MYCOTAXON

Volume 96, pp. 31–39 *April–June 2006*

Annulate *Pluteus* species, a study of the genus *Chamaeota* in the United States

Andrew M. Minnis[1*], Walter J. Sundberg[1], Andrew S. Methven[2], Sedonia D. Sipes[1], & Daniel L. Nickrent[1]

**drew3@siu.edu*
[1]Department of Plant Biology, Southern Illinois University at Carbondale Carbondale, IL USA 62901-6509

[2]Department of Biological Sciences, Eastern Illinois University Charleston IL 61920-3099

Abstract—*Chamaeota* is a rare agaric genus traditionally classified in the *Pluteaceae*. The present work offers a treatment of the two species described from the United States. Historical records, morphological and anatomical examinations of type and other collections, and molecular data from nuclear LSU rDNA sequences were studied. Typifications of *Annularia mammillata* and *A. sphaerospora* are offered for nomenclatural stability. *Chamaeota sphaerospora* is synonymized with *C. mammillata* and the new combination *Pluteus mammillatus* is proposed.

Key words—*Agaricales*, taxonomy, *Hispidoderma*, lectotype, epitype

Introduction

The genus *Chamaeota* (W.G. Sm.) Earle, typified by *C. xanthogramma* (Ces.) Earle, a poorly known fungus, is typically classified in the *Pluteaceae* Kotl. & Pouzar (Singer 1986) because the better known species have pink basidiospores, free lamellae, a convergent lamellar trama, and an annulus. The genus shares the first three characters with *Pluteus* Fr. and *Volvariella* Speg., the other members of the family, but differs in the last (Singer 1986). Recent molecular evidence, however, suggests that *Pluteus* and *Volvariella* together do not form a clade (Moncalvo et al. 2002). The relationship of *Chamaeota* to other agarics and its potential use as an outgroup for *Pluteus* was unknown.

Chamaeota is a small genus consisting of approximately ten species worldwide. Species of *Chamaeota* have been infrequently reported in the United States from a limited number of sites (Longyear 1902, Peck 1906, Murrill 1917, Murrill 1943, Kauffman 1918, Beardslee 1939). Species delineation remains unclear and several authors (Kauffman 1918, Beardslee 1939, Murrill 1943, Singer 1978) have commented on the need to clarify the range of variation and boundaries of individual species. Two species, *Chamaeota sphaerospora* and *Chamaeota mammillata* are the only species originally described from the United States. Their interpretation is made difficult by the lack of explicitly designated type specimens. The present paper is offered to address these problems in the United States.

Materials and methods

Molecular Study

Genomic DNA was isolated from the *ASM 7916* collection identified by classical morphology and anatomy as *C. sphaerospora* using a version of the method described by Nickrent (1994) scaled down to use less material. Universal primers routinely used in Vilgalys' laboratory at Duke University (http://www.biology.duke.edu/fungi/mycolab/primers.htm) were employed to amplify (5.8SR, LR7) and to obtain (LR5, LR16, LROR, LR3R) a partial nuclear LSU rDNA sequence using standard protocols. The resulting sequence was subsequently aligned manually to the LSU sequence alignment used in the analysis of Moncalvo et al. (2002). A heuristic parsimony analysis similar to that of Moncalvo et al. (2002) was performed to place the sequence on a fungal tree (results not shown). The above analysis placed the sequence within a *Pluteus* clade. A reduced dataset containing 21 taxa was constructed to demonstrate the position of *Chamaeota* within the *Pluteus* clade. *Limacella illinita* (Fr.) Maire, *Volvariella volvacea* (Bull.) Singer, and *Auricularia polytricha* (Mont.) Sacc. were chosen as outgroup taxa because of their positions outside of the *Pluteus* clade. A branch-and-bound analysis was performed with the following parameters: gaps treated as missing data, multistate taxa interpreted as uncertainty, addition sequence furthest, branches collapsed if maximum branch length is zero, MulTrees option in effect, characters weighted using the Moncalvo et al. (2002) stepmatrix. Branch robustness was evaluated with 1000 bootstrap replications using identical search parameters.

Morphological & anatomical study

Fresh collections identified as *Chamaeota sphaerospora* were made, documented, and preserved with standard methods (Smith 1949). Color terms are from Kornerup and Wanscher (1978). Standard techniques were used to prepare material for anatomical study (Smith 1949, Largent et al. 1977). Dried material was wetted with 95% ethanol for 1 min, soaked in water for 1 min, blotted to remove excess water, and sectioned by hand. Material was mounted and viewed in 3% KOH or Melzer's reagent. Basidiospore dimensions of the newly designated lectotype of *Annularia sphaerospora* are based on observation of 30 basidiospores. All other microscopic dimensions are based on observation of at least 10 structures. Length to width ratios are reported as Q. Mean values for length, width, and Q are designated as L^m, W^m, and Q^m respectively. Anatomical data for the lectotype of *A. sphaerospora* are presented separately in the species description because of the importance of this collection. Data from other specimens are presented as a composite description under "**additional data**" where they add to or differ from the type material and original circumscription. Herbarium acronyms are from Holmgren and Holmgren (1998 onwards).

Results

The nuclear LSU rDNA dataset analyzed included 781 characters. Of these, 74 were parsimony-informative. The maximum parsimony analysis produced one shortest tree of 990.3 steps (Fig. 1). The consistency (CI), homoplasy (HI), retention (RI), and rescaled consistency (RC) indices were 0.7157, 0.2843, 0.7698, and 0.5509, respectively.

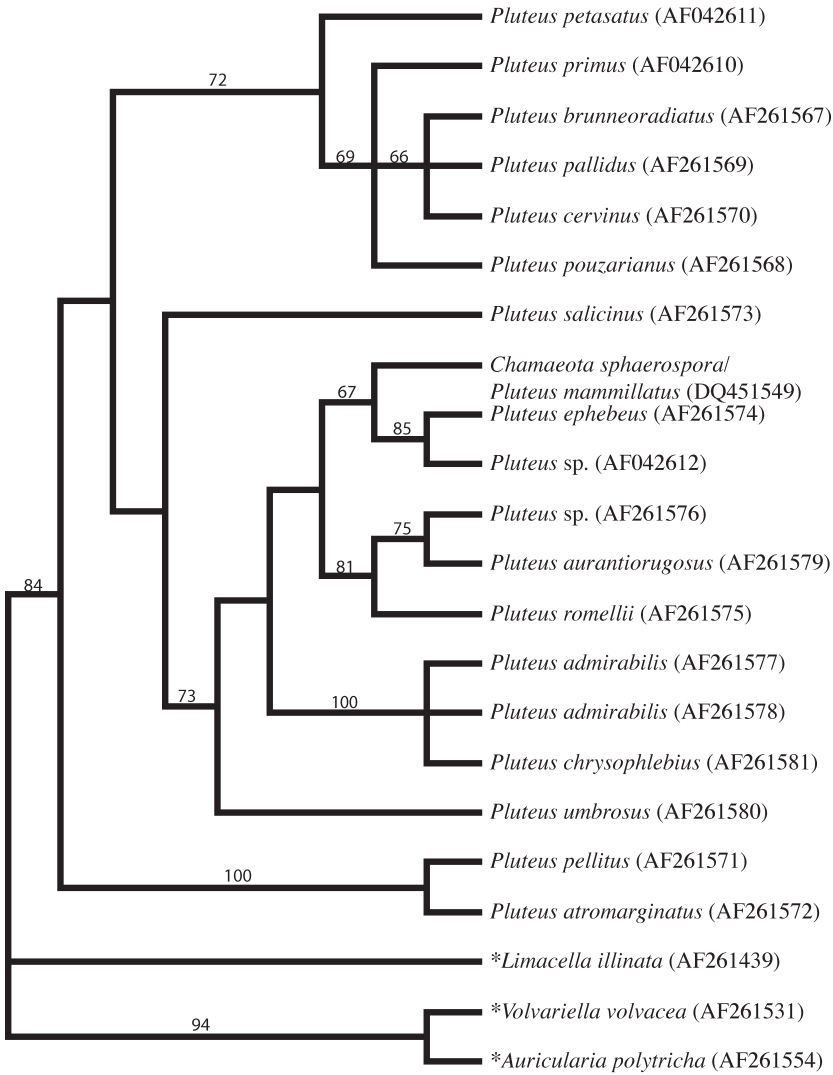


Fig. 1. The single most parsimonious tree based on partial nuclear LSU rDNA sequences. Bootstrap values greater than 50% are indicated above branches. Genbank accession numbers for each sample are shown. All sequences originated in the Moncalvo et al. (2002) study except *Pluteus* sp. (AF042612) from Moncalvo et al. (2000) and *Chamaeota sphaerospora*/*Pluteus mammillatus* from this work. Asterisks denote outgroup taxa.

Pluteus forms a clade (84% BS) with the sequence of the *ASM 7916* collection of *C. sphaerospora* embedded within it. There is moderate bootstrap support (67% BS) for a sister relationship of the *ASM 7916* sequence to the *P. ephebeus* clade.

Typification

Chamaeota mammillata (Longyear) Murrill, N. Amer. Fl. 10: 139. 1917.

≡ *Annularia mammillata* Longyear, Rep. Mich. Acad. Sci. 3: 59. 1902. Type: Pl. I, Fig. 4 in Longyear, Rep. Mich. Acad. Sci. 3: 58. 1902—***Lectotype [icon.] designated here.*** Type: *Annularia mammillata* Longyear, *Pluteus mammillatus* Minnis et al., *ASM 7916* (EIU)—***Epitype designated here.***

When Longyear (1902) named the species, he based it on a single basidiocarp collected from Greeneville, Michigan on a decaying log in woods and did not explicitly designate a type. The original material mentioned in the protologue was not found at Michigan State University, formerly the Michigan Agricultural College (Heather Hallen and Alan Fryday, pers. comm.). Likewise, it is not present at the New York Botanical Garden (NY) (Barbara Thiers, pers. comm.) or the University of Michigan Herbarium (MICH).It is apparently lost. No other authentic material or other specimens are available for study. Since it is allowable to select an illustration as a lectotype, we chose the figure from Longyear's original circumscription. See Fig. 2 for a reproduction of this image.In order to facilitate a more precise taxonomic interpretation, a collection used in this study was chosen as the epitype. All of the specimen data on the herbarium box are given above.

Chamaeota sphaerospora (Peck) Kauffman, The *Agaricaceae* of Michigan 534. 1918.

≡ *Annularia sphaerospora* Peck, Bull. Torrey Bot. Club 33: 216. 1906. Type: *Annularia sphaerospora* Peck, Loc. Detroit, Mich, Leg. O.E. Fischer, Aug. '05 (NYS)—***Lectotype designated here.***

In the protologue, Peck gave O.E. Fischer, a physician, credit for collecting the material used to circumscribe the species. However, unpublished correspondence between Peck and Fischer indicate that a Mr. Burrows of 50 Hazelwood Ave. (Detroit?) was the collector. Fischer sent one of the two collections given to him by Mr. Burrows to Peck. This material mentioned in the protologue was apparently used by Peck to write the circumscription, but he did not clearly designate a type. All of the type specimen data present on the specimen label are given above. An isolectotype not mentioned in the original species description is located in the University of Michigan Herbarium (MICH). According to the herbarium collection manager, Patricia Rogers (pers. comm.), Dr. Pierre-Arthur Moreau noticed some *Hebeloma* material mixed in with this collection. We have not examined this collection.

Taxonomy

Pluteus mammillatus (Longyear) Minnis, Sundb., & Methven, **comb. nov. Figs. 2-7.**

≡ *Annularia mammillata* Longyear, Rep. Mich. Acad. Sci. 3: 59. 1902 (basionym).

≡ *Chamaeota mammillata* (Longyear) Murrill, N. Amer. Fl. 10: 139. 1917.

= *Annularia sphaerospora* Peck, Bull. Torrey Bot. Club 33: 216. 1906.

≡*Chamaeota sphaerospora* (Peck) Kauffman, The *Agaricaceae* of Michigan 534. 1918.

Data based on study of the lectotype of *Annularia sphaerospora*:

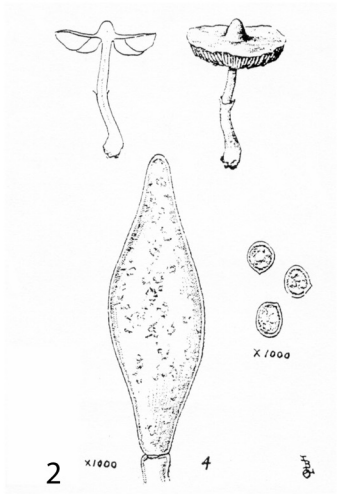
Basidiospores globose to subglobose to short-ellipsoid to slightly ovate in face and profile views, circular in end-view, 5.3–7.0 × 4.8–6.6 µm, Q = 1.0–1.18 (L^m = 6.1 µm, W^m = 5.5 µm, Q^m = 1.11), apiculate, smooth, walls slightly thickened, subhyaline to pale yellow in KOH, with a single large oil drop inside or several smaller drops resulting in a granular appearance. Basidia clavate, tetrasterigmate, 20–23 × 7–9 µm without sterigmata, walls thin and hyaline in KOH. Pleurocystidia abundant, broadly to narrowly fusoid-ventricose with pedicels and short necks with obtuse apices, 37–61 × 14–24 µm, walls thin and hyaline in KOH, no apparent contents. Cheilocystidia abundant, vesiculate to clavate to fusoid-ventricose, 31–58 × 11–22 µm, walls thin and hyaline in KOH, no apparent contents. Lamellar trama convergent. Subhymenium cellular. Pileipellis more or less a cutis with bundles of erect to appressed hyphae that are more abundant towards the disc, hyphae thin-walled occasionally containing light brown intracellular pigment. Context composed of 3–15 µm wide hyphae, thin-walled, hyaline in KOH, no apparent contents. Stipitipellis a cutis of cylindrical hyphae, 3–8 µm wide, thin-walled, hyaline in KOH, no apparent contents. Clamp connections absent. All anatomical features inamyloid in Melzer's reagent.

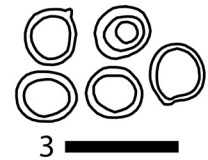
Additional data:

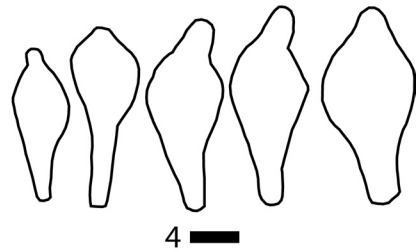
Pileus 0.9-7 cm in diameter, initially conic-campanulate becoming convex-plane, low narrow to broad umbo present or not; margin entire, slightly striate, occasionally appendiculate, initially incurved then becoming decurved; surface dry, radially fibrillose with fibrillose scales on disc, fibrils becoming smaller and more separated towards the margin, fibrils light brown to brown (6D7-D6, 6E8-E6); ground color deep yellow to orange yellow (4A-8-A6 to 4B8-B7). Lamellae free, close, thin, broad, not marginate, white becoming grayish red (7B3) in age. Lamellulae in two tiers. Stipe 0.2–8 cm × 1–14 mm, equal or narrowly clavate, terete, whitish to pale yellow (3A3), surface dry, longitudinally fibrillose or streaked, fibrils concolorous with those on pileus, basal tomentum white. Annulus found on lower 1/2 to 1/3 of stipe, white to yellowish white (4A2,3A2), thin, membranous, sheathing with flaring apex, occasionally evanescent. Flesh of pileus and stipe solid, white. Odor and taste not distinctive.

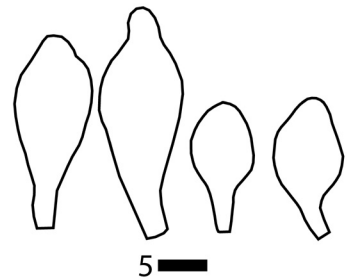
Basidiospores brownish pink in mass, 4.8–7.0 × 3.1–6.6 µm, Q = 1.08–1.88 (L^m = 6.1 µm, W^m = 5.3 µm, Q^m = 1.17). Basidia 17–29 × 6–11 µm, usually with granular contents. Pleurocystidia rarely broadly clavate, 39–99 × 8–30 µm. Cheilocystidia 30–86 × 8–24 µm. Pileipellis hyphae often containing brown intracellular pigment, 5–13 µm wide. Stipitipellis hyphae 4–18 µm wide, with or without brown intracellular pigment. Caulocystidia consist of cylindrical hyphae with obtuse apices, 5–9 µm wide, thin-walled, with brown intracellular pigment. Annulus composed of filamentous hyphae, 3–10 µm wide, thin-walled, hyaline in KOH, no apparent contents.

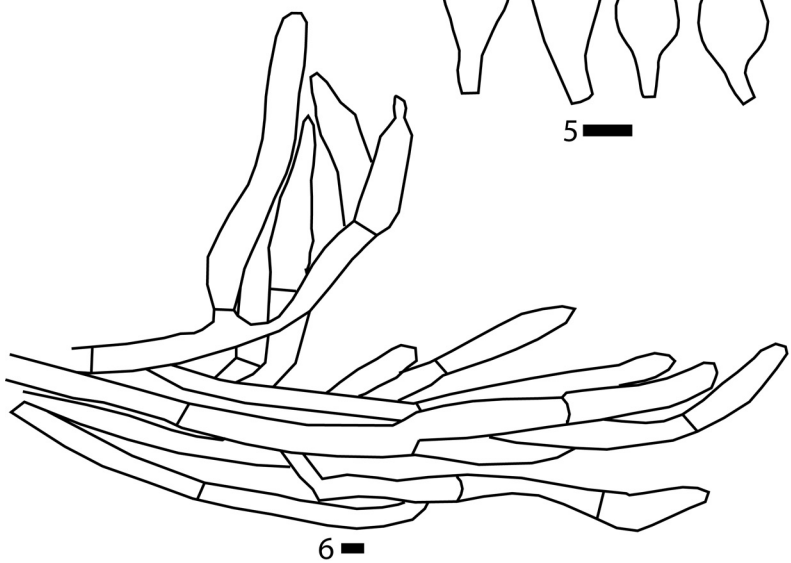
Specimens examined—UNITED STATES. Florida: Seminole Co., Oviedo, 22.XII.1935, coll. HC Beardslee Jr. (*35135*, MICH as *Chamaeota pusilla*); 9.XI.1936, coll. HC Beardslee Jr. (*35135*, MICH as *Chamaeota fenzlii*); Highlands Hammock State Park near Sebring, 1942, coll. R Singer, F244a (F). **Michigan: Wayne Co.**, Detroit, VIII.1904, coll. RH Stevens (F as *Annularia fenzlii*). **Missouri: Wayne Co.**, Mingo National Wildlife Refuge near Puxico, scattered on lignicolous substrates, 20.IX.2003, coll. AM Minnis, *Minnis 3-09-20-1* (SIU) & *Minnis 3-09-20-2* (SIU); 17.IX.1994, coll. AS Methven, *ASM*











Figs. 2-6. *Pluteus mammillatus*. Fig. 2. Lectotype illustration of *Annularia mammillata* from Longyear (1906). Fig. 3. Basidiospores (*A. sphaerospora* lectotype). Fig. 4. Pleurocystida (*A. sphaerospora* lectotype). Fig. 5. Cheilocystidia (*A. sphaerospora* lectotype). Fig. 6. Pileipellis (*Minnis 5-09-20-1*). Scale bars for Figs. 3-6 = 10 μm.

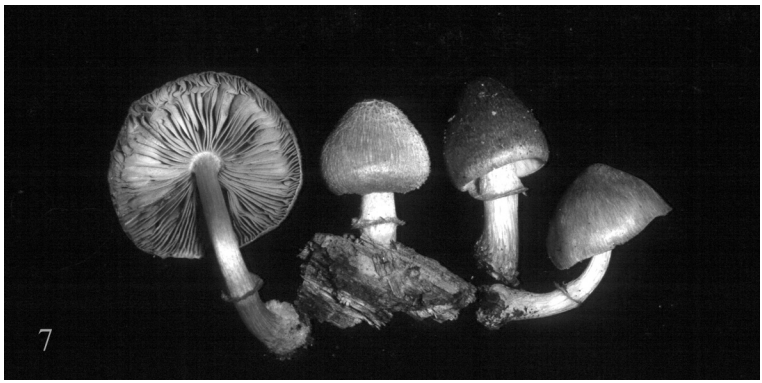


Fig. 7. *Pluteus mammillatus*. Basidiocarps of two collections, *Minnis 3-09-20-1* and *Minnis 3-09-20-2*.

7916, DQ451549 (designated epitype of *A. mammillata*, EIU); 20.IX.2005 coll. AM Minnis, *Minnis 5-09-20-1* (SIU).

Comments—This species is easily recognized in the field by its yellowish pileus, free, dirty-pink lamellae, annulus, and occurrence on decaying deciduous substrates. Microscopically, there is considerable variation in pleurocystidial shape. This variation around the fusoid-ventricose type is present within and among individual basidiocarps. In our experience, this variation is not unusual in *Pluteus*. Kauffman (1918) did not note the presence of pleurocystidia in *Chamaeota sphaerospora*. However, they are present in all of the collections we have examined. Hesler noted the presence of pleurocystidia on an annotation label for the lectotype of *A. sphaerospora*. Despite the misleading species epithet of *C. sphaerospora*, the spores are not always globose.

Murrill (1917) considered *C. mammillata* to be a synonym of *C. sphaerospora*. Kauffman (1918) and Singer (1978) recognized the two as distinct species. Considering the range of variation we have seen in *C. sphaerospora*, Longyear's description (1902) is a match and the two species are synonyms. See discussion for justification of the transfer to *Pluteus*.

Pluteus mammillatus (designated above) is rarely collected in the United States. Even so, it appears to be widespread in the Eastern United States. It has also been reported from Argentina (Moreno and Albertó 1996). Wright and Albertó (2002) included a photograph with their description of an Argentinean collection that appears to be *P. mammillatus*.We have not examined any of the South American collections.

Chamaeota fenzlii (Schulzer) Singer, a European species, is similar. According to Singer's description, it differs from *C. sphaerospora* by possessing yellow-marginate lamellae (1978).

Discussion

Singer (1986) discussed the similarities of *Chamaeota* to *Pluteus* section *Hispidoderma* Fayod. This section is characterized by a pileipellis consisting entirely of elongated

elements and thin-walled cystidia. The species of *Chamaeota* noted by Singer differ primarily by the presence of a partial veil. For convenience in matters of identification, Singer (1986) felt that it was acceptable to maintain the genus as separate at that time despite the number of other agarics where the presence or absence of a veil does not warrant genus level distinction. In contrast, in his seminal work on *Pluteus* in South America, Singer (1958) noted the presence of a rudimentary volva in *Pluteus stephanobasis* Singer and stated that it was acceptable to maintain this species in the genus instead of placing it in *Volvariella*. Characters that are not found in *Volvariella* including metuloid pleurocystidia and the lack of a natural transition from a rudimentary volva to the more complex volvas found in *Volvariella* supported his view (Singer 1958).

Based on the molecular evidence presented in this study, *Chamaeota* is not a good outgroup for *Pluteus* because it is in the *Pluteus* clade. The partial veil of *C. mammillata* is a derived character within this *Pluteus* lineage. Of the taxa examined, *C. mammillata* is most closely related to *Pluteus ephebeus* (Fr.) Gillet, a member of *Pluteus* section *Hispidoderma*. We feel that the partial veil does not warrant exclusion of *C. mammillata* from the genus *Pluteus*. Future descriptions of the genus should be modified to potentially include the presence of a partial veil.

Interestingly, *Chamaeota tropica* Pegler and *Chamaeota subolivascens* Courtec. are described as having cellular pileipelli (Pegler 1983, Courtecuisse 1991). This feature is also found in *Pluteus* section *Celluloderma* Fayod. It would be interesting to know if these species are also derived from within the *Pluteus* lineage. If so, a partial veil is likely to have been derived more than one time in the genus. Future studies on the other species of *Chamaeota* including the type of the genus, *C. xanthogramma*, should be performed. It is our belief that the genus will be rendered obsolete.

Acknowledgements

We thank Dr. Else C. Vellinga and Dr. Scott A. Redhead of the University of California, Berkeley and the Eastern Cereal and Oilseed Research Centre, respectively, for their presubmission reviews of the manuscript. We also acknowlege the New York State Museum (NYS), the University of Michigan Herbarium (MICH) and the Field Museum of Natural History (F) for the loans of collections used in this study. Dr. Raymond Stotler and Dr. Dale H. Vitt are credited for advice on nomenclature. Finally, our gratitude is extended to the Missouri Mycological Society for their support and comraderie.Financial support for this work was provided by the Alexander H. and Helen V. Smith Research Fund.

Literature cited

Beardslee HC. 1939. New and interesting fungi. Mycologia 26: 253-260.

Courtecuisse R. 1991. Eléments pour un inventaire mycologique des environs du saut Pararé (Arataye) et l'inselberg des Nouragues (Guyane française). V. *Pluteaceae*. (*Pluteales*, Basidiomycota). Crypt. Bot. 2:136-152.

Holmgren PK, Holmgren NH. 1998 onwards (continuously updated). Index Herbariorum. New York Botanical Garden. http://sciweb.nybg.org/science2/IndexHerbariorum.asp

Kauffman CH. 1918. The *Agaricaceae* of Michigan. Vol. 1. Wynkoop Hallenback Crawford Co., State Printers, Lansing, Michigan. 924 p.

Kornerup A, Wanscher JH. 1978. Methuen Handbook of Color. 3rd ed. Eyre Methuen, London. 252 p.

Largent D, Johnson D, Watling R. 1977. How to Identify Mushrooms to Genus III: Microscopic Features. Mad River Press, Eureka, California. 148 p.

Longyear BO. 1902. New species of Michigan fungi. Rep. Mich. Acad. Sci. 3: 57-60.

Moncalvo JM, Lutzoni F, Rehner SA, Johnson J, Vilgalys R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49: 278-305.

Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémencon H, Miller Jr. OK. 2002. One hundred and seventeen clades of Euagarics. Mol. Phyl. Evol. 23: 357-400.

Moreno G, Albertó E. 1996. *Agaricales sensu lato* de Argentina. I. Cryptogamie, Mycol. 17: 61-84.

Murrill WA. 1917. (*Agaricales*), *Agaricaceae* (pars), *Agariceae* (pars). N. Amer. Fl. 10: 77-144.

Murrill WA. 1943. Some Southern novelties. Mycologia 35: 422-433.

Nickrent DL. 1994. From field to film: Rapid sequencing methods for field collected plant species. BioTechniques 16: 470-475.

Peck CH. 1906. New species of fungi. Bull. Torrey Bot. Club 33: 213-221.

Pegler DN. 1983. Agaric Flora of the Lesser Antilles. Her Majesty's Stationary Office, London. 668 p. 27 pl.

Singer R. 1958. Contribution toward a monograph of the genus *Pluteus*, especially those of the east slope of the Andes and Brazil. Lloydia 21: 195-299.

Singer R. 1978. Keys for the identification of the species of *Agaricales* II. Sydowia 31: 193-237.

Singer R. 1986. The *Agaricales* in Modern Taxonomy. 4th ed. Koeltz Scientific Books, Koenigstein. 981 p.

Smith AH. 1949. Mushrooms in Their Natural Habitats. Sawyers, Portland. 626 p.

Wright JE, Albertó E. 2002. Hongos. Guía de la Región Pampeana. 1. Hongos con Laminillas. L.O.L.A., Buenos Aires. 279 p.