Characterization of four species including one new species of *Agaricus* subgenus *Spissicaules* from Eastern China

Dai RC\(^1\)\(^2\), Li GJ\(^2\), He MQ\(^1\)\(^2\), Liu RL\(^3\), Ling ZL\(^2\), Wu JR\(^1\) and Zhao RL\(^2\)

\(^1\)Key Laboratory of Forest Disaster Warning and Control in Yunnan Province, Southwest Forestry University, Kunming 650224, China.

\(^2\)State Key Laboratory of Mycology, Institute of Microbiology Chinese Academy of Sciences, Beijing 100101, China.

\(^3\)Nature Reserve Administration Bureau of Zhejiang Province, Lishui City, Jingning County, Jingning 323500, China.


Abstract

Four species of *Agaricus* subgenus *Spissicaules*, including a new species *A. catenariocystidiosus*, are identified from Eastern China based on morphological and molecular data. All of the species are introduced with full descriptions. Phylogenetic relationships were inferred based on the nuclear ribosomal internal transcribed spacer (ITS) region. The known species *A. inthanoensis*, *A. parasubrutilesces* and *A. planipileus* are compared with their original descriptions, and several supplementary morphological characters are provided.

Keywords – Agaricales – Agaricaceae – ITS – phylogeny – taxonomy

Introduction

*Agaricus* L. (Agaricales, Agaricaceae) is a genus that includes more than 400 species (Chen et al. 2012, 2015, Lebel et al. 2012, 2013, Li et al. 2014, Wang et al. 2015, Gui et al. 2015, Zhao et al. 2010, 2011, 2012, 2016). Edible mushrooms are generally low in calories and high in minerals, such as essential amino acids, vitamins and fibers (Mattila et al. 2002). The genus *Agaricus* contains many edible species, and some of them have been cultivated widely, such as *A. bisporus* (Large) Singer (Adams et al. 2008) and *A. subrufescens* Peck (Kerrigan 2005).

The epithet “*Spissicaules*” was first proposed for subsection *Spissicaules* Heimen. of section *Agaricus* based on the type species *A. spissicaulis* F.H. Møller (Heinemann 1978). Then, it was proposed and considered to be a section *Spissicaules* (Kerrigan 1985; Parra 2008) according to the possible affinities of *A. lilaceps* Heimen. *Spissicaules* contains *A. lanipes* (F.H. Møller & Jul.Schäff.) Hlaváček, *A. litoralis* (Wakef. & A. Pearson) Pilát and *A. bresadolanus* Bohus (Parra 2008). Species of section *Spissicaules* are characterized as follows: pileus diameter equal to, or smaller than, the length of the stipe; stipe often ventricose; cylindrical or clavate; mostly bulbous at the base; simple annulus; clearly striate on its upper surface; odor of anise or almond, sometimes very faint; NaOH reaction that is negative or very weak on all surfaces of the basidiome; and cheilocystidia clavate to cylindrical, sometimes septate at base, similar to immature basidia (Parra 2008).
However, section *Spissicaules* has been revealed to be a possible polyphyletic group based on phylogenetic analyses using ITS sequences (Zhao et al. 2011). In the most recent systematic study on *Agaricus* using multigene sequences, subgenus *Spissicaules* (Heinem.) R.L. Zhao & Moncalvo has been proposed to accommodate four sections: Amoeni Callac & R.L. Zhao, Rarolentes Kerrigan, nom prov., Spissicaules (Heinem.) Kerrigan, and Subrutilentes Kerrigan, nom. prov. (Zhao et al. 2016; Kerrigan 2016 in press).

We surveyed macrofungi from southwest of Zhejiang Province and north of Fujian Province, Eastern China, in 2015. Those areas have a subtropical monsoon climate, and their main vegetation is broad-leaved evergreen forests. The morphological and molecular phylogenetic studies of *Agaricus* specimens resulted in the discovery of one new species and three known species from subgenus *Spissicaules*.

**Materials & Methods**

**Morphological analyses**

Specimens were collected and photographed in the field. The macrocharacters were recorded when specimens were fresh, including the features of the pileus, lamella, stipe, annulus, odor and chemical reactions. Specimens were dried overnight in a food drier at 70°C after the morphological description was made. Dry specimens were kept in plastic bags, sealed and deposited in the Herbarium of Mycology, Institute of Microbiology, Chinese Academy of Sciences (HMAS). Microcharacters, such as basidia, basidiospores, cheilocystidia, pileipellis and annulus hyphae, were studied under a microscope (Olympus CX31). At least 20 measurements were made for each feature size for observations of basidiospores, basidia, cheilocystidia, pileipellis and annulus hyphae. Microscopic data were recorded as follows: X = the mean of length by width ± SD; Q = the quotient of basidiospore length to width; and Qm = the mean of Q values ± SD; Q = 1.15-1.3, broad ellipsoid; Q = 1.3-1.6, ellipsoid (Yang 2015). The description of morphological characters and chemical reactions followed Largent’s methods (Largent 1986a, 1986b).

**Phylogenetic analyses**

The D3591-01 forensic kit (OMEGA Company, USA) was used for DNA extraction. The Taq PCR Mix (Beijing Biomed Company, China) was used for PCR amplification. Each amplification reaction volume was 50 µl and contained the following: DNA template, 2 µl; PCR mix, 25 µl; dH2O, 20 µl; and each primer, 1.5 µl. The primers used were ITS1 (5'-GTAGGTTAACCCTTGGG-3') and ITS4 (5'-TCTCCGCTTATTGATATGC-3') (Li et al. 2012). The PCR cycling conditions were as follows: initial denaturation at 94 °C, denaturation at 94 °C for 3 min., annealing at 40 °C for 30 s, extension at 72 °C for 54 s, for 35 cycles, with a final extension at 72 °C for 10 min. The PCR amplicons were sent to Biomed Company for sequencing. Sequences were assembled in SeqMan (Swindel et al. 1997). The dataset used for phylogenetic analysis consisted of the ITS sequences produced from this study as well as additional sequences from GenBank. The dataset was initially aligned by ClustalX 2.0 (Thompson et al. 1997) and then adjusted manually in BioEdit v. 7.0.4 (Hall 2007). The alignment was submitted to TreeBASE (submission ID 18397). The best substitution model was determined by MrModeltest 2.2 (Nylander 2004). The GTR+I+G nucleotide substitution model was used in the analysis and was detected by MrModeltest 2.2 (Nylander 2004). Maximum parsimony (MP) analyses was performed using PAUP*4.0b 10 (Swofford 2004), gaps in alignment were treated as missing data and the tree bisection-reconnection (TBR) algorithm was performed using the heuristic search option. Parsimony bootstrap values were obtained from 1000 bootstrap replicates. The consistency index (CI), retention index (RI), and tree length (TL) were calculated, and the max-trees were set to 1,000,000. MrBayes 3.2 (Ronquist et al. 2012) was used for the Bayesian analysis with four chains (one cold, three incrementally heated) for 10,000,00 generations, and trees were sampled every 100 generations. The procedure was stopped when the tree split deviation frequency value reached 0.01, and the remaining trees were used to calculate the Bayesian posterior probabilities (PP) of individual clades.
Results

MP and Bayesian tree topologies were the same, so the Bayesian tree was used for the results. The dataset contained 79 ITS sequences, of which 17 were produced from this study. The subgenus *Spissicaules* includes 4 clades with good support: sect. *Subbrutilescentes* (clade A) with moderate support (PP/BS = 0.63/72), sect. *Amoeni* (clade B) with strong support (PP/BS = 0.94/61), sect. *Rarolentes* (clade C) with full support (PP/BS = 1/100) and sect. *Spissicaules* (clade D) with moderate support (PP/BS = 0.6/100). In the phylogenetic tree (Fig. 1), specimens ZRL2015038, ZRL20151199 and ZRL20151210 composed a separate clade with full support (PP/BS = 1/100) in Clade A (sect. *Subbrutilescentes*). Specimens ZRL2015060, ZRL20150110, ZRL2015106 and ZRL2015112 nested in the clade of *A. parasubbrutilescentes* Callac & R.L. Zhao with a moderately supported PP/BS value of 0.73/78. Specimens ZRL20151566, ZRL2015166 belong to a branch of *A. inthanensis* L.J. Chen, K.D. Hyde & R.L. Zhao and *A. brunneopileatus* Callac & R.L. Zhao and have strongly supported PP/BS values (0.99/72). Specimens ZRL20151230, ZRL20151244, ZRL20151241, ZRL20151565, ZRL20151193, ZRL20151574, ZRL20151239 and ZRL20151222 join the clade of *A. planipileus* R.L. Zhao with strong support (PP/BS = 1/88).

Taxonomy

*Agaricus catenariocystidiosus* R.C. Dai & R.L. Zhao, **sp. nov.**

**Etymology** – refers to the cheilocystidia are in chains.

**Typus** – China, Zhejiang Province, Lishui City, Jingning County, Caoyutang Forest Park, N 27°58’ E 119°38’, elv.1200 m., 2nd June 2015, Zhao Rui-Lin, ZRL2015038 (HAMAS 280112)

**Diagnosis** – the morphological characters that distinguish *A. catenariocystidiosus* from other *Agaricus* species are its chain-like cheilocystidia, longer basidiospores, NaOH reaction turns weakly green, odor of phenol.

**Description** – *Pileus* 42–98 mm in diam., convex and umbo-nate, cap margin entire, surface dry, covered with appressed, triangular fibrillose squamules, dense at disc, brown to light brown towards the margin, background dirty white. *Context* 1–6 mm thick, white and flesh. *Lamellae* free, crowded, intercalated with lamellulae; 3–5 mm broad; at first white, then grayish white, reddish brown, brown, finally dark brown. *Stipe* apex 4–7 mm, base 6–23 mm in diameter, 68–96 mm in length, cylindrical to long clavate, hollow, surface smooth above the ring, fibrillose scales under the ring, white. *Annulus* single, membranous, superous, edge entire, white, upside smooth, lower side floccose. *Odor* of phenol. Discoloration indistinct on touching and cutting, or turning brown on context exposure after 5 mins.

**Macrochemical reaction** – 10% NaOH reaction weakly green on surface.

**Basidiospores** 5–6 × 3–4 μm [x = 5.2 ± 0.3 × 3.4 ± 0.1, Q = 1.3–1.8, Qm = 1.5 ± 0.1, n = 20], ellipsoid, no germ pore, smooth, brown. *Basidia* 15–24 × 6–9 μm, hyaline, smooth, cylindrical or clavate, 4-spored. *Cheilocystidia* 13–28 × 8–18 μm, smooth, mostly pyriform, clavate, spherical, some in chains, hyaline. *Pleurocystidia* absent. *Pileipellis* cutis composed of hyphae 3–16 μm in width, constricted at the septa, contains brown membranous pigments. *Annulus* composed of hyphae 4–10 μm in width, smooth, clavate, hyaline.

**Habit** – solitary, scattered or gregarious on soil of broad-leaved forests.

**Other specimens examined** – China, Zhejiang Province, Lishui City, Jingning County, Baiyun, Wangdongyang Natural Reserve, N 27°58’ E 119°38’, elv. 1200 m., 11 August 2015, Zhao Rui-Lin, ZRL20151210 (HAMAS 275805); *ibid*, He Mao-Qiang, ZRL20151199 (HAMAS 275804).

Notes – The morphological characters of *A. catenariocystidiosus* match the circumscription of section *Subbrutilescentes* well (Zhao et al. 2016), and the phylogenetic analysis also supports its placement in this section. Compared with the known species of section *Subbrutilescentes* and section *Spissicaules*, *Agaricus catenariocystidiosus* is different from *A. Linzhiensis* and *A. brunneopileatus* by having wider or thinner basidiospores than *A. catenariocystidiosus* in Zhao et al. (2016), *A. Linzhiensis* spores that are broadly ellipsoid, Qm = 1.3; *A. brunneopileatus* spores elongate ellipsoid
Fig. 1 – Phylogeny of *Agaricus* subgenus *Spissicaules* generated from the Bayesian analysis of the ITS sequences. Bayesian posterior probability (PP) values and Bootstrap support (BS) values > 50 % are given at the internodes (PP/BS). *Agaricus campestris* (CA637), A. sp (ADK2171) and A. sp (CA486) were used as an outgroup. T indicates the type species. Sequences produced from this study are in bold. Four sections are indicated.

Qm = 1.7). *Agaricus impudicus* develops strong red or reddish brown discoloration on its gills when bruised, while *A. catenariocystidiosus* lacks such a discoloration. *Agaricus planipileus* and *A. inthanonensis* differ by developing a distinct yellow discoloration upon cutting. Comparing with *A. parasubrutilescens*, *A. catenariocystidiosus* has chain-like cheilocystidia and a 10% NaOH reaction leads it to be weakly green on the surface. However, in *A. parasubrutilescens*, its cheilocystidia are pyriform without in chains and the 10% NaOH reaction is indistinct.

The most similar species to *A. catenariocystidiosus* in morphology is *A. subrutilescens* because both have a greenish discoloration when reacted with NaOH, which is rare within *Agaricus* species (Kerrigan, 1986). However, the latter species differs by its dark brown, reddish brown scales on the pileus and simple cheilocystidia. The phylogenetic analysis also supports them as different species.
Fig 2. – *Agaricus catenariocystidiosus: ZRL2015038* (HMAS 280112 type) a-b. Basidiomata in field; c. Pileipellis hyphae; d. Basidiospores; e. Cheilocystidia; f. Basidia.

Description – Pileus 76–91 mm in diam., plane and slightly depressed, cap margin entire, surface dry, covered with appressed, triangular fibrillose-scales, brown scales on a light brown background. Context 5–6 mm thick, flesh. Lamellae free, crowded, intercalated with lamellulae, 6–8 mm broad, brown. Stipe apex 8 mm, base 11–21 mm in diameter, length 73–109 mm, cylindrical, base bulbous, hollow, surface smooth above the ring, fibrillose scales below the ring, white. Annulus single, membranous, superuous, edge entire, persistent, white, upside smooth, lower side floccose with brown pigments. Odor of phenol. Discoloration light yellow on bruising at the surface of pileus.

Macrochemical reaction – 10% NaOH reaction yellow on surfaces of stipe and cap. Basidiospores 5–7 × 3–5 μm [x = 6.0 ± 0.4 × 4.0 ± 0.3, Q = 1.3–1.7, Qm = 1.5 ± 0.1, n = 20], ellipsoid, no germ pore, smooth, brown. Basidia 14–26 × 5–8 μm, hyaline, smooth, cylindrical or clavate, 4-sспорed. Cheilocystidia 9–17 × 6–13 μm, smooth, ellipsoid, clavate or spherical. Pleurocystidia absent. Pileipellis cutis composed of hyphae 4–11 μm in width, constricted at the septa, contains brown membranous pigments. Annulus composed of hyphae 3–11 μm in width, smooth, clavate and hyaline.

Habit – solitary, scattered or gregarious on soil of broad-leaved forests.


Notes – The morphological characters of the specimens from this study generally match those of A. inthanonensis (Zhao et al. 2016), with the exception of the 10% NaOH reaction, which is absent from the original description, but is yellow in the specimens from this study. However, in the phylogenetic tree, they are separated. Currently, we have named these specimens as A. cf. inthanonensis. Information from multigene sequence analyses may be needed for a more precise identification.

Agaricus planipileus R.L. Zhao, Fungal Divers. 2016 in press, Fig. 4

Description – Pileus 52–120 mm in diam., parabolic to convex with truncate disc when young, then convex to plane with a slight depressed disc in age, margin entire, smooth or appendiculate, surface dry, covered with fibrillose-scales in whole surface, triangular shape or not, brown. Lamellae free, crowded, intercalated with lamellulae, 8.4–10 mm broad, white or little pink, grayish white, to dark brown in age. Context 5–9 mm thick, flesh, white. Stipe apex 8–13 mm, base 18–33 mm in diameter, length 69–128 mm, clavate-bulbous, hollow, surface smooth above ring, floccose to heavily squamose below ring, white, some squames with brown tips. Annulus 10 mm diam., single, membranous, superuous, white, upside smooth, lower side floccose, white or light brown fibrillose. Odor of almonds or aniseed. Discoloring on surfaces of pileus and stipe on bruising, handling or scratching; discoloring light orange on context exposure.

Macrochemical reaction – 10% NaOH reaction colored the stipe and cap yellow. Basidiospores 4–7 × 3–5 μm [x = 5.3 ± 0.5 × 3.6 ± 0.5, Q= 1.3–1.7, Qm =1.5±0.2, n =20], ellipsoid or broad ellipsoid, no germ pore, smooth, brown. Basidia 14–23 × 6–9 μm, hyaline, smooth, clavate to broadly clavate, 4-sпорed. Cheilocystidia 9–29 × 9–17 μm, smooth and hyaline, elliptic, spherical, pyriform, clavate, few separated, some specimens lacking or basidia-like. Pleurocystidia absent. Pileipellis cutis composed of hyphae 3–14 μm in width, constricted at the septa, contains brown membranous pigments.

Habit: solitary or in troops of broad-leaved forest.

Specimens examined – China, Zhejiang Province, Lishui City, Jingning County, Baiyun, Wangdongyang Natural Reserve. N 27°58’ E 119°38’, elv.1200 m., 11 August 2015, Zhou Jie-
Fig. 3 – *Agaricus* cf. *inthanonensis*: a, c, e from specimen ZRL20151566 (HMAS 275814); b, d, f–i from ZRL2015166 (HMAS 275816); a–e Basidiomates in the field; f. Pileipellis hyphae; g. Cheilocystidia; h. Basidiospores; i. Basidia.
Fig. 4 – *Agaricus planipileus*: specimen a, b, f–i from ZRL20151193 (HMAS 275808); c, e from ZRL20151565 (HMAS 280114); d from ZRL20151239 (HMAS 275809); f. Pileipellis hyphae; g. Cheilocystidia; h. Basidiospores; i. Basidia.
Fig. 5 – *Agaricus parasubruliscens*: a-f from ZRL2015112 (HMAS 275811); c. Pileipellis hyphae; d. Cheilocystidia; e. Basidiospores; f. Basidia.

Min, ZRL20151193 (HMAS 275808); *ibid*, He Mao-Qiang, ZRL20151241 (HMAS 280113); *ibid*, Zhao Rui-Lin ZRL20151222 (HMAS 280115); *ibid*, Zhao Rui-Lin, ZRL20151239 (HMAS 275809); *ibid*, ZRL20151244 (HMAS 275807); *ibid*, Huangtian Lake, 20 August 2015, He Mao-Qiang, ZRL20151574 (HMAS 275806); *ibid*, Caoyutang, Forest park Natural Reserve, 19 August 2015, He Mao-Qiang, ZRL20151565 (HMAS 280114). China, Yunnan Province, Tengchong county, Datang, Danlonghe Village, 28 July 2011, Zhao Rui-Lin, ZRL2011250 (HMAS 273997 Holotype).
Notes – The morphological characters of the specimens in this study match the original description (Zhao et al. 2016), and the phylogenetic analyses also support their classification as the same species under the name *A. planipileus*. The species has additional 1 bp in ITS sequences than originally reported. In the original description, the 10% NaOH reaction was not detected, but from the examination of our specimens, the NaOH reaction is yellow, which would be a supplementary character for this species definition. The specimens ZRL20151239 and ZRL20151222, are lacking cheilocystidia, maybe the specimens are too young or too old.

*Agaricus parasubrutilescens* Callac & R.L. Zhao, Fungal divers. 2016 in press  

Description – *Pileus* 22–81 mm in diam., convex to planate, cap margin striate, surface dry, covered with triangular fibrillose-scales, brown scales on light brown background. *Context* 4–5 mm thick, flesh, white. *Lamellae* free, crowded, intercalated in lamellulae, 3–4 mm broad, pink when young, then brown, dark brown when old, edges even. *Stipe* apex 7–9 mm, base 14–17 mm in diameter, length 64–102 mm, clavate, base bulbous, hollow, surface smooth, white above ring, fibrillose scales white and light brown towards base below ring. *Annulus* single, membranous, superous, upside smooth and white, lower side floccose. Odor is aniseed. No discoloration on touching; discoloring reddish brown on context exposure of stipe.

Macrochemical reaction – 10% NaOH reaction is indistinct.

*Basidiospores* 4–6 × 3–4 µm [x = 4.7 ± 0.4 × 3.2 ± 0.3, Q= 1.2 – 1.8, Qm =1.5 ± 0.2, n= 20], ellipsoid or broad ellipsoid, no germ pore, smooth, brown. *Basidia* 14–18 × 5–7 µm, hyaline and smooth, cylindrical, 4-spored. *Cheilocystidia* 11–19 × 7–16 µm, smooth and hyaline, some pyriform or clavate, mostly elliptic and spherical. *Pleurocystidia* absent. *Pileipellis cutis*, composed of hyphae 3–12 µm in width, constricted at the septa, contains brown membranous pigments. *Annulus composed of hyphae* 4–9 µm in width, smooth, clavate and hyaline.

Habit – solitary, scattered or gregarious on soil in forest.

Specimens examined – China, Zhejiang Province, Lishui City, Jingning County, Baiyuns, Wangdongyang Natural Reserve, N 27°58′ E 119°38′, elv.1200 m., 4 June 2015, Su Sheng-Yu, ZRL20151112 (HAMAS 275811); *ibid*, ZRL2015110 (HAMAS 275812); *ibid*, He Mao-Qiang, ZRL2015106 (HAMAS 275813); *ibid*, Caoyutang, Forest Park, 2 June 2015, He Mao-Qiang, ZRL2015060 (HAMAS 275810). China, Yunnan Province, Dali City, Cangshan, 24 July 2014, Su Sheng-Yu, ZRL2014076 (HAMAS274000 Holotype).

Notes – the morphological and phylogenetic analyses support the classification of the specimens in this study as the known species *A. parasubrutilescens* (Zhao et al. 2016). This species is widely distributed in tropical and subtropical areas, such as Thailand and southern China (Yunnan and Guangdong Provinces) (Zhao et al. 2016). The discovery of this species in Zhejiang Province is the most northern distribution described to date.

Acknowledgements

Su Sheng-Yu and Zhou Jie-Min are thanked for their help in collecting specimens. This work is financed by the National Natural Science Foundation of China [31000013, 31360014, 31470152, 31500013] and the support of developing subject.

References


Chen J, Zhao R, Parra LA, Guelly AK, De Kesel A, Rapior S, Hyde KD, Chukeatirote E, Callac P. 2015. – *Agaricus* section *Brunneopicti*: a phylogenetic reconstruction with descriptions of


Nylander JAA 2004. – MrModeltest 2.2. Program distributed by the author. Uppsala University, Uppsala: Department of Systematic Zoology, Evolutionary Biology Centre.


Swindell SR, Plasterer TN. 1997. – SEQMAN. Sequence data analysis guidebook, 75-89.


