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Agaricus section *Brunneopicti*: a phylogenetic reconstruction with descriptions of four new taxa

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Abstract

Agaricus is a genus of saprobic basidiomycetes including species of nutritional and medicinal interest. Historically the temperate species have been grouped into eight classical sections. Recent phylogenetic analyses however, revealed that two-thirds of the tropical taxa do not cluster in these sections, but form exclusively tropical clades. Seven (TR I to TR VII) strongly supported tropical clades have been revealed and it was hypothesized that clade TR I might represent *Agaricus* section *Brunneopicti*. This section was initially characterized by the presence of punctiform squamules, the remains of the veil, on the pileus and stipe. The present morphological study and phylogenetic ML, MP and Bayesian analyses based on ITS1+2 sequences show that clade TR I corresponds to *Agaricus* section *Brunneopicti* and includes 16 taxa grouped in four strongly supported subclades and two isolated branches. The six species with punctiform squamules which initially characterized the section constitute one of these subclades. We propose the new replacement name *Agaricus brunneopunctatus* for the illegitimate name *Agaricus brunneopictus*. All 16 species are discussed, full descriptions are provided for five, among them, *A. brunneosquamulosus*, *A. niveogranulatus*, *A. sordidocarpus* and *A. toluenolens* are described as new species. We also report on certain members of section *Brunneopicti* traits which generally characterize species belonging to other sections. These shared characters raise the issue of their origin and complicate the systematics and the identification of the tropical *Agaricus* species. An artificial dichotomous key is presented for species identification. Section *Brunneopicti* is the first reconstructed section of tropical *Agaricus*. Its known geographical distribution range is strictly palaeotropical. We predict that the species richness of other somewhat forgotten or new tropical sections will also increase in coming years.

Key words: Basidiomycota, phylogeny, systematics, tropical biodiversity

Introduction

Agaricus L. is a genus of saprobic fungi within the order Agaricales (Basidiomycota) including more than 400 species worldwide (Zhao *et al.* 2011). This genus includes species of nutritional and medicinal interest, such as *A. bisporus* (J.E. Lange) Imbach or *A. subrufescens* Peck. Taxa from temperate regions are grouped into eight commonly recognized sections based on morphological and organoleptic traits, as well as macrochemical reactions (Cappelli 1984; Parra 2008, 2013). Species identification in *Agaricus* however, remains problematic due to a limited number of taxonomically relevant morphological features (Challen *et al*. 2003; Zhao *et al*. 2011). However, the circumscription of species and sections has been improved by exploiting the polymorphism of the nuclear ribosomal ITS (Internal transcribed spacer) DNA sequences (Challen *et al*. 2003; Kerrigan *et al*. 2005, 2008). Compared with temperate areas, knowledge of species diversity is less-developed in tropical regions. Zhao *et al*. (2011) found that two-thirds of

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tropical species did not belong in any of the eight traditional sections. Although, numerous tropical species have been described, only few sections to accommodate these taxa have been proposed (Heinemann 1956, 1978, 1980; Singer 1986; Pegler 1977; Peterson *et al*. 2000). Phylogenetic analyses of Zhao *et al*. (2011) revealed seven (TR I to TR VII) strongly supported tropical clades in addition to the clades of the eight traditional sections (two being polyphyletic). To what extent these tropical clades can correspond to the sections proposed by Heinemann remains unresolved. Zhao *et al*. (2011) suggested that clade TR I was the best candidate to represent the tropical *Agaricus* section *Brunneopicti* Heinem. (Heinemann 1956) because it included a collection identified as *A. brunneopunctatus* (a *nom. nov*. for the illegitimate name *A. brunneopictus* Heinem. & Gooss.-Font., the type species of the section). Unfortunately the examined specimens of this collection were not mature enough to confirm the identification. Moreover, sequencing of the type specimen of *A. brunneopunctatus* failed (Olivier Raspé personal communication). Heinemann also included *A. bingensis* Heinem. and *A. kivuensis* Heinem. in section *Brunneopicti*; however analyses including the sequence of a type specimen revealed that *A. kivuensis* belongs to the unrelated clade TR III (Zhao *et al*. 2011).

With the aim of assessing whether clade TR I represents section *Brunneopicti*, an enlarged sample including six specimens identified as *A. bingensis* were incorporated in a phylogenetic analysis of this clade. Moreover, each taxon belonging to clade TR I was compared to the following description of section *Brunneopicti* proposed by Heinemann (1956): small brown punctiform scales on pileus and surface of the lower part of the stipe remaining from the general veil; medium to large sporocarps; whitish or yellowish brown pileus; solid or fistulose long stipes with rounded bulb at base; often with "benzoilée" (almond) odor; short pileipellis hyphae; and weak or negative Schäffer's reaction. Since Heinemann acknowledged that this section was not morphologically well-characterized (Heinemann 1984), we used both morphological and phylogenetic criteria to delimit the section. Finally, we confirmed that clade TR I is equivalent to section *Brunneopicti.* The reconstructed section includes 16 species which are commented upon or fully described; four new taxa are proposed.

Materials and methods

Sampling

The 43 specimens used in the present study are listed in Table 1. Among them, 25 were recently collected in Thailand or Togo and deposited in Herb. MFLU (Mae Fah Luang University Herbarium), BBH (BIOTEC Bangkok Herbarium) and TOGO (Herbier du Laboratoire de Botanique et Ecologie Végétale de la Faculté des Sciences de l'Université de Lomé). The 18 remaining samples have already been used in analyses of Zhao *et al*. (2011) in which they belonged to 15 putative species classified as follows: ten belonged to the strongly supported clade TR I; one was unclassified and located on an isolated branch closely related to clade TR I (ADK4732/*A. subsaharianus* L.A. Parra, Hama & De Kesel); the remaining four species (CA820/*Agaricus* sp., ZRL2132/*Agaricus* sp., CA186/*A. freirei* Blanco-Dios, and LAPAG 531/*A. bohusii* Bon) respectively represent the four clades most closely related to clade TR I: clade TR a, clade TR b, clade/section *Xanthodermatei* Singer, and one of the clades of the polyphyletic section *Sanguinolenti* Jul. Schäff. & F.H. Møller ex L.A. Parra. These four samples are used as outgroup taxa.

Morphological study

Photographs were taken at the collecting site, and odor and color change on bruising were recorded in the field. The macroscopic characters, including chemical testing were determined according to the methodology described by Largent (1986). Schäffer's reaction is a cross reaction between aniline and nitric acid on the surface of pileus with a positive reaction typically orange-red in sections *Arvenses* and *Minores* (Parra 2008). KOH reaction was performed with 5% KOH solution on both pileus and stipe surface of fresh specimens. Colour terms are according to those of Kornerup and Wanscher (1978). Micromorphological features were examined from dried specimens following the protocols of Largent (1986) including anatomy of lamellae, pileipellis and partial veil, and features of basidiospores, basidia and cystidia. Measurements of anatomical features (spores, basidia and cheilocystidia) were presented based on at least 20 measurements, and include x, the mean of length by width ± SD; Q, the quotient of basidiospore length to width, and Q_m, the mean of Q-values ± SD.

Nucleic acid preparation, PCR and sequencing

Two methods were used depending on the laboratory. At the Institut National de la Recherche Agronomique (INRA), DNA was isolated following a CTAB protocol as described by Zhao *et al*. (2011). At Southwest Forestry University

TABLE 1. Collections included in the phylogenetic analyses.

Sample No.	Identification	Origin area	Date	Collector[b]	Location[c]	Habitat	Herb.[d]	GenBank
Sect. *Brunneopicti*								
PYP009	*A. niveogranulatus*	Thailand	2011/6/30	PA	Chiang Mai, MRC temple	In forest	MFLU	KJ540960
LD201125	*A. niveogranulatus*	Thailand	2011/9/1	JC	Chiang Rai, Doi Pui Site2	In forest	MFLU	KJ540957
LD201223	*A. niveogranulatus*	Thailand	2011/9/1	JC	Chiang Rai, Doi Pui Site2	In forest	MFLU	KJ540958
LD201124	*A. niveogranulatus*	Thailand	2011/9/1	JC	Chiang Rai, Doi Pui Site2	In forest	MFLU	KJ540959
C3182	*A.* sp. 5	Togo	2010/6/5	AG	Lomé, Campus of University	In urban park	TOGO	KJ540956
LD201127	*A.* sp. 4	Thailand	2011/9/1	JC	Chiang Rai, Doi Pui Site1	In forest	MFLU	KJ540955
NTS113[a]	*A. chiangmaiensis*	Thailand	2010/7/27	SK-JG	Chiang Mai, University P	In grassland	MF+CG	JF514513
ADK2564[a]	*A. brunneopunctatus*	Bénin	1999/6/8	AK	Niaouli, Plateau	In forest	BR	JF514518
ADK1992	*A. bingensis*	Bénin	1997/8/25	AK	Boukombe, Atacora	In grassland	BR	KJ540954
C3195	*A. bingensis*	Togo	2012/5/11	AG	Lomé, Campus of University	In urban park	HMT	KJ540953
C3184	*A. bingensis*	Togo	2010/5/6	AG	no data	no data	HMT	KJ540952
C3155	*A. bingensis*	Togo	2010/5/25	AG	Lomé, Campus of University	In urban park	HMT	KJ540950
C3190	*A. bingensis*	Togo	2010/6/5	AG	Lomé, Campus of University	In urban park	HMT	KJ540951
C3181	*A. bingensis*	Togo	2010/6/4	AG	Lomé, Campus of University	In urban park	HMT	KJ540949
CA926	*A. toluenolens*	Thailand	2011/6/11	PC-SR-SK	Chiang Mai, Lampang	In grassland	MF+CG	KJ540948
CA911	*A. toluenolens*	Thailand	2011/6/10	PC-SR-SK	Chiang Rai, University P	In grassland	MF+CG	KJ540947
NTT117[a]	*A.* sp. 3	Thailand	2010/7/27	KW	Chiang Mai, University P	In grassland	MFLU	JF514534
ADK4732[a]	*A. subsaharianus*	Burkina-Faso	2004/7/25	EM	Ouagadougou	In urban park	BR	JF440300
LD201237	*A. sordidocarpus*	Thailand	2012/6/6	JC	Chiang Mai, University P	In grassland	MFLU	KJ540946
NTS115[a]	*A. megacystidiatus*	Thailand	2010/7/27	SK	Chiang Mai, University P	In grassland	MFLU	KC971098
NTS116[a]	*A. megacystidiatus*	Thailand	2010/7/27	SK-JG	Chiang Mai, University P	In grassland	MF+CG	JF514532
LD2012179	*A. megacystidiatus*	Thailand	2012/8/28	ARB	Phitsanulok, SW	In grassland	MFLU	KF305946
LD2012168	*A. megacystidiatus*	Thailand	2012/8/5	JC	Chiang Mai, University P	In grassland	MFLU	KF305947
NT019[a]	*A.* sp. 2	Thailand	2009/7/22	SK-KW	Chiang Rai, Doi Tung Site3	In forest	MFLU	JF727844
NTT34[a]	*A. duplocingulatus*	Thailand	2010/6/19	KW	Chiang Mai, MRC	In forest	MFLU	JF514536
LD2012177	*A. duplocingulatus*	Thailand	2012/8/19	JC	Chiang Rai, Road to Phaya	In forest	MFLU	KJ540961
CA903	*A. duplocingulatus*	Thailand	2011/6/5	PC-SR-SK	Hua Kok Ma	In forest	CGAB	KJ540962

.....continued on the next page

TABLE 1. (Continued)

Sample No.	Identification	Origin area	Date	Collector [b]	Location [c]	Habitat	Herb. [d]	GenBank
LD201275	*A. duplocingulatus*	Thailand	2012/7/5	JC	Chiang Mai, TSW	In forest	MFLU	KJ540964
ZRL3064[a]	*A. duplocingulatus*	Thailand	2006/6/13	TO	Chiang Mai, DSPNP	In forest	BBH	KJ540966
LD201274	*A. duplocingulatus*	Thailand	2012/7/5	JC	Chiang Mai, TSW	In forest	MFLU	KJ540963
LD201233	*A. duplocingulatus*	Thailand	2012/6/4	JC	Chiang Mai, 3 km far to TL	In forest	MFLU	KJ540965
ZRL3031[a]	*A. duplocingulatus*	Thailand	2006/6/7	DD	Chiang Mai, DSPNP	In forest	BBH	JF691550
LD201218	*A. duplocingulatus*	Thailand	2012/6/1	KW	Chiang Mai, 3 km far to TL	In forest	MFLU	KJ540967
LAPAF1[a]	*A.* cf. *inoxydabilis*	Togo	2010/5/12	LP	Ola	In corn field	LAPAF	JF727841
LD2012105	*A. brunneosquamulosus*	Thailand	2012/7/18	JC	Chiang Mai, University P	In grassland	MFLU	KJ540968
NTT118[a]	*A. brunneosquamulosus*	Thailand	2010/10/27	KW-SK-JG	Chiang Mai, University P	In grassland	MFLU	KJ540970
LD201238	*A. brunneosquamulosus*	Thailand	2012/6/6	JC	Chiang Mai, University P	In grassland	MFLU	KJ540969
ZRL4017[a]	*A. brunneosquamulosus*	Thailand	2007/5/15	PS	Chiang Mai, Mae Taeng	In forest	BBH	JF691549
CA800[a]	*A.* sp. 1	Thailand	2010/7/25	JG-GB	Chiang Mai, University P	In grassland	MF+CG	JF727862
Clade "TR a"								
CA820[a]	*A.* sp.	Thailand	2010/7/27	JG-GB-SK	Chiang Mai, University P	In grassland	MF+CG	JF727861
Clade "TR b"								
ZRL2132[a]	*A.* sp.	Thailand	2005/8/21	ZR	Chiang Mai, Mae Taeng	In forest	BBH	JF691558
Sect. *Xanthodermatei*								
CA186[a]	*A. freirei*	France	2002/11/9	JG	Le Verdon	Under *Cupressus*	CGAB	DQ185553
Sect. *Sanguinolenti*								
LAPAG531[a]	*A. bohusii*	Czech Rep.	2002/9/15	OJ	Tremosnice near Caslav	Under *Carpinus*	LAPAG	JF797180

[a] Specimens previously used by Zhao *et al.* (2011)

[b] AK, A. De Kesel; ARB, A.R. Bandara; DD, D. Desjardin; GB, G. Barroso; JC, J. Chen; JG, J. Guinberteau; LP, L.A. Parra; KW, K. Wisitrassameewong; OJ, O. Juhasz; PC, P. Callac; PS, P. Sysouphanthong; SK, S. Karunarathna; SR, S. Rapior; ZR, R.L. Zhao

[c] DSPNP, Doi Suthep Pui National Park; P, Park or National Park; TL, Tharnthong Lodges; TSW, Thep Sadet Waterfall;W, Waterfall

[d] Herbarium: MF+CG = MFLU, CGAB (Collection du Germplasm des Agarics à Bordeaux)

(SWFU), commercial DNA extraction kit (E. Z. N. A. Forensic Kit, D3591-01, Omega Bio-Tek) was used for DNA extraction from dried specimens. Protocols for PCR by using primers ITS4 and ITS5 followed those of White *et al*. (1990) with some modifications (Zhao *et al*. 2010). Sequencing was performed on ABI Prism Genetic analyzer (Applied Biosystems) at Beckman Coulter Genomics, England or on ABI 3730 XL DNA analyzer (Applied Biosystems) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China. Twenty-five novel ITS1+2 sequences used in this study were deposited in GenBank database under accession numbers KJ540946 to KJ540970 (Table 1).

Sequence alignment and phylogenetic analyses

The original sequences produced from this work plus the other sequences retrieved from GenBank were initially aligned using T-coffee ver 8.99 (Notredame *et al*. 2000), then manually adjusted in BioEdit v. 7.0.4 (Hall 2007). The alignment has been submitted to TreeBase (submission ID 15483).

Maximum parsimony (MP) analysis, was performed using PAUP* 4.0b10 (Swofford 2004), by heuristic searches with unordered characters, random addition of sequences, gaps treated as missing data, and the tree bisection-reconnection (TBR) branch swapping. Maximum likelihood (ML) analysis was performed on Phylogeny.fr platform (http://www.phylogeny.fr/). The phylogenetic tree was constructed using the ML method implemented in the PhyML ver 3.0 (Guindon & Gascuel 2003). The HKY85 substitution model was selected with an estimated proportion of invariable sites of 0.674 and assuming 4 gamma-distributed rate categories. The gamma shape parameter 1.366 was directly estimated from the data. Bootstrap values (BS) were obtained from 1000 or 100 replicates for MP or ML tree, respectively. Bayesian analysis was performed with MrBayes 3.1.2 (Ronquist & Heulsenbeck 2003). One million generations using a GTR+I+G model nucleotide substitution detected by MrModeltest 2.2 (Nylander 2004) were run for six Markov chains and sampled every 100th generation resulting in 10,000 trees. Those trees sampled prior to searches reaching a split deviation frequency value reaching 0.01 were discarded as the burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (PP) of the individual clades. Trees were viewed in TreeView or TreeDyn and exported to graphics programs (Page 1996; Chevenet *et al*. 2006).

Species-specific ITS markers

Comparisons were made between 43 sequences used in this study. Positional data were derived from the sequences of a species. Characters that were unique (within the section) to all representatives of a given taxon are indicated by uppercase type and are given with flanking sequences on both sides. IUPAC codes as Y does not indicate heteromorphisms or ambiguity but that T or C are found at this position depending on the sequence. It should be noticed that these unique data is based on currently available sequences, and with more taxa, reassessment might be needed.

Results

Phylogenetic analyses

The 43 sequences varied in length from 647 to 661 nts. The final alignment contained 643 characters, of which 457 were constant, 42 were parsimony-uninformative and 103 were parsimony-informative characters. The phylogenetic trees generated by ML, MP and Bayesian methods exhibited very similar topologies. Differences are only noted in the branching pattern of the positions of samples NTT 117 and CA800. The Most Likelihood (ML) tree is shown in Fig. 1, with bootstrap support values above 50% and Bayesian posterior probability values above 90% are shown. In all analyses the four outgroup taxa group at the base of the tree.

Clade TR I is relatively well-supported (ML/MP/PP = 88/70/1; Fig. 1) and contains 16 entities. Four subclades: A, B, C and D are revealed with high bootstrap values in both analyses. Subclade A is strongly supported (99/98/1) and includes three species: *A. brunneosquamulosus* sp. nov., *A. duplocingulatus* Heinem. and *A.* cf. *inoxydabilis* Heinem. Subclade B is fully supported (100/100/1) and includes two species: *A. megacystidiatus* Karunarathna, Guinb. & K.D. Hyde and *Agaricus* sp. 2/NT019. Subclade C is well-supported (95/89/1) and includes *A. sordidocarpus* sp. nov. and *A. subsaharianus*. Subclade D which is well-supported (72/83/0.92) includes six species: *A. bingensis*, *A. brunneopunctatus* nom. nov., *A. chiangmaiensis* Karunarathna, Guinb. & K.D. Hyde, *A. niveogranulatus* sp. nov., *Agaricus* sp. 4/LD2011027 and *Agaricus* sp. 5/C3182. The three remaining species *Agaricus* sp. 1/CA800, *Agaricus* sp. 3/NTT117 and *A. toluenolens* sp. nov. are on isolated branches. Even though four subclades were well-supported in whatever the used method, due to the variable positions of *Agaricus* sp. 1/CA800 and *Agaricus* sp. 3/NTT117, the phylogenetic relationships between these two species and the subclades remains unresolved within clade TR I.

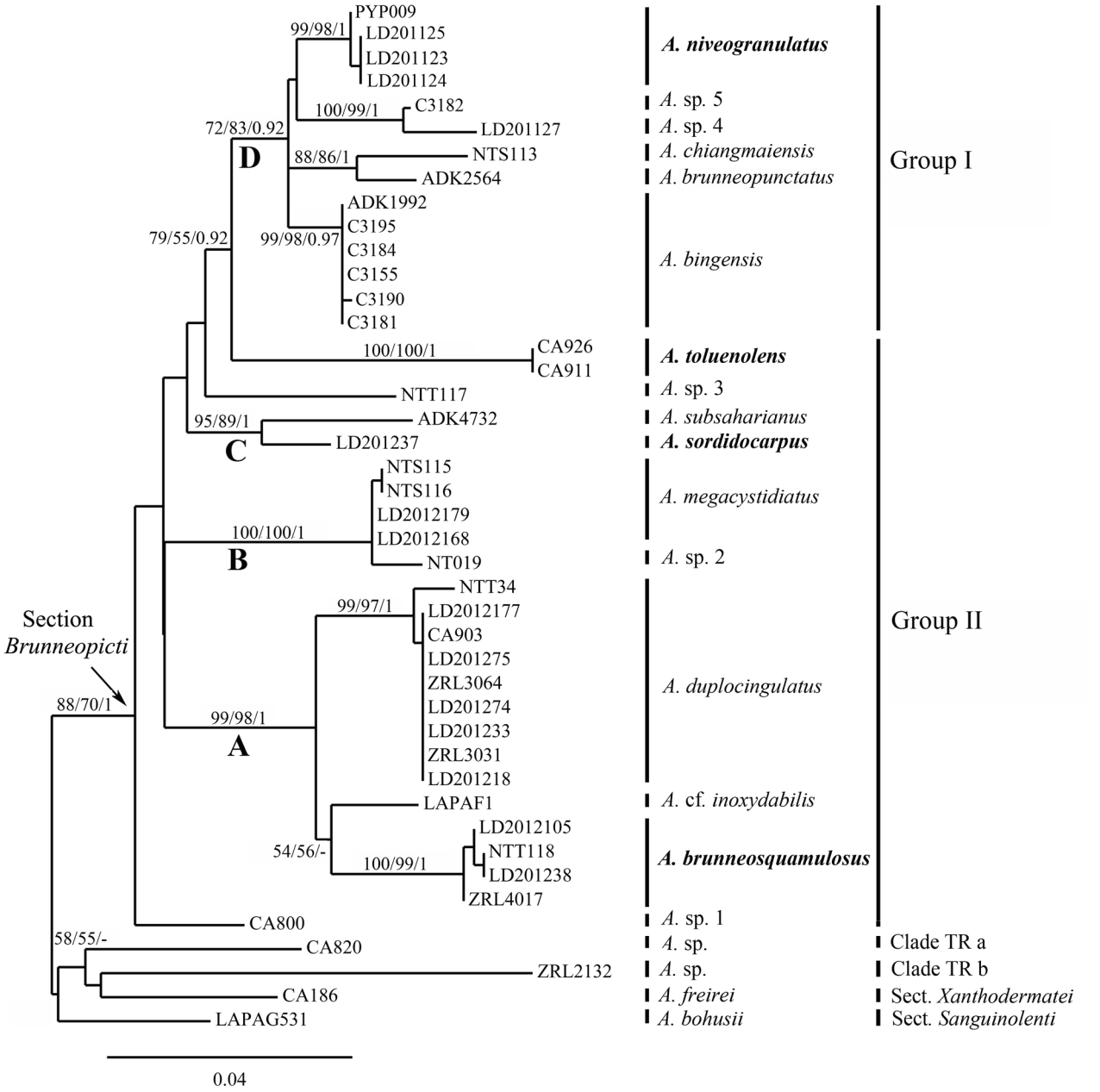

FIGURE 1. Maximum likelihood (ML) phylogram of *Agaricus* section *Brunneopicti* drawn from dataset of 43 ITS1+2 sequences belonging to 16 species of *Brunneopicti* and 4 outgroup species of related clades or sections TR a, TR b, *Xanthodermatei* and *Sanguinolenti* respectively. Bootstrap support values above 50% and Bayesian posterior probability values above 90% are shown (ML/MP/PP). The four novel species described in the present paper are in boldface. Subclades A–D and two morphological groups are shown. Group I refers to species exhibiting punctiform squamules on both surface of pileus and stipe; Group II refers to species exhibiting larger (non-punctiform) squamules on the pileus surface only.

In comparison with the previous analyses of Zhao *et al*. (2011), the inclusion of 25 recent collections in the analyses allowed us to distinguish six more species in the clade TR I. Moreover ADK4732 (*A. subsaharianus*) which remained unclassified on an isolated branch closely related to clade TR I is now included in this clade. In contrast, samples NTT34 and ZRL3031 previously considered as belonging to two sister putative species now belong to the same species (*A. duplocingulatus* Heinem). Finally, the clade TR I comprises 16 species.

Section Brunneopicti

Our analyses showed that *Agaricus brunneopunctatus* (a new name for *A. brunneopictus*, the type species of *A.* section *Brunneopicti*) and *A. bingensis*, the two species initially placed in section *Brunneopicti*, belong to subclade D within clade TR I. Zhao *et al*. (2011) did not establish the link between section *Brunneopicti* and clade TR I because no specimens

of *A. bingensis* were examined and the examined sporocarps of the herbarium collection of *A. brunneopunctatus* (ADK2564) were immature. These two problems were resolved in the present study. On the one hand, six specimens whose macro and microscopical characters completely agreed with the original concept of *A. bingensis* were included in the analyses. On the other hand, we received a dried sporocarp of ADK2564 other than those examined by Zhao *et al*. (2011); this sporocarp exhibited mature spores and other microscopical characters (in addition to its macroscopical ones) in agreement with those of the original description of *A. brunneopunctatus*. Consequently, TR I, a major clade of *Agaricus*, corresponds to section *Brunneopicti*, as it contains *A. brunneopunctatus*, the type species of this section. Based on morphological characters, two groups of species are considered below: the first one is monophyletic since it corresponds to subclade D; the second one is paraphyletic with respect to subclade D since it includes subclades A, B, C and the three isolated branches.

Taxonomy

Agaricus section ***Brunneopicti*** Heinem. Bull. Jard. Bot. État Bruxelles 26: 71 (1956).

MycoBank MB 808477

Orig. Diag.: *Velum universale ex squamulis parvis punctiformibus constans; elementa veli brevia, crasse tunicata, asperulata. Annulus membranaceus, amplus.*

Type:—*Agaricus brunneopictus* Heinem. & Gooss.-Font., Bull. Jard. Bot. État Bruxelles 26: 74 (1956).

Delimitation of Agaricus section Brunneopicti:—Schäffer's reaction negative or rarely weakly positive, KOH reaction positive but usually faint. Pileus roughly covered with either punctiform squamules from the universal veil, or brownish larger squamules. Stipe cylindrical to clavate or sub-bulbous, often with round base. Double or complex annulus with scales and cortinate fibrils on the lower surface. Context discoloration when bruised faint to strong: yellow, orange, rufescent, brownish rufescent or red. Odor from pleasant bitter almond to unpleasant like phenol or solvent used in marker pen. Cheilocystidia usually present, pyriform to broadly clavate. Absence of unique unifying characters in ITS 1+2. *Agaricus kivuensis* originally placed in this section is excluded here because it belongs to the unrelated clade TR III (Zhao *et al*. 2011). Geographical distribution range: known only from palaeotropics.

Two groups are considered: Group I is characterized by brownish punctiform squamules on the pileus surface and concolor or whitish punctiform squamules on the stipe surface close to the base; Group II is characterized by brownish non-punctiform squamules or appressed scales only on the pileus surface.

Species included in Group I:

Agaricus bingensis Heinem., Bull. Jard. Bot. État Bruxelles 26: 72 (1956). Fig. 7A

Species-specific ITS marker:—gtcaCAytat @ 222–223.

Comments:—*Agaricus bingensis* is a common species largely distributed in Africa and characterized by a large pileus (up to 25 cm diam.) with crowded punctiform squamules and large spores (8.3–10 × 5.1–5.7 µm). This species has been originally described by Heinemann (1956) from Binga (Democratic Republic of the Congo) and placed in *Brunneopicti* based on its morphology and Schäffer's reaction negative. Pegler (1977) reported this species later from Uganda but accommodated it in section *Agaricus* because of the slightly reddening discoloration of the context on exposure; moreover section *Brunneopicti* was not retained in his classification. Our collections from Togo and Bénin correspond well with the description of Heinemann. According to the phylogenetic analyses, it belongs to section *Brunneopicti* and exhibits morphological affinities to *A. brunneopunctatus*. Edibility: this species is used for food, particularly by the Acholi tribe from Uganda (Pegler 1977). Geographical distribution range: known only from Africa (Bénin, Democratic Republic of the Congo, Togo, Uganda).

Agaricus brunneopunctatus L.J. Chen, Callac & L.A. Parra, *nom. nov.* Fig. 7B

≡*Agaricus brunneopictus* Heinem. & Gooss.-Font., Bull. Jard. Bot. État Bruxelles 26: 74 (1956) [replaced synonym] *nom. illeg*., Art. 53.1, *non Agaricus brunneopictus* Berk. & Broome, J. Linn. Soc., Bot. 11(56): 533 (1871) [*brunneo-pictus*] *qui est Pluteus brunneopictus* (Berk. & Broome) Sacc., Syll. Fung. 5: 669 (1887) [*brunneo-pictus*].

Mycobank MB 808479

Species-specific ITS marker:—caac[CCC]ctta @ 459–461.

Comments:—*Agaricus brunneopunctatus* was described from material collected on grassland in Democratic Republic of the Congo and designated as the type species of section *Brunneopicti* (Heinemann 1956). Original diagnosis: *Pileus carnosus, hemisphaericus deinde convexus, brunneolus, centro saturior, squamulis furfuraceis. Stipes elongatus, plenus, cylindraceus deorsum incrassatus, albidus deinde brunneolus, deorsum brunneo-furfuraceus; annulus amplus, albus, margine laceratus. Lamellae confertae, latiusculae, liberae, albae, deinde roseae, denique atrobrunneae. Caro alba, fracta brunneola; sapor ut odor gratus, amygdalinus. Sporae atrobrunneae, 7,6–8,5 µ × 4,9–5,3 µ, ellipticae. Cheilocystidiae piriformes, 18–35 µ × 10–16 µ*. Later, this species was also reported from Singapore (Heinemann 1980). Our collection ADK2564 from Bénin, with basidiospore size 7,3–7,8–8,45 × 4,4–4,9–5,4 µm agrees well with the protologue. This species was described with a medium-sized pileus (10 cm diam.) with an almond odor which was not noted in the collection from Singapore (Heinemann 1956, 1980). A strong phenol-like odor was detected in our collection from Bénin. Edibility is unknown. Geographical distribution range: known only from the palaeotropics.

Agaricus chiangmaiensis Karunarathna, Guinb. & K.D. Hyde, Chiang Mai J. Sci. 41(4): 773 (2014).

Species-specific ITS markers:—atccCacct @ 78; tgaaGgcac @ 167; gcacGgctg @ 172; ctgtTctCtact @ 178, 181; aaagTgggc @ 483.

Comments:—*Agaricus chiangmaiensis* was described from material collected on grassland in Thailand (Karunarathna *et al*. 2014). This species can be recognized by its relatively large sporocarps (up to 17 cm diam.), light brown, punctiform, innate squamules, on the pileus surface, white stipe with powdery granules on its lower one-third surface, clearly visible annulus with a cog-wheel on its lower surface close to its margin, and relatively large spores (7–8.5 × 3–4 µm). According to the phylogenetic tree of Fig. 1, *A. chiangmaiensis* and *A. brunneopunctatus* are sister species. Edibility of this species is unknown. Geographical distribution range: known only from Asia (Thailand).

Agaricus niveogranulatus L.J. Chen, R.L. Zhao, Callac & K.D. Hyde *sp. nov.* Fig. 2, 7C–G

MycoBank MB 808172

Etymology:—*niveogranulatus* referring to the white colored granulose squamules.

Original description:—*Macroscopical characters*: Pileus 10–16 cm diam., 4–8 mm broad, parabolic to hemispherical when young, then convex, sometimes more or less truncated or slightly depressed at disc, finally applanate; margin straight, appendiculate; surface dry, covered with granulose or punctiform squamules which are white except at disc where they are brownish (5B2); surface usually splitting in radial interwoven bands; Lamellae free, crowded, ventricose, lamellulae with more than 5 series, 5–10 mm broad, first white, then pink, brownish orange (7C3), light brown (7D4), brown, to finally dark brown with age. Stipe 70–165 × 7–17 (base 10–25) mm, cylindrical to clavate, occasionally slightly bulbous, surface smooth, while with white punctiform scales close to base, occasionally with short rhizomorphs, white, hollow; slowly bruising light brown to brownish red. Annulus superous, white, with two layers: a smooth membranous upperside and a woolly floccose underlayer connected by cortinate fibrils to the stipe, then breaking in radial tatters which can form a cogwheel near the stipe or can remain attached to the pileal margin, wide and finally hanging down over a length of 13–27 mm. Context, firm, white, color not changed by cutting. Odor phenol-like.

Macrochemical reactions: KOH reaction slightly yellow. Schäffer's reaction negative.

Microscopical characters: Spores 7.0–8.8(–9.3) × 3.8–5.1 µm, [x = 8.1 ± 1.1 × 4.5 ± 0.7, Q = 1.45–2.3, Qm = 1.79 ± 0.32, n = 20], ellipsoid to oblong, rarely cylindrical, smooth, dark brown, thick-walled. Basidia 16–24 × 6.8–8.5 µm, clavate, hyaline, smooth, 4-spored. Cheilocystidia 10–21 × 7–14.5 µm, pyriform or sphaeropedunculate, vesicular, sometimes shortly catenulate, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis composed of hyphae of 9.5–27 µm diam., globose to shortly cylindrical, catenulate, deeply constricted at the septa. Annulus hyphae 5–8 µm in diam., apex inflated to 10–13 µm in diam., cylindrical to long clavate, hyaline, smooth.

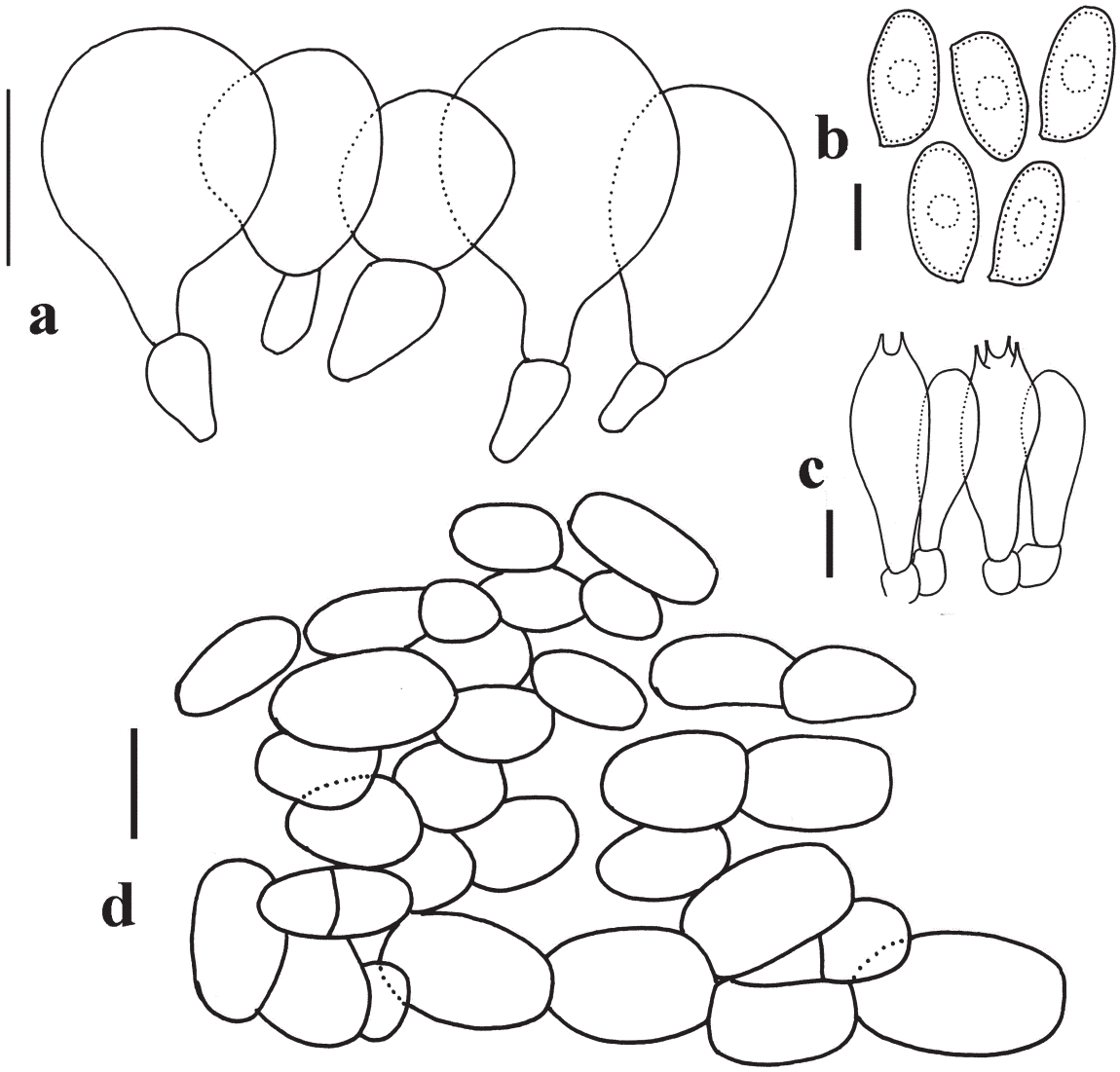

FIGURE 2. Microcharacters, *A. niveogranulatus* (MFLU11 1307, holotype), a. Cheilocystidia. b. Spores. c. Basidia. d. Pileipellis. Scale bars: a, c = 10 µm; b = 5 µm; d = 20 µm.

Habitat:—scattered or gregarious, on soil, in open areas of *Dipterocarpus* forest; bamboo woods, or rich soil mixed with rotted leaves of grassland, it also grows in parks and gardens.

Geographical distribution range:—known only from Asia (Thailand).

Species-specific ITS markers:—ggatTtgca @ 154; tttgCtgta @ 473.

Type:—THAILAND. Chiang Rai Province: Doi Pui site (2), UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 1 September 2011, *Jie Chen*, *LD201124* (holotype MFLU11 1307!, isotype SWFC!).

Additional specimens examined:—THAILAND. Chiang Rai Province: Doi Pui site (2), UTM-N2379744.485281, UTM-E653217.753714, alt. 1600m, 1 Septemper 2011, *Jie Chen*, *LD201123*, *LD201125* (MFLU11 1306!, MFLU11 1308!); Mae Fah Luang University, 26 July 2012, *Jie Chen*, *Phongeun Sysouphanthong*, *LD2012140* (MFLU12 0972!); Khun Korn Waterfall, 31 July 2012, *Jie Chen*, *LD2012147* (MFLU12 0979!); Chiang Mai Province: Pathammikaram Temple, 30 June 2011, *Pamela Alva*, *PYP009* (MFLU11 1404!).

Comments:—*Agaricus niveogranulatus* is characterized by its generally large sporocarps, pileus surface with white, well-defined, innate, punctiform squamules; double layered annulus, white punctiform scales on the lower part of stipe surface, relatively large basidiospores and catenulate pileipellis hyphae formed by globose or shortly cylindrical cells; but the most remarkable trait of this species is the aspect taken by the pileus surface during its development when it splits in radial interwoven bands and remains pure white except at the disc (Fig. 7. C–D). We also observed that with age and/or dryness the pileus surface can also crack in larger brownish scales at the disc and show

squamules elsewhere; in wet conditions the pileus can have pink tones. The most phenetically similar species are *A. chiangmaiensis* and *A. brunneopunctatus*, however the former differs in small flakes on the center of pileus surface and smaller spores (7–8.5 × 3–4 µm), and *A. brunneopunctatus* differs in larger cheilocystidia (18–35 × 10–16 µm) and almond odor (Karunarathna *et al*. 2014; Heinemann 1956). *Agaricus bingensis* also shares some traits, but it could be easily distinguished by its larger basidiospores with truncated apex (Heinemann 1956).

Agaricus sp. 4/LD201127

Species-specific ITS markers:—ttacAtggc @ 184; ccacAgaat @ 192; aattCatat @ 225; tcaaAggtc @ 520.

Comments:—A single collection of this entity was collected from Thailand in a forest clearing. Phylogenetic analyses (Fig. 1) and morphological traits indicate this species belongs to section *Brunneopicti*: the grayish-brown punctiform squamules on both surfaces of pileus and stipe base, pinkish discoloration of the context when cut and odor of bitter almonds. Unfortunately samples were immature for a complete description. In section *Brunneopicti*, this species is sister to *Agaricus* sp. 5/C3182.

Agaricus sp. 5/C3182

Species-specific ITS marker: ttacGtggc @ 184.

Comments:—This single collection was found under *Azadirachta indica* in Togo. According to the phylogenetic analyses this sample is a sister species to *A*. sp. 4/LD201127. Morphologically, it is relatively similar to *A. bingensis* with punctiform squamules observed on the stipe. However, the squamules of the pileus surface which are concentrically arranged, densely to scattered from the center to the margin, are typically punctiform at the disc only. Light brownish discoloration has been noted on the stipe surface when bruised. The pileus diameter exceeds 10 cm. This is certainly a good species but more collections are needed to circumscribe it.

Species included in Group II:

Agaricus brunneosquamulosus L.J. Chen, R.L. Zhao, K.D. Hyde & Callac *sp. nov.* Fig. 3, 8A–D

MycoBank MB 808173

Etymology:—referring to the brownish colored squamules.

Original description:—*Macroscopical characters*: Pileus 3–6 cm in diam., 3–4 mm broad, convex and more or less truncated at the top to plano-convex when young, finally applanate when mature; margin straight; surface dry, with ferruginous brown appressed or occasionally slightly erect triangular squamules (about 1 mm in width) regularly distributed on a white background, more dense towards the disc which is entirely ferruginous brown. Lamellae free, crowded, ventricose, lamellulae with more than 5 series, 3–4 mm broad, pink, to brownish gray (6C2), dark brown with age. Stipe 33–75 × 6–10 (base 9–12) mm, subcylindrical or clavate, surface smooth, white, hollow; bruising light yellow, orange and then becoming light brown within few minutes. Annulus superous, double and complex with woolly scales and cortinate fibrils on the lower surface, white. Context firm, white, slightly pinkish by cutting. Odor phenol-like.

Macrochemical reactions: KOH negative or slightly yellowish (only observed on collection LD2012105); Schäffer's reaction negative.

Microscopical characters: Spores 5.3–6.8 × 3.4–4 µm, [x = 5.7 ± 1.1 × 3.7 ± 0.3, Q = 1.39–1.72, Q_m = 1.52 ± 0.2, n = 20], ellipsoid, smooth, brown, thick-walled. Basidia 18–20 × 7.4–8.5 µm, clavate, hyaline, smooth, 4-spored. Cheilocystidia rarely present, 12–25 × 7–15 µm, pyriform to vesicular, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis composed of hyphae of 5–9 µm diam., cylindrical, light brown, smooth, slightly constricted at the septa.

Habitat:—solitary on soil of grassland, in garden.

Geographical distribution range:—known only from Asia (Thailand).

Species-specific ITS markers:—tctgCtggg @ 103; ggtcTgcct @ 125; tttcCatcg @ 206; ggagActat @ 214.

Type:—THAILAND. Chiang Mai Province: Chiang Mai University, 18 July 2012, *Jie Chen*, *LD 2012105* (holotype MFLU12 0943!, isotype SWFC!).

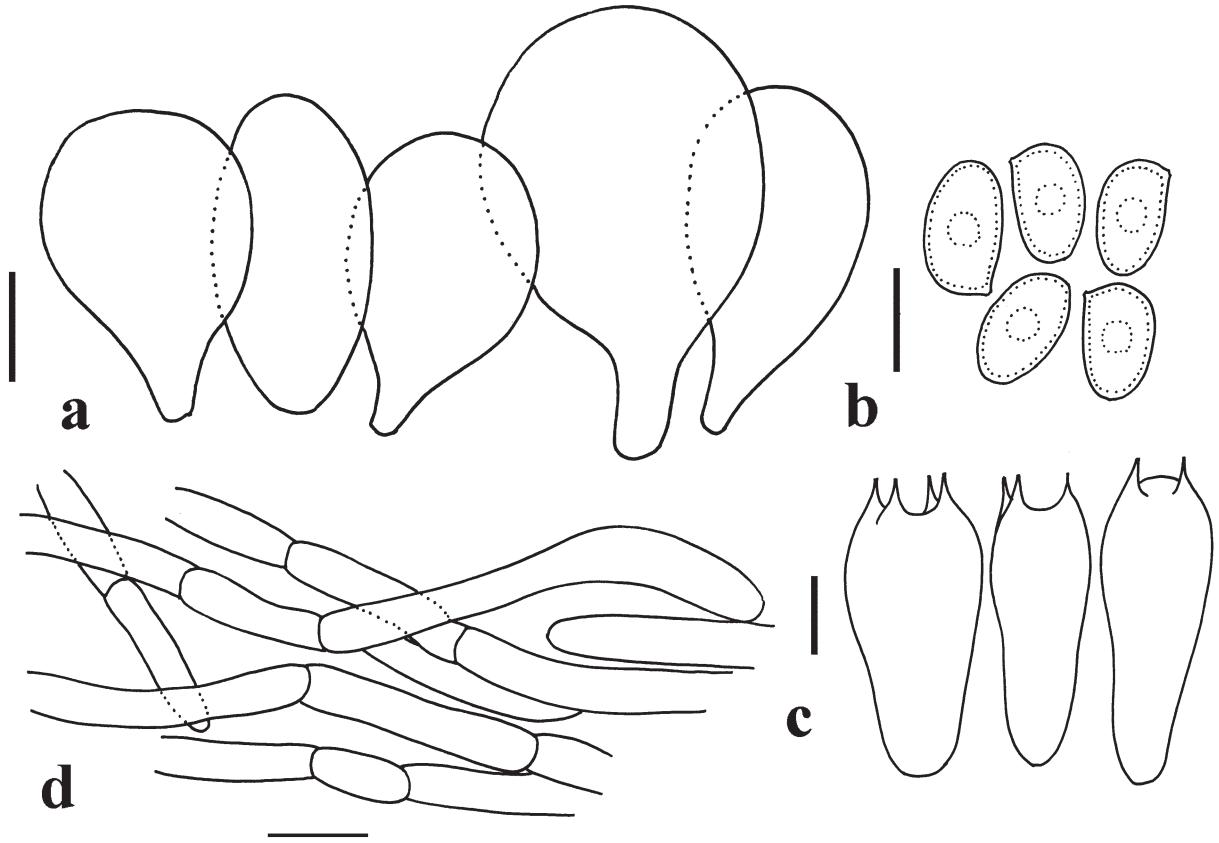

FIGURE 3. Microcharacters, *A. brunneosquamulosus* (MFLU12 0943, holotype), a. Cheilocystidia. b. Spores. c. Basidia. d. Pileipellis. Scale bars: a, b and c = 5 µm; d = 20 µm.

Additional specimens examined:—THAILAND. Chiang Mai Province: Mae Taeng, 15 May 2007, *Phongeun Sysouphanthong*, *ZRL4017* (MFLU10 0697!); Chiang Mai Province: Chiang Mai University, 27 July 2010, *Komsit Wisitrassameewong*, *NTT118* (MFLU14 0029!); same location, 6 June 2012, *Jie Chen*, *LD201238* (MFLU12 0882!).
Comments:—*Agaricus brunneosquamulosus* is characterized by small sporocarps, tiny ferruginous brown appressed triangular squamules on the pileus surface, complex annulus with woolly scales and cortinate fibrils on its lower surface. Both *A. inoxydabilis* and *A. duplocingulatus* exhibit ferruginous brown squamules on the pileus. However *A. inoxydabilis* has larger spores (5.5–7 × 3.7–4.5 µm) and a membranous annulus. *A. duplocingulatus* typically has two distinct annulus and catenulate cheilocystidia (Heinemann 1980 and this study). *Agaricus megacystidiatus* differs from *A. brunneosquamulosus* by much larger spores (8–9.5 × 4–5 µm) and cheilocystidia (30–45 × 8–25 µm).

The ITS1+2 sequence of the collection LD201238 has three heteromorphisms, collection LD2012105 which was collected later in the same region has only one, while the third collection ZRL4017 differs at three positions: two heteromorphisms and a deletion. However, excluding the heteromorphic positions with shared alleles, the samples do not differ from each other at more than one position. In the absence of significant morphological differences among the three collections we considered that they belong to the same species.

Agaricus duplocingulatus Heinem., Bull. Jard. Bot. Natl. Belg. 50(1–2): 32 (1980). Fig. 4, 8E–F

Pileus 4–6 cm diam., 3 mm broad, plano-convex with umbo or truncated at the top disc; margin straight, exceeding the lamellae; surface dry, with brown ferruginous appressed fibrillose squamules on a cream white background; squamules congregated on the disc and concentrically arranged on elsewhere. *Lamellae* free, crowded, ventricose, lamellulae with more than 5 series, 3 mm broad, pink to orange gray (5B2), then grayish brown (8D3), finally brown with age. *Stipe* 54–62 × 5–5.5 (base 8–10) mm, cylindrical with enlarged base, surface smooth, white, occasionally with short rhizomorphs, hollow; color change to light orange first, then become to reddish brown when bruised. *Annulus* double, the above one membranous, upper surface smooth, lower surface fibrillose-woolly, connected with stipe by cortinate fibrils; the below one bracelet-like, easily broken when mature, movable, white. *Context* firm, cream white. *Odor* phenol-like.

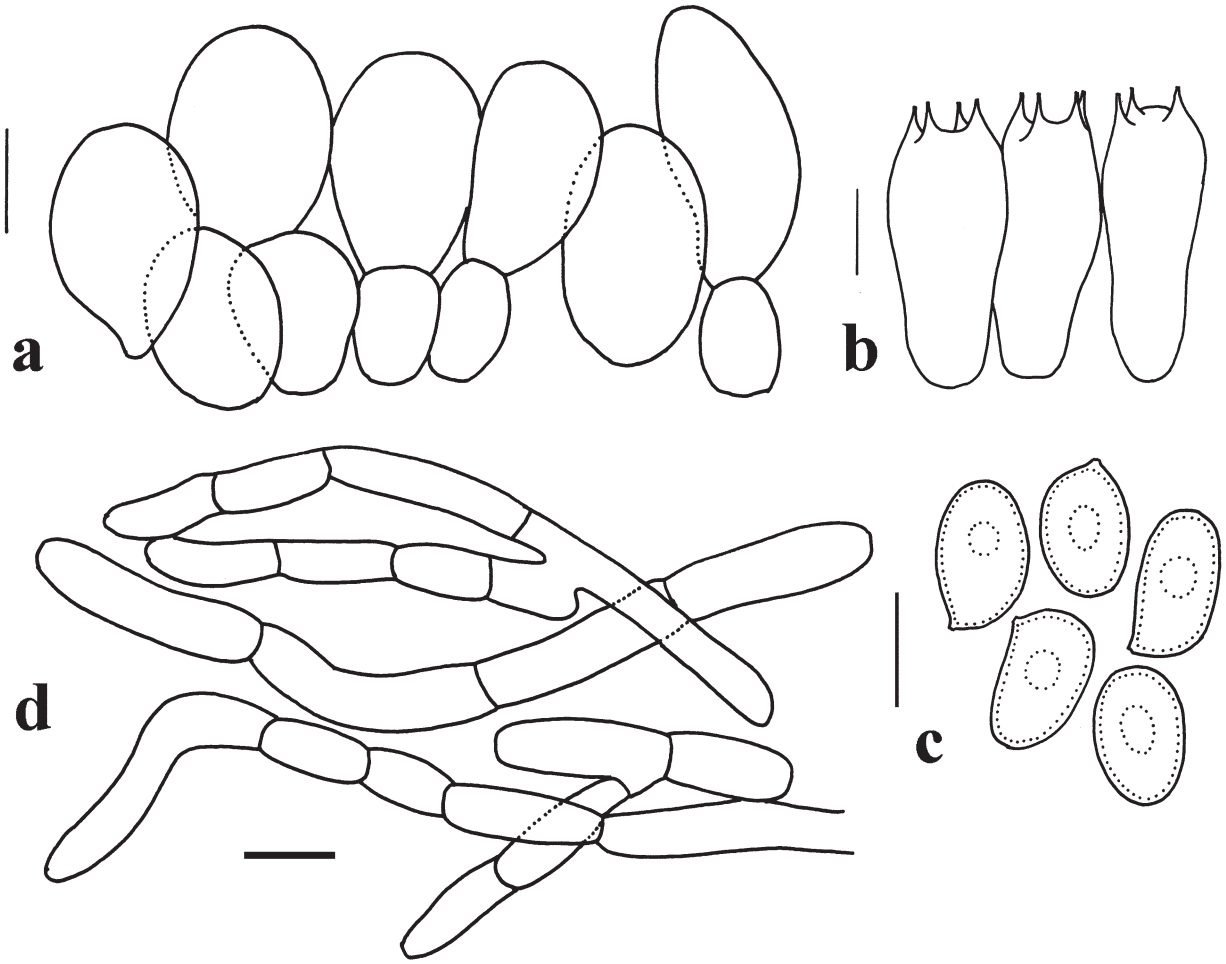

FIGURE 4. Microcharacters, *A. duplocingulatus* (MFLU12 0914), a. Cheilocystidia. b. Basidia. c. Spores. d. Pileipellis. Scale bars: a and d = 10 μm; b and c = 5 μm.

Macrochemical reactions: KOH reaction no reaction on pileus, slightly yellow on context. Schäffer's reaction negative.

Spores 4.8–5.4–6(–6.6) × 3.5–4.5 μm, [x = 5.4 ± 0.6 × 3.9 ± 0.6, Q = 1.25–1.46, Q_m = 1.39 ± 0.14, n = 20], ellipsoid, smooth, brown, thick-walled. *Basidia* 12–17.5 × 6.5–7.8 μm, clavate to broadly clavate, hyaline, smooth, 4-spored. *Cheilocystidia* 12–24 × 9.5–17.5 μm, pyriform, vesicular, sometimes shortly catenulate, hyaline, smooth. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 4.3–9.5 μm diam., cylindrical, light brown, smooth, slightly constricted at the septa, rarely branched.

Habitat:—solitary on rich soil covered with leaf litter, in forest.

Geographical distribution range:—known only from Asia (Thailand, Singapore).

Species-specific ITS markers:—ytgcGgtgy @159; ccytTtact @ 467.

Specimens examined:—THAILAND. Chiang Mai Province: Doi Suthep, 07 June 2006, *Tim*, *ZRL3031* (BBH19547!); same location, 13 June 2006, *TO, ZRL3064* (BBH19580!); MRC, 19 June 2010, *Komsit Wisitrassameewong*, *NTT34* (MFLU14 0028!); 3 km down the road from Tharnthong Lodges, 1 June 2012, *Komsit Wisitrassameewong*, *LD 201218* (MFLU12 0862!); 3 km far from Tamthong Lodges, 4 June 2012, *Jie Chen*, *LD201233* (MFLU2012 0877!); Thep Sadet Waterfall, 5 July 2012, *Jie Chen*, *LD 201274*, *LD 201275* (MFLU12 0913!, MFLU12 0914!); Chiang Rai province: Road to Phayao, 5 July 2012, *Jie Chen*, *LD2012177* (MFLU12 1002!).

Comments:—*Agaricus duplocingulatus* was originally described from Singapore and it is characterized by brown ferruginous appressed squamules on the pileus surface, double annulus with the lower one movable, ochraceous to reddish discoloration when cut or bruised and catenulate cheilocystidia (Heinemann 1980). The Thai material agrees with the original description, except for a slightly wider range of spore size (5.3–6.1 × 3.5–4.1 μm, Heinemann). Intraspecific variability is also remarkable among the ITS 1+2 sequence data of the nine studied samples since there are 16 polymorphic positions (Table 2). In Zhao *et al*. (2011), ZRL3031 and NTT034 were considered as two putative

sister species because they differ at six positions. However, with a larger sampling, nucleotide characters at these positions appear to be alleles shared by different samples; more particularly LD2012177 is heteroallelic at these six positions (Table 2, in boldface). Such a level of intraspecific variability is very rare in the genus *Agaricus* and should require more investigation to know to what extent *A. duplocingulatus* should represent a complex of species. Presently we do not detect major geographical, ecological or morphological differences that could be correlated with the genetic divergence among the samples.

TABLE 2. Polymorphism at 16 positions within ITS 1+2 rDNA sequences of nine samples of *Agaricus duplocingulatus*.

Sample	Positions in the ITS 1+2 alignment (654 nts)															
	109	146	164	165	197	207	209	224	235	272	387	465	516	519	553	613
ZRL3031	T[a]	T	G	**A**[b]	**C**	**A**	C	T	**T**	T	G	T	**C**	T	C	**T**
NTT34	T	T	G	**C**	**T**	**G**	Y	Y	**C**	T	G	T	**T**	T	C	**C**
LD2012177	Y	T	G	**M**	**Y**	**R**	C	T	**Y**	T	G	T	**Y**	T	Y	**Y**
ZRL3064	C	T	G	A	C	A	C	T	T	T	G	T	T	C	C	T
LD201218, LD201274	C	Y	R	A	C	A	C	T	T	T	G	T	Y	Y	C	T
LD201275	C	Y	G	A	C	A	C	T	T	Y	G	T	Y	Y	Y	T
LD201233	Y	T	G	A	C	A	C	T	T	T	G	T	C	T	Y	T
CA903	Y	T	G	R	C	A	C	T	T	T	R	Y	Y	Y	C	T

[a] Characters homoallelic (A = A/A, C = C/C, G = G/G, or T = T/T) or heteroallelic (M = A/C, R = A/G, or Y = C/T)

[b] Characters in boldface are those which differ at six positions between ZRL3031 and NTT034 but which are all heteroallelic in LD2012177.

When Heinemann (1980) described *A. duplocingulatus*, he noted that the systematic position of this species is unclear because the lower ring is typically found in section *Xanthodermatei*, while catenulate cheilocystidia is generally observed in section *Arvenses*. Because of its reddish discoloration, this species also keys out in section *Sanguinolenti* (Heinemann 1980). At this time it could not be placed in section *Brunneopicti* that was limited to species exhibiting punctiform squamules.

Agaricus cf. ***inoxydabilis*** Heinem., Bull. Jard. Bot. Natl. Belg. 50(1–2): 18 (1980).

Species-specific ITS markers:—tcggGgcat @ 42; tcagActct @ 127; gggcCttga @ 267.

Comments:—*Agaricus inoxydabilis* is a tropical species originally recorded from Singapore and also cited from Indonesia and Malaysia (Heinemann 1980). However the identification of the Malaysia collection might be doubtful because the material was not in good condition. *A. inoxydabilis* is characterized by a medium sized pileus (8 cm diam.) covered with brownish fibrillose squamules; large annulus with ochraceous flakes on its lower surface; context white, color unchanged, spore size 5.5–7 × 3.7–4.5 µm and pyriform cheilocystidia. Our collection showing a negative Schäffer's reaction and a weakly positive KOH reaction globally agrees with the original description. Curiously, Heinemann (1980) did not indicate the odor and placed this species in section *Sanguinolenti*, while a light aromatic pleasant odor differing from almond or aniseed odor has been noticed for our collection from Togo. From morphological examination this collection likely belongs to *A. inoxydabilis*. However, ITS sequence data of collections from Asia where this species was initially described are required to definitely confirm the identification, knowing that until now such a distribution on both continents has not ever been confirmed for any species of the section. According to the phylogenetic analyses *A.* cf. *inoxydabilis* is a sister species to *A. brunneosquamulosus* in section *Brunneopicti*. Edibility of the species is unknown.

Agaricus megacystidiatus Karunarathna, Guinb. & K.D. Hyde, Chiang Mai J. Sci. 41(4): 775 (2014).

Species-specific ITS markers:—absent.

Comments:—*Agaricus megacystidiatus*, recently described from Thailand, is a tropical species usually found in grassland, solitary or sometimes gregarious. It is characterized by medium sized sporocarps, yellowish-brown scaly pileus up to 5 cm in diameter; 8–9.5 × 4–5 µm sized oblong basidiospores; relatively large sized pyriform to broadly clavate cheilocystidia and distinctively reddish discoloration on stipe surface when bruised (Karunarathna *et al*. 2014).

From the ITS 1+2 sequence data, the closest relative is one undescribed species (*Agaricus* sp. 2/ NTF019) which differs at eight nucleotide positions and together formed a strongly supported clade. Edibility of this species is unknown.

Agaricus sordidocarpus L.J. Chen, Callac & K.D. Hyde *sp. nov.* Fig. 5, 8G–H

MycoBank MB 808174

Etymology:—*sordidocarpus* in reference to the dirty appearance of the pileus given by the fibrillose squamules and their gray tint particularly after rain.

Original description:—*Macroscopical characters*: Pileus 4 cm diam., 0.5 cm broad, convex and more or less truncated at the top; margin eroded and appendiculate; surface dry, with brownish gray (6E2) appressed squamules (about 2 mm wide) on a white background; squamules densely congregated on the disc, while sparse to the margin. Lamellae free, crowded, ventricose, lamellulae in more than 5 series, 5 mm broad, pink, to brownish gray (6C2), dark brown with age. Stipe 48 × 6–7 mm, cylindrical and slightly enlarging toward the base, surface smooth, white, hollow; color change to slightly orange, later dark brown when bruised. Annulus superous, double and lower surface with brownish gray colored flakes, white. Context firm, white. Odor phenol-like. Edibility of this species is unknown.

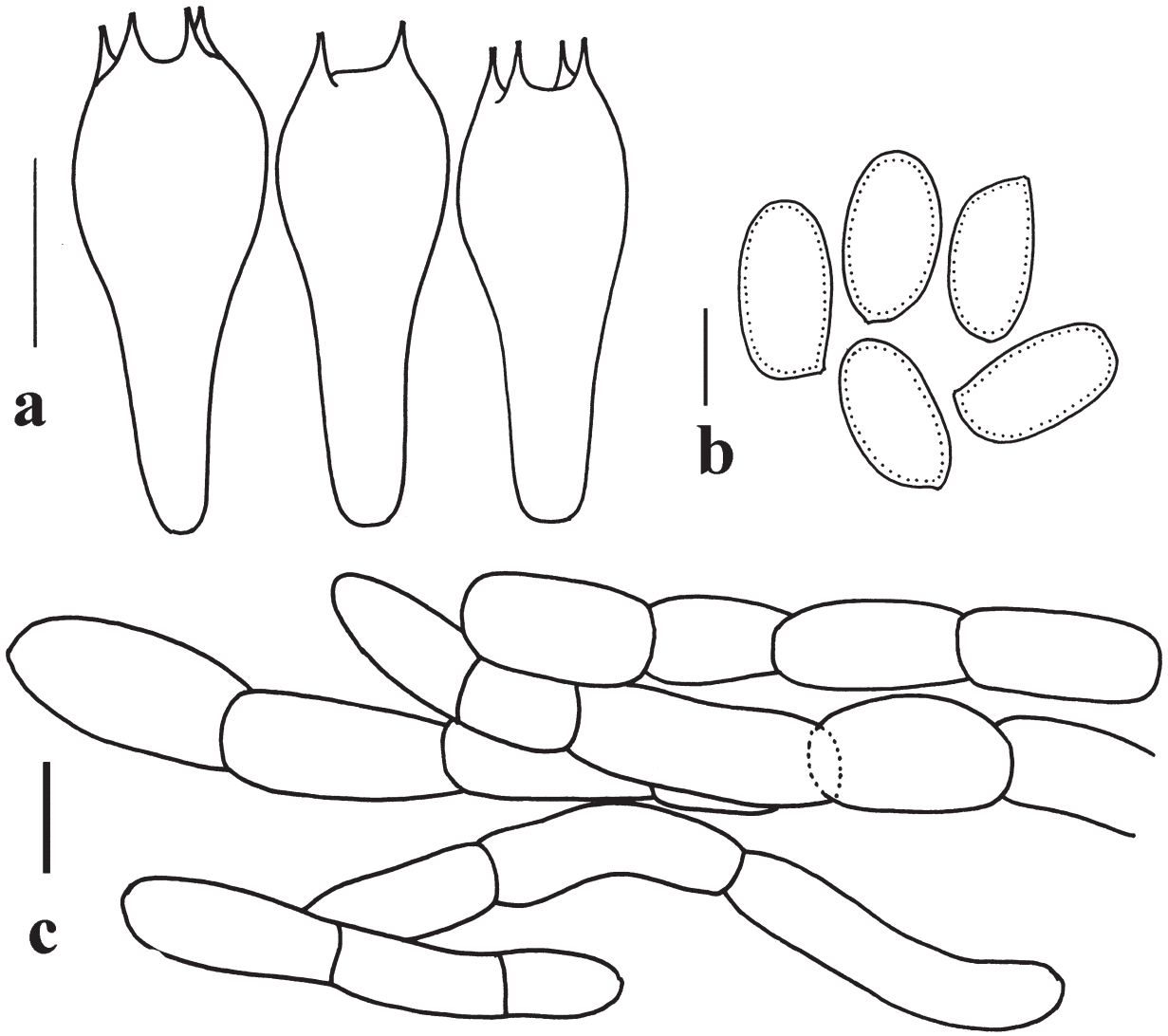

FIGURE 5. Microcharacters, *A. sordidocarpus* (MFLU12 0881, holotype), a. Basidia. b. Spores. c. Pileipellis. Scale bars: a and c = 10 µm; b = 5 µm.

Macrochemical reactions: KOH reaction yellow; Schäffer's reaction negative.

Microscopical characters: Spores 7.9–9.6 × 4.6–6.0 µm, [x = 8.5 ± 1.1 × 5.1 ± 0.9, Q = 1.48–1.84, Q_m = 1.66 ± 0.18, n = 20], ellipsoid to elongate, smooth, brown, thick-walled. Basidia 19–25 × 8.5–9.8 µm, clavate, hyaline,

smooth, 4-spored. Cheilocystidia absent. Pleurocystidia absent. Pileipellis a cutis composed of hyphae of 6–12 µm diam., cylindrical, with brown pigment, smooth, constricted at the septa, rarely branched.

Habitat:—solitary in soil of grassland, in park.

Geographical distribution range:—known only from Asia (Thailand).

Species-specific ITS markers:—gatgAgttg @ 22; gcacAtatt @ 88; ttgtCaaag @ 477.

Type:—THAILAND. Chiang Mai Province: Chiang Mai University, 06 June 2012, *Jie Chen*, *LD201237* (holotype MFLU12 0881!, isotype SWFC!).

Comments:—*Agaricus sordidocarpus* is characterized by brownish gray triangular-shaped fibrillose squamules on the pileus surface, brownish gray colored flakes on the lower surface of annulus and large spores. This species morphologically resembles *A. megacystidiatus* but the latter has particularly large cheilocystidia (30–45 × 8–25 µm; Karunarathna *et al*. 2014). A collection identified as *A. variegans* F.H. Møller that Heinemann reported from Singapore (1980) also has brownish triangular scales and exhibits pale pinkish discoloration when bruised, but it can be distinguished by brightly red discoloration on lamellae when broken and smaller spores (4.5–7.5 × 3–4.5 µm).
In our phylogenetic analyses, *A. sordidocarpus* appears as a sister species to *Agaricus subsaharianus*; the two species are morphologically quite different and their ITS1+2 sequences differ at 26 positions which is much higher than the divergence generally observed within a species or a species complex in the genus *Agaricus*.

Agaricus subsaharianus L.A. Parra, Hama & De Kesel, Cryptog. Mycol. 31: 223 (2010).

Species-specific ITS markers:—gtctAgatt @ 61; gtctTtgtc @ 101; tgtcAgg-t @ 106; tcagTctat @ 130; gatcAgcag @ 160; aatcAtttt @ 204 ; cttgCtgt- @ 527; acaaCtttt @ 664.

Comments:—*Agaricus subsaharianus* was described from Niger, Burkina Faso and Tanzania (Hama *et al*. 2010). It usually grows in groups or clustered with a preference for sandy soils moderately enriched with organic matter. Remarkable characters: large sized white sporocarps reaching 13 cm diam.; upturned, triangular, large, concentrically arranged scales; stipe cylindrical or subbulbous with short rhizomorphs at the base, surface smooth; annulus at maturity composed of two parts, upper part with small whitish to light brown flakes on its outer surface, lower part narrow and appressed to the stipe, almond odor and positive Schäffer's reaction. As a consequence of these morphological features, *A. subsaharianus* was provisionally placed in section *Spissicaules*. It was not placed in *Arvenses* or *Minores* in which Schäffer's reaction is instantaneously positive in both young and mature specimens, while its reaction was positive orange to red after 40 seconds in mature specimens and negative in young specimens. Based on ITS 1+2 sequence data, it clearly belongs to section *Brunneopicti* and is closely related to *A. sordidocarpus* even though they are morphologically quite different. The white color of its scales is atypical in the group II. *A. subsaharianus* is consumed by some local population and in Niger; it is used by men for attracting bees in hives (Hama *et al*. 2010).

Agaricus toluenolens Callac, L.J. Chen & K.D. Hyde *sp. nov.* Fig. 6, 8I–J

MycoBank MB 808175

Etymology:—*toluenolens* in reference to the toluene-like odor.

Original description:—*Macroscopical characters*: Pileus 3–6 cm diam., 3–4 mm broad, convex-plane; margin straight and not exceeding the lamellae; surface dry, covered with brownish gray concentrically arranged squamules on white background; squamules densely congregated on the disc and more scattered towards the margin. Lamellae free, crowded, ventricose, with more or less crenate edge, lamellulae in more than 5 series, 3–4 mm broad, pink, to brownish gray, brown with age. Stipe 35 × 6–10 (base 9–12) mm, clavate with slightly swollen at the base, surface smooth, white, hollow; bruising pale yellow. Annulus superous, pendant, white, with gray flakes on its border of lower surface. Context firm, white. Odor strong unpleasant, like solvent used in marker pen (toluene). Edibility of this species is unknown.

Macrochemical reactions: KOH reaction unknown. Schäffer's reaction negative.

Microscopical characters: Spores 8.2–9.4 × 4–5.3 µm, [x = 8.6 ± 0.8 × 4.6 ± 0.7, Q = 1.68–2.11, Q_m = 1.88 ± 0.23, n = 20], ellipsoid to elongate, smooth, brown, thick-walled. Basidia 16–21 × 7–8.5 µm, clavate, hyaline, smooth, 4-spored. Cheilocystidia 21–58 × 11–28 µm, various, pyriform with short or long cylindrical base, or vesicular, or broadly clavate, hyaline, smooth. Pleurocystidia absent. Pileipellis a cutis composed of hyphae of 4.5–8.5 µm diam., cylindrical, brown, smooth, slightly constricted at the septa, with vacuolar pigments.

Habitat:—solitary in grassland.

Geographical distribution range:—known only from Asia (Thailand).

Species-specific ITS markers:—acctAtctg @ 57; gtatCgagg @ 111; atgtAGggat @ 148–149; tyacCttgA @ 184, 188; ttttCcctg @ 203; ctgcTggag @ 209; gtcaTcata @ 223; attgAaata @ 287; ccttCtact @ 466; tgtaCaagG @ 479, 483; gactTtgga @ 495.

Type:—THAILAND. Chiang Mai Province: Chiang Mai University Park, in grass, 10 June 2011, *Philippe Callac*, *Sylvie Rapior* and *Samantha Karunarathna*, *CA911* (holotype MFLU14 0025!).

Additional specimens examined:—THAILAND. Chiang Mai Province: Lampang, Park, in grass under *Samanea samam*, 12 June 2011, *Philippe Callac*, *Sylvie Rapior* and *Samantha Karunarathna*, *CA 926* (MFLU14 0026!).

Comments:—*A. toluenolens* is characterized by brownish gray squamules concentrically arranged on the pileus, double annulus with gray flakes on the margin of lower surface, large spores and relatively large cheilocystidia. Because of its distinctive odor, this species can easily be recognized from other *Agaricus* species in the field.

Agaricus sp. 3/NTT117

Species-specific ITS markers:—tcatCttca @ 70; ggatCtgag @ 146; gtcaCAgaat @ 246–247; ctctAatac @ 464; gtcgGggtc @ 519; aaatAcatt @ 538; tgctTtccg @ 606; gttcTgctt @ 616.

Comments:—A single collection of this entity was collected on grassland of Chiangmai University in Thailand. It is characterized by its pileus (8 cm diam.) covered with brownish upturned squamules, a cylindrical stipe enlarging toward the base, and relatively large spores (8−9.4 × 4.7−5.4 µm). Unfortunately, only little information is available: odor, context discoloration and Schäffer's reaction are lacking. From phylogenetic analyses, this entity belongs to section *Brunneopicti* on an isolated branch of the tree.

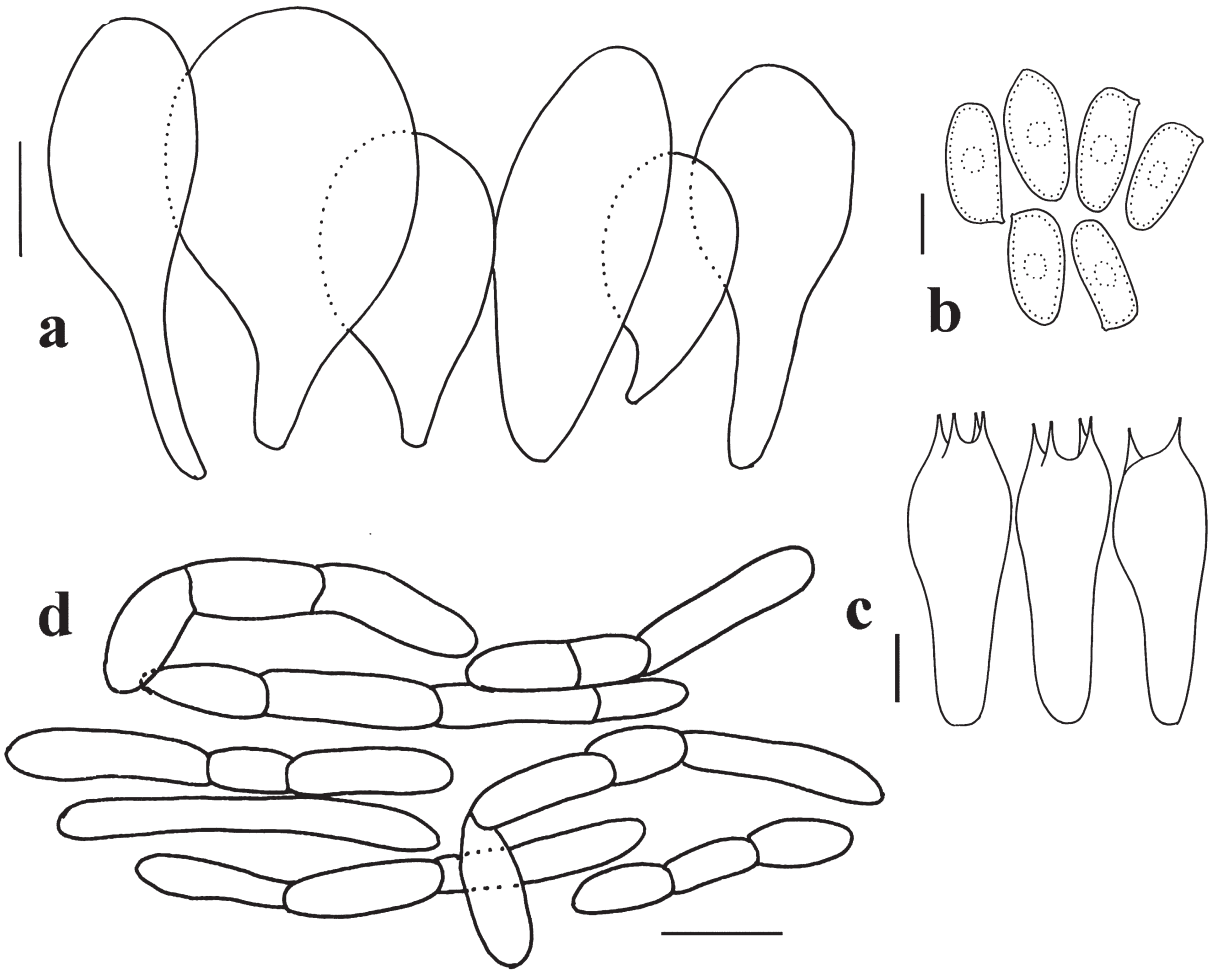

FIGURE 6. Microcharacters, *A. toluenolens* (MFLU14 0025, holotype), a. Cheilocystidia. b. Spores. c. Basidia. d. Pileipellis. Scale bars: a and d = 20 µm; b and c = 5 µm.

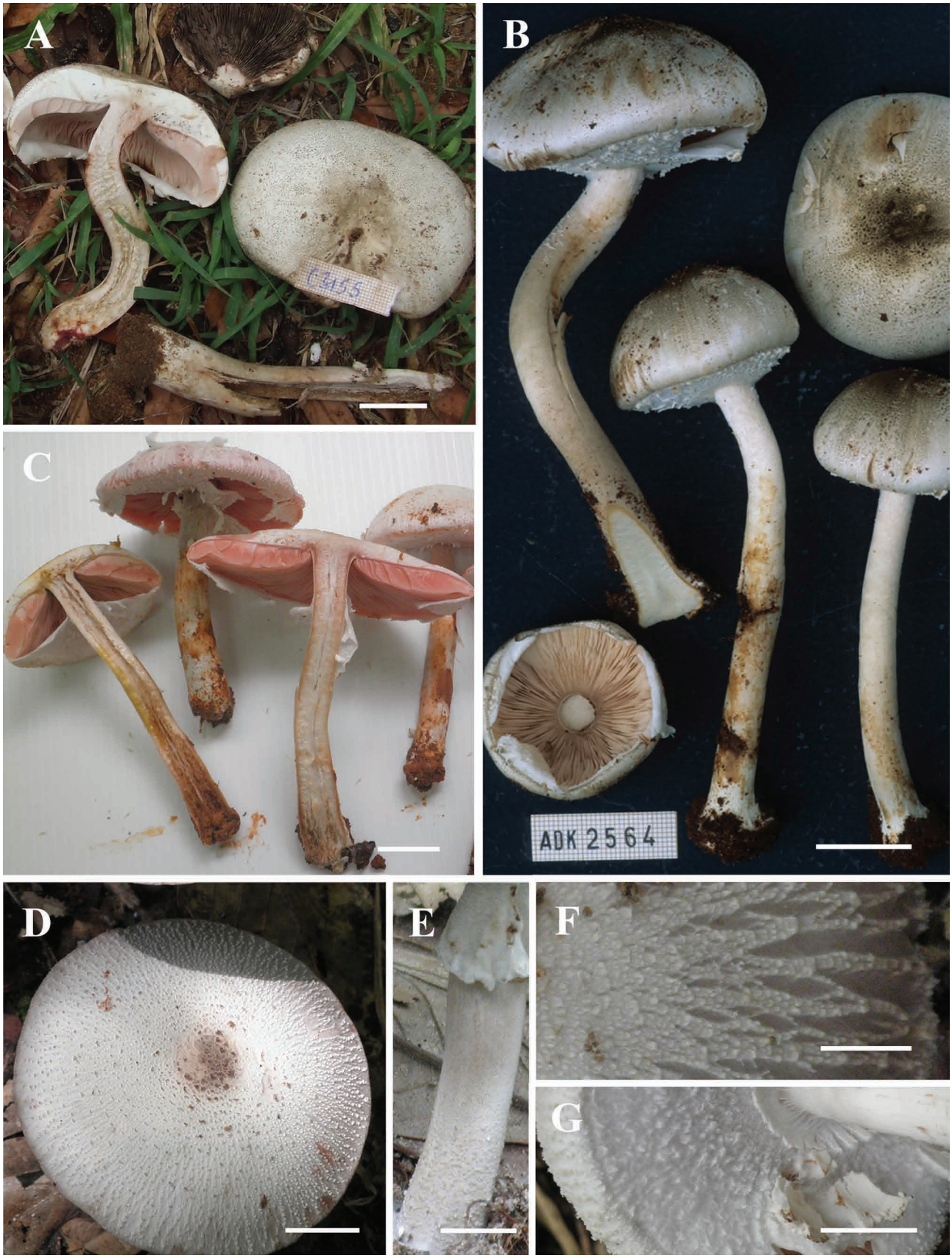

FIGURE 7. *Agaricus bingensis* (A = C3155), *A. brunneopunctatus* (B = ADK2564) and *A. niveogranulatus* (C = LD2012140, D–G = MFLU11 1307 holotype, E = LD201123). A. sporophores of *A. bingensis in situ*. B. sporophores of *A. brunneopunctatus*. C. section view of *A. niveogranulatus* in laboratory. D. punctiform squamules on pileus surface. E. punctiform squamules on stipe surface. F. surface splitting in radial interwoven bands. G. annulus. Scale bars: A–E = 20 mm, F–G = 10 mm.

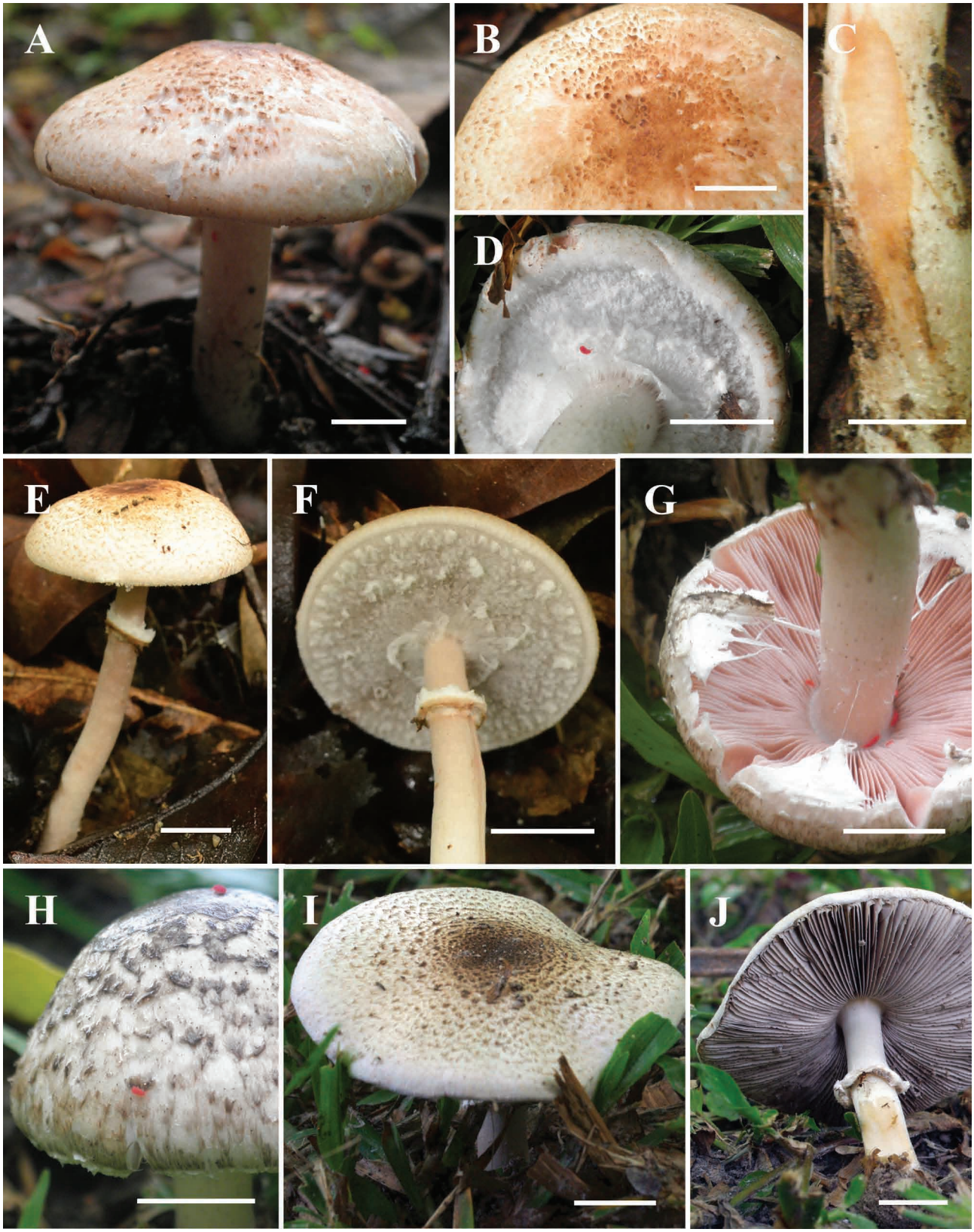

FIGURE 8. *Agaricus brunneosquamulosus* (A, B, C = MFLU12 0943 holotype, D = LD201238), *A. duplocingulatus* (E–F = LD201218), *A. sordidocarpus* (G–H = MFLU12 0881 holotype) and *A. toluenolens* (I–J = MFLU14 0025 holotype). A. sporophore of *A. brunneosquamulosus in situ*. B. ferruginous brown appressed squamules on pileus. C. discoloration on stipe surface by bruising. D. annulus. E. sporophore of *A. duplocingulatus in situ*. F. double annulus. G. annulus of *A. sordidocarpus*. H. brownish gray squamules on pileus. I. sporophore of *A. toluenolens in situ*. J. annulus and discoloration on stipe surface by bruising. Scale bars: A–J = 10 mm.

Agaricus sp. 2/NT019

Species-specific ITS markers:—aaaaAttgt @ 285.

Comments:—This entity is represented by a single specimen collected in forest in Thailand. It has a medium-sized pileus (9.5 cm diam.) covered with brownish triangular squamules. Microscopically, pyriform or sphaeropedunculate cheilocystidia and relatively small spores (6−7.2 × 4−4.6 µm) are observed. In the phylogenetic analyses it is a sister species to *A. megacystidiatus*. However, since the field material is not in good condition, future collections are needed for a complete description.

Agaricus sp. 1/CA800

Species-specific ITS markers:—agaaAgtca @ 239; gcttGtatg @ 266; tagcAaggg @ 592.

Comments:—A single specimen was collected on the grassland of a university park in Thailand. Macroscopically, it closely resembles to *A. sordidocarpus*, with a pileus (8 cm diam.) covered with brownish triangular scales. However, collection CA800 shows strong red discoloration when bruised, smaller spores (4.8−5.5 × 3.0−4.0 µm) and lacking cheilocystidia. According to our phylogenetic analyses, this entity is on an isolated branch with low bootstrap value. Its evolutionary relationship with other taxa inside of the section remains unresolved.

Artificial key to section *Brunneopicti*

1. Pileus generally ≥ 10 cm diam.; brownish punctiform squamules on the pileus surface and concolor or whitish punctiform squamules on the stipe surface close to the base (Group I) 2
- Pileus generally < 10 cm diam.; brownish non-punctiform squamules or appressed scales on the pileus surface only (Group II) 5

2. On the entire pileus surface, the punctiform scales are not uniformly brownish 3
- On the entire pileus surface, the punctiform scales are brownish 4

3. Pileus center yellowish white, brownish punctiform squamules distributed elsewhere ..... ***A. chiangmaiensis***
- Pileus center brownish, whitish punctiform squamules distributed elsewhere ..... ***A. niveogranulatus***

4. Basidiospores of large size, 8.3–10 × 5.1–5.7 µm, with an apical endosporal thickening ..... ***A. bingensis***
- Basidiospores smaller, 7.6–8.5 × 4.9–5.3 µm, without an apical endosporal thickening ..... ***A. brunneopunctatus***

5. Schäffer's reaction late and weakly positive; pileus surface with white triangular scales ..... ***A. subsaharianus***
- Schäffer's reaction negative; pileus surface with brownish appressed squamules 6

6. Cheilocystidia absent ..... ***A. sordidocarpus***
- Cheilocystidia present 7

7. Double annulus, with lower one movable; cheilocystidia shortly catenulate ..... ***A. duplocingulatus***
- Double annulus, lower one fixed; simple cheilocystidia 8

8. No discoloration on stipe surface by bruising ..... ***A. inoxydabilis***
- Discolored on stipe surface by bruising 9

9. Toluene-like odor (solvent of marker pen) by bruising ..... ***A. toluenolens***
- Phenol-like odor by bruising the pileus surface 10

10. Basidiospores large, on average 8.5 × 4.7 µm; large cheilocystidia up to 30–45 × 8–25 µm, pyriform to broadly clavate ..... ***A. megacystidiatus***
- Basidiospores smaller, on average 5.7 × 3.7 µm; smaller and infrequent cheilocystidia, 12–25 × 7–15 µm, pyriform to vesiculose ..... ***A. brunneosquamulosus***

Discussion

The present phylogenetic reconstruction confirmed that the major clade TR I of Zhao *et al.* (2011) is equivalent to the section *Brunneopicti* which is therefore monophyletic. This section currently comprises 16 species which is

much more than the three species initially included (Heinemann 1956): *A. brunneopunctatus* (as *A. brunneopictus*, the type species of the section), *A. bingensis* and *A. kivuensis*. We confirmed the placement of *A. bingensis* in section *Brunneopicti* although Pegler (1977) classified it in section *Agaricus*. *Agaricus kivuensis* however, has been excluded from clade TR I and consequently from section *Brunneopicti* by Zhao *et al*. (2011) who showed that it belongs to the unrelated clade TR III. Three species previously placed in other sections are currently included: *A. duplocingulatus* that Heinemann (1980) could not surely classify in section *Xanthodermatei* or in section *Arvenses*, *A.* cf. *inoxydabilis*, a species classified in section *Sanguinolenti* (Heinemann 1980), and *A. subsaharianus* which was provisionally included in section *Spissicaules* (Hama *et al*. 2010). Among the 11 remaining members of this section, two (*A. chiangmaiensis* and *A. megacystidiatus*) have been recently described as belonging to clade TR I (Karunarathna *et al*. 2014), four (*A. brunneosquamulosus*, *A. niveogranulatus*, *A. sordidocarpus* and *A. toluenolens*) are described in the present study, and five require more collections to be formally described. This brief history of the members of section *Brunneopicti* shows that confusion has been possible with five of the eight traditional sections of the genus *Agaricus* and even with the major clade TR III recently revealed by Zhao *et al*. (2011).

The modification and extension of the initial concept of the section *Brunneopicti* were needed to include all species of the clade TR I. Indeed, 13 of the 16 species are distributed in four strongly supported subclades (A, B, C and D) but only the six species of the subclade D agree with the initial concept of the section mainly characterized by punctiform decorations remaining from the general veil and located on pileus and stipe. The concept of the section was enlarged for the ten species of the Group II that do not exhibit such decorations but larger brown squamules only present on their pileus. However even in this group not all the species perfectly exhibit the brown color of the punctiform squamules since they are white in *A. niveosquamulosus* except on the disc and very pale in *A. chiangmaiensis*.

Although they may not be strongly reliable, two other traits seem correlated with the presence/absence of punctiform squamules that characterize the two groups: the sporocarps size which is larger in Group I with a maximum pileus diameter exceeding 10 cm in all the species and reaching up to 25 cm for *A. bingensis*, while sporocarps are small or medium-sized in Group II with maximum pileus diameter reaching from 4 to 10 cm except *A. subsaharianus* reaching 13 cm. The second trait is the spore size which is relatively large with minimum and mean length greater than or equal to 7 and 8 µm respectively in all species of Group I while only half of the ten species have such larger spores in Group II.

The identification of section *Brunneopicti* is particularly difficult because some exceptions are found for each of the main features making almost impossible the generalization of shared characters. In Table 3 we briefly report the Schäffer's reaction, the flesh discoloration by bruising, and the odor for the 16 species of *Brunneopicti* and for six of the eight traditional sections of *Agaricus*. The two remaining sections (*Bivelares* and *Chitonioides*) are omitted because they differ mainly by the structure of their annulus and also they are poorly represented in tropical areas particularly in Thailand where we have never collected them. With the exception of Clade I, the tropical clades of Zhao *et al.* (2011) are also omitted in Table 3 since they have not been morphologically investigated yet. The three traits used in this table are generally considered as relevant to distinguish the six sections between them. Table 3 shows that only the negative Schäffer's reaction is a constant trait in section *Brunneopicti* with the exception of *A. subsaharianus* for which a late reaction (only on mature specimens, see Hama *et al*. 2010) makes possible confusion with section *Spissicaules*. In any case Schäffer's reaction allows rejecting sections *Arvenses* and *Minores*. The two other traits are very variable within section *Brunneopicti*. Species having almond odor might be confused with section *Spissicaules* and those having phenol-like odor with section *Xanthodermatei*; moreover with a pleasant odor *A.* cf. *inoxydabilis* might be misclassified in section *Agaricus* and *A.* sp. 1/CA800 which exhibits a red discoloration might be in section *Sanguinolenti*. However we note that (i) most of the species with almond odor are in Group I and thus distinguished from the *Spissicaules* by their punctiform squamules, and (ii) most of the species with phenol-like odor are in Group II and do not exhibit the typical yellow discoloration of section *Xanthodermatei* but more or less pronounced orange or brown color. Consequently, species of section *Brunneopicti* can be excluded from the other sections by using combination of traits. But without DNA sequence data the certainty that newly discovered taxa belong to this section will remain difficult for the same reason that the diversity of this section has been underestimated until now.

From the sharing of various traits between section *Brunneopicti* and other sections, the questions arise about the possible ancestral origin of these traits. From the Table 3 the discoloration when the pileus or the stipe surfaces are bruised appears as a variable balance between different tints (mainly yellow, red, and brown) and the odor as a variable balance mainly between almond and phenol-like. In contrast, these traits seem more fixed in the traditional sections. The discoloration process has been studied only in *A. bisporus*. Tyrosinases and peroxydases are involved in the late brown discoloration of this species which results of oxidation of phenolic substrates into quinones (Jolivet *et al*. 1998; Weijin *et al*. 2013). For the odor, almond and phenol-like odors are typical of sections *Minores*/*Arvenses* and *Xanthodermatei*

respectively in which they result of the presence of volatile components as benzaldehyde and phenol respectively (Wood and Largent 1999; Petrova *et al*. 2007). Such components could be both present in section *Brunneopicti*. Moreover, the proportions of different volatile components can make the interpretation of the odor partly subjective. This is the case in section *Arvenses* where the individual judgment is sensitive to the ratio between benzaldehyde and benzyl alcohol. As a result, for example in *A. augustus* some people detect almond odor while others detect aniseed smell (Wood *et al*. 1990). It will be interesting to see to what extent future volatile organic compound analysis and odor panel tests would support this viewpoint applied to almond and phenol odors in section *Brunneopicti*. According to Callac *et al*. (2005) the phenolic substances would derive from an evolutionary ancestral biochemical shift and its maintaining all along the evolution of section *Xanthodermatei* indicates that this trait could represent a defense mechanism as for example a feeding barrier. In the tree of Zhao *et al.* (2011) section *Brunneopicti* appeared as the more ancient among the described section of the genus. Although this remains to be confirmed because the phylogeny at the sectional level was not well-supported, this would agree with the expression of multiple plesiomorphic traits in this section.

TABLE 3. Morphological comparison between species of section *Brunneopicti* and other sections of *Agaricus*

Taxon (clade)	Schäffer's reaction	Discoloration by bruising	Odor
Sect. *Brunneopicti* Group I			
A. niveogranulatus (D)	−	brownish-red	phenol
A. sp. 5/C3182 (D)	unknown	brownish	unknown
A. sp. 4/LD201127 (D)	−	pinkish	almond
A. chiangmaiensis (D)	−	faint rufescent	benzene/unpleasant
A. brunneopunctatus (D)	−	brown	almond
A. bingensis (D)	−	slightly reddish	almond
Sect. *Brunneopicti* Group II			
A. toluenolens	−	pale yellow	toluene
A. sp 3/NTT17	unknown	light brown	unknown
A. subsaharianus (C)	−	yellow	almond
A. sordidocarpus (C)	−	slightly orange	phenol
A. megacystidiatus (A)	−	reddish-brown	phenol/aniseed
A. sp. 2/NT019 (A)	unknown	unknown	unknown
A. duplocingulatus (B)	−	brownish-red	phenol
A. cf. *inoxydabilis* (B)	−	unchanged	pleasant [c]
A. brunneosquamulosus (B)	−	yellow orange	phenol
A. sp. 1/CA800	−	strongly red	unpleasant [d]
Sections			
Brunneopicti	+/−[a]	yellow, pink, red, brown	almond, phenol, or distinct
Xanthodermatei	−	unchanged, yellow, pink, red	phenol, iodine or rarely not so
Sanguinolenti	+/− [b]	pink, red, brown	mushroomy or hardly distinct
Agaricus	−	unchanged, pink	mushroomy, pleasant
Spissicaules	+weak/−	yellow, pink	almond, scleroderma-like
Arvenses+*Minores*	+	yellow	almond, aniseed

[a] negative in young sporocarp but positive in mature one

[b] positive violaceous purple only in *A. bohusii*

[c] weak aromatic (not indicated in description of *A. inoxydabilis*)

[d] odor of old unventilated cellar, *A. bernardi*-like

Unlike the traditional sections, the known geographical distribution range of section *Brunneopicti* is limited to the palaeotropics. Five of the six other major clades exclusively tropical revealed by Zhao *et al*. (2011) are also distributed whether in palaeotropics or in neotropics exclusively indicating that species diversification occurred independently in these two areas and that migration between these areas have been extremely limited. Our data reinforce the hypothesis

of Zhao *et al*. (2011) that geography and climate did had a major impact throughout the evolution of the genus. What are the factors which limit the migration of these species and which physiological process and genetic determinants are implicated in their cold tolerance remain unresolved questions on crucial aspects of the evolution and the adaptation of these saprobic fungi (Largeteau *et al*. 2011; Navarro *et al*. 2014). At a smaller scale similar questions arise to know if the species of section *Brunneopicti* are distributed in both Asian and African continents. We note that two of the four subclades contain both species initially described from Asia and Africa. On the other hand, we note that among the 16 species only two are reported from both continents: *A. inoxydabilis* and *A. brunneopunctatus* initially described from Asia and Africa respectively. However, in both cases this geographical range has not been confirmed by comparing sequences of specimens from both continents. This is the reason for which we consider that ITS sequence data of Asian collections are required to confirm the identification of our African specimen LAPAF 1 to *A. inoxydabilis*.

Agaricus species are considered with high nutritional and medicinal values, besides this several wild species are appreciated by human. In section *Brunneopicti*, *A. bingensis* and *A. subsaharianus* are consumed by local people. However, unless future experiments confirm their edibility, we do not recommend the consumption of the species with phenol-like or solvent odor. It remains also to confirm that the phenol-like odor is really due to the presence of this component as this has been done for the species of section *Xanthodermatei* (Gill and Strauch 1984; Petrova *et al*. 2007). Moreover the phenol is likely responsible for the poisoning (gastrointestinal symptoms) following consumption of these species (Kerrigan *et al*. 2005; Petrova *et al*. 2007).

In retrospect, it appears that since its establishment over 57 years ago no species has been introduced in section *Brunneopicti*. It is currently the first reconstructed section of tropical *Agaricus* and it already contains 16 species exclusively from palaeotropics. Although combinations of morphological traits can help to reject other sections and to identify species of section *Brunneopicti*, ITS sequence data remain essential to establish new species in this section. We believe that through such an approach some species previously placed in traditional sections could join the section *Brunneopicti* as that was the case for three species in the present study. From the study of Zhao *et al*. (2011) as from the present study it can be predicted that the species richness of other somewhat forgotten or new tropical sections will also increase in coming years.

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