Notes on Methodology for Study of *Amanita* (Agaricales)

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The organizers of the first presentation of *Seminario sobre Amanita* (at the Mycological Laboratory, Universidad Autónoma de Tlaxcala, Tlaxcala, México) asked me to discuss every step of my collecting and note taking process as well as other methodological points in regard to the study of *Amanita* Pers. This resulted in production of a detailed syllabus (Tulloss, 1994a). It seemed to be a profitable thing to do despite the fact experienced attendees at the seminar were very familiar with practices common to collecting agarics in general. The seminar's syllabus has evolved through three additional editions. This paper is intended to share the methodological part of the seminar with a broader audience; it is a "work in progress."

The paper is divided into three sections entitled: "In the Field," "In the Laboratory—Macroscopic Characters," and "In the Laboratory—Microscopic Characters with Comments on Their Observation."

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* (E. J. Gilb.) Veselý *emend.* Corner & Bas. Reading this document was an enlightening experience for me. It changed the direction of my life and gave me 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 is dedicated to Dr. Bas to whom I wish to express my profound gratitude for his work, his mentoring, his wisdom, his subtle criticism, his generosity, his humor, his encouragement, and his discipline.

This paper is full of information that I have learned by the study of documents and collections given or loaned to me by correspondents and herbaria around the world. My ignorance would be far greater if it were not for these friends.

Finally, one must especially acknowledge the recent, outstanding work on *Amanita* by Z. L. Yang (1997) whose work promises to advance the taxonomic study of this fascinating genus into the next academic generation.

In the Field

Photographing. If a camera with macro or other close-up lens has been taken into the field, I make color slides of the whole fruiting body and 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). I 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 features, I use reflectors (made from aluminum foil wrapped around sheets of cardboard) to light the side of the specimen away from the sun. I groom natural settings so that twigs, grass, etc. don't block the camera's view of the mushroom. After getting a spore print, a photo of the fruiting body in longitudinal section is often helpful (see below).

Collecting. I remove the fruiting body from the soil carefully. Having the whole fruiting body is often necessary for determination of 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 missed 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 I use a large-bladed knife or a narrow garden trowel. I have seen an aluminum tent peg used to good advantage for the same purpose.

My basket design. For carrying *Amanita* collections in the field, I use a very deep basket that is cross-laced with strings so that many rectangular "compartments" are outlined by the strings. I got this idea from Dr. David T. Jenkins, University of Alabama, Birmingham. *Amanita* specimens are wrapped in 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 something that's a lot easier to measure than it might have been otherwise.

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 expecting to collect with a borrowed basket, I bring a set of cardboard dividers from inside a carton of U.S. tomato ketchup bottles. I simply expand the dividers and insert them in the borrowed basket. They can be folded to fit baskets of various dimensions. It seems that U.S. ketchup bottles are very well-proportioned as far as *Amanita* collecting goes (Fig. 1).

Field Annotation. "Field notes" is a poor term for what is intended to be entered on my note form; some of these notes are best made after returning from the field (see the following section). A copy of any form mentioned in this paper is available from the author.

In the field, I find it useful to note collector's names, collection number, locality, date, quantity and distribution of fruiting bodies, soil, and habitat.

I do my best 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 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.

In the Laboratory—Macroscopic Characters

This section follows the outline of my note-taking form that appears as Appendix A2 of (Tulloss, 1998a)

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—must be measured 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 string. Pileus thickness, breadth of lamellae, and dimensions of the stipe are all best measured after making a longitudinal section of the fruiting body. Therefore, I hold off on making these measurements until a spore print has been obtained (unless a spore deposit is not to be obtained—see "triage," below).

I treat the length of the stipe above the bulb (if one is present) and the length of the bulb 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. I am wary with regard to measurements in the literature because I find bulb length sometimes included in stipe length and sometimes not included—even within a single work. There is no true bulb in species of section *Vaginatae* (Fr.) Quél., and the apparent bulb in species of section *Amidella* (E. J. Gilb.) Konr. & M. is usually only a very thick volval sac.

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 basidiocarps in a collection; and, if one of the ratios is especially high or low in another basidiocarp of the same collection, data for that basidiocarp should be recorded as well.

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 oppressed 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. Sometimes a species with deeply pigmented stipe fibrils will also have marginate lamellae.

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.) Pers. and of *A. hemibapha* (B. & Br.) Sacc. and on its Western Hemisphere relatives (e.g., *A. jacksonii* Pomerleau, see 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.

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 basidiocarp. *Photo*: R. E. Tulloss

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).

Colors. Colors can be expressed in your own terms, but it will be much easier to communicate about them if a color book is 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. 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 (now selling in the range of $US 400 - 500), one shouldn't hesitate to use it.

The color of the universal veil and lamellae may change as a fruiting body 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 basidiocarp of this group is usually correlated to that in a young one. For example, the pale orangish white volva of one New Jersey Pine Barrens species (*Amanita sp. 49*) retains a faint orangish tint as it becomes gray; and the brilliant yellow-orange volva of a species collected in Maine (*Amanita sp. N29*) becomes red-brown. I check colors of the universal veil, annulus (if present), and lamellae in both young and mature fruiting bodies. Colors of the lamellae are recorded both in mass (viewed from directly below the pileus) and in side view (after longitudinal sectioning of the basidiocarp).

Bruising or staining reactions on the surfaces or in the context of an *Amanita* can be important for determination. However, in at least one case (Tulloss, unpub. results), a species that does not normally change color when cut [*A. subsolitaria* (Murr.) Murr.] will turn brilliant yellow occasionally—apparently due to some invasive agent. Because of this observation, an investigation of spores size and shape and anatomy should be undertaken in cases in which yellow staining occurs—before settling on a determination quickly. In *A. subsolitaria*, no mature spores have been found on yellow staining fruiting bodies; moreover, the lamellae are usually covered with budding yeast cells.

Odor and taste. When it comes to odor and taste, one is on one's own. I 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. I usually taste specimens clearly assignable to section *Vaginatae*, but never swallow the material tasted.

Spore deposit. I obtain a spore print if at all possible (but see under "triage," below) for every taxon studied. I 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. If field set-up is impractical, I 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.

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. I select at least one fresh fruiting body (it is best, if time allows, to test both an immature and a mature specimen) and slice it longitudinally. Using a razor or a very sharp knife, I slice off a "silhouette" of the mushroom about 2 or 3 mm thick (if possible given the size of the stipe) from the exposed inner surface of one of the two half-mushrooms. I divide this silhouette down the center and place each half-silhouette 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, mix, etc. (For all types of macrochemical tests, I always dry the parts of the mushroom that I don't use for the test. On the herbarium label for such material I indicate 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.)

Procedure for the spot tests: 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. I suggest the use of the note form presented in Appendix A3 of (Tulloss, 1998a). 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 collec-

tion date and collection number so that the record of results can be correlated with the collection and your other notes.

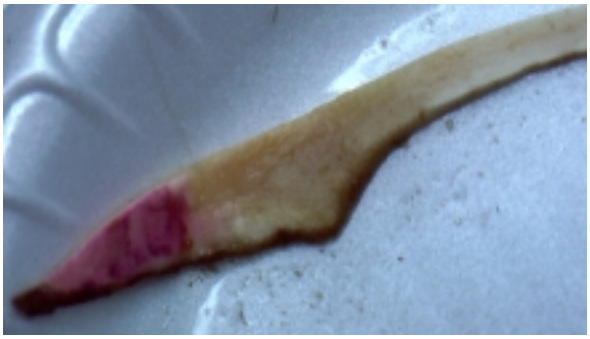


Fig. 3. Positive (lavender) reaction for laccase in the radicating bulb of *A. subsolitaria.* The yellow tint in the stipe context is the color of unreacting syringaldazine. *Photo*: R. E. Tulloss

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 from 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).

Sulfuric acid spot tests. In the literature, only a supposed purple reaction on the lamellae of *A. phalloides* is commonly mentioned. In the mid-90s, the late Dr. L. J. Tanghe, G. Lincoff, and I experimented with concentrated H_2SO_4 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 H_2SO_4 spot tests, see Appendix A4 of (Tulloss, 1998a). I suggest that the tests only be performed with concentrated acid.

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

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 in central Mexico of what appears to be *A. bisporigera* often produce faint reactions or none at all. Color photographs are important for unusual color reactions or for varying shades of yellow on taxa usually not reported to be reactive.

Testing with ammonium hydroxide. Almost nothing is known about reactions in *Amanita*. Experiments are needed. In the present state of knowledge, it would be inappropriate to define a taxon based on a reaction to NH_4OH.

Melzer's reagent. If one wants to test macroscopically for amyloidity of spores (I never do this, but some do), it can't be done effectively on the spore print. 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 for carrying 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 hyphae in various parts of fruiting bodies; however, insufficient information is available to make this a useful taxonomic tool at present. Experiments are needed.

Triage. If I am limited on time, I don't eliminate all of the steps related to collection and photography if the most important steps can be managed at all. The steps I 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 fruiting bodies) can be photographed and dried, keeping notes only on locality, date, collector, etc. Collections of common species with specimens of unusual size can be photographed and should have notes taken on the dimensions of the fruiting bodies. Collections of common material in poor condition that are not needed to provide vouchers for mapping projects (for example) can be discarded.

Drying the specimens. I prefer to dry material rapidly because, in my experience, this preserves delicate structure (such as the lamella trama) best. For example, a forced air vegetable dryer with stacking trays can be used. When temperature regulation is possible, a forced air dryer should be set to operate at 55° - 60° C (130° - 140° F). 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. I have built 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 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. 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.

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 laboratory and herbarium facilities, preservation in liquid is 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 art 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.

In the Laboratory—Microscopic Characters with Comments on Their Observation

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. Among recent works that are useful are those of Tulloss et al. (1992), Tulloss (1993, 1994, 1998a), and Yang (1997).

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. Examples of such elements are filamentous, undifferentiated hyphae; vascular hyphae; and acrophysalides. Spore data are treated next. Finally, 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 in the pileipellis.

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

I. Characters of elements common in many tissues.

A. Filamentous, undifferentiated hyphae [see (Tulloss et al., 1992)].

1. Range of width
2. Range of wall thickness
3. Frequency of branching
4. Frequency of septa
5. Wall color or color of intracellular pigment (colorless or yellowish or sordid yellowish or brown or other)
6. Fasciculate?
7. Dominant orientation (e.g., in many taxa, but not in all, subradial in pileipellis)
8. Relative frequency—as opposed to frequency of acrophysalides or other inflated cells
9. Form (e.g., coiling, branched, constricted at septa)
10. Decoration internally or externally

B. Vascular hyphae [see (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 insoluble in water and aqueous solutions of KOH and NH_4OH.

1. Range of width
2. Frequency of branching
3. Color (especially if not yellow)
4. Presence in fascicles of filamentous, undifferentiated hyphae
5. Frequency
6. Peculiarities of form (e.g., coiling, branched, tangled in knots, etc.)

C. Acrophysalides and other inflated cells.

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 basidiocarp. 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 is important to note 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, I have not used the term “acrophysalide” for such cells. Regrettably, I have not had an

opportunity to discuss this point with Dr. Bas, although he has never objected to my usage. 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 apparently circular, Dr. Bas and I 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.).

1. Range of size (at least largest seen and top of range in which most observed cells lie)
2. Color
3. Range of wall thickness
4. Relative frequency as opposed to that of filamentous, undifferentiated hyphae in same tissue
5. Range of shapes (noting if wall thickness or size is relatively common for a given shape)
6. Terminal or intercalary?
7. Occurring singly or in chains (if latter, give range of lengths of chains in number of cells per chain observed)
8. Decoration internally or externally (External decoration is very rare. A truly warted exterior is known only from the universal veil of one New Zealand species of *Amanita* section *Amanita* (Tulloss, unpub. data)—*A. nehuta* Ridley, apparently phenetically related to *A. friabilis* (Karst.) Bas and *A. farinosa* Schw.

II. Spores [see (Tulloss et al., 1992) and (Tulloss, 1994)].

Terminology and notation:

L = the average spore length computed for one specimen examined
L' = the average spore length computed for all spores measured
W = the average spore width computed for one specimen examined
W' = the average spore width computed for all spores measured
Q = the ratio of length/width for one spore
Q = the average value of Q computed for all spores of one specimen examined
Q' = the average value of Q computed for all spores measured

Note: Spores should always be measured in lateral view with apiculus and both ends of the spore all in focus.

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
d = the largest value measured.

When presenting spore data for a taxon, I 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.

1. Measure 20 per specimen (if that many can be found) - I do not use data from specimens for which less than 7 spores could be found.
2. Spores should only be measured when both ends of the spore are in focus.

3. 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 the 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. (i.e., 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, I have observed that the effort pays off in taxonomic usefulness of the resulting data.

4. Compute individual length/width ratio (Q) for each spore

5. Compute average length (**L**), average width (**W**), and average Q (**Q**) for each specimen

6. Compute overall averages of length, width, and Q (**L'**, **W'**, and **Q'** respectively) for each taxon. It is no harm to compute standard deviation for these averages; however, for a user of your data with only a small number of specimens before him/her, it is more useful to provide the ranges within which you have found the averages to fall. (See item 7.)

7. Report ranges for length, width, Q, **L**, **W**, and **Q**. A taxonomist with extended experience with a taxon may have measured spores from dozens of basidiocarps in many stages of development and in varying conditions dependent upon the stage of development when dried, the condition when dried, the speed of drying, the quality of preservation in an 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. The variation is most easily used by, and, hence, most useful to, later workers when the range of the raw data is available

8. Report ranges by indicating lowest and highest values observed (extremes) and the range in which 90% of values fall using the notation "(*a*-) *b* - *c* (-*d*)."

9. To aid in evaluating whether a given specimen may have spores of unusually small or large size, I record the spore data according to spore length in columns labeled by length ranges (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 article by Tulloss & Gminder on *A. lactea* Malenç., Romagn. & Reid in the present volume.

10. Hyaline or opaque?

11. Color. The term "hyaline" means "transparent, like glass." It should not be taken to mean "colorless" as well.

12. Wall thickness or decoration (see crassospore discussion, below)

13. Presence or absence of adaxial flattening

14. Irregularity of form (swollen at one end, constricted, "Y"-shaped, shaped like a planarian, etc.)

15. Presence of "giant spores"

16. 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. The latter are never amyloid in *Amanita* to my knowledge. They may sometimes have dextrinoid contents (e.g., in *A. mutabilis* Beardslee). Also, the spores may be compared to air bubbles in the mount.

17. 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.

18. Form of contents [e.g., monoguttulate, multiguttulate, granular, monoguttulate with additional small granules, etc.]

III. Tissue by tissue analysis.

A. Pileipellis.

1. Present or not? In a number of taxa of section *Lepidella* [notably, in most taxa of subsection *Vittadiniae* Bas, *A. rhoadsii* (Murr.) Murr. var. *rhoadsii*, and *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 my experience in all cases to date that the universal veil is being described in a species that lacks a well-developed pileipellis.

   a. If present: thickness

   b. If not present: relationship of pileus context to universal veil, characteristics of transitional region

2. Gelatinized to partially gelatinized suprapellis (thickness, color)

   a. Is the gelatinous material caused by breakdown of hyphal cell walls?

   b. Is the gelatinous material a matrix in which whole hyphae interweave loosely?

3. Predominantly ungelatinized subpellis (thickness, color)

4. Filamentous, undifferentiated hyphae

5. Presence (if any) and character of inflated or partially inflated intercalary segments of hyphae

6. Vascular hyphae (See I.B.)

7. Clamps

B. Pileus context.

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.

1. Filamentous, undifferentiated hyphae

2. Acrophysalides (See I.C.)

3. Vascular hyphae (See I.B.)

4. Clamps

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

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

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.

Caution: 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.

1. Form taken by bilateral tissues, range of angles of divergence of basal elements of subhymenial tree

2. Central stratum

   a. Measure w_{cs} (always measure if at least some rehydration is possible!) - Always state the quality of rehydration achieved. It is valuable information albeit qualitative.

   b. Filamentous, undifferentiated hyphae

   c. Intercalary inflated cells (See I.C.)

   d. Vascular hyphae (See I.B.)

3. Subhymenial base

   a. Organization, structure

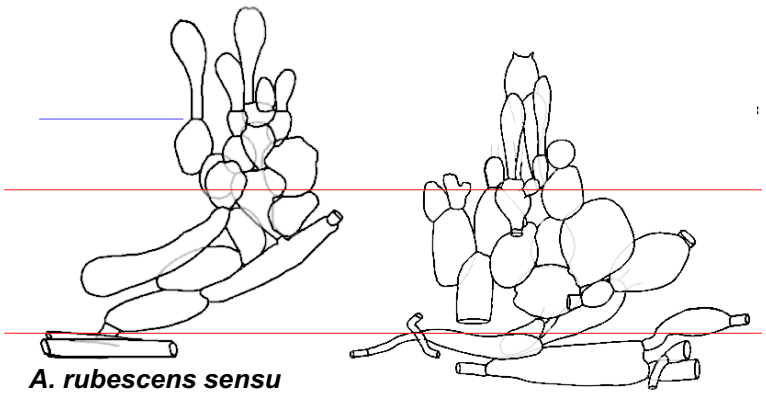
   b. Filamentous, undifferentiated hyphae (See I.A)

   c. Inflated cells (terminal? intercalary?) (See I.C.)

   d. Vascular hyphae (See I.B.)

   e. Clamps (See V.)

f. Subhymenial tree—make drawing of hymenium and subhymenial tree inclusive of edge of central stratum if possible. See examples in Fig. 4.



A. rubescens sensu **eastern U.S. authors**

A. rubescens **(Pers.:Fr.) Pers.**

Fig. 4. Comparison of subhymenial tree in true European *A. rubescens* (right) and *A. rubescens sensu* eastern U.S. authors. Note the values of w_{st}-near are larger in the U.S. material (compare distance from bottom red line to blue line vs. distance between two red lines) and the size of the inflated cells in the subhymenial tree are larger in the true rubescens.

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

Caution: Divergent, terminal cells appear to be quite rare in the lamella trama of some species of *Amanita* despite the literature to the contrary. Many times cells that appear terminal were in fact intercalary before being displaced 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.

1. If divergent, terminal, inflated cells present,...

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

2. In all cases,...

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

   b. Describe types and forms of cells from which basidia arise

   c. General characterization of form (cellular or pseudoparenchymatous, coralloid, ramose, etc.). Often, it seems better to write a description of the drawing (see above) rather than limited a description to selection of one or more of the terms introduced by Bas (1969).

   d. Clamps (See V.)

E. Basidia.

1. Range of size
2. Relative proportion of 4-sterigmate, 2-sterigmate, etc. basidia
3. Size of sterigmata (width at base and length) and any oddities of form
4. Wall thickness
5. Crassobasidia (see below)
6. Include in drawing with subhymenial tree and hymenium (above, and, e.g., in Fig. 4)
7. Clamps (See V.)
8. Oddities of structure/form [e.g., branched (rare)].

F. Universal veil.

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.

1. Draw tissue either from pileus or stipe base; if tissues of layers are very varied, draw those layers that are not essentially composed of filamentous, undifferentiated hyphae or such hyphae and vascular hyphae.
2. On the pileus
   a. Identify layers and treat each separately.
   b. Filamentous, undifferentiated hyphae (See I.A)
   c. Inflated cells (See I.C.)
   d. Vascular hyphae (See I.B.)
   e. Clamps
3. On the stipe base
   a. Identify layers and treat each separately.
   b. Filamentous, undifferentiated hyphae (See I.A)
   c. Inflated cells (See I.C.)
   d. Vascular hyphae (See I.B.)
   e. Clamps

G. Stipe context.

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 concentration of vascular hyphae at the stipe apex.

1. Filamentous, undifferentiated hyphae (See I.A)
2. Acrophysalides (See I.C.)
3. Vascular hyphae (See I.B.)
4. Clamps

H. Partial veil.

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. In my descriptions, I only refer to the latter inflated cells in descriptions of partial veil tissue. While a subradial orientation of elements is common in the partial veil, I check for a cross-weave of other elements or a possible layering. A drawing should be made of the tissue of the partial veil.

1. Filamentous, undifferentiated hyphae (See I.A)

2. Inflated cells (See I.C.)

3. Vascular hyphae (See I.B.)

4. Clamps

IV. Thoughts on determining material from anatomy.

It is often necessary for me to review spores, lamella trama, universal veil, and partial veil in order to have a hope of coming up with a definitive determination 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 possibly the presence or absence of clamps). To be able to do efficient work in determining taxa, notes on macroscopic characters are necessary. The difference between an hour and a day spent on a single specimen is significant. As more and more taxa are described, the process is bound to become more difficult. Good notes on fresh material and good photographs will become more (not less) important.

V. Finding basidial clamps.

Portions of lamellae that are least mature are the most likely places to find basidial clamps.

1. Stain with Congo Red or other cell wall stain.

2. Obtain a good section.

3. Check near lamella edge.

4. Check part of lamella nearest pileus margin.

5. Check for "V"-shaped bases of basidia in more mature regions of lamella (called "proliferated clamps" by Bas (1969)).

VI. Crassospores and crassobasidia.

My observations of crassospores and crassobasidia are based on taxa from subgenus *Amanita* (*A. morenoi* Raithelhuber and *A. pseudospreta* Raithelhuber) both collected in the same, very limited region of Argentina. A similar phenomenon has been reported (as rare) in several European taxa. I have seen the same phenomenon in a few North American taxa, but very rarely. See (Tulloss and Halling, 1997).

1. Basidia and spores with thickened walls.

2. Walls of basidia thicken as they approach maturity and sterigmata appear.

3. By the time apophyses appear, the thickened wall becomes distinctly decorated.

   a. The thin outermost wall layer separates from the inner layer(s) and retains its undecorated form.

   b. The remaining layer or layers bulge inward here and there forming roughly evenly distributed hemispherical depressions.

4. As spores form, they develop similarly decorated walls.

5. Cause is as yet unknown.

VII. Parasitized *Amanita* specimens.

A. *Hypomyces hyalinus* (Schw.:Fr.) Tul. parasitization.

1. Taxa involved include at least the following, all in *Amanita* section *Validae* (Fr.) Quél.:
   a. *Amanita rubescens sensu* eastern North American authors
   b. *Amanita flavoconia* Atk. var. *flavoconia*
   c. *Amanita sp. 10*, known from deciduous woods in New Jersey
   d. *Amanita sp. 39*, known from Pine Barrens in New Jersey
2. Some research questions.
   a. Range of *H. hyalinus* on *Amanita*.
   b. Apparent limitation to species of section *Validae.*

B. *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 me. I would welcome information concerning its biology.

C. Yellow-staining specimens of *A. subsolitaria*.

1. Often found among normal specimens.
2. Rapidly staining yellow when cut. (See Fig. 5.)
3. Sometimes exuding orange fluid when cut.
4. Lamellae covered with dividing yeast cells.
5. Spores distorted and small.
6. Some research questions.
   a. Isolation of causative agent(s).
   b. Mechanism by which such agent(s) cause(s) the staining reaction.
   c. Relation to yellow-staining mechanism in taxa accepted as unparasitized, *e.g.*, *A. cinereoconia* var. *croceescens* Bas, *A. crassifolia* Bas *nom. prov.*, and *A. rhoadsii* var. *flavotingens* Bas [placed with *A. subsolitaria* in stirps *Rhoadsii* by Bas (1969)].

Fig. 5. Parasitized specimen of *A. subsolitaria* showing yellow-staining reaction in context. *Photo*: R. E. Tulloss

D. Specimens of *A. polypyramis* (B. & C.) Sacc. with cheese odor.

1. Established as related to parasitization by a hyphomycete by Morales-Torres et al. (1999). Similar infestation was found in other Mexican exsiccata of *Amanita* section *Lepidella* that had had an odor of cheese when fresh.
2. *Amanita alexandri* Guzmán is, consequently, a synonym of *A. polypyramis*. The holotype of the former is infested by the hyphomycete.
3. Some research questions.
   a. Isolation of causative agent.
   b. Mechanism by which agent alters the odor of the basidiocarp.

E. The case of *A. aminoaliphatica* Filippi *nom. inval*.

1. Odor “of aliphatic amines.”
2. Micromorphologically very similar to *A. ovoidea* (Bull.:Fr.) Link.

3. Stipe context in some basidiocarps largely replaced by hyphae like those that parasitize *A. polypyramis*.

4. Can "*A. aminoaliphatica*" be a result of parasitization of *A. ovoidea* or *A. proxima* Dum.?

5. Some research questions.

   a. Isolation of causative agent.

   b. Mechanism by which agent alters the odor of the basidiocarp.

   c. Relation to agent and mechanism in *A. polypyramis* (above).

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