universal veil material on the lower stipe and bulb (if one exists). Attempt to fully utilize the macrolens—getting close to characters to be illustrated so that they fill the frame. Depth of field is increased by slowing the lens speed (if that is what can be controlled on the camera) or by stopping down the lens as much as is feasible. To avoid shadows that hide key fea-

tures, use reflectors (made from aluminum foil wrapped around sheets of cardboard) to light the side of the specimen away from the sun. Groom natural settings so that twigs, grass, etc. don't block the camera's view of the mushroom. Use of a tripod and a delay feature (if such are available) are useful to reduce any vibration of the camera during an exposure. After getting a spore print, a photo of the basidiome in longitudinal section is often helpful.

In the laboratory—macroscopic characters

In general, this section follows the note-taking form of Appendix A1. On the other hand, we do not feel it necessary to discuss every word or entry space on the form.

1. The need for macroscopic annotation—comments on determining material from microscopic anatomy alone

It is often necessary to review at least spores, pileipellis, lamella trama, universal veil, and partial veil in order to have a hope of coming up with a definitive determination of dried material when macroscopic data is lacking. Microscopic characters are plentiful and very valuable in *Amanita*, and future keys should be available that are based on anatomy of these tissues and the presence or absence of clamps in them. Today, to be able to do efficient work in determining taxa, notes on macroscopic characters are necessary. The difference between an hour vs. a day spent on a single specimen is significant. The process is bound to become more difficult as the next several hundred taxa of *Amanita* are described. Good notes on fresh material and good photographs will become more (not less) important.

2. Dimensions

In order to make a meaningful ratio of the length of pileus striations to the diameter of the pileus, the pileus diameter must be measured along the pileus surface—as though the pileus were expanded to a fully planar condition. Such a measurement can be done by draping a piece of string or thread or a strip of paper over the pileus, holding the points on the string (for example) that are precisely at the opposing pileus margins, and then measuring the straightened (but unstretched) string. (Dra. E. Pérez-Silva, Universidad Nacional Autónoma de México, suggested this method to the first author.) Pileus thickness, breadth of lamellae, and dimensions of the stipe are all best measured after making a longitudinal section of the basidiome. Therefore, we suggest holding off on making these measurements until a spore print has been obtained (unless a spore deposit is not to be obtained—see “triage,” below).

In the literature, bulb length is sometimes included in stipe length and sometimes not included—even within a single work. Valuable information can be lost in this way and confusion is created in the literature. The length of the stipe above the bulb (if one is present) and the length of the bulb should be treated as two separate dimensions. The overall length of the mushroom is then computable from the thickness of the pileus, the length of the stipe, and the length of the bulb. There is no true bulb in species of section *Vaginatae* (Fr.) Quéél., and the appearance of a bulb in species of section *Amidella* (E.-J. Gilbert) Konrad & Maubl. is usually only the result of a very thick volval sac.

3. Important ratios

Two ratios that are important are the ratio of the length of the striations on the pileus margin to the radius of the pileus and the width of the central cylinder of the stipe to the overall width of the stipe. These should be recorded at least for the largest and smallest basidiomes in a collection; and, if one of the ratios is especially high or low in another basidiome of the same collection, data for that basidiome should be recorded as well.

4. Colors.

Colors can be expressed in your own terms, but it will be much easier to communicate about them if terms defined by a standard color book are used—such as the ones published by Methuen (Kornerup & Wanscher 1978) and Munsell. The set of soil colors published by Munsell (1975) is a good supplement (largely browns and grays) to the wide color range in Methuen (Bas, pers. corresp.). Since Ridgway (1912) colors can be translated into the Munsell code (Hamly 1949), even though Ridgway's publication is a rather rare book these days, the color names can be made meaningful to readers who lack it. If one has a copy of Ridgway, one shouldn't hesitate to use it. On the other hand, one must be aware that Ridgway's handmade color chips vary from copy to copy (Hamly 1949); and Hamly's work has a small number of apparent typographical errors.

The color of the universal veil and lamellae may change as a basidiome ages. This is particularly notable in the taxa of section *Vaginatae* having a friable universal veil. The tendency in these taxa is for the universal veil to become grayer, browner, or even black with age. The lamellae tend to become significantly grayer also. The color of the universal veil in an old basidiome of this group is usually correlated to that in a young one. For example, the pale orangish white volva of one undescribed New Jersey Pine Barrens species (the first author's *Amanita* sp. 49) retains a faint orangish tint as it

becomes gray; and the brilliant yellow-orange volva of an undescribed species collected in Maine (the first author's *Amanita* sp. N29) becomes red-brown. One should check colors of the universal veil, annulus (if present), and lamellae in both young and mature basidiomes. Colors of lamellae should be recorded both in mass (viewed edge-on) and in side view (after longitudinal sectioning of the basidiome).

Bruising or staining reactions on the surfaces or in the context of an *amanita* can be important for determination. However, a species that does not normally change color when cut [e.g., *A. subsolitaria* (Murrill) Murrill] will turn brilliant yellow occasionally—apparently due to some invasive agent. Because of this observation, an investigation of spore size and shape and anatomy should be undertaken in cases in which yellow staining occurs—before settling on a determination. In *A. subsolitaria*, no well-formed mature spores have been found on some yellow staining basidiomes; moreover, the lamellae may become noticeably thickened and are often covered with budding yeast cells.

Basidiocarp aging or exposure to direct sunlight can cause significant changes in pileus coloration [e.g., *A. flavoconia* var. *inquinata* Tulloss, Halling & Ovrebo (= *A. flavoconia* var. *sinapicolor* Tulloss, Halling & Ovrebo), *A. mortenii* Knudsen & Borgen, *A. muscaria* subsp. *flavivolvata* Singer, etc.]; therefore, when a large fruiting illustrates such a phenomenon, it is an important opportunity for collections and their annotations to reflect the details thoroughly.

In some taxa, intense pigment development does not occur until after a basidiocarp is well-expanded (e.g., *A. spreata* (Peck) Sacc., which may be nearly white although having reached a robust stage of expansion, although eventually becoming virgate with fine “appressed fibrils” of a shade of gray or brown).

When the color to the human eye is the result of the presence of multiple pigments, these may not all develop in some individual basidiocarps or may not all develop at the same rate or may develop only in segments or patches of the pileipellis. Careful observation of unusually colored pilei over a few days can be valuable in avoiding undesirable generation of useless infraspecific taxa. Common examples of uneven or partially failed pigment development are *A. muscaria* (L.:Fr.) Lam. and *A. muscaria* subsp. *flavivolvata* Singer.

5. Decoration of the stipe surface

Very often, the stipe surface of an *Amanita* will be longitudinally striatulate (at least in age). This is a reflection of the longitudinally acrophysalidic character of the stipe context probably made manifest by the drying and collapse of surface tissues. Other forms of decoration are numerous.

Near the stipe apex on a number of species, the surface is pubescent, farinose, or pulverulent. In some taxa of section *Vaginatae* (e.g., *A. arctica* Bas, Knudsen & Borgen in Knudsen & Borgen), a thin, subfelted layer may be appressed to the upper stipe; the anatomy of such layers often suggests a poorly formed partial veil. In many members of section *Vaginatae* with exannulate stipes, the surface of the lower two-thirds of the stipe may be fibrillose; the fibrils may be concolorous with the (pallid) ground color or may range from subtle orangish white to orange or various shades of brown or gray or black. Often a species with deeply pigmented stipe fibrils will also have marginate lamellae because of the proximity of the edges of the lamellae to the stipe surface in the early development of the basidiome.

Species with a friable or felted *limbus internus* of the universal veil often deposit such material on the stipe surface (below the annulus if there is one). This is the origin of the orange patches on the stipe of *A. caesarea* (Scop.:Fr.) Pers. and of *A. hemibapha* (Berk. & Broome) Sacc. and the latter's Western Hemisphere relatives (e.g., *A. jacksonii* Pomerleau, Fig. 2). Similarly, the *limbus internus* appears to be the origin of the ragged and collapsing false (second or lower) annulus on the stipe of the taxon called “*A. caesarea*” by authors of the southwestern U.S.A.

In a variety of taxa, the stipe surface may be decorated by warts or patches of the universal veil or by large or small recurved scales where the context splits (apparently due to an adhesive(?) effect of adnate patches of universal veil that prevent areas of stipe surface from expanding even though the underlying tissue expands). Dramatic examples of species having a bulb with recurved scales include *Amanita cokeri* (E.-J. Gilbert & Kühner) E.-J. Gilbert, *A. concentrica* Oda et al., and *A. eijii* Zhu L. Yang.

In very rainy weather, the stipe may become quite watersoaked as one would expect; and such specimens may not be worth collecting; however, we know of at least one species in which the normal condition of the stipe is to suggest a tallow candle—*A. calyptroides* Peck. This may have something to do with a particularly moist outerlayer of the stipe which is sometimes noticeable as a darker layer in exsiccata. The small annulus of this species seems to melt into the stipe when it collapses. The annulus eventually disappears.



Fig.2. *Amanita jacksonii* as collected in north-central New York state. Note the unusual situation of the felted extension of the *limbus internus* of the universal veil being drawn up the stipe on the edge of the annulus on the larger basidiome.
Photo: R. E. Tulloss

6. Odor and taste.

When it comes to odor and taste, one is on one's own. We try to be as explicit as possible and use terms for odors and tastes that are likely to be terms for experiences common to many people. Since people are unlikely to taste amanitas in sections of the genus in which there are numerous poisonous taxa, taste is not as important a character to record as is odor. We usually taste specimens clearly assignable to section *Vaginatae*, but never swallow the material tasted.

7. Spore deposit.

We obtain a spore print if at all possible (but see under “triage,” below) for every taxon studied. We set up for spore prints in the field if possible—by placing a white card under the cap of a specimen before wrapping it and inserting it into the basket. This seems to be especially important in regions where collection takes place at a significantly higher altitude than the location of the laboratory where collections will be studied (Rossman *et al.* 1998). If field set-up is impractical, We set up for spore prints immediately upon returning from the field. In many cases a satisfactory spore print is obtained by taking an index card the breadth of which exceeds the pileus diameter, cutting out a slot for the stipe to be slipped into, and then hanging the stipe in a tall glass or cup. In this way the plant is exposed as little as possible to drying of internal tissues. The whole construction can have wax paper wrapped around its top so that evaporation from the pileus surface is reduced. Experimentation may lead to better techniques especially for very small and very large specimens.

8. Macrochemical spot tests.

8.1. Phenoloxidase spot tests. Phenoloxidase tests (spot tests for laccase and tyrosinase) are often valuable and likely to become more so—at least for some taxa. The first author has employed the test on well over 100 different taxa. The tester selects at least one fresh basidiome (it is best, if time allows, to test specimens at different stages of maturity) and slices it longitudinally. Using a razor or a very sharp knife, a “silhouette” of the mushroom about 2 or 3 mm thick (if possible given the size of the stipe) is sliced off the exposed inner surface of one of the two half-mushrooms. This silhouette is divided in half down the center, and each half-silhouette is placed on a non-reactive surface like a white dinner plate, a plastic picnic plate, a pane of glass, or a porcelain-coated laboratory tray. The two pieces could be on the same surface, but they must be far enough apart so that the liquids that are going to be placed on them don't run together and mix. For all types of macrochemical tests, the first author dries the parts of the mushroom that aren't used for the test. On the herbarium label for such material is indicated that the collection is a voucher for a spot test. The collection can be checked later in case a mistake in its determination is suspected.



Fig.3. Positive (lavender) reaction for laccase in the radicating bulb of *A. subsolitaria*. The yellow tint in the stipe context is the color of unreacting syringaldazine. Photo: R. E. Tulloss

Procedures for paracresol spot tests were originated by Marr (1979) and Marr *et al.* (1986). On one half-silhouette, drip syringaldazine solution until the whole half-silhouette is wetted. Mark down the time. Treat the other half-silhouette in the same way, but with paracresol solution. Note the time that this is done. For 15 to 20 minutes note down the color changes (if any) as they occur on both half-silhouettes. It is suggested that you use the note form presented in Appendix A3 of (Tulloss 1998a) [??add to this paper??](#). It originated with Marr *et al.* (1986) and was modified for use with amanitas by M. en C. Adriana Montoya Esquivel (Universidad de Tlaxcala, Mexico). For each change in color, note the time and location of the change on the half-silhouette. Alternatively, make a note on the colored (reacting) regions of each half-silhouette every minute. This is easier than it sounds—especially if one uses simple abbreviations. On the chemical test form, for a half-silhouette of a mushroom in which you observe a positive reaction, shade in the area of the appropriate

half-silhouette drawing corresponding to the visible reaction occurring on your specimen. Mark the time that you stopped observing for each test when you stop. This is sufficient. The other side of the form need not be filled out at this time—except for indication of the collection date and collection number so that the record of results can be correlated with the collection and your other notes.

A positive test for laccase (syringaldazine) is in the range of pinkish lavender (Fig. 3) to purple. The ethanol solvent in the syringaldazine solution can sometimes accelerate an oxidation reaction that occurs naturally (*e.g.*, the pinkening reaction in some species of *Amanita* section *Amidella*). This phenomenon can be confirmed by using ethanol alone as a control. In cases in which a particularly strong oxidation reaction obliterates the sometimes pale purplish reaction due to laccase, the purple color can sometimes be seen in excess reagent adjacent to the material being tested.

A positive test for tyrosinase is in the range of orange-red to orange-brown to rather dark brown. In a number of mushrooms, if the tyrosinase test set-up is left standing for some time (an hour or more), the dark pigment, melanin, will start to form; and areas where reaction has occurred may become nearly black. This terminal part of the reaction does not need to be recorded. Recipes for the reagents and a more extended discussion of applying them and recording test data can be found in (Marr 1979) and (Marr *et al.* 1986).

8.2. *Sulfuric acid spot tests.* In the literature, only a supposed purple reaction on the lamellae of *A. phalloides* is commonly mentioned as a result of spot testing with sulfuric acid. In the mid-90s, the late Dr. L. J. Tanghe, G. Lincoff, and the first author experimented with concentrated H₂SO₄ on the lamellae and other parts of a variety of species. A pink or pinkish lavender reaction is very common (unpub. results) on lamellae and elsewhere and is not even restricted to *Amanita* section *Phalloideae* (Fr.) Quél. For a draft data recording form for H₂SO₄ spot tests, see Appendix A4 of (Tulloss 1998a) **??add to this paper??**. It is suggested that tests only be performed with concentrated acid.

8.3. *Testing with iron salts.* Almost nothing is known about reactions with iron salts in *Amanita*. Experiments are needed. In the present state of knowledge, we believe it would be inappropriate to define a taxon based on a reaction to iron salts.

8.4. *Testing with KOH.* While the yellow reaction on the pilei of *A. bisporigera* Atk. and *A. virosa* Lamarck is well-known, a survey of reactions to KOH has not been made. Experimentation is needed. In particular, yellow reactions on “normally” non-reacting species (*A. magnivelaris* Peck and *A. verna* (Bull.:Fr.) Lamarck) is a topic on which further research undoubtedly will be done. The hue and the intensity of the color reaction should be assessed in all cases. Collections in the southwestern U.S. and Mexico of what appears to be normal strongly reactive *A. bisporigera* often produce faint reactions or none at all. The absence of a color reaction in *A. verna* may have entered the literature when *A. phalloides* var. *alba* was tested and reported as *A. verna*. In recent years, efforts by correspondents of the first author to obtain non-reactive *A. verna* in France have not been fruitful (F. Massart, pers. corresp.)—all material collected has produced a bright yellow reaction to KOH. Color photographs are important for recording unusual color reactions or for varying shades of yellow on taxa usually considered not reactive.

8.5. *Testing with ammonium hydroxide.* Almost nothing is known about reactions in *Amanita*. Experiments are needed. In the present state of knowledge, we believe it would be inappropriate to define a taxon based on a reaction to NH₄OH.

8.6. *Melzer's reagent.* If one wants to test macroscopically for amyloidity of spores (we never do this, but some do), it can't be done effectively on spore prints on paper. Paper will produce a dark amyloid reaction all by itself. One must scoop up a bit of material from the spore print and place it on a glass or ceramic surface in order to carry out the test. A simpler procedure is to place a glass slide under the pileus while the spore print is being made and let some of the spore print be made directly on it. A drop of Melzer's reagent on a patch of white spores will produce a very distinct reaction (distinct to the naked eye) if the spores are strongly amyloid. Unfortunately, there are a few amanitas with weakly amyloid spores. In these cases, microscopic examination of spores in Melzer's reagent is required (see below).

Melzer's reagent has also been used to test for amyloidity in the plasma of hyphae in various parts of basidiomes of *Amanita* species (Kotilová-Kubièková 1982); however, insufficient information is available to make this a useful taxonomic tool at present. Neville and Poumarat (2004) have carried out experiments on a number of European taxa, and this work may encourage similar experiments elsewhere.

9. Triage.

If time is limited, don't eliminate all of the steps related to collection and photography if the most important steps can be managed at all. The steps we sacrifice are the following (in the order in which they would be abandoned): 1) tests for amyloid reaction of spores (can always be done with dried specimen), 2) phenoloxidase tests (a few tests per taxon will suffice for current studies), 3) photography in the field, 4) spore print (as long as spore color is demonstrated a few times...spores can be measured from lamellae of dried material), 5) recording odor and taste, 6) photography in the lab (when a collection belongs to a commonly collected taxon). Taking of detailed notes should always begin with the most unfamiliar, taxonomically problematic, rare, or fragile taxa.

If the number of collections is so large that more drastic measures must be taken. The more common species (especially collections of these that do not include exceptionally large or exceptionally small basidiomes) can be photographed and dried, keeping notes only on locality, date, collector, etc. However, one should give thought to whether such collections will have value for current or future research. A reasons to keep such material, for example, is maintenance of vouchers for new localities. Collections of common species with specimens of unusual size can be photographed and should have notes taken on the dimensions of the basidiomes. Collections of common material in poor condition that are not needed to provide vouchers for mapping projects (for example) should be discarded.

10. Preserving specimens.

10.1. Drying specimens. It is necessary to emphasize the importance of drying specimens properly and as soon as possible after macroscopic annotation (Yang 2000b). Numerous collections of *Agaricales* deposited in the herbaria of the world can't be used for serious taxonomic, systematic and molecular phylogenetic studies because they were poorly dried and/or poorly preserved. We have both found that many type specimens of *Amanita* were poorly dried, and thus rehydrate very poorly. Consequently, their anatomical and taxonomic characters no longer can be traced (Tulloss 1994b; Yang and Doi 1999). If dried specimens become moldy or damaged by insects due to poor preservation, their scientific value will be reduced dramatically. In particular, some hyphomycetes seem to have a preference for attacking spores and the exposed ends of basidia and basidioles on hymenial surfaces, with obvious negative effects.

We prefer to dry material rapidly because this best preserves delicate structures such as the lamella trama. When temperature regulation is possible, a forced air dryer should be set to operate at 55° - 60° C (130° - 140° F). If electricity is accessible, a forced air vegetable dryer with stacking trays can be used. If slower drying is necessary due to available equipment, the specimens should be cut in an orderly manner (*e.g.*, longitudinally sectioned in quarters or eighths) and placed in a well-ventilated place with heat low enough so that the mushrooms don't cook. If commercial drying equipment is not available, one can build a plywood cabinet with removable trays over four 200 watt light bulbs. The trays are simply frames onto which are stapled fiberglass screening. The light bulbs can be wired to be turned on and off individually. If the cabinet is placed in a dry spot (*e.g.*, in a moderately air conditioned building), a satisfactory result can be obtained. A commercial dryer without forced air is also an option, for example, the SIGG Dörrex dryer which is used by both of us. Less expensive dryers with plastic (rather than metal) frames are available for drying fruits and vegetables; they also work well for mushrooms—although the trays are sometimes very shallow.

In some remote regions, electricity is not available; and a good alternative is a kerosene burning dryer (Fig. 4). The second author has built a cabinet with removable trays. Under the trays, a kerosene stove may be placed. The fire can be regulated manually. Satisfactory results have been obtained during collecting trips in China.

10.2. Preservation in liquid. When working in humid climates without available desiccants and tightly closed collection boxes and especially when such work is at a considerable distance from laboratory and herbarium facilities, preservation in liquid may be the only alternative. If this is not done, the most important and most fragile parts of *Amanita* anatomy are not likely to survive until it is possible to thoroughly examine the specimen. It is not necessary to preserve whole specimens in liquid; but a wedge-shaped part of the pileus with attached lamellae, a piece of partial veil, and a piece of universal veil (especially from species with limbate or saccate partial veils) can be preserved in small, separate containers—while the remainder of a collection is dried. This worked very well for the first author and his co-authors as reported in (Tulloss *et al.* 2001).

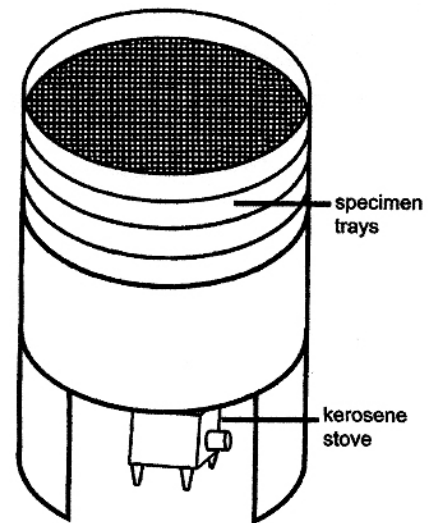


Fig.4. Schema for drying mushrooms over kerosene heater. Drawing: Z. L. Yang.

In the laboratory—microscopic characters

1. Core literature.

Before discussing study of *Amanita* anatomy, it is important to acknowledge the fundamental importance of the anatomical approach of Bas (1969). Bas' work should be reviewed thoroughly by any student of the genus along with (Corner

and Bas 1962). Among recent works that are useful are those of Tulloss *et al.* (1992), Tulloss (1993, 1994, 1998a), and

Table 1. Comparison of terminology used by Tulloss and Yang

Terms used by Tulloss (and his co-authors)	Terms used by Yang (and his co-authors)
Filamentous, undifferentiated hyphae	Filamentous hyphae
Acrophysalides	Inflated terminal cells
L = the average spore length computed for one specimen examined	n. p. (not provided)
L' = the average spore length computed for all spores measured	n. p.
W = the average spore width computed for one specimen examined	n. p.
W' = the average spore width computed for all spores measured	n. p.
Q = the ratio of length/width of one spore	Q = the ratio of length/width of one spore
Q = the average value of Q computed for all spores of one specimen examined	n. p.
Q' = the average value of Q computed for all spores measured	Q = average of Q for all spores measured \pm sample standard deviation
Suprapellis (of pileipellis)	Upper layer (of pileipellis)
Subpellis (of pileipellis)	Lower layer (of pileipellis)
w _{CS} , w _{st} -near, w _{st} -far, w _{ex} -near, w _{ex} -far of lamella	n. p.
Central stratum of lamella	Mediostratum of lamella
Subhymenial base of lamella	Lateral stratum of lamella
Subhymenium	Subhymenium
Subhymenial tree	Lateral stratum and subhymenium
Universal veil	Volva (or volval remnants)
Partial veil	Annulus

Yang (1997).

2. Terminology.

Terminology used by Tulloss and his co-authors and Yang and his co-authors in describing anatomy in *Amanita* sometimes differs. We both regard our choices of terminology and of anatomical characters in descriptions as in a process of on-going development. The following table provides a comparison of terminology we use. Because of the greater detail of Tulloss' terminology, the latter is applied in this paper.

3. Rehydration and sectioning.

Usually, we use dried specimens for microscopic studies. Specimens in some herbaria may be sectioned for study by light microscopy using a stereo dissecting microscope without special preparations. However, specimens from other collections may be very dry and fragile. It is difficult to section such specimens without first moistening them. A method of moistening with least mechanical impact on the tissues is depicted in Fig. 5. The specimen is placed in a small culture

disc that is floated on water in a larger dish. The pair are then covered to achieve high humidity. Adequate moistening occurs in one to three minutes. The specimen can then be sectioned normally.

Sections of lamellae often coil in 5% KOH. It is recommended to use water as a mounting medium at first. When the cover slip is in place, 10% NH₄OH or 5% KOH can be added at one edge of the cover slip while water is taken up by absorptive paper at the opposite edge. The concentration of the reagent can be gradually increased in this manner and the coiling effect avoided.

The first author has described other freehand methods for sectioning, but these are to be rejected when a stereo dissecting microscope is available.

Note regarding thickness of cell walls: Measurements of cell wall thickness should be made at 1000× or greater magnification. Optical artifacts may falsely suggest that walls are thickened when viewed at lower magnification.

4. Details of microscopic anatomy.

The remainder of this section is presented in list or outline form with added commentary. Data on elements common to many tissues are catalogued first (under heading 1). Examples of such elements are filamentous, undifferentiated hyphae; vascular hyphae; and acrophysalides. Spore data are treated next (under heading 2). Then (under heading 3) elements to be examined are organized on a tissue by tissue basis with discussion of characters specific to each tissue, such as the thickness of gelatinized suprapellis and ungelatinized subpellis of the pileipellis. Suggestions for locating basidial clamps (if any are present in a specimen) are provided under heading 4. A discussion of crassospores and crassobasidia is found under heading 5. A brief discussion of parasitized (and possibly parasitized) specimens of *Amanita* is given under heading 6.

1. Characters of elements common in many tissues.

1.1. Filamentous, undifferentiated hyphae [See (Tulloss *et al.* 1992).]

1.1.1. Range of width

1.1.2. Range of wall thickness

1.1.3. Frequency of branching

1.1.4. Frequency of septa

1.1.5. Wall color and/or color of intracellular pigment (colorless or yellowish or sordid yellowish or brown or other)

1.1.6. Plenitude and size/complexity of fascicles of hyphae

1.1.7. Dominant orientation (*e.g.*, in many taxa, but not in all, subradial in the pileipellis when observed at mid-radius)

1.1.8. Relative frequency—as opposed to frequency of acrophysalides or other inflated cells

1.1.9. Form (*e.g.*, coiling, branched (with indication of frequency of branches), constricted at septa)

1.1.10. External or internal decoration—if any.

1.2. Vascular hyphae (Tulloss 1994).

Caution: Care must be taken to distinguish vascular hyphae from filamentous, undifferentiated hyphae with colored walls or subrefractive walls. Vascular hyphae have few or no septa; often are sinuous; often have an irregular outline; and, when broken or cut, often exude an approximately concolorous substance that is apparently insoluble or poorly soluble in water and aqueous solutions of KOH and NH₄OH.

1.2.1. Range of width

1.2.2. Frequency of branching

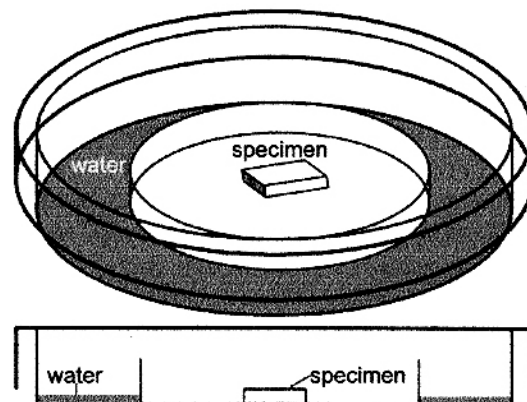


Fig.5. Schema of technique for rehydration of fragile herbarium specimens—viewed from above and from side. *Drawing:* Z. L. Yang

- 1.2.3. Color (especially if not yellow or yellowish)
- 1.2.4. Presence in fascicles otherwise comprising filamentous, undifferentiated hyphae
- 1.2.5. Frequency
- 1.2.6. Peculiarities of form (*e.g.*, coiling, branched, tangled in knots, etc.)

1.3. Acrophysalides and other inflated cells.

1.3.1. *Terminology*: The term “acrophysalide” was originated by Bas (1975). It applies to terminal, inflated cells that are present both in the primordium and, in a “second generation” in the developing basidiome. Bas applied this term to the terminal, inflated cells of the pileus and stipe contexts and, arguably, to similar cells in other tissues, especially since Bas proposes that his term is synonymous with “protocyst” as used by Malençon (1955) in the latter’s discussion of *Torrendia*. It should be noted that intercalary cells that are at least partially inflated may also be found in these tissues—sometimes in chains.

Since the lamella trama in *Amanita* has its own unusual and separate ontogeny (*e.g.*, Reijnders 1963; Reijnders and Stalpers 1992; Yang and Oberwinkler 1999), the first author has not used the term “acrophysalide” for inflated cells in that tissue. The reader is strongly encouraged to read the full text of (Bas 1975).

Because the definition of the stipe tissue in *Amanita* as “*Amanita* tissue” is logically circular, Dr. Bas and Tulloss agreed to use the phrase “longitudinally acrophysalidic” to describe this tissue. Numerous illustrations of this tissue can be found in figures of Bas (1969, 1975, etc.) and Yang (1997).

1.3.2. *Data to be collected.*

- 1.3.2.1. Range of size (at least largest seen and top of range in which most observed cells lie)
- 1.3.2.2. Color
- 1.3.2.3. Range of wall thickness
- 1.3.2.4. Relative frequency as opposed to that of filamentous, undifferentiated hyphae in same tissue
- 1.3.2.5. Range of shapes (noting if wall thickness or size is relatively common for a given shape)
- 1.3.2.6. Indication if any similar cells are intercalary
- 1.3.2.7. Occurrence of such intercalary cells singly or in chains—giving the range of lengths of chains in number of cells per chain observed
- 1.3.2.8. Description of internal or external decoration (External decoration is very rare in inflated cells of *Amanita*. A truly warted exterior is known only from inflated cells of the universal veil of one New Zealand species of *Amanita* section *Amanita* (Tulloss, unpub. data)—*A. nehuta* G. S. Ridl., apparently phenetically related to *A. friabilis* (Karst.) Bas and *A. farinosa* Schw.

2. Spores (Tulloss 1994; Tulloss *et al.* 1992).

2.1. *Notation*. When presenting a range of measurements of the form (*a*-) *b* - *c* (-*d*), the numbers have the following meanings:

- a* = the smallest value encountered
- b* = the greatest measured value such that at least 95% of all spores measured yielded a number greater than or equal to *b*
- c* = the least measured value such that at least 95% of all spores measured yielded a number less than or equal to *c* (*i.e.*, the value of *c* is the 95th percentile of spore length)
- d* = the largest value measured.

When presenting spore data for a taxon, we follow Bas (1969) in placing three values in the format “[*m/n/p*]” prior to the data. These numbers have the following meanings: *m* is the number of spores measured; *n* is the number of specimens from which spores were measured; *p* is the number of collections from which those specimens came.

2.2. *Procedure*.

- 2.2.1. Measure 20 spores per specimen (if that many can be found). We do not use data from specimens for which less than 7 spores could be found.
- 2.2.2. At least, in cases in which *Q* is under about 1.7, spores should be measured in lateral view only (apiculus in view and in focus together with both ends of the spore being in focus)—otherwise the value of *Q* will vary too much due to variation in spore profile when viewed from different angles and will be of less value taxonomically. Because of lack of a method such as this, subglobose spores are often reported as globose; ellipsoid spores, as broadly ellipsoid; etc.—spores are reported with *Q* value too low. The more nearly globose the spore or the more a spore is asymmetrical, the more patience is required in following this procedure; however, we have observed that the effort pays off in taxonomic usefulness of the resulting data.
- 2.2.3. Do NOT include the apiculus in any measurement.
- 2.2.4. Compute individual length/width ratio (*Q*) for each spore
- 2.2.5. Compute average length (**L**), average width (**W**), and average *Q* (**Q**) for each specimen
- 2.2.6. Compute overall averages of length, width, and *Q* (**L'**, **W'**, and **Q'** respectively) for each taxon. The second author prefers to report standard deviation for an average rather than a range. The first author presents data differently as indicated in Table 1. Sample standard deviation is designed to convey information that omits singular (outlying) data points. It is a question of

choosing between a briefer statistical presentation and presentation of more details of the raw data for “engineering” or “application” purposes (see next item).

- 2.2.7. If ranges are to be reported, report them for length, width, **Q**, **L**, **W**, and **Q**. A taxonomist with extended experience with a taxon may have measured spores from dozens of basidiomes in many stages of development and in varying conditions of preservation—dependent upon the stage of development when dried, the condition when dried, the speed of drying, the quality of preservation in a herbarium, etc. All these variables can alter spore sizes and shapes. The averages computed by such a worker may be spread out over a considerable range. In the first author’s view, the information about variation developed in such a case is arguably most easily used by, and, hence, most useful to, later workers when the range of the raw data is available.
- 2.2.8. If ranges are to be reported, report ranges by indicating lowest and highest values observed (extremes) and the range in which approximately 90% of values fall using the notation “(a-) b - c (-d)” described above.
- 2.2.9. To aid in *evaluating whether a given specimen may have spores of unusually small or large size or a skewed distribution of spore sizes*, record the spore data according to spore length in columns labeled by length ranges in μm (7.5 - 8.5, 9-10, 10.5-11.5, etc.). By measuring at least 20 spores per specimen, the columns taken together will either suggest a normal distribution (bell) curve or will demonstrate multiple peaks, skewing or long tails on the distribution. Appropriate cautionary comments can then be made in a description based on specimens with skewed spore size distribution. Skewed spore size can be caused by a specimen having been senescent when dried, having partially dried *in situ* prior to collecting, having partially dried between collection and placement in a dryer, having been in initial stages of sporulation when dried, etc. For example, see the discussion of spores of the type and other collections of *A. lactea* Malenç., Romagn. & Reid by Tulloss and Gminder (2000).
- 2.2.10. Indicate the following:
 - 2.2.10.1. Wall opacity (hyaline or opaque)
 - 2.2.10.2. Color [The term “hyaline” means “transparent, like glass.” For greatest accuracy, it should not be taken to mean “colorless” as well.]
 - 2.2.10.3. Wall thickness or decoration (See V. for discussion of crassospores.)
 - 2.2.10.4. Presence or absence of adaxial flattening
 - 2.2.10.5. Irregularity of form (swollen at one end, constricted, “Y”-shaped, shaped like a planarian, etc.)
 - 2.2.10.6. Presence of “giant spores”
 - 2.2.10.7. Reaction to Melzer's reagent and/or Cotton Blue [In cases of weak amyloid reactions, compare spores on an hymenial surface with the background color of the basidia and basidioles. Also, the spores may be compared to air bubbles in the mount.]
 - 2.2.10.8. Position and shape/size of apiculus [Typical shapes are cylindric and truncate-conic. To describe the shape accurately, it is necessary to examine the apiculi of spores in lateral view. The apiculus is almost always sublateral. If it appears to be otherwise positioned, the spore being observed may not be being observed in lateral view.]
 - 2.2.10.9. Form of contents [*e.g.*, monoguttulate, multiguttulate, granular, monoguttulate with additional small granules, etc.]

3. Tissue by tissue analysis.

3.1. Pileipellis.

- 3.1.1. Presence [In a number of taxa of section *Lepidella* [notably, in most taxa of subsection *Vittadiniae*, in *A. rhoadsii* (Murrill) Murrill var. *rhoadsii*, and in *A. magniverrucata* Thiers & Ammirati] a well-defined pileipellis is not present. When a pileipellis of an *Amanita* has been described as comprising upstanding rows of cells, it has been our experience in all cases to date that the universal veil is being described in a species that lacks a well-developed pileipellis.]
 - 3.1.1.1. If present: thickness
 - 3.1.1.2. If not present: relationship of pileus context to universal veil, characteristics of transitional region
- 3.1.2. Gelatinized to partially gelatinized suprapellis (thickness, color)
 - 3.1.2.1. Is the gelatinous material caused by breakdown of hyphal cell walls?
 - 3.1.2.2. Is the gelatinous material a matrix in which whole hyphae interweave loosely?
- 3.1.3. Predominantly ungelatinized subpellis (thickness, color)
- 3.1.4. Filamentous, undifferentiated hyphae (See 1.1.)
- 3.1.5. Presence (if any) and character of inflated or partially inflated intercalary or terminal segments of hyphae
- 3.1.6. Vascular hyphae (See 1.2.)
- 3.1.7. Presence/absence of clamps. (See 4.)

3.2. Pileus context.

- 3.2.1. *Special attention*: In some taxa, there is a definite concentration of vascular hyphae near the stipe apex within the pileus context or in the apex of the stipe. The pileus context should be sampled both near to and distant from the stipe apex. Similarly, the context of the stipe apex should be checked.
- 3.2.2. Filamentous, undifferentiated hyphae (See 1.1.)
- 3.2.3. Acrophysalides (See 1.3.)

3.2.4. Vascular hyphae (See 1.2.)

3.2.5. Presence/absence of clamps. (See 4.)

3.3. Lamella trama [see (Tulloss 1993; 1994; 1998a)].

3.3.1. *Drawing*: The lamella trama provides so many valuable characters for *Amanita* taxonomy, it should be described at length; and, if rehydration permits, the trama should be drawn in sufficient detail to convey all elements and their organization. If rehydration permits, the area of tissue illustrated should extend from the central stratum to the basidia

3.3.2. *Terminology*: The following terminology was defined in order to name certain characters of the lamella trama that current research indicates are of taxonomic value:

w_{cs} = the width of the central stratum of a lamella, measured *ca.* midway from pileus context to lamella edge at about mid-radius of the pileus

$w_{st-near}$ = the distance from an outer margin of the central stratum to the nearest base of a basidium/-ole, measured *ca.* midway from pileus context to lamella edge at about mid radius of the pileus.

w_{st-far} = the distance from an outer margin of the central stratum to the farthest base of a basidium/-ole on the same side of the central stratum, measured *ca.* midway from pileus context to lamella edge at about mid radius of the pileus.

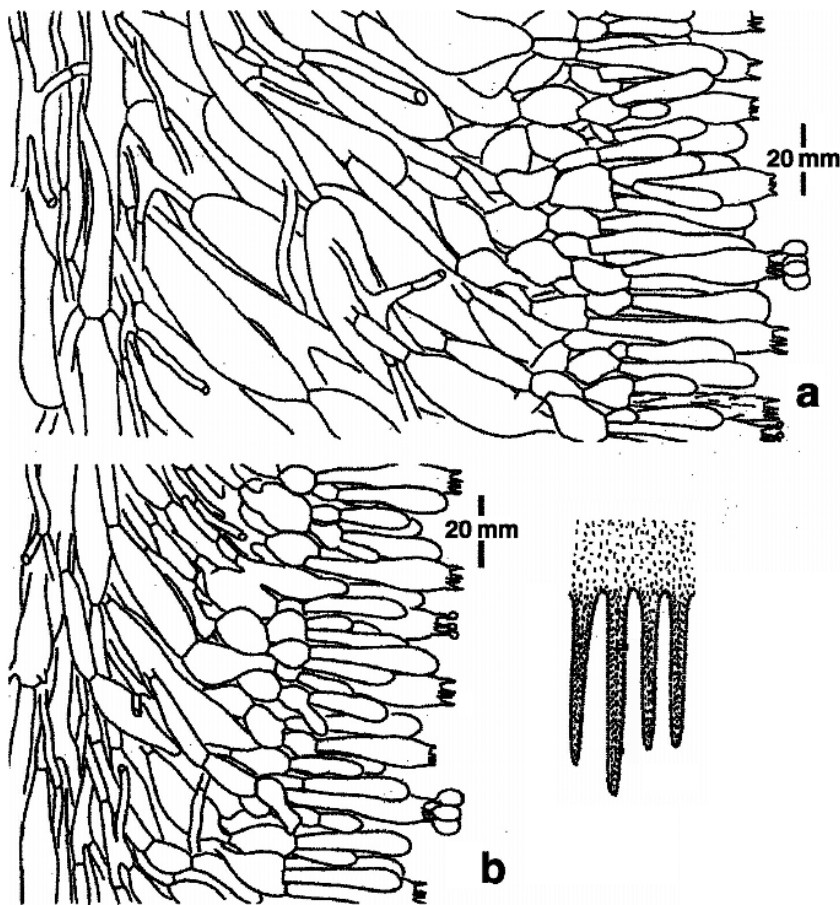


Fig.6. Cross-sections of lamella of *Amanita excelsa*: (a) from between pileus context and lamella mid-width, (b) from between lamella mid-width and lamella edge. (Drawing: Z. L. Yang.)

$w_{ex-near}$ = the distance from the outermost point on a terminal, inflated cell of the subhymenial base to the nearest base of a basidium/-ole, measured *ca.* midway from pileus context to lamella edge at about mid radius of the pileus.

Restricted in use to cases in which terminal, inflated cells are rather frequent in the lamella trama.

w_{ex-far} = the distance from the outermost point on a terminal, inflated cell of the subhymenial base to the most distant base of a basidium/-ole, measured *ca.* midway from pileus context to lamella edge at about mid radius of the pileus.

Restricted in use to cases in which terminal, inflated cells are rather frequent in the lamella trama.

The term “subhymenial tree” is used for that portion of the lamellae comprising the diverging elements between the central stratum and the hymenium. The term “subhymenial base” denotes the portion of the subhymenial tree exclusive of the subhymenium.

3.4. Details.

3.4.1. *Cautions.* It is important to examine the lamella trama at about the midpoint of the width of the sectioned lamella. Because the cross-section of a lamella tapers from its attachment to the pileus to its free edge (Fig. 6), inconsistency in selection of the area to section will lead to highly variable results that cannot be usefully compared with lamella cross-sections from other specimens.

Divergent, terminal inflated cells are uncommon in the lamella trama of some taxa of *Amanita* despite the literature to the contrary. Many times cells that appear terminal were in fact intercalary before being displaced or separated by sectioning. Careful examination of the apex of such a cell will frequently reveal an opening made in sectioning or the remains of a cell that was attached at that point. Care must be taken to distinguish between cells and gaps between cells, which can sometimes be taken for cell outlines.

3.4.2. Describe the form taken by the bilateral tissues, range of angles of divergence of basal elements of subhymenial tree.

3.4.3. Describe the central stratum including

3.4.3.1. The value of w_{cs} [Always provide this value if at least some rehydration is possible. State the quality of rehydration achieved—it is valuable information albeit qualitative.]

3.4.3.2. The filamentous, undifferentiated hyphae (See 1.1.)

3.4.3.3. Any intercalary inflated cells (See 1.3.)

3.4.3.4. Any vascular hyphae (See 1.2.)

3.4.3.5. Presence/absence of clamps. (See 4.)

3.4.4. Describe the subhymenial base including

3.4.4.1. Its organization/structure

3.4.4.2. Its filamentous, undifferentiated hyphae (See 1.1.)

3.4.4.3. Its inflated cells (indicating whether they are terminal or intercalary) (See 1.3.)

3.4.4.4. Its vascular hyphae (See 1.2.)

3.4.4.5. Presence/absence of clamps. (See 4.)

3.5. Subhymenium [see (Tulloss 1993; 1994; 1998a)].

3.5.1. Give the thickness of the subhymenium in μm or, if it is pseudoparenchymatous, by giving the number of layers of cells in the subhymenium.

3.5.2. If divergent, terminal, inflated cells are present in subhymenial base and if rehydration permits, measure w_{ex} -near and w_{ex} -far. Always state the quality of rehydration achieved. It is valuable information albeit qualitative.

3.5.3. In all cases, do the following:

3.5.3.1. If rehydration permits, measure w_{st} -near and w_{st} -far. Always state the quality of rehydration achieved. It is valuable information albeit qualitative.

3.5.3.2. Describe the types and forms of cells from which basidia arise.

3.5.3.3. Give a general characterization of form (cellular or pseudoparenchymatous, coralloid, ramose, etc.). Sometimes, it seems better to write a description of the drawing (see above) rather than limit a description to selection of one or more of the terms introduced by Bas (1969).

3.5.3.4. Indicate the presence/absence of clamps. (See 4.)

3.6. Basidia.

3.6.1. Indicate the range of size observed.

3.6.2. Give the relative proportion of 4-sterigmate, 2-sterigmate, etc. basidia.

3.6.3. Provide the maximum observed size of sterigmata (width at base and length).

3.6.4. Provide wall thickness.

3.6.5. Report the presence of crassobasidia (See 5.)

3.6.6. Include the basidia in the drawing of the subhymenial tree and hymenium (*e.g.*, in Fig.6)

3.6.7. Indicate the presence/absence of clamps (See 4.)

3.6.8. Describe any oddities of structure/form [*e.g.*, branched (rare)].

3.7. Universal veil.

3.7.1. *Cautions and observations:* Layering of the universal veil is common. This may range from the case in which there are a few more filamentous, undifferentiated hyphae in the base of a wart than in the apex to the development of as many as five distinct layers [*e.g.*, in *A. volvata* (Peck) Lloyd]. Each layer should be described separately. Since the frequency of inflated cells with respect to the frequency of hyphae often differs between universal veil material left on the pileus and such material left on the stipe base, tissue from both regions should be investigated. It should be more widely appreciated that there are groups of species in which gelatinization of the pileipellis doesn't occur or occurs only after the expansion of the pileus (*e.g.*,

in *A. farinosa* Schw., *A. basiana* Tulloss & M. Traverso (2001), *A. friabilis*, *A. nehuta*, etc.). In these taxa, the universal veil is, even for a time at maturity, connected to the pileipellis by ungelatinized hyphae.

- 3.7.2. *Drawings*: Draw tissue from pileus or stipe base. If the volval tissue on the pileus has a distinct orientation of its elements, this may be more valuable to convey in a drawing than disordered tissue from the stipe base. Variation in color of inflated cells may be different at the stipe base from that on the pileus; in such a case, illustrations of both tissues may be of value. If tissues of layers vary, draw at least those layers that are not essentially composed of filamentous, undifferentiated hyphae or such hyphae and vascular hyphae. Illustration of the interface between the universal veil and the pileipellis or (if there is no pileipellis or only a very limited one) the pileus context can be very valuable.
 - 3.7.3. On the pileus
 - 3.7.3.1. Identify layers and treat each separately.
 - 3.7.4. Filamentous, undifferentiated hyphae (See 1.1.)
 - 3.7.5. Inflated cells (See 1.3.)
 - 3.7.6. Vascular hyphae (See 1.2.)
 - 3.7.7. Presence/absence of clamps. (See 4.)
 - 3.7.8. On the stipe base
 - 3.7.8.1. Identify layers and treat each separately.
 - 3.7.9. Filamentous, undifferentiated hyphae (See 1.1.)
 - 3.7.10. Inflated cells (See 1.3.)
 - 3.7.11. Vascular hyphae (See 1.2.)
 - 3.7.12. Presence/absence of clamps. (See 4.)
- 3.8. Stipe context.
 - 3.8.1. *Cautions*: A sample of this tissue should be taken in such a way that it does not represent only the stipipellis or only the material from the stipe's central cylinder. Remember to check for a concentration of vascular hyphae at the stipe apex.
 - 3.8.2. Filamentous, undifferentiated hyphae (See 1.1.)
 - 3.8.3. Inflated cells (See 1.3.)
 - 3.8.4. Vascular hyphae (See 1.2.)
 - 3.8.5. Presence/absence of clamps. (See 4.)
 - 3.9. Partial veil.
 - 3.9.1. *Cautions*: Quickly collapsing inflated cells that serve to separate the partial veil from the edges of the lamellae are often found on the upper surface of the partial veil. In young material, such cells may be in good condition. These should not be mistaken for inflated cells that are intrinsic to the partial veil. We only refer to the latter inflated cells in descriptions of partial veil tissue. The others may be described as deciduous cells from the interface between the edges of lamellae and the partial veil. While a subradial orientation of elements is common in the partial veil, we also check for a cross-weave of other elements or a possible layering.
 - 3.9.2. *Drawing*: A drawing should be made of the tissue of the partial veil.
 - 3.9.3. Filamentous, undifferentiated hyphae (See 1.1.)
 - 3.9.4. Inflated cells (See 1.3.)
 - 3.9.5. Vascular hyphae (See 1.2.)
 - 3.9.6. Presence/absence of clamps. (See 4.)
4. Finding basidial clamps.
 - 4.1. *Comments*: Bas (1969) made considerable use of basidial clamps as a taxonomic character in *Amanita*. In discussions with us, Dr. Bas discussed the possibility that too great an emphasis may have been placed on clamps; however, we do not see any reasons to discourage the use of their presence/absence in *Amanita* taxonomy. Furthermore, because of the difficulty of using macroscopic characters to place species of *Amanita* section *Lepidella* to subsection or stirps, we would not propose alteration of the taxonomic approach of Bas' monograph. Our observations support Bas' statement that if clamps are found anywhere in an *Amanita* basidiome, they will be found at the bases of some basidia.
 - 4.2. *Cautions*: It is important to understand that clamps may be small, difficult to observe, disappear as a basidiome matures, become converted to "proliferated clamps," etc. Portions of lamellae that are least mature are the most likely places to find basidial clamps. Be demanding of yourself in recognizing clamps. Damage during sectioning or bits of displaced cells can masquerade as clamps.
 - 4.3. Details
 - 4.3.1. Stain a bit of lamella with Congo Red or other cell wall stain.
 - 4.3.2. Obtain a good section.
 - 4.3.3. Check for clamps near the lamella's lower edge.

- 4.3.4. Check for clamps in the part of the lamella nearest the pileus margin.
- 4.3.5. Check for distinctly “V”-shaped bases of basidia in more mature regions of lamellae (indications of what Bas (1969) called “proliferated clamps”).
5. Crassospores and crassobasidia.
- 5.1. *Comments*: Our observations of crassospores and crassobasidia are based on taxa from subgenus *Amanita* (*A. morenoi* Raithelhuber and *A. pseudospretta* Raithelhuber) both collected in the same, very limited region of Argentina (Tulloss and Halling 1997). A similar phenomenon has been reported (as rare) in several European taxa (Kotilová-Kubiěková and Pouzar 1988). The first author has seen the same phenomenon in a few North American taxa, but very rarely.
- 5.2. Details
- 5.2.1. Crassobasidia and crassospores have abnormally thickened walls.
- 5.2.2. Walls of basidia thicken as they approach maturity and sterigmata appear.
- 5.2.3. By the time apophyses appear, the thickened wall becomes distinctly decorated.
- 5.2.3.1. The thin outermost wall layer separates from the inner layer(s) and retains its undecorated form.
- 5.2.3.2. The remaining layer or layers bulge inward here and there forming roughly evenly distributed hemispherical depressions.
- 5.2.4. As spores form, they develop similarly decorated walls.
- 5.2.5. Crassospores are apparently not liberated with normal spores and remain attached to crassobasidia. They can become detached in the process of preparing material for microscopic study.
- 5.2.6. The cause is as yet unknown. What may be spherical viral particles were noted in transmission electron micrographs of crassospores (Tulloss and Halling 1997).
6. Parasitized *Amanita* specimens.
- 6.1. *Hypomyces hyalinus* (Schw.:Fr.) Tul. parasitization.
- 6.1.1. Our direct experience with taxa involved includes at least the following, all in *Amanita* section *Validae* (Fr.) Quél.:
- 6.1.1.1. *Amanita rubescens sensu* eastern North American authors
- 6.1.1.2. *Amanita flavoconia* Atk. var. *flavoconia*
- 6.1.1.3. The first author’s *Amanita sp. 10*, a probably undescribed taxon known from deciduous woods in New Jersey and apparently phenetically related to *A. flavoconia*
- 6.1.1.4. The first author’s *Amanita sp. 39*, a presumably undescribed taxon known from Pine Barrens in New Jersey
- 6.1.2. Some research questions.
- 6.1.2.1. What is the range of *H. hyalinus* on *Amanita*?
- 6.1.2.2. Is the apparent limitation to species of section *Validae* a real one?
- 6.2. *Mycogone rosea* Link parasitization.
- This species is reported on *A. caesarea* and is sometimes said to be an anamorph of a species of *Hypomyces*, but is not reported as such by Rogerson & Samuels (1994). It is not familiar to us. We would welcome information concerning its biology.
- 6.3. Yellow-staining specimens of *A. subsolitaria*.
- 6.3.1. They are often found among normal specimens.
- 6.3.2. Characteristically, they rapidly stain yellow when cut. (See Fig.7.)
- 6.3.3. Sometimes they exude orange fluid when cut.
- 6.3.4. Their lamellae are often malformed or covered with yeast cells.
- 6.3.5. Their spores are almost always distorted and abnormally small.
- 6.3.6. Some research questions.
- 6.3.6.1. What is/are the causative agent(s)?
- 6.3.6.2. What is the mechanism by which such agent(s) cause(s) the staining reaction?
- 6.3.6.3. Is there a relation to the yellow-staining mechanism in taxa currently accepted as unparasitized? In North America, these taxa include *A. cinereoconia* var. *croceescens* Bas, *A. crassifolia* Bas *nom. prov.*, and *A. rhoadsii* var. *flavotingers* Bas [placed with *A. subsolitaria* in stirps *Rhoadsii* by Bas (1969)]?
- 6.4. Specimens of *A. polypyramis* (B. & C.) Sacc. and other species with an odor suggesting cheese.
- 6.4.1. The odor of cheese was established as related to parasitization by a hyphomycete by Morales-Torres *et al.* (1999).
- 6.4.2. *Amanita alexandri* Guzmán is a synonym of *A. polypyramis*. The holotype of *A. alexandri* is a specimen of *A. polypyramis* that is infested by a hyphomycete. Similar infestation was also found in other Mexican exsiccata of *Amanita* section *Lepidella* that had had an odor of cheese when fresh.



Fig.7. Parasitized specimen of *A. subsolitaria* showing yellow-staining reaction in context. Photo: R. E. Tulloss

- 6.4.3. Some research questions.
 - 6.4.3.1. What is/are the causative agent(s)?
 - 6.4.3.2. What is the mechanism by which the agent alters the odor of the basidiome?
- 6.5. The case of *A. aminoaliphatica* Filippi *nom. inval.*
 - 6.5.1. The material is reported to have the odor “of aliphatic amines”—a cheese-like odor (J. White, pers. corresp.)
 - 6.5.2. Micromorphologically the material is very similar to *A. proxima* Dum.
 - 6.5.3. Stipe context in some basidiomes of putative type material has been largely replaced by hyphae morphologically similar to those that parasitize *A. polypyramis* (Tulloss 1998). (See 6.4.)
 - 6.5.4. Can “*A. aminoaliphatica*” be a result of parasitization of *A. proxima* Dum.?
 - 6.5.5. Some research questions.
 - 6.5.5.1. If “*aminoaliphatica*” is a case of parasitization, what is/are the causative agent(s)?
 - 6.5.5.2. What is the mechanism by which such agent(s) alter(s) the odor of the basidiome?
 - 6.5.5.3. Is there a relation to the agent(s) and mechanism(s) in the case of the cheese-odor specimens of *A. polypyramis*? (See 6.4.)

Acknowledgments

It is important to acknowledge at the outset the great debt that is owed to the work of Dr. Cornelis Bas, particularly to his thesis (Bas 1969) on *Amanita* section *Lepidella*. Reading this document was an enlightening experience for us. It gave us the best foundation for the study of *Amanita* for which one could have hoped and a clear model of excellence in taxonomic scholarship.

This paper contains much information we have learned by the study of documents and collections given or loaned to us by correspondents and managers of herbaria around the world, and we express our deep sense of gratitude to all these friends.

The original version of this paper was prepared by the first author as part of a syllabus to support a seminar on *Amanita* hosted by the Mycological Laboratory of the Autonomous University of Tlaxcala. He is very grateful for the support of this institution; Dr. Arturo Estrada Torres, then head of the Laboratory; M. en C. Adriana Montoya Esquivel; Biol. Alejandro Kong Luz; and their colleagues. They supported the development and delivery of the seminar in every way necessary. Subsequently, the syllabus was revised with the support of the Boston Mycological Club, the National Autonomous University of Mexico, and the Mexican Mycological Society. Dra. Evangelina Pérez-Silva was instrumental in arranging the support in Mexico. The fourth edition was extensively reworked and recast with new software tools and was made available both as a printed book and as hypertext viewable with Adobe® Acrobat® tools. This would not have been possible without motivation from, and the support of, the North American Mycological Association and the Mycological Society of San Francisco. The preparation and revision of this paper would not have been completed if not for the help of Mary Tulloss, to whom the first author is, as always, especially grateful.

The first author's opportunity to collect in Costa Rica was made possible by Dr. Roy E. Halling and the Institute of Systematic Botany of the New York Botanical Garden. The present work was partially supported by the Funds for Young and Middle-aged Talents in Science and Technology, Yunnan Province, China (2000YP09) for the second author.

Literature cited

- Bas, C. 1969. Morphology and subdivision of *Amanita* and a monograph of its section *Lepidella*. *Persoonia* 5: 285-579.
- Bas, C. 1975. A comparison of *Torrendia* (Gasteromycetes) with *Amanita* (*Agaricales*). *Studies on the Higher Fungi*. H. Bigelow & H. D. Thiers, ed. *Beih. Nova Hedwigia* 51: 53-60, pl. 11.
- Bas, C. 2000. A broader view on *Amanita*. *Boll. Gruppo Micol. G. Bresadola* 43(2): 11-12.
- Buyck, B. 1994. Ubwoba: Les champignons comestibles de l'Ouest du Burundi. *Publ. Agaricoles (Bruxelles)* 34: 1-123.
- Coker, W. C. 1917. The amanitas of the eastern United States. *J. Elisha Mitchell Sci. Soc.* 33: i + 1-88.
- Corner, E. J. H. and C. Bas. 1962. The genus *Amanita* in Singapore and Malaya. *Persoonia* 2: 241-304.
- Fraiture, A. 1998. Les amanitopsis d'Europe (genre *Amanita*, *Agaricales*, Fungi) Synthèse critique de la littérature. *Opera Belg. Bot.* 5: 1-130.
- Gilbert, J.-E. 1940-41. *Amanitaceae*. *Iconogr. Mycol. (Milan)* 27, suppl.: i-xx, 1-427, pl. 1-73.
- Hamly, D. H. 1949. The Ridgway color standards with a Munsell notation key. *J. Optic. Soc. Amer.* 39: 592-599.
- Jenkins, D. T. 1977. A taxonomic and nomenclatural study of the genus *Amanita* section *Amanita* for North America. *Biblioth. Mycol.* 57: 1-126.
- Kornerup, A. and J. H. Wanscher. 1978. *Methuen handbook of colour*. (Methuen, London). 252 pp.
- Kotilová-Kubièková, L. 1982. Occurrence of amyloid substance in the plasma in hyphae of basidiocarps of some *Amanita* species (*Agaricales*). *Èeská Mykol.* 36: 114-117.
- Kotilová-Kubièková, L. and Z. Pouzar. 1988. Three types of basidiospores in *Amanita*. *Èeská Mykol.* 42: 65-70.
- Malençon, G. 1955. Le développement du *Torrendia pulchella* Bres. et son importance morphogénétique. *Rev. Mycol. (Paris)* 20: 81-130.
- Marr, C. D. 1979. Laccase and tyrosinase oxidation of spot test reagents. *Mycotaxon* 9: 244-276.

- Marr, C. D., D. W. Grund and K. A. Harrison. 1986. The taxonomic potential of laccase and tyrosinase spot tests. *Mycologia* 78: 169-184.
- Miller, O. K., Jr. 1992a [“1991”]. New species of *Amanita* from Western Australia. *Canad. J. Bot.* 69: 2692-2703.
- Miller, O. K., Jr. 1992b. Three new species of *Amanita* from Western Australia. *Mycologia* 84(5): 679-686.
- Montoya Esquivel, A. 1997. Estudio etnomicológico en San Francisco Temezontla, Estado de Tlaxcala. (Tesis M. en C., UNAM). x+133 pp.
- Morales-Torres, E., M. Villegas, J. Cifuentes and R. E. Tulloss. 1999. Revision of *Amanita alexandri* and its similarity to *Amanita poly-pyramis*. *Mycotaxon* 73: 477-491.
- Munsell Color. 1975. Munsell soil color charts. Baltimore. unpaginated.
- Neville, P. and S. Poumarat. 1996. Révision critique des taxons spécifiques et infraspécifiques de la sous-section *Solitariae* Bas du genre *Amanita* Pers. en Europe et leur extension extra-européenne. *Doc. Mycol.* 26(101): 1-88.
- Neville, P. and S. Poumarat. 2004. *Amaniteae*. *Fungi Europaeae* 9: 1-1120.
- Reid, D. A. 1978. New species of *Amanita* (fungi) from Australia. *Victorian Naturalist* 95: 47-49.
- Reid, D. A. 1980. A monograph of the Australian species of *Amanita* Persoon ex Hooker (Fungi). *Austral. J. Bot. Suppl. Ser.* 8: 1-96.
- Reijnders, A. F. M. 1963. Les problèmes du développement des carpophores des Agaricales et de quelques groupes voisins. (Uitgev. Junk, Den Haag). xvi+412 pp. + 55 pl.
- Reijnders, A. F. M. and J. A. Stalpers. 1992. The development of the hymenophoral trama in the Aphyllophorales and the *Agaricales*. *Stud. Mycol.* 34: 1-109.
- Ridgway, R. 1912. Color standards and color nomenclature. (Published by the author, Washington, D. C.). iv+44 pp. + 43 pl.
- Rogerson, C. T. and G. J. Samuels. 1994. Agaricolous species of *Hypomyces*. *Mycologia* 86: 839-866.
- Rossman, A. Y., R. E. Tulloss, T. E. O’Dell and R. G. Thorn. 1998. Protocols for an All Taxa Biodiversity Inventory of Fungi in a Costa Rican Conservation Area. (Parkway Publ., Boone, N. Carolina). xviii+195 pp.
- Tulloss, R. E. 1993. *Amanita pachysperma*, *Amanita subvirginiana*, and *Amanita virginiana* (taxonomy and distribution) with notes on description of the lamella trama in *Amanita*. *Mycotaxon* 49: 449-475.
- Tulloss, R. E. 1994a. Seminario sobre *Amanita* (Supporting Materials for a Seminar on *Amanita*). (Univ. Autón. Tlaxcala). 127 pp.
- Tulloss, R. E. 1994b. Type studies in *Amanita* section *Vaginatae* I: Some taxa described in this Century (studies 1-23) with notes on description of spores and refractive hyphae in *Amanita*. *Mycotaxon* 52: 305-396.
- Tulloss, R. E. 1998a. Syllabus for a Seminar on *Amanita*, 4th ed. (N. Amer. Mycol. Assoc. & Mycol. Soc. San Francisco). vi+186 pp.
- Tulloss, R. E. 1998b [“1997”]. Provisional world key to species closely related to *Amanita hemibapha* with notes on the slender Caesar’s mushrooms of eastern North America. *McIlvainea* 13(1): 46-53. [A revision of this paper may be downloaded from the following URL: <<http://pluto.njcc.com/~ret/amanita/hemibkey.html>>.
- Tulloss, R. E. 2000. Note sulla metodologia per lo studio del genere *Amanita* (*Agaricales*). *Boll. Gruppo Micol. G. Bresadola* 43(2): 45-59.
- Tulloss, R. E. and H. R. Bhandary. 1992. *Amanita chepangiana*—a new species from Nepal. *Mycotaxon* 43: 25-31. [Note: revised by Yang (1997).]
- Tulloss, R. E. and T. Borgen. 1996. *Amanita mortenii*—a correction: Emendation was inappropriate. *Mycotaxon* 59: 419-425.
- Tulloss, R. E. and A. Gminder. 2000. *Amanita lactea*: stato attuale dell conoscenze su una specie relativamente isolata dell sezione *Vaginatae*. *Boll. Gruppo Micol. G. Bresadola* 43(2): 279-285.
- Tulloss, R. E. and R. E. Halling. 1997. Type studies of *Amanita morenoi* and *Amanita pseudospreta* and a reinterpretation of crasso-spores in *Amanita*. *Mycologia* 89: 278-288.
- Tulloss, R. E., S. H. Iqbal, A. N. Khalid, R. P. Bhatt and V. K. Bhatt. 2001. Studies in *Amanita* (*Amanitaceae*) from southern Asia. I. Some species of Pakistan’s Northwest Frontier Province. *Mycotaxon* 77: 455-490.
- Tulloss, R. E., C. L. Ovrebo, and R. E. Halling. 1992. Studies on *Amanita* (*Agaricales*) from Andean Colombia. *Mem. New York Bot. Gard.* 66: 1-46.
- Wood, A. E. 1997. Studies in the genus *Amanita* (*Agaricales*) in Australia. *Austral. Sys. Bot.* 10: 723-854.
- Yang, Z. L. 1997. Die *Amanita*-Arten von Südwestchina. *Biblioth. Mycol.* 170: i-ii, 1-240.
- Yang, Z. L. 2000a. Species diversity of the genus *Amanita* (Basidiomycetes) in China. *Acta Bot. Yunnanica* 22: 135-142.
- Yang, Z. L. 2000b. On taxonomic studies of the Chinese amanitae. *Mycosystema* 19: 435-440.
- Yang, Z. L. and Y. Doi. 1999. A contribution to the knowledge of *Amanita* (*Amanitaceae*, *Agaricales*) in Japan. *Bull. Natn. Sci. Mus., Tokyo*, Ser. B 25: 107-130.
- Yang, Z. L. and F. Oberwinkler. 1999. Die Fruchtkörperentwicklung von *Amanita muscaria* (Basidiomycetes). *Nova Hedwigia* 68: 441-468.

Appendix A1: Collecting notes for species of *Amanita*

Developed by R. E. Tulloss, P.O. Box 57, Roosevelt, NJ 08555-0057.

Suggestions and improvements are solicited.

Suggested mode of use: Copy pages 1 and 2 (reduced) on one side of a sheet of letter paper and pages 3 and 4 on the reverse side of the same sheet.

Date of Collection:

Name of Collector(s):

Collector's Collection No.:(if assigned)

Field Diagnosis:

Locale of Collection:Park/Town/Borough/etc.

County State/Province Country

Type of Soil:

Species of Trees and other vegetation around collecting site:

Other comments on site ecology, collecting conditions, etc.:

PILEUS: Diam.: Thickness. Color(s):

Shape (describe changes during expansion if possible, include inflexed or decurved margin, umbo, etc.):

Striate? Y N Length of Striation [Give a range of values when dealing with multiple basidiocarps. It is convenient to provide the information as a fraction (without computing the result) such as 14/43, where 43 is the radius of the pileus with 14 mm long striation.]:

Appendiculate? Y N

Form of appendiculate material:

Dry/Viscid/Tacky/Shiny/Dull?

Odor? (Give it a name if possible.) Taste? (Give it a name if possible.)

Surface staining or bruising reactions?

Context color: Context staining:

Describe how the context thins from stipe to margin. (For example, ``slowly at first, then rapidly to a membrane for the last 1 cm nearest margin."")

General comments about pileus other than Universal Veil.

Universal Veil on Pileus: Color. Form. Texture.

Adnate or Easily removed?

Membranous/Submembranous/Felted/Floccose/Pulverulent/Other?

Staining or bruising reaction?

LAMELLAE: Color in mass: Color side view:

Breadth:

Free/Adnate/Narrowly adnate/Other?

Decurrent line on stipe?

Staining or bruising reaction?

Form of Lamellulae (truncate, subtruncate, rounded truncate, subattenuate, attenuate, attenuate in steps, other, evenly distrib., unevenly distrib., 1-length, 2-length, div. lengths, plentiful, uncommon).

Other comments on lamellae (forking, anastomosing, distant, subdistant, close, subcrowded, crowded, relation of thickest portion to stipe, other).

STIPE: Length (bottom of pileus context to top of bulb):

Width at midstipe:

Length of bulb: Width of bulb (at broadest point):

Shape of bulb (globose, subglobose, ovoid, fusiform, napiform, rooting, turbinate, carrot-shaped, other; or note if stipe simply clavate).

(Note there is no bulb in Section *Vaginatae*. Don't confuse presence of volval sac with the presence of a bulb.) A small drawing is sometimes helpful in conveying bulb shape.

Color:

Staining or bruising:

Narrowing upward, narrowing downward, cylindrical, other?

Flaring at apex?

Decoration on outer surface:

Presence, position, color, form, staining of annulus:

Color of context: Staining or bruising of context:

Hollow/Stuffed/Solid? Diam. central cyl.:

Form of stuffing material:

Color in worm or insect tunnels in context:

Universal veil material on stipe base:

a. Sac: Distance from stipe base to highest point of limb:

Thickness at midpoint between top and attachment:

Texture. Color. Layered?

Tough/Flimsy?

When longitudinal section is made, is a little, inner limb present? If so, make a drawing showing how and where the inner limb is attached to the outer limb and where the latter is attached to the stem.

b. Not evident.

c. In warts. (Describe size*, color, placement, etc. Do they seem to cause the presence of recurved scales on the top of the bulb?)

d. In a collar as in *Amanita pantherina*. (Describe size*, color, placement, etc.)

e. In broken collars as in *A. muscaria*. (Describe etc.)

f. In a loose limb against stipe. (Describe etc.) Give distance from base to topmost point of limb.

g. In loose patches easily left in the soil. (Describe etc.)

h. Other.

SPORE PRINT: Color:

Other comments:

Were color slides taken? Y N What identifying marks are on these slides?

Were digital photos taken? Y N What file numbers were generated for the images?

Appendix A3: Record Form for Phenoloxidase Spot Testing

APPENDIX A3: LACCASE, TYROSINASE TEST DATA

COLLECTION DATA:

NAME: _____ DATE: _____

COLL. NO. _____ COLLECTORS: _____ PHOTOS: _____

LOCATION & HABITAT: _____

SPOT TEST DATA:

TEST TIME: _____ COLOR OF CUT SURFACE: _____

BRUISING: _____

LACCASE:

YOUNGEST SPOROCARP

____ (- -) TOTALLY NEGATIVE

____ (- +) MILD REACTIONS

____ (+ +) TOTALLY POSITIVE

____ (+ -) SPECIFIC AREAS POS.
(E.G. GILLS)

INTERMEDIATE

____ (- -)

____ (- +)

____ (+ +)

____ (+ -)

OLDEST SPOROCARP

____ (- -)

____ (- +)

____ (+ +)

____ (+ -)

TYROSINASE:

YOUNGEST SPOROCARP

____ (- -) TOTALLY NEGATIVE

____ (- +) MILD REACTIONS

____ (+ +) TOTALLY POSITIVE

____ (+ -) SPECIFIC AREAS POS.
(E.G. GILLS)

INTERMEDIATE

____ (- -)

____ (- +)

____ (+ +)

____ (+ -)

OLDEST SPOROCARP

____ (- -)

____ (- +)
















____ (+ +)

____ (+ -)

LACCASE: DEVELOPMENTAL PATTERNS

TYROSINASE: DEVELOPMENTAL PATTERNS

COMMENTS:

TEST	YOUNGEST SPOROCARP		INTERMEDIATE SPOROCARP		OLDEST SPOROCARP	
	L	W	L	W	L	W
CONTROL # 1 distilled water						
CONTROL # 2 95 % Methanol						
TYROSINASE TEST - 5 % L-tyrosine						
TYROSINASE TEST 1 % p-urecol						
LACASE TEST - 1 % Syringaldazine						

Appendix A4: Record Form for Sulfuric Acid Spot Testing

H₂SO₄ Spot Tests on Amanita

Form version: May 22, 1993

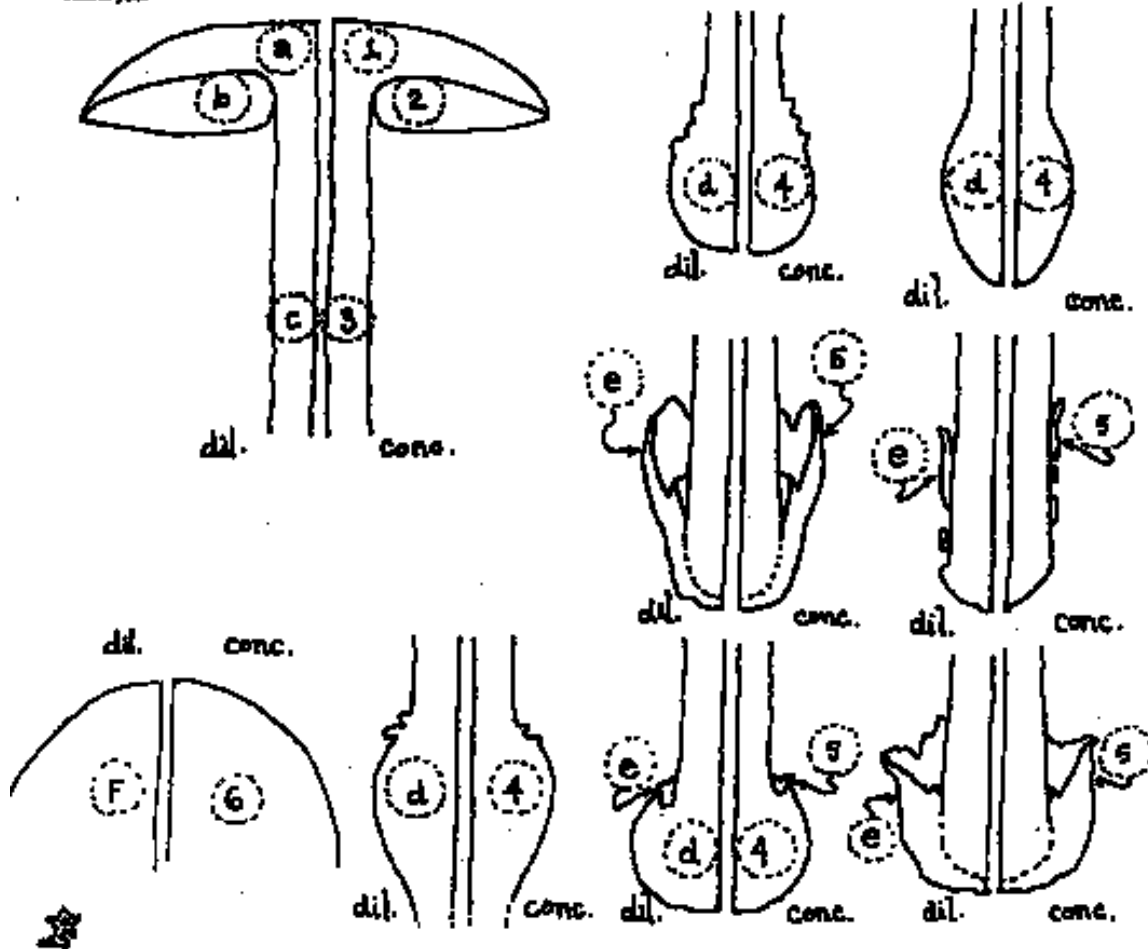
Amanita _____ date: _____

Collector: _____ Collector's no.: _____

Locality: _____

Make a thin longitudinal slice of the mushroom at its broadest part. Lay the slice on a surface that will not be damaged by acid. With a razor or sharp knife cut the mushroom "silhouette" into two equal halves. One half is to be tested with concentrated H₂SO₄ and the other half with dilute H₂SO₄ (18% by volume in water). Drops are to be placed on the pileus center, the edge centered at about the midpoint, the bulb (if there is one) at about the midpoint, the limb of the gills (if there is one) at mid-height, the surface of the lamellae. Since the reaction may be quick and fleeting, the results of each drop should be recorded before passing on to the next one. Take one eighth to one quarter of the portion of the pileus not used in the test of the longitudinal section. Attempt to peel the pileipellis from this place and record to what degree the pileipellis can be peeled. If the pileipellis cannot be peeled from the underlying context, remove a piece of the pileipellis carefully leaving as much of the context in place as possible. Make two more spot tests: One on a part of the pileipellis that is still attached to a piece of the pileus and one on the context that has been bereft by removing a piece of pileipellis. Record the results fully. Dry the remainder of the specimen and label it consistent with the data at the top of this sheet. Package the dried specimen carefully to protect from the Postal Service and send it with this completed form to:

Thank you.



NOTES

The front cover of this book was designed by Mary Macher (M C Macher LLC)

The layout was designed by the author with the assistance of Mary Macher.

The text is set in Times Ext Roman, Ludivicos, Arial, and Symbol fonts.

Page layouts were created with Adobe FrameMaker vers. 9.0p237.

Illustrations were prepared with Adobe Photoshop vers. 6.0.

Final copy was prepared for the printer with Adobe Acrobat Pro vers. 9.3.4.

