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The genus *Amanita* should not be split

Rodham E. Tulloss [a1], Thomas W. Kuyper[b], Else C. Vellinga[c], Zhu Liang Yang[d], Roy E. Halling[e], József Geml[f], Santiago Sánchez-Ramírez[g], Susana C. Gonçalves[h], Jaqueline Hess[i], Anne Pringle[j]

[a]P. O. Box 57, Roosevelt, New Jersey 08555-0057, USA

[b]Department of Soil Quality, Wageningen University, P. O. Box 47, 6700 AA Wageningen, the Netherlands

[c]Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, California 94720-3102, USA

[d]Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[e]Institute of Systematic Botany, New York Botanical Garden, 2900 Southern Boulevard, Bronx, New York 10458, USA

[f]Naturalis Biodiversity Centre, P. O. Box 9517, 2300 RA Leiden, The Netherlands

[g]Environmental Genomics Group, Max Planck Institute for Evolutionary Biology, August-Thienemann-Str. 2, 24306 Plön, Germany

[h]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

[i]Department of Biosciences, University of Oslo, P. O. Box 1066 Blindern, N-0316 Oslo, Norway

[j]Department of Botany, University of Wisconsin, Madison, Wisconsin 53706, USA

Correspondence to: retamanita@comcast.net, Thom.Kuyper@wur.nl, ecvellinga@comcast.net, fungi@mail.kib.ac.cn, rhalling@nybg.org, József.Geml@naturalis.nl, santiago.snchez@gmail.com, scgoncal@ci.uc.pt, jaqueline.hess@gmail.com, apringle2@wisc.edu

Abstract—Recently the well-known genus *Amanita* has been split into two genera, *Amanita*, a genus of putatively ectomycorrhizal fungi, and *Saproamanita*, a genus of putatively saprotrophic fungi. We disagree with this generic split and argue why *Amanita* should not be split. The proposal to split the genus does not conform to the recently proposed guidelines for publishing new genera. Concise amended characterizations are provided for the monophyletic family *Amanitaceae* and its two monophyletic genera *Amanita* and *Limacella*.[2] The characterization of *Amanita* rests on a single, unique synapomorphy—schizohymenial ontogeny in its agaricoid and secotioid taxa. We propose a minimal reorganization of *Amanita*—removal of stirps *Hesleri* from subsection *Vittadiniae*. Some open issues in *Amanita* systematics are discussed. *Amanita* is an emblematic genus and the focus of diverse research programs. Taxonomists and users of taxonomic and systematic products are used to, and rely on, *Amanita* as a genus with meaningful, morphologically defined subdivisions, easy to teach and easy to use. Splitting the genus is unnecessary and would prove costly—degrading our ability to communicate with each other and complicating connections to past literature. We argue that the current use of next-generation sequencing in studies of fungal ecology does not necessitate the splitting of *Amanita*.

Key words—*Amanita* subgenus *Lepidella* sensu Bas, *Saproamanita*, mycorrhizae, saprotrophy, rDNA, trophic mode, monophyly, next-generation sequencing

Introduction

From the first large nrLSU-based tree (Weiß et al., 1998) for *Amanita* Pers. (Persoon 1797) up to the present, phylogenetic hypotheses have consistently supported monophyly of the genus (e.g., Drehmel et al. 1999, Moncalvo et al. 2000, Moncalvo et al. 2002, Zhang et al. 2004, Wolfe et al. 2012b), although sometimes with small sample size. The only exception that we are aware of is the phylogeny, based on nrLSU, by Vizzini et al. (2012), where the asymbiotic clade (clade Φ) is sister to

1. Research Associate (hons.), New York Botanical Garden, Bronx, New York, U.S.A.

2. Just prior to completion of this article, we learned of a proposed phylogenetic hypothesis for the *Amanitaceae* comprising five genera—including *Catatrama* and dividing *Limacella* into three genera corresponding to the present sections of the genus (Yang et al. in press).

Limacella, and where *Limacella* plus Φ are sister to the remainder of *Amanita*. We consider that phylogenetic hypothesis as unlikely. It also lacks, as we will detail below, morphological support. In the Wolfe et al. (2012b) hypothesis, the *Amanita* and *Limacella* Earle (Earle 1909) clades are clearly sisters with strong support. The number of *Amanita* taxa sampled for the Wolfe phylogeny was the largest at the time and a significant effort was made to extensively sample *Amanita* sect. *Lepidella* sensu Bas (1969). As a result of this focus, a novel element in the phylogeny was its treatment of *Amanita* as two monophyletic clades and a third element with poor support that may not be a clade. We call this latter element (grade, clade) Φ.

The Wolfe et al. (2012b) hypothesis quickly gave rise to proposals to split the genus *Amanita* into two genera (Vizzini et al. 2012)—*Amanita* and *Aspidella* E.-J. Gilbert (Gilbert 1940). When it turned out that *Aspidella* E.-J. Gilbert was illegitimate, being a homonym of the fossil genus *Aspidella* E. Billings, the new generic name *Saproamanita* was proposed instead (Redhead et al. 2016). We oppose the splitting of the genus *Amanita* for several reasons. We do not see any compelling reasons to split, and in fact, think that the proposal does not seem in agreement with the guidelines proposed by Vellinga et al. (2015). Our purpose in this article is to argue against the split, clarify some misinterpretations of the Wolfe et al. (2012b) data, and to motivate and support a minimal change in *Amanita* systematics. *Amanita* is a fascinating and diverse set of organisms—the target of research in many fields. We indicate practical considerations supporting maintenance of an unsplit genus.

Materials and methods

Authorial citations follow Kirk and Ansell (2015). The version of the International Code of Nomenclature for Algae, Fungi and Plants (ICN) used is the "Melbourne Code" (McNeill et al. 2012). Journal name abbreviations follow Botanico-Periodicum-Huntianum (BPH*Online*) < http://fmhibd.library.cmu.edu/fmi/iwp/cgi?-open >.

Alignments used to provide genetic distance data were performed using MUSCLE (Edgar 2004), as implemented in Geneious® 8.1.8. Trees for long branch attraction experiments were calculated using PhyML (Guindon and Gascuel 2003) as implemented in the same tool set.

The systematic proposal for *Amanita* sect. *Lepidella* sensu Bas was constructed from the bottom up. For the first level of grouping above species, Bas used an informal, typeless, unauthored rank—stirps. This is a Latin word (plural "stirpes") meaning "group". The only other supraspecific ranks used by Bas were subsection, section, and subgenus. This conservative approach contributed to his creation of a meticulous, understandable, applicable, and widely used systematic proposal. We utilize the term "stirps" following Bas in our proposals and discussion.

Road map to this paper

The sections of this paper play distinct, interrelated roles. Since we hope for a broad audience, it seems useful to explain our view of the functional role played by each of the following numbered sections. The core of the argument against splitting *Amanita* runs through sections 1, 2, 5, and 7. Sections 3 and 4 address misconceptions that are associated with some arguments for splitting. Section 6 looks at the current state of section *Lepidella* sensu Bas with a view to identifying areas of future work.

1. In the first section, we provide a concise characterization for the *Amanitaceae*, *Amanita*, and *Limacella*. Our purpose is to diminish the use and influence of the diffuse, confusing characterizations of these taxa that have been common up to the present.
2. In the second section, we demonstrate that the poorly supported element, Φ, should not be split off as a separate genus according to guidelines for such actions recently proposed (Vellinga et al. 2015). We also demonstrate why Redhead et al. (2016) did not adhere to the guidelines as proposed in that paper.
3. In the third section, we explain why the view (Vizzini et al. 2012) that Φ is equivalent to *A.* subsection *Vittadiniae* Bas (Bas 1969) is not supported by the Wolfe et al. (2012b) hypothesis.
4. In the fourth section, we address the misconception that the Wolfe et al. (2012b) phylogeny definitively separates a set of mycorrhizal species from a set of saprotrophs. We repeat evidence from the original paper and provide supporting, more recent evidence for more extensive variation in modes of carbon access (trophic modes) in the genus *Amanita.*
5. In the fifth section, we propose the single systematic change we feel is presently justified—removal of Bas' stirps *Hesleri* (Bas 1969) from subsect. *Vittadiniae*.
6. In the sixth section, we describe open problems posed by our current understanding of the systematics of *Amanita*, especially with regard to *A.* sect. *Lepidella* sensu Bas.

7. In the final section, we provide a sampling of the breadth of multidisciplinary study of the genus *Amanita* and argue for the considerable practical value of maintaining name stability for amanitas.
8. An appendix provides a brief comparison of sampling rates for sections of the genus *Amanita* in the Vizzini et al. (2012) nrLSU gene tree and the Wolfe et al. (2012b) four gene phylogeny. This illustrates the difference in experimental design between the two approaches.

1. Characterizing *Amanita*

1.1. Traditional mycological characterizations of genera are not easily converted to operational tests for species membership.

Traditional mycological descriptions of genera are not concise operational tests for determining membership in the taxon being defined; and, once introduced, these descriptions are subsequently used over and over, with additions or subtractions of content. Eventually, descriptions often develop into a mixture of errors and tautology-like statements and persist in lacking a precise and clearly identified characterization of the taxon in question.

In the case of *Amanita,* errors found in a sampling of characterizations and implied characterizations of the genus include: All lamellae are free from the stipe; all lamellae are white; lamellae are never forked; spore deposits are always white; a partial veil is always present; a universal veil is always membranous; the stipe's bulb and the universal veil are one and the same structure; and cheilocystidia are present. None of these statements is true. For example: (a) There are numerous species of sect. *Caesareae* Singer having yellow gills and a bulbless stipe with its base enclosed in a membranous, saccate volva; (b) the definition of cheilocystidia excludes elements of a uniform tissue as well as elements developing in the interior of a basidiome; and both these characters are true of the lamella edge tissue of all agaricoid amanitas; (c) many taxa of *Amanita* have lamellae that are more or less broadly attached to the apex of the stipe.

Many of the most extensive descriptions take the form of a list of character state ranges such as "spores range from globose to cylindric." Often, these characterizations also include tautology-like statements or phrases along the lines of, "for all amanitas, either 'A' is true; or 'not A' is true." One common example is "spores amyloid or inamyloid." While such phrases contain information—that two character states are present among species of the genus, rather than one or three—and while the phrases are not true tautologies, the phrases do not offer any defining characters. After testing for reaction of a mushroom's spore walls to iodine, we can learn that it is probably not an *Amanita* (if its spores are dextrinoid) but cannot learn if it is an *Amanita* (if it's spores are not dextrinoid). Leading publications of the last half century include the components of a precise diagnosis mixed with much other information all presented as of equal importance (e.g., Bas 1969, Jenkins 1986, Yang 1997). Such characterizations are difficult to put to practical use. Generic diagnoses that are imprecise are confusing; and, for *Amanita*, they are unnecessary.

1.2. Characterizations of *Amanitaceae* and *Limacella*

We begin by providing a preferred characterization for the *Amanitaceae* E.-J. Gilbert (Gilbert 1940) and for the genus *Limacella*.

Amanitaceae E.-J. Gilbert

CHARACTERIZATION: A species of the Agaricales is a member of the *Amanitaceae* if and only if it satisfies one of the following conditions:

1. It has an agaricoid basidiome, and the stipe tissue is longitudinally acrophysalidic. A useful, supportive diagnostic character shared by all these species is a bilateral, divergent lamella trama.
2. It has a secotioid basidiome, and the stipe tissue is longitudinally acrophysalidic.
3. It has a hypogeous basidiome and is a member of a list determined by molecular means with current members being *A. grandispora* (G. W. Beaton et al.) Justo (Justo 2010) and *A. oleosa* (Bougher & T. Lebel) Justo (Justo 2010). It is possible that the truncate columella or other context not closely associated with hymenial tissue could be treated as acrophysalidic; however, we do not have sufficient personal experience at this time to make that judgment. Also we note that in the original treatment of these taxa in *Amarrendia* Bougher and T. Lebel (2002), taxa "with affinity to the *Russulales*" potentially with inflated cells homologous to the sphaerocysts of, say, *Russula* were included in *Amarrendia* (Hallen et al. 2004) along with *A. grandispora* and *A. oleosa*. We do not yet know if we could make the distinction between the two types of inflated cells in taxa formerly assigned to *Amarrendia*.

Limacella Earle

MycoBank: 17978

CHARACTERIZATION: A species of the *Amanitaceae* is a member of the genus *Limacella* if and only if it satisfies both the following conditions:

1. It has an agaricoid basidiome not produced by schizohymenial ontogeny.
2. It has a fertile lamella margin and a gluten bearing cap with gluten held in place by anticlinally oriented elements.

1.3. Preferred characterization of *Amanita*

The genus *Amanita* [including the taxa proposed for *Aspidella* (Vizzini et al. 2012) and *Saproamanita* (Redhead et al. 2016)] possesses a unique diagnostic character or synapomorphy—schizohymenial ontogeny of the basidiome (Bas 1969, Reijnders 1963, Yang and Oberwinkler 1999). Note "schizohymenial ontogeny" is commonly used (and is used by us) to refer to development of the entire basidiome and not just the development of hymenial surfaces.

Most agarics begin basidiome development by developing a proto-stipe, from which a pileus is initiated, followed by lamellae growing away from the proto-pileus into empty space. The development of stipe, pileus, and lamellae of *Limacella* is of the typical agaric type as described in detail by Reijnders (e.g., 1963, 1979). A fundamental transformation of ontogeny distinguishes *Amanita*.

Every eventually agaricoid or secotioid basidiome of *Amanita* begins growth within a solid primordium—an undifferentiated mass of tissue. Individual tissues (veil or veils, pileus with or without a pileipellis, lamellae, stipe, bulb) develop in position without any empty spaces for separation of developing parts. When the developing basidiome nears initiation of sporulation, the solid mass is torn apart to create a mushroom. This requires specialized tissues (e.g., between the edges of lamellae and the stipe and/or the partial veil). Tissues must break apart easily, or collapse, gelatinize, or disintegrate (Bas 1969). The unique chemistry needed to enable development in this manner must be extraordinary; for an agaricoid or secotioid *Amanita* uses the blunt instrument of hydrostatic pressure to accurately rip an incompletely developed mushroom out of a truffle-like tissue mass in such a way that there is no interruption of development of a mature, spore-bearing agaric or secotioid basidiome.

As an alternative to traditional characterizations of *Amanita*, we propose the following (Tulloss 2015d):

Amanita Pers. nom. conserv.

MYCOBANK: 17045

CHARACTERIZATION: *Amanita* comprises those species of the family *Amanitaceae* satisfying exactly one of the following conditions:

1. It has a hypogeous basidiome.
2. It has an agaricoid or secotioid basidiome produced by schizohymenial ontogeny. Note that there are a number of equally diagnostic characters that may be used: Basidiomes of taxa fitting this characterization will also exhibit character states related to the distinctive ontogeny—e.g., sterile edges of *Amanita* lamellae or absence of gluten and gluten-retaining hyphae on *Amanita* pilei (e.g., Tulloss and Rodríguez-Caycedo 2011).

2. Φ and the six simple guidelines for introducing new genera of fungi

The hypothesis of Wolfe et al. may be represented schematically as shown in Fig. 1

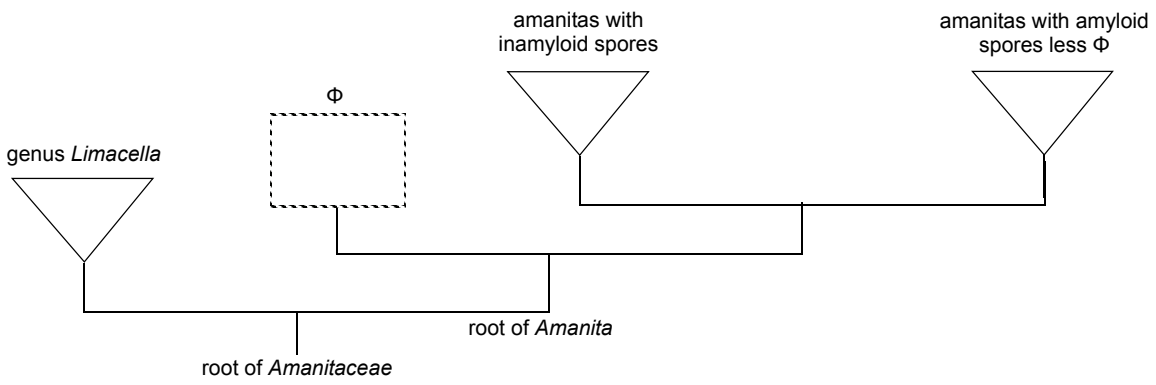

Figure 1. Schematic version (not to scale) of the large phylogeny of Wolfe et al. (2012b).

Φ (and we prefer the use of Φ rather than *Saproamanita* as we consider the monophyly of Φ has not yet been established) is sister to a monophyletic clade. That fact does not imply that Φ is, itself, a monophyletic clade. Monophyly must be established independently in both sister elements.

We previously mentioned that Φ is poorly supported. The maximum likelihood bootstrap (mlb) value given in the (Wolfe et al. 2012b, fig. 2) hypothesis is 66%. In our view this low support implies that Φ cannot be unambiguously treated as a clade. In fact, other interpretations of the basal part of the phylogeny are also possible. One, in our view equally likely, interpretation is that Φ is indeed a basal grade, consisting of three to four well-supported clades. Redhead et al. (2016) noted this possibility – using the term "clade" for Φ between inverted commas, and suggesting that the ectomycorrhizal (EM) species (an unambiguous clade) arose from within this grade. They also entertained the possibility that arguments of monophyly would necessitate the breaking up of Φ into two of three genera.

Table 1. Wolfe et al. (2012b) sampling of *Amanita* subsection *Vittadiniae* Bas (= Φ plus stirps *Hesleri*)

stirpes of subsect. *Vittadiniae* Bas (Bas 1969)	no. of recognized and probable taxa	number sampled (% of subsect.)	geographic range sampled (not sampled)
stirps *Hesleri*	3	1 (33)	North America (China, South Africa)
stirps *Hondurensis* (Tulloss unpub.)	1	0(0)	- (Mesoamerica)
strips *Inopinata* (Tulloss unpub.)	1	1 (100)	Presumed introduced in Europe, presumed endemic in New Zealand
stirps *Nana* (Bas 1969)	1	0 (0)	- (south central Asia)
stirps *Nauseosa* (Bas 1969)	6	2 (33)	North America, Pacific islands (Mesoamerica, South America, central Africa, South Africa, eastern Asia, southern Asia)
stirps *Thiersii* (Bas 1969)	5	1 (20)	North America (central Africa, South Africa, Argentina, India)
stirps *Vittadinii* (Bas 1969)	18	8(44)	North America, Mediterranean Europe, eastern Brazil (North America, South America, Caribbean Islands, South Africa)
stirps presently unassigned	2	1 (50)	New Zealand (Chilean Pacific island)
Totals	37	14 (38)	

All branches other than Φ shown in Fig. 1 have an mlb value of 99-100% except one with a value of 91% (the branch marked "root of *Amanita*").

There are two additional aspects of the case that lead us to question whether Φ is a clade:

- Φ intermixes easily distinguishable morphological groups.
- The sampling size for Φ is rather small and unevenly distributed geographically (Table 1, above).

2.1. Topology of Φ vs. the delineation of Bas' stirpes

The species named on the leaves of Φ include the following: two species that were not treated by Bas [*A. inopinata* D. A. Reid and Bas (Reid 1987, Tulloss 2015e) and *A. sp-Ridley-2* (Tulloss 2015g)], two species of stirps *Nauseosa* [*A. manicata* (Berk. & Broome) Pegler (Pegler 1986) and *A. nauseosa* (Wakef.) D. A. Reid (Reid 1966)], one species of stirps *Thiersii* [*A. thiersii* Bas (Bas 1969, Wolfe et al. 2012a, Tulloss 2015b)], and the remaining eight falling into the much larger stirps *Vittadinii* {*A. armillariiformis* Trueblood & Dav. T. Jenkins (Miller et al. 1990), *A. codinae* (Maire) Bertault (Bertault 1955, Bas 1969), *A. grallipes* Bas & de Meijer (Bas and de Meijer 1993), *A. prairiicola* Peck (Peck 1897), *A. pruittii* Tulloss et al. (Tulloss et al. 2014), *A. singeri* Bas (Bas 1969), *A. subcaligata* [(A. H. Sm. & P. M. Rea) A. H. Sm. ex Tulloss (Volk and Burdsall 1995)], *A. vittadinii* (Moretti) Vitt. (Vittadini 1826)}. The stirpes are described in a manner making them easy to distinguish. Φ looks like a tree for stirps *Vittadinii* into which the other taxa have been inserted. The species of stirps *Nauseosa* are near each other, but both are sister to groups including members of stirps *Vittadinii*. *Amanita inopinata* is sister to a grouping with all members in stirps *Vittadinii*. *Amanita sp-Ridley-2* and *A. thiersii*—the only two sampled species lacking clamps on the basidia, a character of great importance in Bas' taxonomy of the lepidellas—are sisters and sole members of a group sister to *A. vittadinii*. The latter is a clamp-bearing taxon of stirps *Vittadinii*.

2.2. The sampling breadth of Φ

Wolfe et al. (2012b) treated 14 taxa of subsection *Vittadiniae* that are presently recognized or treated as probable taxa by Tulloss and Yang (2015). These are listed in Table 1. From the point of view of number of taxa sampled, the overall value of 38% of taxa sampled might be satisfactory if there were not so much variation in morphology and if the stirpes designated by Bas were more similar in size. As it is, one stirps (stirps *Vittadinii*) strongly dominates the subsection, including 18 of 37 taxa. Table 1 illustrates the limited nature of sampling from a geographical point of view.

2.3. Conformance with the six guidelines of Vellinga et al.?

Vellinga et al. (2015) proposed six guidelines for introducing a new genus. They actually apply to any new taxon proposal. We repeat the basic guidelines for the convenience of the reader and urge the reader to review the full treatment of Vellinga et al.

1. All genera that are recognized should be monophyletic, not only the one that is the focus of the [a] study, but also the group from which it is separated and the group to which it is added.
2. The coverage of the phylogenetic tree has to be broad.
3. The branching of the phylogenetic trees should have sufficient and strong statistical support.
4. A list of options should be discussed, different options should be tested, and arguments for the final decision given.
5. The phylogenetic evidence has to be based on more than one gene, preferably protein coding genes in addition to gene regions of the SSU-ITS-LSU repeat.
6. All supporting evidence and background information should be included in the publication in which the new taxa are proposed; and secondly, this publication should be peer-reviewed.

The splitting off of Φ as a new genus (or subgenus) would not be acceptable on the basis of the six guidelines.

- Guideline 1: In sections 2.1 and 2.2, we demonstrate that we cannot be certain that Φ is monophyletic and suggest that it may not be. As noted by Redhead et al. (2016) the EM species may well be nested within grade Φ, rather than being sister to it. There is no compelling reason why saprotrophy (the plesiomorphous state shared with *Limacella*) should be the basis for monophyly. Similar cases, where the EM species are nested in a monophyletic group, exist – such as *Entoloma*, *Peziza* and *Ramaria*. It is doubtful whether splitting of these genera would serve a useful purpose. In that respect the Redhead et al. (2016) comparison of the present case with the (old) genus *Coprinus* is misleading. *Coprinus* was polyphyletic; but the splitting of the part within the *Psathyrellaceae* was probably premature, as subsequent investigations have shown that *Psathyrella* was not monophyletic.
- Guideline 2: In section 2.2, we demonstrate that sampling is inadequate because of the limited number of known taxa in some stirpes and because of the number of geographic regions that are poorly covered.
- Guideline 3: In section 2, we noted that Φ does not have sufficient and strong statistical support.

- Guideline 4: In the present paper, we discuss splitting *Amanita*; and, given the foregoing three points, decide not to do so. Redhead et al. (2016) provided one additional argument why, in their opinion, their new genus was justified. That argument is based on a putative association between genera, identified by next-generation sequencing of (parts of the) ITS barcode, and trophic mode. But there is no reason why genus-level designations cannot accomodate diversity in trophic modes. In cases in which the ITS barcodes have sufficient resolving power, it is simple to indicate a trophic mode for every molecular taxon. And if the resolution is insufficient, there may be even larger benefits to keep taxa of diverse trophic modes under the common generic denomination. The only thing that is relevant for assignment of trophic modes to molecular taxa is that authors, who wish to do so, are aware of the current literature on diversity of trophic modes in certain groups – and we think that the work of Wolfe et al. (2012b) initiates the development of such information in *Amanita*.
- Guideline 5: The best available phylogenetic evidence (Wolfe et al., 2012b) is based on four genes. In that research, only a tree exclusively based on nLSU suggested that the basal clade Φ was monophyletic (Hess and Pringle, 2014), and this dominated the final phylogeny. None of the four loci are protein coding—all four are regions of either the nuclear or mitochondrial rDNA repeat. Investigating a large and representative assemblage of *Amanita* species for protein coding genes remains an important challenge. Substantive preliminary steps have been taken in recent research on *A.* sect. *Caesareae* (Sánchez-Ramírez et al., 2015b).
- Guideline 6: The Wolfe et al. (2012b) and Redhead et al. (2016) articles were peer-reviewed.

3. Φ and *Amanita* subsect. *Vittadiniae*

In Vizzini et al. (2012), *Amanita hesleri* is included in the genus *Aspidella*. The species was not included in *Saproamanita* by Redhead et al. (2016) and was not mentioned by the latter authors. We think it worthwhile to provide the following argument that we supplied to Redhead prior to publication of the latter article.

3.1. Historical note

Bas (1969) hypothesized *Amanita* had what would now be called an early-diverging group that included a number of taxa apparently occurring without a mycorrhizal symbiont. Bas grouped these taxa in subsect. *Vittadiniae* based on their unique universal veil; however, it was clear to him that many of these taxa shared other common traits such as predilection for habitats such as lawns, pastures, natural grasslands, and xeric areas—without obvious EM symbionts. From before the initiation of the research of Wolfe et al., subsect. *Vittadiniae* was their focus of interest; and Bas' rarely mentioned "phylogeny-like" diagram (Bas 1969: 337, fig. 25) was a source of inspiration. Over decades, Tulloss has repeatedly returned to study of species assignable to subsect. *Vittadiniae*; data and analysis appears on relevant taxon pages of (Tulloss and Yang 2015).

3.2. Confidence in the data of Wolfe et al.

Since the publication of (Wolfe et al. 2012b), Tulloss has re-examined odd placements of taxa in the phylogeny. The sequences derived from a collection labeled "*A. magniverrucata*" were in fact from an undescribed species of *Amanita* sect. *Amanita*. One species has been redetermined as a different, closely related one [*A. eriophora* (Berk.) E.-J. Gilbert (Gilbert 1941, Bas 1969, Tulloss 2015c)]. Errors have been corrected on the relevant GenBank sequence pages; and no error affected the phylogeny. The errors were not cases of irreproducible or otherwise "bad" data. Collaboration of Tulloss with various labs since the publication of Wolfe et al. (2012b) have produced derivations of nrLSU sequences for some of the species treated by Wolfe et al., and new sequences are essentially identical to the sequences employed in developing the Wolfe et al. phylogeny (e.g., Tulloss et al. 2014).

3.3. Φ is not equivalent to *Amanita* subsect. *Vittadiniae*

None of the proposed stirpes of subsect. *Vittadiniae* include one or more species in clade Φ and another species outside of that clade. All stirpes are either (a) absent from the Wolfe et al. hypothesis (the monotypic stirps *Nana*), (b) included in the phylogeny but absent from clade Φ (stirps *Hesleri*), or (c) represented entirely in clade Φ [stirpes *Inopinata* (monotypic), *Nauseosa*, *Thiersii*, and *Vittadinii*]. As noted previously, stirps *Vittadinii* is by far the largest stirps of the six and includes the subsection's type. In addition, Bas's figure cited above indicates that he did not see a strong morphological connection between stirps *Hesleri* and the remainder of subsect. *Vittadiniae* except that they shared the very distinctive form of the universal veil by which Bas defined the subsection.

There appear to be three taxa included in stirps *Hesleri*. In addition to *A. hesleri* Bas (Bas 1969), there are *A. veldiei* D. A. Reid & Eicker ex Redhead (Tulloss and Possiel 2015, Redhead 2016)[3] from South Africa and *A. zangii* Zhu L. Yang et al. (Yang et al. 2001) from Japan and tropical China. *Amanita hesleri* may be EM (Wolfe et al. 2012b) and has been repeatedly collected in association with *Quercus* and in mixed forests containing *Quercus* and *Pinus* (Tulloss 2015a). According to their protologs, *A. veldiei* was collected under an alien *Quercus* species; and *A. zangii* is found in forests where there are EM species of the *Fagaceae*, *Pinaceae*, and *Dipterocarpaceae*.

Sequences of nrLSU have been derived for *A. hesleri* and *A. zangii*. From preliminary studies (Tulloss unpub.), we think it likely that *A. hesleri* and *A. zangii* are sister taxa attached at approximately the position of *A. hesleri* in the Wolfe et al. (2012b) phylogeny. Hence, existing evidence indicates that stirps *Hesleri* can be considered to be represented in the Wolfe et al. (2012b) hypothesis by *A. hesleri*.

We considered the possibility that *A. hesleri* might be segregated from Φ in the Wolfe et al. (2012b) hypothesis due to the effect of long branch attraction (LBA). We observed recent evidence (e.g., Cai et al. 2014) suggesting that LBA might be affecting *Amanita* phylogenies utilizing and possibly dominated by nrLSU. Tulloss (unpub. data) followed the suggestions of Bergsten (2005: 186) to identify LBA among nrLSU sequences of *Amanitaceae*—specifically, among the long terminal branches that are common in some clades inclusive of taxa considered to fall in sect. *Lepidella* sensu Bas. This attempt utilized a one-gene (nrLSU) tree including over 230 species of the *Amanitaceae* with a 63 species outgroup from which long branches were excluded per Bergsten (2005). Although our search was not exhaustive, we identified several probable long branch attractors (LBAs) in sect. *Lepidella* sensu Bas using the Bergsten approach. For these LBAs we identified corresponding distorting impacts on the tree of the experiment (again following Bergsten's suggestions), and we removed the LBAs from our tree one at a time as we progressed through the experiment. We did not demonstrate any impact related to the relative positions of *A. hesleri* and Φ in the Wolfe et al. (2012b) phylogeny. Hence, we failed to find any evidence that would justify combining clade Φ and stirps *Hesleri* in a monophyletic unit when attempting to create a systematic approach to the *Amanitaceae* supported by the Wolfe et al. (2012b) hypothesis. That is to say, *Amanita* subsect. *Vittadiniae* is paraphyletic according to the latter hypothesis.

The Wolfe et al. (2012b) data suggests the possibility that long branch attractors evolved after the introduction of EM, perhaps facilitated by consequent enhanced speciation, genomic rearrangement, evolution of transposable elements, etc. The branches in Φ are all quite short; this raises the possibility that it may be very hard, if not impossible, to demonstrate the monophyly of Φ.

We have not dealt with stirps *Nana* and its sole species—*Amanita nana* Singer (Singer 1941). All known material of this species is 50 or more years old and in poor condition (e.g., due to attack by mold). The known collections are from Pakistan and Kazakhstan. No genetic data are available, and none may become available soon. Bas (1969) judged the closest affinity of *Amanita nana* was to stirps *Vittadinii*. As far as condition of the most recently collected material allowed, Tulloss (2015f) confirmed Bas' observations. There is no evidence related to placement of stirps *Nana* in our developing revision of *Amanita* systematics other than that presented by Bas; hence, we provisionally assign stirps *Nana* to the group of stirpes represented in Φ.

In sum, we interpret Φ as comprising the stirpes of subsect. *Vittadiniae* less stirps *Hesleri*.

4. The subset of cellulose degrading enzymes present in a given *Amanita* species does not identify its trophic mode.

In this section we address a common misunderstanding of the data on cellulases in (Wolfe et al. 2012b)—that there are only two trophic modes possible among species of *Amanita*—"amycorrhizal" is equivalent to "asymbiotic."

The latter may be based on an incorrect assumption that presence/absence data on three genes encoding a suite of cellulose degrading enzymes are sufficient evidence to segregate asymbiotic and EM amanitas or to otherwise characterize clades. This is not the case. One outcome of the analysis of these genes is that it is suggestive of trophic diversity in Φ—arguing against its recognition as a clade. The element Φ includes one definitively saprobic taxon [*A. thiersii* (Bas 1969, Wolfe et al. 2012a. Tulloss 2015b)]. The trophic modes of the other taxa have not been studied in detail.

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[3] As published, *A. veldiei* (D. A. Reid & Eicker. 1991. *Mycol. Res.* 95: 93, figs. 35-37, 44) was not valid because the holotype's location was not designated unambiguously. The intended type collection was split between K and PRUM. After communication with curators at both herbaria, Tulloss found that the material in PRUM had been destroyed by insects. The material in K survived (Tulloss and Possiel 2015). Redhead (2016) selected the material from K as holotype without offering a reason.

Together, the phylogeny and cellulase presence/absence data of Wolfe et al. (2012b) support the observation that extant species of *Amanita* show an overall trend towards loss of the three cellulases.

A trend is not a dichotomous division. Consider the set of possible presence/absence combinations of the three cellulases (Table 2)—this table is a summary of data presented species-by-species on the right hand side of (Wolfe et al. 2012b fig. 2). No species can correspond to more than one triplet (i.e., one row of Table 2). Clearly, "all cellulases absent" (Table 2 line 2) does not segregate the apparently EM group from the apparently non-EM group. "All cellulases present" (Table 2 line 1) also fails to segregate the two groups. In fact no row of the table and no combination of rows can be used to segregate the two groups. This is simply a matter of the data and logic.

Table 2. Cellulase presence/absence triplets from Wolfe et al. 2012b. The data are not sufficient to define a taxon's niche.

endoglucanase (eg1)	cellobiohydrolase (cbhI-I)	homologs of β-glucosidase	group of taxa defined
+	+	+	subgroup of apparently non-EM taxa
-	-	-	subgroup of apparently EM taxa & some apparently non-EM taxa
-	-	+	subgroup of apparently EM taxa & some apparently non-EM taxa
-	+	-	subgroup of apparently non-EM taxa
+	-	-	none
-	+	+	subgroup of apparently non-EM taxa
+	+	-	none
+	-	+	none

Examining the data in more detail, we observe: Complete loss is evident for endoglucanases and cellohydrobiolase in EM taxa; several EM *Amanita* species (especially in subgen. *Amanita*, far less so in subgen. *Lepidella* sensu Bas (Subg. *Amanitina* in Redhead et al. 2016) retained β-glucosidase. The pattern in the asymbiotic species of *Amanita* is more complex: some species retained almost the full complement (*A. manicata* and *A. thiersii*), while other species (*A. inopinata*) lost both endoglucanases and cellohydrobiolase and even β-glucosidase. This pattern is difficult to reconcile with the monophyly of the asymbiotic amanitas, but seems consistent with the claim that gene loss may have preceded the switch towards an EM lifestyle, implying that the EM clades of *Amanita* are nested within *Amanita*, a possibility explicitly entertained by Redhead et al. (2016); and in general, EM amanitas appear to lack cellulases more commonly found among non-EM Amanita. However, some of the cellulases (homologs of β-glucosidase) are sometimes present in EM species.

Amanita genomes house tens of thousands of functional genes (Hess and Pringle, unpublished), including many genes involved in the degradation of plant cell wall material that were not studied by Wolfe et al. (2012b). Until these data are more carefully analyzed (Kohler et al. 2015) and until genomes are available for more than a handful of species, accurate determinations of the subset of genes found only in EM or only in non-EM species will be difficult. Moreover, until the natural histories of species within Φ are more carefully described, Φ cannot be identified as entirely asymbiotic, even if none of the species are EM (Wolfe et al. 2012b).

5. Restructuring, not splitting

Since splitting *Amanita* is not justified, at the moment we propose nothing except removing stirps *Hesleri* from subsection *Vittadiniae* Bas.

5.1. Necessary amendment

Amanita subsection *Vittadiniae*

AMENDED DIAGNOSIS: This subsection contains those species of *Amanita* (a) having all of the following: amyloid spores, an appendiculate pileus margin, a universal veil dominated by inflated cells that are large, cylindric or elongate-fusiform or clavate, and often concatenated and (b) lacking the combination of clampless basidia and thin-walled spores with length > 10 μm and average length:width ratio (**Q'**) > 1.6.

6. Remaining open issues regarding sect. *Lepidella* sensu Bas

We decided to make no changes to section *Lepidella* sensu Bas other than movement of stirps *Hesleri* treated above. We took into consideration (1) lack of molecular data, (2) indications that future work may move away from use of rDNA in phylogenies [e.g., our observations on LBAs and the recent research of Sánchez-Ramírez et al. (2015b) into singly occurring, protein encoding loci] and the nascent state of relevant research, (3) the potentially short life of taxa that we might erect, and (4) our desire to maintain stability of communications among researchers and those persons reliant upon the product of current research. Also Bas (1969) himself called attention to the number of monotypic stirpes in subsect. *Limbatulae* and some stirpes containing a small number of species which he could have chosen to split into monotypic stirpes. Hence, there is evidence of lack of information critical to a well-informed analysis of genetic relationships among taxa assigned to sect. *Lepidella* sensu Bas.

We choose a table format to avoid giving the impression that there is sufficient data to support an unambiguous phylogenetic hypothesis. We choose to retain relevant supraspecific names without any alteration of rank.

Table 3. Current subdivisions of *Amanita* sect. *Lepidella* sensu Bas.

taxon	type species	species count [a]	number seq'd. (%)	observations [b]
subsect. *Solitariae*	*solitaria*	113	23 (21)	includes majority of taxa in the section; dominates large early-diverging clade of subgenus *Lepidella*; worldwide in temperate and tropical regions; nrLSU sequences include demonstrable LBAs.
subsect. *Vittadiniae*	*vittadinii*	37	15 (41)	our proposal makes this subsection equivalent to Φ. It is discussed extensively, above.
subsect. *Limbatulae*	*limbatula*	17	4 (24)	comprises small stirpes that could well be further divided into monotypic groupings as noted by Bas (1969); in "leaky" or Mediterranean ecosystems of S Europe, N Africa, SW Australia, SE N. America, and SW N. America; nrLSU sequences include demonstrable LBAs.
subsect. *Gymnopodae*	*gymnopus*	5	1 (20)	Bas did not divide this small subsection into stirpes because of their considerable mutual similarity; distribution limited to Australia, E Asia, Mesoamerica.
stirps *Hesleri*	—	3	2 (67)	Distribution in isolated regions—SE N. America, Mesoamerica (may include undescribed taxon), E Asia, S. Africa.

a. Counts follow (Tulloss and Yang 2015) and include probable taxa known by provisional names or temporary codes.
b. Compass bearings are written without a period except when parts of geographic names.

7. Name stability

7.1. Taxonomists decide how to use the rank of genus

According to the ICN, genus is a level of organization (rank) in a nearly neutral system for the organization of information about living organisms named in the full title of the ICN. The ICN tells us how to form the name of a genus and how to form the names of the things of the lower rank called "species." Among other things, the ICN is a product of extended and intensive labor and is constantly altered to improve the ability to organize data about organisms and their relationships and to improve communication about the organisms, the relationships, the history of these things, and the history of their study. The ICN is a guide to communication, but not to all the specifics of content.

The ICN can be understood to be focused on improving communication not only among research specialists, but also communication between specialists and nonspecialists and between nonspecialists of many sorts. If we consider rules associated with conserving names, the ICN even takes into account such issues as the use of specialist-generated names in business and government. We fully understand that the nomenclatural concept of conservation does not apply to the present paper. Nevertheless, it is worth noting that the ICN itself is concerned with preserving communication external to a group of specialists; and if that is the case, surely teaching, developing research goals, and circulating research results must be functions of equal importance.

7.2. Communicating with the complex customer-base for taxonomic knowledge

We regard the stability of names combined in *Amanita* as valuable for communications with many colleagues having a wide variety of technical specialties. Their research includes investigations of biodiversity of diverse ecosystems on public and private lands worldwide, forestry and forest ecology (Bruns et al. 2002), genomics (Chaib de Mares et al. 2014, Hess et al.

2014, Kohler et al. 2015), evolution and variation of mycorrhizae and other trophic forms (Wolfe et al. 2012b), invasion biology and range expansions (Pringle et al. 2009a, 2009b; Wolfe et al. 2012a), phylogenetics and phylogeography (Geml et al. 2008, 2010; Cai et al. 2014; Sánchez-Ramírez et al. 2015a, 2015b), genomic searches for new loci to improve phylogenetic results (Sánchez-Ramírez et al. 2015b), nrITS heterogeneity (Hughes et al. 2013, Hughes and Tulloss unpub. data), sequestrate form evolution (Justo et al. 2010), taxonomy and publication of new species (e.g., Bojantchev and Davis 2013; Davison et al. 2013, 2015; Deng et al. 2014; Li et al. 2015; Menolli et al. 2009; Tang et al. 2015; Tulloss and Franco-Molano 2008; Tulloss et al. 2011, 2014; Wartchow et al. 2009, 2015; Zhang et al. 2010), the Agaricales Diversification project (Nagy et al., unpub.), the North American Mycoflora project (Bruns 2011), methodologies for assay of toxins (Garber et al. unpub. data), toxicology and public health (Yarze and Tulloss 2012), toxin evolution (Hallen et al. 2007), heavy metal hyperaccumulation and environmental remediation (e.g., Borovička et al. 2007), etc. Research benefits from the stability inherent in avoiding the splitting of a genus when, as in the present case, there is no compelling reason to do so.

As many readers will be aware, avoiding confusion in the scientific community is not the sole motivation for name stability. There are others having to do with the communication of data, hypotheses, and practical knowledge to a much broader audience (Anonymous 2015, Tulloss and Yang 2015). Various "name standardization" efforts (e.g., within governmental agencies such as the U.S. Dept. of Agriculture) are explicitly motivated, at least in part, by practical interests in name stability. Name stability facilitates and improves communication with the public at large as well as with the mycologically educated public, especially citizen scientists. From personal experience, we know that avoidable name instability is a threat to maintenance, coherence, compatibility, and general utility of valuable resources such as databases (including herbarium catalogs); websites; computerized teaching tools; keys, checklists, and field guides whether on paper or online; etc. Such taxonomic and taxonomy-related products represent large investments of effort and support large customer-bases. To contribute to confusion in these resources seems unwise. Nomenclatural stability, rather then nomenclatural inflation, in the absence of hard evidence that groups are polyphyletic (as in the *Coprinus* case), may be a great and common good.

Amanita as a genus of moderate size with clearly defined subgenera and sections has proved easy to teach to beginning mushroomers. This is evidenced by the broad and growing use of sectional and subsectional classifications of the genus in widely accessed networking groups such as the communities using the increasingly international mushroomobserver.org site and the "Amanitas of North America" facebook page.

If a justifiable systematic hypothesis can be created to maintain a genus, as in the present case, costs inherent in a split can be avoided. As persons deeply involved in *Amanita* research, on our own and in collaboration with individuals and laboratories on all continents except Antarctica, maintenance of *Amanita* seems very clearly the best path forward.

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Appendix A. Some notes on phylogenetic analyses in Vizzini et al. (2012)

The nrITS locus evolves too quickly to provide alignments useful for phylogenies even below the generic level (e.g., Bruns 2001), in other words, many judgments are required, and extensive manipulation (including deletion) of data may be required to manually align sequences. Moreover a single species may house divergent copies of the locus (e.g., Lindner and Banik 2011, Hughes et al. 2013, Tulloss et al. unpub. data). Hence, the Vizzini et al. (2012) nrITS-tree is of questionable value.

In selecting nrLSU sequences, Vizzini et al. (2012) discard all Wolfe et al. *Amanita* sequences except those that fall in Φ. Hence, *A. hesleri* is omitted. Because Wolfe et al. (2012b) sequenced many taxa of sect. *Lepidella* for the first time, there is no *"*second source*"* for this data; and the approach of Vizzini et al. reduces the number of taxa most closely related to the species of subsect. *Vittadiniae* to only three, with one species each from North America, eastern Asia, and Europe. Wolfe et al., in contrast, emphasized coverage of sect. *Lepidella* in sampling for their tree in hopes of clarifying as much as possible relationships among the species of the section, including Φ. In doing so they sampled 40 species of sect. *Lepidella* other than those included in subsect. *Vittadiniae*. The contrast in experimental design is illustrated in Table A-1.

Table A-1. Comparison of sampling rates for sections of the genus *Amanita* in the Vizzini et al. nrLSU gene tree and the Wolfe et al. four gene phylogeny [a]

source	sect. *Amanita*	sect. *Caesareae*	sect. *Vaginatae*	sect. *Amidella*	sect. *Lepidella* excluding the *Vittadiniae*	sect. *Phalloideae*	sect. *Validae*	subsect. *Vittadiniae*
Species counts per Tulloss & Yang (2015)	139	83	236	31	159	60	121	37
Sampling effort (%): Wolfe et al. (2012b)	11 (8)	6 (7)	6 (3)	3 (10)	40 (25)	4 (7)	16 (14)	14 (38)
Sampling effort (%): Vizzini et al. (2012)	8 (6)	3 (4)	9 (4)	5 (16)	4 (3)	8 (13)	8 (7)	13 (35)

a. The number of species in each section sampled by each article is provided in the form number of species sampled (percent of group species count sampled).

The smallest section-level sampling by Vizzini et al. is that of sect. *Lepidella* excluding subsect. *Vittadiniae* (3%). By decreasing the number of species of sect. *Lepidella* outside of subsect. *Vittadiniae* in their sampling, the effect of sister taxa on the resulting topology is significantly reduced contrary to the author's stated goals for their work. In consequence, the gene tree is less likely to be an accurate estimator of relationships than is the tree of Wolfe et al. The data regarding *Lepidella* need not have been omitted from the nrLSU-tree. The nrLSU sequences for the omitted species were posted to GenBank by Wolfe et al. and were available to Vizzini et al.

Omissions of data contribute to problems in the Vizzini et al. phylogenies. For one thing, the *Amanitaceae* are not monophyletic in their nrLSU tree, and *Limacella* is not sister to *Amanita*. As previously noted, *Amanita* and the *Amanitaceae* have been consistently supported as monophyletic.

As for the idea that *Limacella* could be other than sister to *Amanita* including the former subsect. *Vittadiniae*, Vizzini et al. state that the morphology of subsect. *Vittadiniae* indicates that it may have evolved from a morphology like that now seen in *Limacella*. We agree. We suggest that the evidence they offer on this point could be extended from the bilateral lamella trama and acrophysalidic stipe context (their examples) to other organs of the *Amanita* basidiome. For example, the volva of concatenated narrow cells in section *Vittadiniae* is a probable homolog of the erect, gluten-retaining elements on the pileus in *Limacella*.