

FULL PAPER

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Cleistocybe, a new genus of Agaricales

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Abstract A new clitocyboid genus of Agaricales, *Cleistocybe*, is described to accommodate the isolated evolutionary position of the new species *Cleistocybe vernalis* inferred by a previously published multigene phylogenetic study. *Cleistocybe* is distinguished from other clitocyboid lineages by a combination of morphological characters, including lamellae that become gray in age, an interwoven hymenophoral trama with divergent elements when young, strongly interwoven pileipellis with pigmented and encrusted hyphae, white spore deposit, a distinct or ephemeral fibrillose to submembranous partial veil, and smooth, inamyloid basidiospores that are inequilateral in profile view. *Cleistocybe* encompasses two species, *C. vernalis* and *Clitocybe gomphidioides*, based on morphological comparisons with *C. vernalis*. *Clitocybe subvelosa* is confirmed as conspecific with *C. gomphidioides* based on morphological and ITS sequence comparisons of type collections. *Cleistocybe* is known only from western North America in coniferous forests and appears most closely related to the ectomycorrhizal genus *Catathelasma* and the saprotrophic genera *Callistosporium*, *Macrocybe*, and *Pleurocollybia* based on nLSU-rDNA phylogenetic analysis. Together these lineages constitute the *Catathelasma* clade.

Key words Basidiomycota · Fungi · Mushroom · Phylogeny · Systematics

Introduction

The clitocyboid habit refers to members of the Agaricales that produce fruit bodies (basidiomata) with decurrent lamellae and convex to funnel-shaped pilei (Bas et al. 1998). At least seven independent clitocyboid mushroom lineages have been identified recently in a multigene phylogenetic analysis of the Agaricales (Matheny et al. 2006). Three of these lineages include (1) *Ampulloclitocybe* Redhead, Lutzoni, Moncalvo & Vilgalys, closely related to the Hygrophoraceae; (2) *Cantharocybe* H.E. Bigelow & A.H. Sm., which appears related to the Pleurotaceae; and (3) *Infundibulicybe* Harmaja, which is currently unresolved in the Agaricales (Bigelow and Smith 1973; Redhead et al. 2002; Harmaja 2003). Four additional independent clitocyboid lineages also are distributed in the tricholomatoid clade, one of six major clades of Agaricales identified in Matheny et al. (2006): (4) *Neohygrophorus* Singer (see Redhead et al. 2000); (5) *Clitocybe* (Fr.) Staude sensu stricto (typified by *C. nebularis* (Batsch: Fr.) Quél.), possibly the sister-group to the Entolomataceae and Lyophyllaceae; (6) a group including *Clitocybe candicans* (Pers.: Fr.) P. Kumm. and *C. subditopoda* Peck; and (7) what was initially determined by Matheny et al. (2006) as *Clitocybe subvelosa* A.H. Sm. & D.E. Stuntz (Smith and Stuntz 1950).

Based on a comparison of material identified as *C. subvelosa* in Matheny et al. (2006) with the holotype of *Clitocybe subvelosa*, we determined that the former constitutes a new species that is distinct from but phenetically similar to *C. subvelosa*. Given the unique phylogenetic position of this species in an inclusive 25S rRNA analysis presented here and in Matheny et al. (2006), we describe the new genus *Cleistocybe* and the new species *Cleistocybe vernalis* to accommodate it. Two other taxa are subsumed in *Cleistocybe*: *Clitocybe gomphidioides* A.H. Sm. and *Clitocybe subvelosa*. The latter is reported here as conspecific with *C. gomphidioides*.

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Materials and methods

Field collections, morphology, and microscopy

Materials were collected fresh and air-dried with a food dehydrator. The gross morphology of basidiomata was documented in fresh condition. Fresh basidiospore deposits were tested for an amyloid reaction with Melzer's solution. Basidiospores were mounted in Cotton Blue to test for a cyanophilous reaction. KOH (2%–3% solution) was applied to the surface of the pileus to note any color changes. Fresh and dried materials were compared with the types of *C. subvelosa* and *C. gomphidioides* and five other collections of these species received on loan from MICH (for herbarium abbreviations, see Holmgren et al. 1990). Sections of dried material were rehydrated in 3% KOH in preparation for anatomical studies with light microscopy. Capitalized colors are from Ridgway (1912). Other color notations are our own approximations. Gross morphological descriptions of *C. subvelosa* and *C. gomphidioides* are taken from Bigelow (1985). Microscopic descriptions are modified from Bigelow (1985) based on our observations of the types and supplementary materials. Mean spore dimensions are calculated from measurements of twenty spores per collection and are italicized.

Phyloinformatics

Phylogenetic analyses of a six-gene region supermatrix in Matheny et al. (2006) demonstrated support for the monophyly of "*Clitocybe subvelosa*," *Callistosporium* Singer, and *Catathelasma* Lovejoy. This grouping was labeled the *Catathelasma* clade and received a significant Bayesian posterior probability (PP) of 1.0 and a maximum parsimony (MP) bootstrap proportion (BP) of 44%. To examine relationships of these and additional taxa in more detail, we assembled a 25S rRNA gene data set of members of the *Catathelasma* clade and the callistosporioid clade of Moncalvo et al. (2002) and produced four new 25S and internal transcribed spacer (ITS) sequences for the paratype of *Cleistocybe vernalis* and *Clitocybe* aff. *fellea* (EF416916–19) following DNA extraction, polymerase chain reaction (PCR), and sequencing protocols outlined in Matheny et al. (2007). ITS sequences for type and supplementary collections of *Clitocybe gomphidioides* and *C. subvelosa* from MICH were produced (EF457510–19) by sequencing ITS1 and ITS2 separately using primers ITS1F/ITS2 and 5.8SR/ITS4. Relationships of these lineages to additional sequences were assessed by BLAST searches of the nucleotide database at the National Center for Biotechnology Information (NCBI <http://www.ncbi.nlm.nih.gov/>). As a result, 25S sequences of the genera *Callistosporium*, *Catathelasma*, *Macrocybe*, and *Pleurocollybia* were pooled from Pegler et al. (1998), Moncalvo et al. (2002), Matheny et al. (2006), and unpublished sequences at NCBI. Sequences of *Mycena galericulata* (Scop.: Fr.) Gray and *M. amicta* (Fr.: Fr.) Quél. (Mycenaceae) were chosen for outgroup purposes based on results from Matheny et al. (2006). Sequences

were aligned initially in ClustalX 1.83 (Thompson et al. 1997) and manually adjusted in MacClade 4.0 (Maddison and Maddison 2000).

All positions were included for phylogenetic analysis except for the nonuniformly sampled ends of the sequences. The 25S data set was analyzed using MP in PAUP* (Swofford 2003) with the branch and bound search algorithm and furthest addition sequence and subjected to 500 bootstrap replicates, also with the branch and bound search algorithm. Gaps were treated as missing data. MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used to do a Bayesian analysis under a general time reversible model of evolution incorporating rate heterogeneity parameters. Two independent runs were performed for 1 million generations each, saving one tree every 100 generations. Convergence between runs was assessed by the mean standard deviation of split frequencies. Trees that converged in the posterior distribution were used to calculate posterior probabilities. The alignment is available at TreeBASE (<http://www.treebase.org>) (M3187, S1746). Comparison of ITS sequences was done in PAUP* using the proportional or "p" distance calculation.

Taxonomy

Cleistocybe Ammirati, A. D. Parker & Matheny, gen. nov.

Basidiomata clitocybiforma; stipes centralis, solidus; velum praesens vel ephemerum; lamellae decurrentes, roseobrunneae, demum griseo-brunneae; trama lamellarum divergens vel intertextum, hyphis fibulatis; basidia clavata; cystidia nulla; spores albae, laeve, acyanophilae, inamyloideae.

Species typicae: *Cleistocybe vernalis* Ammirati, A.D. Parker & Matheny

Habit clitocyboid, veil present or ephemeral, thin to fibrillose or membranous and leaving a more or less distinct annulus, pileus convex to plane becoming depressed, margin enrolled to incurved or decurved, surface nonviscid or viscid, appressed fibrillose to squamulose or with agglutinated fibrils, nonhygrophanous, margin opaque, color brick red to light reddish brown with margin light pinkish cinnamon, buff, or paler, or with vinaceous brown fibrils over a pinkish-gray ground color; context of pileus thick but tapered abruptly to margin, confluent with stipe context, solid in stipe, pale pinkish gray to pale vinaceous buff; odor and taste strongly farinaceous; lamellae decurrent to long decurrent, close, crowded or subdistant to distant, relatively narrow, sometimes forked, light pinkish brown, pale pinkish gray to light gray, darkening in age to grayish or brown gray; stipe central, equal to clavate or tapered slightly downward, surface dry, appressed fibrillose to squamulose below, base cottony, color pale pinkish gray, pale vinaceous gray to vinaceous buff. Basidiospores white in deposit, in face view usually elliptical to elliptical oblong, in profile view elliptical to ovate or subfusoid to subcylindrical, often strongly

inequilateral, smooth, distinctly apiculate, inamyloid, not cyanophilous, relatively thin-walled. Basidia narrowly clavate to clavate, sterigmata prominent, usually 4-spored but sometimes 1–3-spored, typically colorless or with granular contents; hymenophoral cystidia absent; pileipellis interwoven, nongelatinous or gelatinous in 3% KOH, hyphae cylindrical, narrow, often flexuous, sometimes encrusted, colorless, yellowish, orange or orange-brown; pileal trama interwoven, hyphae cylindrical or less commonly broadly cylindrical, colorless to yellowish, some encrusted, thin-walled or slightly thickened, walls refractive; lamella trama interwoven to subparallel but more or less divergent when young, hyphae cylindrical, with a tendency to undulate, colorless; veil hyphae similar to those of pileipellis, cylindrical, parallel to interwoven, colorless to yellowish and refractive; oleiferous hyphae present; clamp connections present.

Terrestrial, solitary to caespitose, or in clusters on soil under mixed conifers (*Abies*, *Larix*, *Pseudotsuga*, *Thuja*) or conifers mixed with Betulaceae (*Alnus*, *Betula*).

Type species *Cleistocybe vernalis* Ammirati, A.D. Parker & Matheny (PBM 1856) (18S rRNA sequence: DQ092913, 25S rRNA sequence: AY647208, ITS sequence: DQ486692), Washington, USA (WTU).

Etymology: *Cleistos* (Greek: closed, shut) and *-cybe* (Greek: head) in reference to the veiled basidiomata.

Cleistocybe vernalis Ammirati, A.D. Parker & Matheny, sp. nov. Figs. 1–7

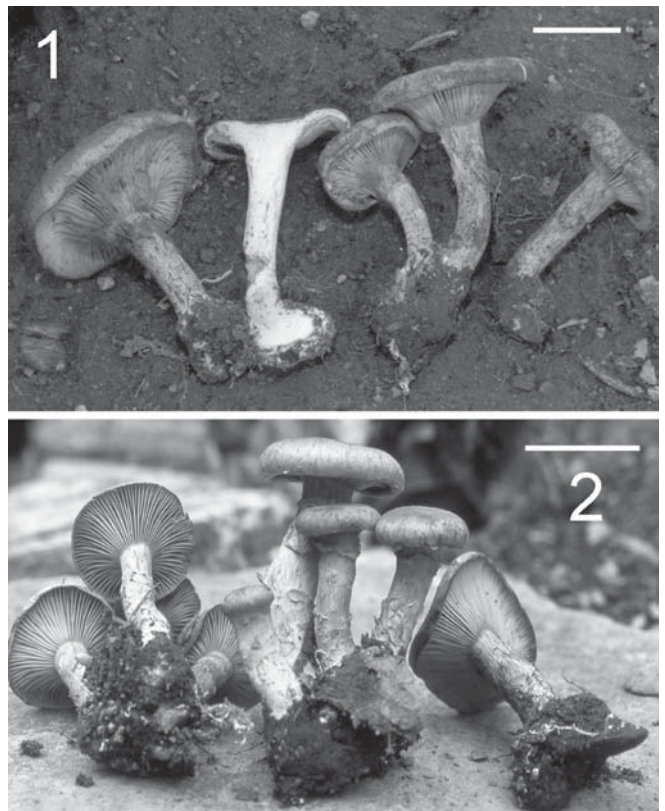
Misapplied name: *Clitocybe subvelosa* sensu Matheny et al., Mycologia 98(6):992, 2006, non A.H. Sm. & D.E. Stuntz (1950).

Pileus 2–6 cm latus, convexus demum depressus, margine incurvus demum decurvus, non viscidus, fibrillosus vel squamulosus, vinaceo-bruuneus vel pallide incarnato-griseus; odor et sapor farinaceus; lamellae pallide incarnato-griseae, confertae, decurrentes; stipes 3–6 cm longus, 7–15 mm crassus, aequalis, solidus, intus pallide incarnato-griseobrunneus, externe vinaceo-bruuneus vel pallide incarnato-griseus, squamulosus; superne membranoso-annulatus; spora (6.3–) 7.4–10.4 × 3.7–4.8 μm, inamyloideae, ellipticae vel amygdaliformes.

Holotypus PBM 1856 (WTU), (48°49.850'N, 117°23.217'W), ad 3.2 km meridiem e Metaline Falls, Pend Oreille County, Washington, 650 m alt., May 13, 2000, leg. A.D. Parker.

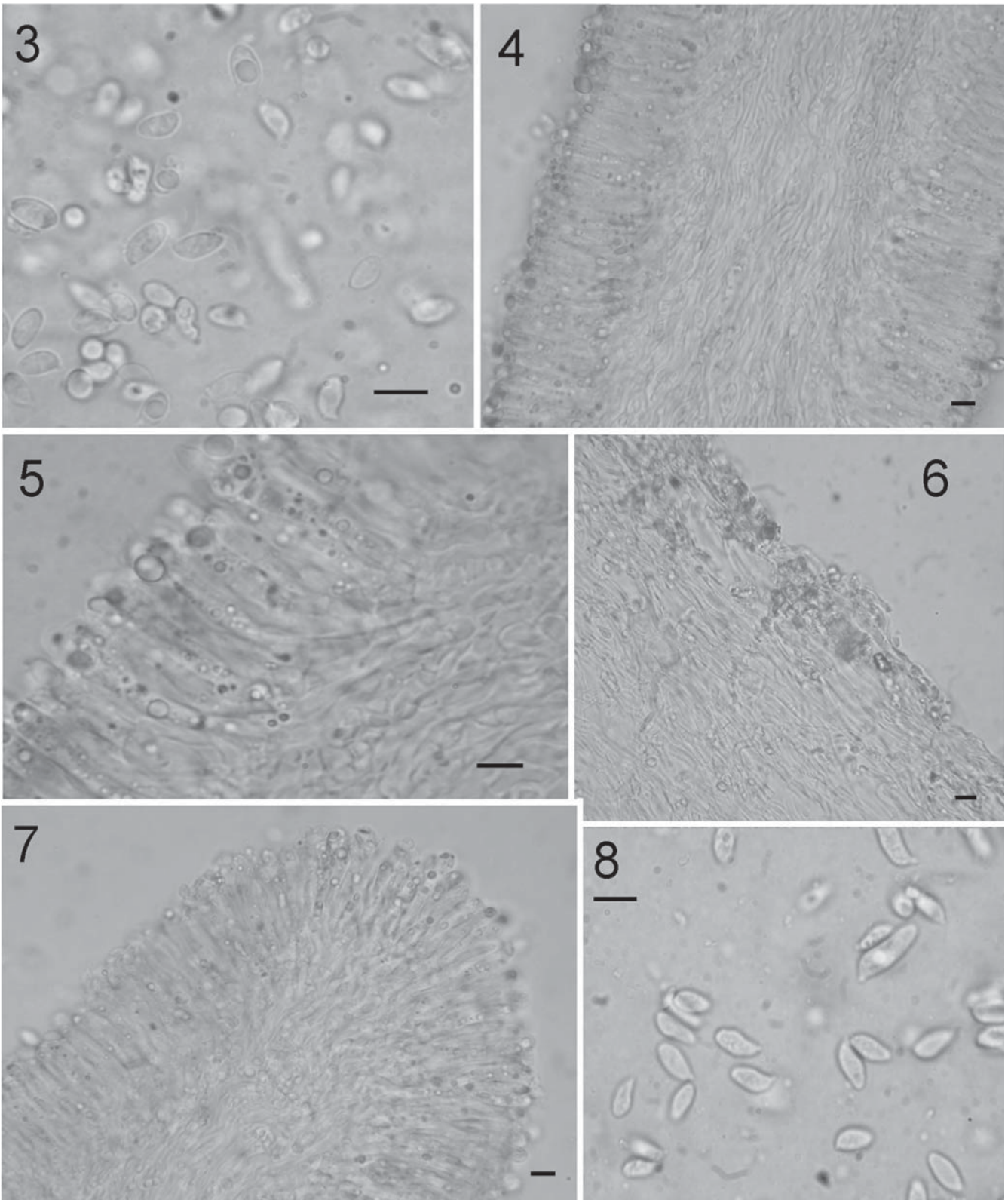
Etymology: *vernalis* (Latin: occurring in spring).

Pileus 2.0–6.0 cm in diameter, convex at first with an incurved margin, becoming depressed with decurved margin in age; surface dry, not viscid, appressed fibrillose to squamulose with vinaceous brown fibrils like “kidskin” over a pale pinkish gray ground, becoming slightly more ochraceous brown in extreme age or on drying, at times cracked-areolate and/or margin with scattered patches of submembranous veil remnants; context pale pinkish gray brown, up to 12 mm thick near the stipe apex, tapering to a point at the margin, not changing color where cut, firm, odor strongly farinaceous; cap surface greenish to pale green when KOH applied on fresh material. Lamellae



Figs. 1–2. Basidiomata of *Cleistocybe vernalis*. 1 PBM 1856 (holotype). 2 ADP 29–04–00–1 (paratype). Bars 1, 2 3 cm

decurrent, close (40–70 reach the stipe), more or less equal number of lamellulae, narrow, up to 10 mm broad near the stipe, pale pinkish gray. Stipe 3.0–6.0 cm long, 7–15 mm wide, equal to slightly tapering downward, concolorous with pileus, surface fibrillose above the annular zone, with coarse irregular patches of veil tissue below, at times several (two to four) basidiomata arising from a common bulbous base; annulus submembranous, superior, pale pinkish gray. Basidiospores (6.3–)7.4–8.4–10.4 × 3.7–4.6–4.8 μm, in face view elliptical to somewhat ovate in outline, in profile view somewhat variable, a few shorter and broad, some reniform, often more elliptical, typically more or less inequilateral and tending to be amygdaliform in outline, apiculus well developed, more or less thin-walled, colorless, empty or with droplets and granules, inamyloid. Basidia 37–44 × 5–6 μm, 4-spored, narrowly clavate, colorless, some with granular materials. Pleurocystidia and cheilocystidia absent. Lamella edges fertile. Lamella trama interwoven to strongly interwoven, more parallel in some areas, somewhat divergent in the compact subhymenium with elements similar to those of *Hygrophorus*, hyphae mostly 3–12 μm diameter, straight to twisted, walls refractive, thin to somewhat thickened, smooth, only rarely seen with incrustations, colorless to slightly yellowish (walls), no granules, pigments, or oleiferous hyphae seen. Pileipellis interwoven to strongly interwoven, in a few places hyphae more radially arranged (similar in cross section), mostly 3–4.5 μm in diameter, cylindrical, smooth to encrusted, some colorless, often



Figs. 3–8. Microscopic features of *Cleistocybe*. **3** Basidiospores of *C. vernalis* (ADP 29-04-00-1). **4** Interwoven lamella trama of *C. vernalis* (PBM 1856, holotype). **5** Subhymenial elements of *C. vernalis* (PBM 1856, holotype). **6** Pileipellis of *C. vernalis* (PBM 1856, holotype). **7** Edge of lamella of *C. vernalis* (PBM 1856, holotype). **8** Basidiospores of *C. gomphidioides* (AHS s. n., Oct. 12, 1941). Bars **3–8** 10µm

yellowish, not embedded in a gelatinous matrix; pileal trama interwoven to strongly interwoven in cross section, in some places hyphae more radially oriented, cylindrical to broadly cylindrical, at times more enlarged, mostly colorless, wall somewhat thickened, refractive, colorless to yellowish, smooth, rarely encrusted, no oleiferous hyphae seen. Clamp connections present throughout basidiomata.

Habit and habitat: Solitary or clustered on soil at edge of dirt road and on sloped road bank, in mixed conifer forests of *Abies*, *Betula*, *Larix*, *Pseudotsuga*, and *Thuja*.

Specimens examined: USA. Washington. Pend Oreille County (48°49.850'N, 117°23.217'W): PBM 1856 (holotype; 18S rRNA sequence: DQ092913, 25S rRNA sequence: AY647208, ITS sequence: DQ486692), collected by A.D. Parker, about 3.2 km south of Metaline Falls on the east side of the Pend Oreille River, about 0.5 km north of Wolf Creek, and about 1 km south of Pocahton Creek, USFS road 3310, elevation 650 m, May 13, 2000 (WTU); ADP 29-04-00-1 (paratype), same locality and collector, April 29, 2000 (WTU); ADP 05-05-06-1 (paratype; 25S rRNA sequence: EF416916, ITS sequence: EF416917), same locality and collector, May 5, 2006 (WTU).

Distribution and phenology: Pacific Northwest, USA, known only from the type locality in Washington where it has been collected or observed from late April to mid-May.

Discussion: *Cleistocybe vernalis* is known from a single locality where it consistently produces basidiomata each spring, and it appears to be a rare species. The pileipellis is not gelatinous, in contrast to *C. gomphidioides* (see following), and the pileus surface turns green with KOH when fresh. Whether the KOH color reaction is similar for *C. gomphidioides* is unknown because specimens were not tested with KOH by A.H. Smith. The basidiospores are somewhat smaller, and more consistently elliptical in profile view than in *C. gomphidioides*, but these features cannot be evaluated from only a single population of *C. vernalis*. ITS sequences of *C. vernalis* and *C. gomphidioides* (including sequences of type collections) differ at 76 nucleotide positions (excluding gaps) among 605 non-gapped sites (648 total aligned sites). The genetic or “p” distance between the two species at the ITS locus is 12.5%. Thus, the vernal occurrence, nongelatinous pileipellis, and ITS data serve to distinguish *C. vernalis* readily from the otherwise morphologically similar *C. gomphidioides*. *Cleistocybe vernalis* was earlier misinterpreted as *C. subvelosa* (a later synonym of *C. gomphidioides*, see following) by Matheny et al. (2006).

Cleistocybe gomphidioides (A.H. Sm.) Ammirati, A.D. Parker & Matheny, comb. nov. Fig. 8

≡ *Clitocybe gomphidioides* A.H. Sm., Bull. Torrey Bot. Club 71:401. 1944. (basionym)

= *Clitocybe subvelosa* A.H. Sm. & D.E. Stuntz, Mycologia 42:82. 1950.

Pileus (3–) 5–9 (–10) cm broad, plane at first with an enrolled margin or very slightly arched, margin at times fringed with veil remnants, becoming broadly convex with disc depressed or plane, color reddish-brown (Mikado

Brown), brick-red to light reddish brown (Orange Cinnamon) to pale Sayal Brown on disc, paler and more buff or pinkish toward margin (Orange Cinnamon, Pinkish Cinnamon, Light Pinkish Cinnamon), in age becoming duller and more yellowish on disc (Sayal Brown), sometimes duller tan, surface not pellucid striate but with streaks of agglutinated fibrils or scaly or finely tomentose then glabrous in age, viscid, central portion with minute spot-like scales or minutely areolate; context thick, firm, tapered abruptly to margin, pale Vinaceous Buff, whitish, or watery Avellaneous (watery spotted), in stipe solid, Pale Vinaceous Buff within or the base darker. Odor and taste very strongly rancid farinaceous. Lamellae pinkish buff to light pinkish brown young (Light Pinkish Cinnamon), light gray to gray (Vinaceous Buff, Avellaneous, Wood Brown), darkening with age to brownish-gray or subfuliginous; short or long decurrent, close to crowded or subdistant, narrow to moderately broad (3–8 mm), often somewhat thickened, intervenose or wrinkled, often forked. Stipe 3–9 cm long, 8–15 mm thick, equal or clavate, color pale gray (Pale Vinaceous Fawn, Vinaceous Buff), base darkening where handled, surface appressed fibrillose below an annular zone, base cottony, partial veil apical, thin, fibrillose to submembranous, at times annulate or collapsed, appressed in age, or ephemeral and no longer macroscopically evident, concolorous with stipe, at times glabrescent in age. Basidiospores white in deposit, (6.0–)7.5–10.0–11.8(–15.5) × (3.5–)4.0–4.5–5.5 μm, elliptical or sometimes more or less elliptical oblong in face view, usually elliptical or ovate but sometimes subfusoid to subcylindrical and inequilateral in profile view, smooth, inamyloid, not cyanophilous. Basidia 35–60 × 6–10 μm, usually 4-spored or rarely 1–3-spored, sterigmata prominent, up to 8 μm long. Pileipellis subgelatinous to gelatinous in KOH, yellowish, orangish or orangish brown, pigment in thickened walls or very finely encrusted, hyphae cylindrical, 2.5–8 μm in diameter, often flexuous and contorted; pileal trama colorless or tinged orangish in KOH, hyphae cylindrical, 2.5–10 μm in diameter. Lamella trama of interwoven hyphae but with some tendency to be undulate subparallel, 1.5–6 μm in diameter. Veil hyphae cylindrical, parallel to interwoven, more or less 3 μm in diameter, colorless to yellowish and refractive. Oleiferous hyphae present. Clamp connections present.

Habit and habitat: Solitary to gregarious under conifers (*Thuja*, *Tsuga*), mixed conifers and alder (*Alnus*), or gregarious on wet soil under *Oplomanax horridus* Miq. (Araliaceae).

Specimens examined: USA. Colorado. San Juan County: *Clitocybe subvelosa* AHS 52631 (ITS sequence: EF457516) (MICH), collected N.J. Smith, Woods Lake, August 22, 1956. Idaho. Boundary-Bonner County: *Clitocybe subvelosa* AHS 76924 (ITS sequence: EF457514) (MICH), leg. A.H. Smith, Upper Priest River, September 13, 1968; Idaho County: *Clitocybe subvelosa* AHS 70321 (ITS sequence: EF457517) (MICH), leg. A.H. Smith, Papoose Creek, Seven Devils Mountains, September 8, 1964. Washington. Pierce County: *Clitocybe subvelosa* AHS 31182 (holotype; ITS sequence: EF457511–12), collected by D.E. Stuntz, Mount Rainier National Park, Lower Tahoma, September 12, 1948

(MICH); Clallam County, AHS17504 (paratype; ITS sequence: EF457513), leg. A.H. Smith, Olympic Hot Springs, Olympic Mountains, October 2, 1941 (MICH); Jefferson County, no collection number (MICH scan number 00055602, paratype; ITS sequence: EF457518–19), leg. A.H. Smith, Lake Mills near Wolf Creek, Olympic Mountains, October 12, 1941 (MICH); AHS17682 (holotype; ITS sequence: EF457510), leg. A.H. Smith, Olympic Hot Springs, Olympic National Park, October 10, 1941 (MICH).

Distribution and phenology: Pacific Northwest, USA, known from Colorado, Idaho and Washington, August to October.

Discussion: Bigelow (1985) considered *Clitocybe subvelosa* and *C. gomphidioides* to be rare species, and indeed there have been no collections of these species seen in recent decades. Bigelow observed about the two species: “The two are very close in all characteristics, differing principally by the presence of a partial veil in *C. subvelosa*. This naturally leads to speculation that the two could be identical and that the veil, or its absence, might have been influenced by a particular set of environmental conditions.” This idea was also considered by Smith and Stuntz (1950). An examination of the lower stipe surface of *C. gomphidioides* material also revealed a covering of narrow, interwoven hyphae that are very similar to those seen in the veil of *C. vernalis* and *C. subvelosa*.

ITS sequences of three collections of *C. subvelosa* and four collections of *C. gomphidioides* (including holotypes and paratypes) are identical except at position 146 in ITS1, which is polymorphic (A/G) for four of the accessions (AHS 17682, AHS 31182, AHS 76924, AHS s.n. October 12, 1941); also, two collections are inferred to share an ITS2 length polymorphism (AHS s.n. October 12, 1941, AHS 52631). Thus, for originally labeled *C. gomphidioides* material it is now possible to construe that the partial veil was not macroscopically discernible.

Key to *Cleistocybe* species

- 1a. Occurring in spring (April, May); pileipellis non-gelatinous *Cleistocybe vernalis*
- 1b. Occurring in fall (August, September, October), pileipellis subgelatinous to gelatinous *Cleistocybe gomphidioides*

Phylogenetic results and constituents of the *Catathelasma* clade

In the multigene Bayesian analyses of Matheny et al. (2006), *Cleistocybe* is sister with significant support to the callistosporioid clade (represented by *Callistosporium* only) and *Catathelasma*. This result was the basis of taxon selection for an inclusive 25S rRNA data set for the study here. This data set is composed of 17 taxa and 1373 included characters, of which 142 are parsimony informative. In Bayesian analyses, the average standard deviation of split frequencies

reached less than 0.01 after 105000 generations. Thus, the last 8950 trees were used to calculate posterior probabilities from the two independent runs for a total of 17900 trees. One sequence for *Callistosporium luteo-olivaceum* (Berk. & M.A. Curtis) Singer (AF042627 in Moncalvo et al. 2000) proved to be an outlier in initial analyses and showed a 98% sequence similarity with species of *Cystoderma* (Cystodermataceae, agaricoid clade). This sequence (AF042627) was pruned from subsequent analyses. *Callistosporium* sp. of Matheny et al. (2006) represents *C. graminicolor* Lennox (Lennox 1979), and molecular data support the distinction of this species from *C. luteo-olivaceum*. Redhead (1982) earlier considered *C. graminicolor* conspecific with *C. luteo-olivaceum* on the basis of morphological comparisons.

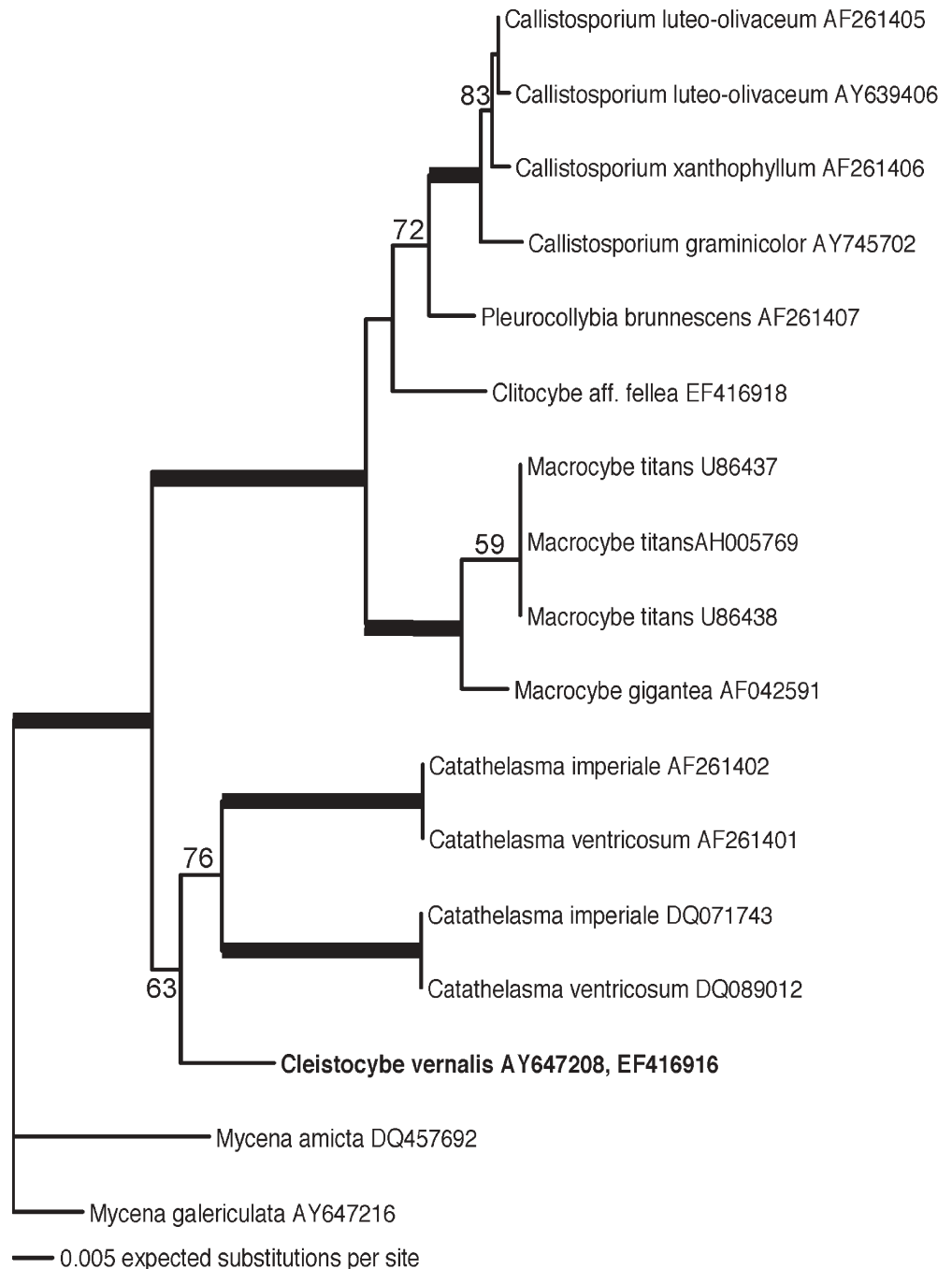
Cleistocybe vernalis forms a weakly supported (63% BP/0.66 PP) monophyletic group with four sequences of *Catathelasma* (Fig. 9). *Catathelasma*, however, is composed of two highly divergent clades, which are not monophyletic in automated MP and neighbor-joining trees produced by the phyloinformatic tool *mor* (<http://mor.clarku.edu>) (Hibbett et al. 2005). North American *Catathelasma ventricosum* (Peck) Singer is not distinguishable from European *C. imperiale* (Fr.) Singer based on 25S rRNA sequences. Sister to *Cleistocybe* and *Catathelasma* is a saprotrophic clade including the tropical genus *Macrocybe* and lignicolous species of *Pleurocollybia* and *Callistosporium*. This group of three genera is strongly supported and has been referred to as the callistosporioid clade by Moncalvo et al. (2002). The specimen with affinities to *Clitocybe fellea* was documented on rotten wood. We are presently attempting to determine the relationship of our material with that of *C. fellea*. Bigelow (1985) considered *C. fellea* as *incertae sedis* in *Clitocybe*.

These constituents of the *Catathelasma* clade are characterized by a white spore deposit, smooth, hyaline spores, which are uninucleate in *Catathelasma* and *Callistosporium*, absence of cheilocystidia, and a cutis-type of pileipellis. Clampless species occur only in the clade containing *Pleurocollybia* and *Callistosporium*. Species of *Catathelasma*, *Macrocybe*, and at least one species of *Pleurocollybia* are edible (at least after cooking), and most species have a farinaceous, bitter, or mild taste (see Bigelow 1985; Singer 1986; Pegler et al. 1998).

Phylogenetic relationships in the *Catathelasma* clade

Both Bigelow (1985) and Smith and Stuntz (1950) debated the relationship of *Cleistocybe* (viz. *C. gomphidioides* and *Clitocybe subvelosa*) to *Clitocybe* as well as other genera such as *Armillaria* and *Hygrophorus*. Bigelow (1985) stated that the basidiospores of *C. subvelosa* and *C. gomphidioides* were unusual in *Clitocybe* because of their subfusoid to subcylindric and inequilateral outline in profile view. This would appear to be a reliable characteristic of this group when combined with other features, such as the strongly interwoven lamella trama with divergent elements in the subhymenium when young, the long narrow basidia, and

Fig. 9. 25S rRNA gene phylogeny of members of the *Catathelasma* clade based on Bayesian and MP bootstrap analyses. The 50% majority rule consensus tree of the Bayesian analysis is shown, including branch lengths. Branches that are significantly supported by posterior probability (PP) and bootstrap proportion (BP) values greater than 0.95 and 70%, respectively, are indicated with *thickened black lines*. BP values greater than 50% are shown for branches that receive nonsignificant PP values. *Cleistocybe* is indicated in *bold type*. The Mycenaceae is used to root the tree



the strongly interwoven pileipellis with pigmented and incrustated hyphae. They also discussed the issue of the divergent lamella trama since A. H. Smith had seen this in fresh material. This was dismissed by these authors after reexamining dried specimens. Our study of the lamella trama of young specimens of *C. vernalis* shows a tendency to be divergent (Fig. 5) as Smith originally observed. This feature is easily missed because of the strong tendency for the hymenophoral trama to become irregularly interwoven (Fig. 4). Singer (1986) and Smith et al. (1979) describe the structure of the lamella trama in *Catathelasma* as becoming interwoven by maturity, and Singer mentions the trama being divergent when young; in a footnote Singer discusses

this as an intermediate structure that might make a connection to the *Clitocybe* type lamella trama. Nevertheless, the presence of a partial veil, divergent to interwoven lamella trama, decurrent lamellae, confluent pileus and stipe, firm flesh, and growth on soil appear to be characters that *Cleistocybe* and *Catathelasma* share in common. *Cleistocybe* is terrestrial and often occurs in the vicinity of ectomycorrhizal plants (Pinaceae, Betulaceae), but it has not been determined if it is ectomycorrhizal or saprotrophic. The two genera differ in that the spores of *Catathelasma* are amyloid and its veil is double (Smith et al. 1979).

The remaining members of the *Catathelasma* clade are saprotrophs, lack a partial veil, and have regular hyme-

nophoral trama. *Macrocybe*, *Pleurocollybia*, and *Callistosporium* have cyanophilous or weakly cyanophilous spores, which are inamyloid. Species of *Macrocybe* are large and fleshy (similar to *Catathelasma*), but the genus is gymnocarpic, nonmycorrhizal with a tropical and subtropical distribution, has sinuate lamellae, and cyanophilic, inamyloid spores (Pegler et al. 1998). *Pleurocollybia* and *Callistosporium* generally are lignicolous and do not produce large, fleshy basidiomata. Ultimately, the relationship between *Cleistocybe* and *Catathelasma*, as well as other genera in the *Catathelasma* clade, requires further morphological, phylogenetic, and ecological study.

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