Mycoscience (2012) 53:300–311
DOI 10.1007/s10267-011-0169-5

FULL PAPER

Agaricus flocculosipes sp. nov., a new potentially cultivatable species from the palaeotropics

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Received: 12 July 2011 / Accepted: 9 December 2011 / Published online: 4 January 2012

Abstract *Agaricus flocculosipes* is described as new from northern Thailand and Mayotte based on morphology and ITS sequence analysis. The new species is compared with similar taxa, and a comprehensive description, line drawings, and colored photographs are provided. *Agaricus flocculosipes* is distinguished among the species in section *Arvenses* by its relatively large sporocarps (110–180 mm in diameter), erect floccose squamules on the surface of the stipe, and relatively small basidiospores (less than 6.5 μm long). Phylogenetic analyses based on ITS sequence data using maximum likelihood, parsimony, and Bayesian analyses all support *A. flocculosipes* as being a distinct species within section *Arvenses*. The new taxon is potentially cultivatable as it is presumably heterothallic, exhibits extensive genetic diversity, and occurs in section *Arvenses*, a lineage that contains other edible cultivatable taxa such as *Agaricus arvensis* and *Agaricus subrufescens*.

Keywords *Agaricus* section *Arvenses* · New species · Phylogeny · rDNA · Taxonomy

Introduction

Our studies of the basidiomycetes from northern Thailand have resulted in documenting many new species and a surprising wealth of biodiversity (Desjardin et al. 2009; Kerekes and Desjardin 2009; Wannathes et al. 2009; Zhao et al. 2010). We have been concentrating recently on potentially cultivatable and edible groups (Karunarathna et al. 2010), and this article reports a new, potentially edible and cultivatable species in the genus *Agaricus* L.: Fr. Species of this genus can be recognized in the wild from mature basidiomes with brown free lamellae, a dark brown to nearly black spore print, and the presence of a partial veil during basidiome development that forms an annulus around the stem (Singer 1986). Species of this cosmopolitan genus are common in tropical areas (Zhao et al. 2011).

Although *Agaricus* species are important cultivated mushrooms, the circumscription and delimitation of species in the genus is complex. In all, 386 species of *Agaricus* have been reported (Zhao et al. 2011). However, most reports concerning *Agaricus* species are from temperate areas, such as North America, Europe, and New Zealand

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(Murrill 1912, 1918, 1941; Hotson and Stuntz 1938; Smith 1944; Möller 1950; Huijsman 1960; Orton 1960; Bohus 1975, 1990; Freeman 1979a, b; Pegler 1983, 1990; Cappelli 1984; Kerrigan 1985, 1986, 1989, 2005; Kerrigan et al. 2006; Callac 1993; Callac et al. 1993, 2003; Alberto 1998; Esteve-Raventós 1998; Mitchell and Walter 1999; Nauta 1999, 2000, 2001; Lanconelli 2002; Parra 2003, 2008; Lacheva and Stoichev 2004; Callac and Guinberteau 2005; Geml et al. 2007). Species diversity is high in tropical areas (Heinemann 1953, 1956a, b, c, 1957a, b, 1961, 1962a, b, c, d, 1965, 1971, 1974a, b, 1977, 1978, 1980, 1982, 1986a, b, 1987, 1988, 1990a, b, 1993; Pegler and Rayner 1969; Pegler 1977, 1986; Saini et al. 1997; Valenzuela et al. 1997; Peterson et al. 2000; Natarajan et al. 2005), and the most commonly referenced monographs of *Agaricus* species in tropical areas are those of Heinemann (especially in 1978 and 1986). Knowledge of *Agaricus*, however, is still very limited in the tropics. For example, in Thailand only *A. campestris*, *A. bisporus*, *A. bitorquis*, *A. trisulphuratus*, and *A rufolanosus* have been reported (Høiland and Schumacher 1982; Ruksawong and Flegel 2001; Desjardin et al. 2004). Molecular studies have also been conducted in this genus (Challen et al. 2003; Mitchell 1999; Robinson et al. 2001), however, these studies included samples mostly from temperate regions.

A project to survey *Agaricus* species and allied genera was initiated in northern Thailand in 2004 and has resulted in many interesting observations (Zhao et al. 2010). We have broadened the project to include other tropical habitats, and the objective of this article is to describe a new potentially cultivatable species of *Agaricus* from Thailand and Mayotte, an overseas French department.

Materials and methods

Morphological character examination

Collections were made in the field in northern Thailand between 2004 and 2010 and in Mayotte in 2011. Specimens were photographed in situ, then gathered and wrapped in aluminum foil or kept separately in a box to avoid mixing or crushing, and returned to the laboratory for treatment. Odor and color change upon bruising were recorded at the time of collection. The description of macrocharacters, chemical testing, and further photography of fresh samples were carried out as soon as possible after return from the field following the methodology described by Largent (1986). Color terms follow those of Kornerp and Wanscher (1978). Specimens were dried overnight in a food dryer, sealed in plastic bags, and deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU), with duplicates deposited in the BIOTEC Bangkok Herbarium (BBH) and the H.D. Thiers Herbarium at San Francisco State University (SFSU). Herbarium acronyms are those of Thiers (http://sweetgum.nybg.org/ih/, continuously updated). Strains isolated from sporocarps or from spore deposits of some collections are kept in the culture collection of Mae Fah Luang University and at INRA (CGAB: Collection du Germplasme des Agarics à Bordeaux). One dry specimen, which was collected in Africa by D. Thoen, was loaned by BR.

Micromorphological features were documented from examination of dried specimens following the protocols of Largent (1986) and Nauta (2001). Particular attention was given to the anatomy of the pileipellis, stipitipellis, and partial veil, and features of the hymenophoral trama, basidiospores, basidia and cystidia. Measurements of anatomical features (spores, basidia, and cheilocystidia) are presented based on at least 20 measurements, and include *X*, all spores length by width; av*X*, the mean *X* of all spores ± SD (standard deviation); *Q*, the quotient of all basidiospores length and width; and *avQ*, the mean *Q* of all spores ± SD.

Molecular phylogenetic analyses

DNA extraction, PCR, and sequencing

Protocols for DNA extraction, polymerase chain reaction (PCR), and sequencing generally followed those of Zhao et al. (2010) with some modifications. DNA extraction from dried fungal specimens involved the use of a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01; Omega Bio-Tek). The PCR reactions and sequencing were performed using primers internal transcribed spacer (ITS)4/ITS5 or ITS1F/ITS4B (White et al. 1990; Gardes and Bruns 1993).

Sequence alignment and phylogenetic analysis

Sequences were initially aligned using Clustal X with default settings (Thomson et al. 1997), then manually adjusted in BioEdit v. 7.0.4 (Hall 1999), and gaps were introduced to improve the alignment. The alignment has been submitted to TreeBase (submission ID 11596).

Unweighted maximum parsimony (UP) and maximum likelihood (ML) phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford 2004). Heuristic searches were performed with unordered characters, random taxon addition of sequences, gaps treated as missing data, and the tree bisection-reconnection (TBR) branch swapping. Bootstrap values (BS) were obtained from 1,000 replicates from UP analysis. The best nucleotide substitution models for ML and Bayesian analyses were chosen by using MrModeltest 2.2 (Nylander 2004). ML analysis start trees were obtained via stepwise addition, random sequence addition, TBR branch swapping, with Multree option used, and MaxTrees was set to 1,000,000.

Bayesian phylogenetic inference was performed with MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2001; Ronquist and Heulsenbeck 2003). Four Markov chains were run for 1×10^6 generations and sampled every 100th generation, resulting in 10,000 trees. The trees sampled before searches reaching a split deviation frequency value less than 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 and exported to graphics programs (Page 1996).

A distance tree of *A. flocculosipes* individuals was constructed using the neighbor-joining (NJ) method with POPULATIONS ver 1.2.32 (Langella 1999–2010) using the shared allele distance (SAD; Jin and Chakraborty 1993), defined as one minus half the average number of shared alleles per locus. Statistical support values were obtained with 1,000 bootstrap replicates.

Results

The ITS sequence dataset on which the phylogenetic analyses were performed comprised 30 sequences (Table 1), which represent 25 *Agaricus* species belonging to the eight sections of *Agaricus* subgenus *Agaricus* as in Parra (2008). *Agaricus* aff. *trisulphuratus*, belonging to subgenus *Lanagaricus*, was chosen as the outgroup. Of the 690 characters, 460 characters were constant, 87 were variable but parsimony uninformative, and 143 were parsimony informative. The ML tree was produced after 18,980 rearrangements, and the log-likelihood of best tree found was −3,510.91344. Three trees were generated from parsimony analyses, and they are not different from each other and the ML tree except for the positions of *A. albolutescens*, *A. fissuratus*, *A. macrocarpus*, and *A. litoralis.* The Bayesian tree showed a topology identical to the ML tree. Therefore, the ML tree was chosen to represent the phylogenetic position of the new species (Fig. 1).

In the phylogenetic tree, section *Arvenses* forms a monophyletic clade with strong statistical support (100% BS, 100% PP). The five samples of the new species *A. flocculosipes* form a clade with 100% BS and 100% PP support that falls in sect. *Arvenses*, where they are sister to several undetermined *Agaricus* species but with low support.

Alignment of all *A. flocculosipes* sequences revealed 14 intraspecific polymorphic positions (Table 2). The sequences of three samples collected at Doi Inthanon from 2006 to 2009 are identical. Two of the four samples collected at Ban Mae Sae are also identical. In contrast, ITS1 + 2 sequences of samples collected in different sites always differ. At 13 of the 14 polymorphic positions, heteromorphisms were observed and confirmed on the two strand sequences. Heteromorphisms in at least six positions (98, 170, 182, 284, 555, and 581) clearly reflect heteroallelisms because each allele is present alone in sequences of different collections. Some evidence for recombination is observed at several pairs of linked loci; for example, at positions 170 and 555, ZRL2127 is C–G, NTF15 is T–T, and NTL33 is C–T. Taking into account the small size of our sample, the absence of the fourth possible genotype T–G is in agreement with the low allelic frequencies in the Thai population of T at position 170 and G at position 581.

Hypothesizing that all the observed polymorphisms are allelic, the following points can be noted from Table 2. (1) At each of the 14 polymorphic positions, the most frequent allele is found in both Thai and Mayotte populations, whereas the other alleles are found in a single one of these two populations (private alleles). The Thai collection NTL33 has the particularity to carry all the frequent alleles; (2) a matrix of genotypes at 14 loci was constructed based on the polymorphisms reported in Table 2 with two alleles (001 and 002) and three possible genotypes at each position; moreover, (3) at position 581 three alleles (corresponding to T, C, or –) and more possible genotypes were considered. The radial distance tree (Fig. 2) resulting from the NJ analysis shows distances between individuals. Only the relatively long branch separating the two specimens of Mayotte from those of Thailand is strongly supported through bootstrap replicates, in agreement with the nonrandom distribution of the alleles among these two geographic populations.

Taxonomy

Agaricus flocculosipes R.L. Zhao, Desjardin, Guinb. & K.D. Hyde, sp. nov. Figs. 3, 4

MycoBank number 561690

Etymology: Refers to the floccose stipe.

Pileus 110–180 mm in diameter, hemispherical to convex or plano-convex, disc applanate; surface smooth or radially ridged in some cases, covered with small, grayish-brown (6D3) to brownish-orange (5C5) or light brown (6D5) grain-like squamules against a white to cream-colored background; squamules closely congregated on the disc and sparse at the margin, punctiform or acute, appressed; lubricous and turning pink in wet condition; margin with sparse appendiculate remnants of the partial veil. Context 8 mm thick, firm, white throughout when young, becoming pale yellowish ochraceous to dark brown in age at the

Table 1 Collections included in the phylogenetic analyses

Sample	Taxon	Date (collector)	Location (habitat)	GenBank accession number
CA101[a]	*Agaricus aridicola* (syn. *Gyrophragmium dunalii*)	05/11/1997 (JG)	France, Oléron Island (dune)	JF797195
CA640[a]	*A. arvensis*	16/10/2008 (JG, PC)	France, Villenave d'Ornon (under *Pinus radiata*)	JF797194
CA377	*A. brunneolus* (syn. *A. porphyrizon*)	22/11/2005 (PC)	Portugal, Aljezur (under *Pinus pinea*)	JN204435
ZRL3028[b]	*A. flocculosipes*	05/06/2006 (RZ)	Thailand, Doi Inthanon	JN664954
ZRL2053	*A. flocculosipes*	27/06/2005 (DD)	Thailand, Doi Inthanon	Identical to JN664954
ZRL2127	*A. flocculosipes*	16/08/2005 (KH)	Thailand, Chiang Mai, Mae Taeng	JF691547
CA798	*A. flocculosipes*	28/07/2010 (SK, JG)	Thailand, Chiang Mai, near Huai Nam Dang	JN204430
NT46	*A. flocculosipes*	05/09/2009 (SK)	Thailand, Doi Inthanon	JN662352
T13	*A. flocculosipes*	22/07/2009 (SK)	Thailand, Chiang Rai, Khun Korn Waterfall	JN662351
NTF15	*A. flocculosipes*	27/06/2010 (SK, JG)	Thailand, Chiang Mai, Mae Taeng, Ban Mae Sae	JN592035
NTS70	*A. flocculosipes*	29/06/2010 (SK)	Thailand, Chiang Mai, Mae Taeng, Ban Mae Sae	Identical to JN592035
NTT30	*A. flocculosipes*	16/06/2010 (KW)	Thailand, Chiang Rai, Doi Tung	JN592037
NTL33	*A. flocculosipes*	09/08/2010 (JC)	Thailand, Chiang Rai, Mae Fah Laug University	JN592036
CA870	*A. flocculosipes*	30/01/2011 (MP)	France, Mayotte	JN204432
CA886	*A. flocculosipes*	13/03/2011 (MP)	France, Mayotte	JN204431
NTS67	*A. flocculosipes*	29/06/2010 (SK)	Thailand, Chiang Mai, Mae Taeng, Ban Mae Sae	JN592034
OR71	*A. flocculosipes*	03/07/2010 (OR)	Thailand, Chiang Mai, Mae Taeng, Ban Mae Sae	JN592038
CA120	*A. litoralis* (syn. *A. spissicaulis*)	30/09/1999 (MM)	France, Gradignan (under *Prunus laurocerasus*)	JN204436
CA358	*A. sylvaticus*	02/11/2005 (JG, PC)	France, Le Verdon (under *Quercus ilex*)	JN204434
Thoen7297[a]	*A.* sp. 1	14/10/1984 (DT)	Senegal	JF514542
Thoen6951	*A.* sp. 2		Senegal	JN204433
ZRL2128	*A.* aff. *trisulphuratus*	03/10/2005	Thailand, Chiang Mai	JN664955

Internal transcribed spacer (ITS) sequences retrieved from GenBank: *Agaricus albolutescens* AY484675, *A. augustus* AY484672, *A. bisporus* DQ404388, *A. bitorquis* AF432898, *A. bresadolanus* DQ185572, *A. campestris* AF432877, *A. cupreobrunneus* DQ182532, *A. endoxanthus* DQ182511, *A. excellens* AY484682, *A. fuscovelatus* AY484677, *A. gennadii* AJ884633, *A. lilaceps* AY484676, *A. macrocarpus* AY484686, *A. subrufescens* AY818651, *A. subrutilescens* AY943973, *A. xanthodermus* DQ182534

DD Dennis E. Desjardin, *DT* D. Thoen, *JG* J. Guinberteau, *KH* Kevin D. Hyde, *KW* Komsit Wisitrassameewong, *MM* M. Mench, *MP* M. Pelissier, *NT* Naritsada Thongklang, *OR* Olivier Raspé, *PC* P. Callac, *RZ* Ruilin Zhao, *SK* Samantha C. Karunarathna

[a] Sample previously used (Zhao et al. 2011)

[b] Holotype

pileus disc and in the stipe. Lamellae free, crowded, 6–10 mm wide, straight to ventricose, white when young, becoming pink, then light brown, reddish brown to dark brown. Stipe 130–170 × 10–20 (at apex) × 20–40 (at base) mm, cylindrical with abruptly bulbous base, with narrowly hollow interior; surface glabrous above the annulus, covered with flake-like to floccose, erect squamules below the annulus, white to yellowish white overall; rhizomorphs copious, coarse, white. Annulus persistent, up to 20 mm broad, membranous, pendant, lower surface floccose, white. Odor pleasant, of almonds. Neither immediate discoloration on touching and cutting, nor a transient ferrugineous reaction on stipe surface on touching; pileus surface spotting red in age.

Macrochemical reaction: Deep yellow with KOH; Schäffer reaction orange (positive).

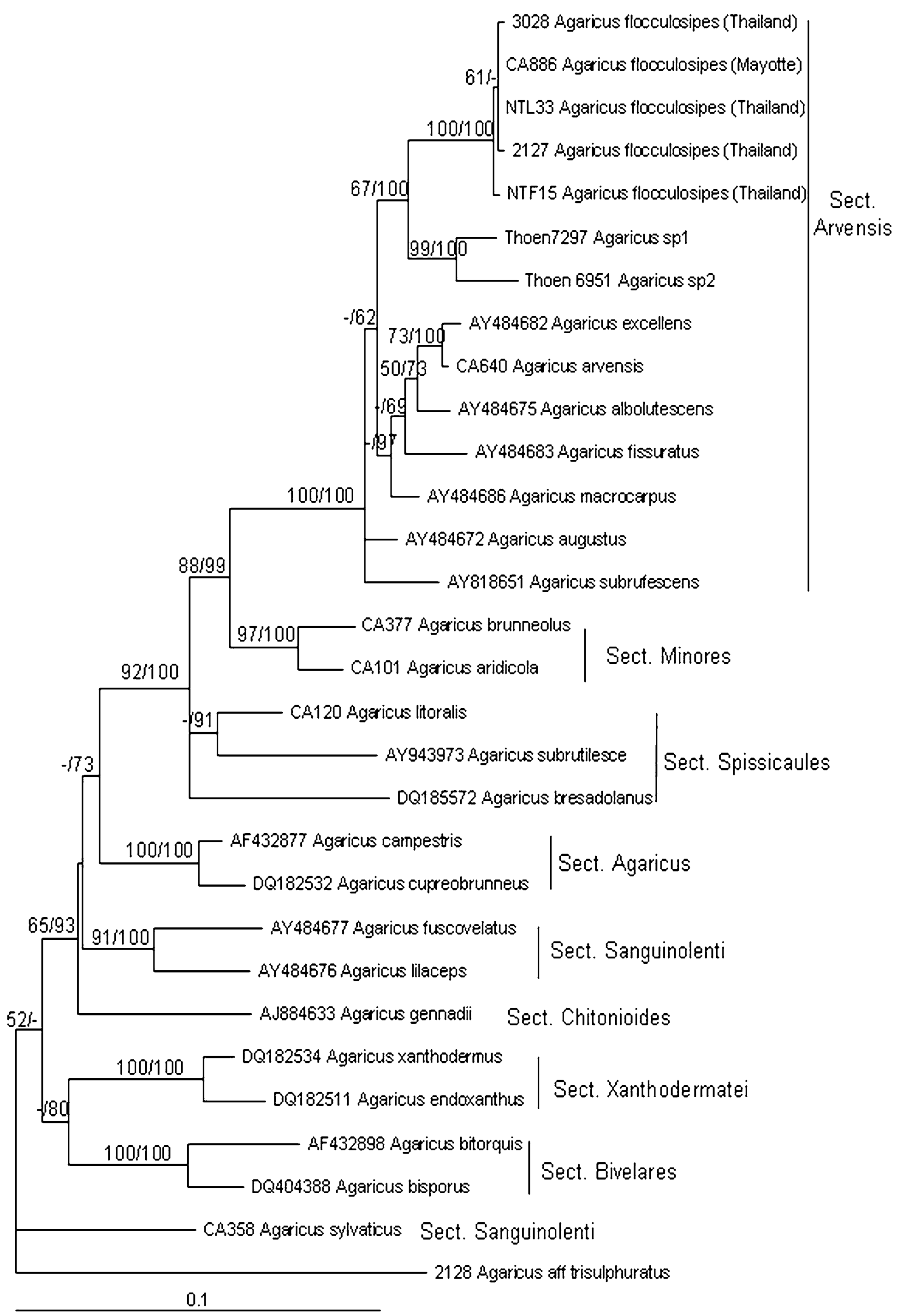

Fig. 1 Phylogeny of *Agaricus* generated from maximum likelihood analysis of ITS sequences rooted with *A.* aff. *trisulphuratus*. Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the *internodes* (BS/PP)

Basidiospores 5–7 (–9) × 3–4.5 µm (av$X = 5.91 \pm 0.2 \times 3.72 \pm 0.4$ µm, $Q = 1.3$–1.91, av$Q = 1.6 \pm 0.2$, $n = 20$ or 30 per specimen, from six specimens), ellipsoid, inequilateral in side view with a prominent oblique apiculus, without germ pore, smooth, reddish brown, thick walled. Basidia 13–17 × 6–7 (–8) µm, clavate, 4-spored. Cheilocystidia 13–23 × 8–15 µm, ellipsoid to subspherical or clavate, single or catenulate, hyaline, smooth, sometimes absent in old sporocarps. Pleurocystidia absent. Pileipellis a cutis; hyphae 5–7.5 µm in diameter, cylindrical, straight or curved, with intracellular yellowish brown pigments or rarely hyaline; squamules composed of interwoven, sometimes agglutinated hyphae 5–7.5 µm in diameter, with cylindrical terminal cells. Stipitipellis (below the annulus) a cutis of hyphae 5–10 µm in diameter, cylindrical to slightly inflated, smooth, hyaline; squamules composed of loosely interwoven, erect hyphae, 5–10 µm in diameter, terminal cells undifferentiated. Annulus hyphae 7–18 µm

Table 2 Polymorphism at 14 positions within ITS1 + 2 rDNA sequences of 14 samples of *A. flocculosipes*

Sample	Site	Position in ITS1 + 2 alignment (665 nts)													
		98–100	126	137	170	178	182	186	187	192	229	284	471–475	555	581/583
Thailand															
ZRL3028[a], ZRL2053	Doi Inthanon	TTT	T	C	C	T	A	G	A	G	T	T	TTTT–	T	CTT
NT46															
ZRL2127[a]	Ban Pha Deng	TTT	C	C	C	T	A	G	A	G	T	T	TTTT–	G	CTT
T13	Khun Korn waterfall	TTT	C	C	C	T	A	G	A	G	T	T	TTTT–	T	YTT
CA798	Huai Nam Dang	TTT	C	C	Y	T	W	G	M	G	T	T	TTTT–	T	CTT
NTF15[a], NTS70	Ban Mae Sae	TTT	C	C	T	T	T	G	A	G	T	T	TTTT–	T	CTT
NTS67	Ban Mae Sae	TTT	C	C	C	T	A	G	A	G	T	T	TTTT–	K	YTT
NTT30	Doi Tung	TTT	C	C	Y	T	T	G	A	G	T	T	TTTT–	T	CTT
NTL33[a]	Mae Fah Luang	TTT	C	C	C	T	A	G	A	G	T	T	TTTT–	T	CTT
OR71	Ban Mae Sae	TTT	C	C	Y	T	W	G	A	S	T	T	TTTT–/ TTTTT	T	CTT
France, Mayotte															
CA870	Convalescence Mamoudzou	TTT/TT–	C	Y	C	Y	A	R	A	G	Y	Y	TTTT–	T	CTT/–
CA886[a]	Idem	TT–	C	C	C	T	A	G	A	G	T	C	TTTT–	T	–

[a] Samples of which ITS1 + 2 sequences were used in the phylogenetic analysis because they differ from each other; they are complete and without any heteromorphism

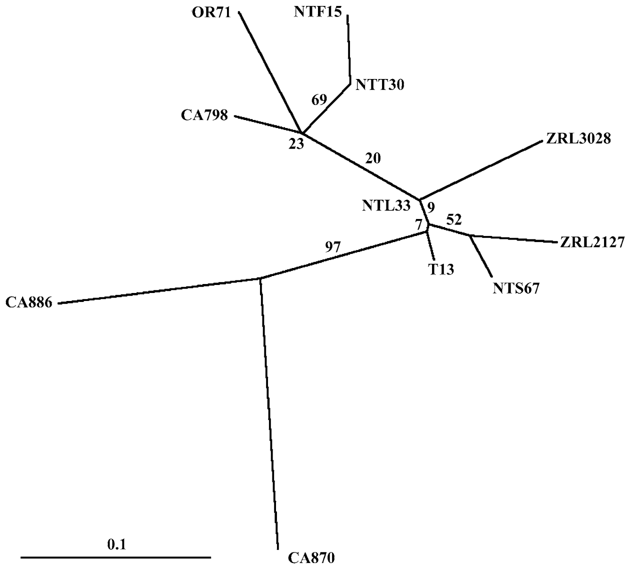

Fig. 2 Radial neighbor-joining (NJ) tree of 11 collections of *A. flocculosipes* with bootstrap support values. The 2 collections from Mayotte (CA870 and CA886) clearly diverge from the 9 remaining collections from Thailand

in diameter, ellipsoid or cylindrical, smooth, hyaline; terminal cells 32–40 × 15–25 µm, ellipsoid to clavate or pyriform, smooth, hyaline. Clamp connections absent.

Cultural behavior: For CA 798, spore germination began after 5 weeks; germination rate was extremely low but mycelium growth rate was excellent because certain subcultures invaded 9-cm-diameter Petri dishes (compost agar medium) after 8 days.

Habit: Solitary, scattered, or gregarious on rich soil or over heavily rotted wood in forests.

Material examined: Thailand, Chiang Mai Prov., Doi Inthanon, junction of Highway 1009 and road to Mae Chem, N19°31.58′, E98°29.64′, elevation 1,700 m, 5 June 2006, collected by Ruilin Zhao, ZRL3028 (BBH 19544 HOLOTYPE, SFSU ISOTYPE); same location, 27 June 2005, collected by Dennis E. Desjardin, ZRL2053 (BBH19437, SFSU); same location, 5 June 2006, collected by Todd Osmundson, ZRL3021 (BBH19538, SFSU); same location, 5 June 2006, collected by Chan Hong Twu, ZRL3022 (BBH19539, SFSU); same location, 28 June 2007, collected by Dirk Stubbe, ZRL4037 (BBH19663, SFSU); same location, 5 September 2009, collected by Samantha C. Karunarathna, NT46 (MFLU10-0110, CA735); Chiang Mai Prov., Mae Taeng District, Ban Pha Deng Village, N19°17.123′, E98°44.009′, elevation 900 m, 16 August 2005, collected by Kevin D. Hyde, ZRL2127 (BBH19507, SFSU); Chiang Rai Province, Tambon Mae Korn and Tambon Huay Chompoo, Muang District, Khun Korn

Fig. 3 *Agaricus flocculosipes.* **a** Specimen ZRL3028; holotype. **b** Specimen ZRL2053; note the well-developed partial veil. **c** Specimen CA886; note the well-developed partial veil and floccose stipe. **d** Specimen CA886; note the pileus squamules. **e** Specimen NTL33; note the well-developed partial veil. **f** Specimen CA798; note the pileus squamules. *Bar* **a** 50 mm

Waterfall, N19°51–54′, E99°35.39′, elevation 1,208 m, moist upper mixed deciduous forest (Royal Forest Department, 1962), 22 July 2009, collected by Samantha C. Karunarathna, T13 (MFLU10-0083, CA718); Chiang Mai Prov., on the way to Huai Nam Dang, N19°18.3, E98°35.9′, elevation 1,530 m, forest dominated by *Pinus*

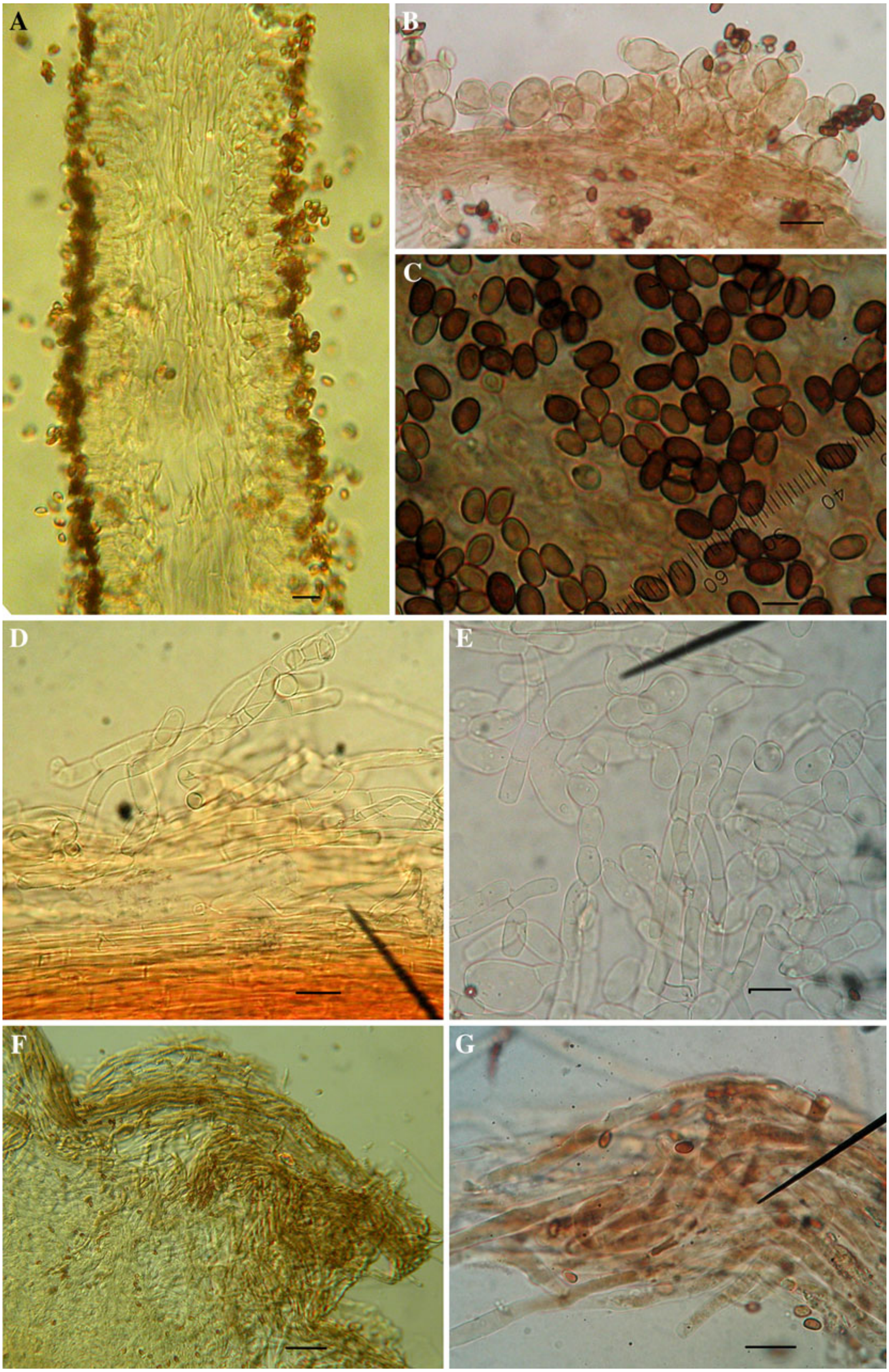

Fig. 4 Microcharacters of *Agaricus flocculosipes* (specimen ZRL3028; holotype). **a** Lamellar trama. **b** Cheilocystidia. **c** Basidiospores. **d** Hyphae from the stipe surface. **e** Hyphae from the annulus. **f**, **g** Hyphae from the pileipellis. *Bars* **a**, **b** 15 µm; **c** 6 µm; **d** 10 µm; **e** 20 µm; **f** 50 µm; **g** 15 µm

keseya, 28 July 2010, collected by Samantha C. Karunarathna and Jacques Guinberteau, CA798 (MFLU10-0970); Chiang Mai Prov., Mae Taeng, Ban Mae Sae village, on Highway 1095 near 50-km marker, rainforest dominated by *Castanopsis armata*, *Castanopsis* sp., *Pinus* sp., and *Lithocarpus* sp., N19°14.599′, E98°39.456′, elevation 962 m, 27 June 2010, collected by Naritsada Thongklang, NTF15 (MFLU10-0670); same location, 29 June 2010, collected by Samantha C. Karunarathna, NTS70 (MFLU10-0663); same location, 29 June 2010, collected by Samantha C. Karunarathna, NTS67 (MFLU10-0662); same location, 3 July 2010, collected by Olivier Raspé, OR71 (MFLU10-0903); Chiang Rai Prov., Mae Sai District, Highway No. 110 to Mae Sai, Doi Tung, deciduous forest dominated by Leguminosae (*Xylia xylocarpa* var. *kerrii*, *Pterocarpus macrocarpus*), *Afzelia xylocarpa*, and *Sindora siamensis* var. *siamensis*), N20°20′45″, E99°50′04″, elevation 950 m, 16 June 2010, collected by Komsit Wisitrassameewong, NTT30 (MFLU10-0675); Chiang Rai Prov., Thasud, Muang District, Mae Fah Luang University park dominated by *Dipterocarpus* sp., *Ficus* sp., and *Tabebuia chrysantha*, N18°05′59.1″, E102°40′02.9″, elevation 488 m, 09 August 2010, collected by Jie Chen, NTL33 (MFLU10-0777); MAYOTTE(an overseas French department located in the Indian Ocean between Madagascar and the republic of Mozambique), Concalescence Mamoudzou, in forest road, 30 January 2011, collected by M. Pelissier, CA870; and same location, 13 March 2011, collected by M. Pelissier, CA886.

Discussion

The ITS sequences analyses indicate that *A. flocculosipes* belongs to subgenus *Agaricus*, section *Arvenses* (Fig. 1). The morphological characters (yellow KOH reaction, almond odor, well-developed pileus squamules and partial veil, and catenulate cheilocystidia) suggest that it is allied with members of subsection *Augusti* in which Heinemann (1978) included *Agaricus brunneolus* (syn. *A. porphyrizon*). However, this subsection is not maintained in the recent monographs of Nauta (2001) and Parra (2008), in which *A. brunneolus* belongs to section *Minores*. In agreement with these recent classifications, our data do not support the subsection *Augusti*, and *A. brunneolus* belongs to section *Minores*.

We note that the floccose lower surface of the annulus characterizes three species of section *Arvenses* having a colored cap: *A. subrufescens*, *A. augustus*, and *A. flocculosipes*, whereas in most of the white species of the section the lower layer of the annulus is split into larger scales or often into a cog-wheel (but *A. urinascens* has a floccose lower surface). *Agaricus* sp. 1 and sp. 2, which were collected from Africa, also belong to section *Arvenses* and are sister to *A. flocculosipes* in our ITS tree. In morphology, *A.* sp. 1 differs from *A. flocculosipes* by its smooth stipe and two-layered annulus with smooth lower surface, and *A.* sp. 2 has a cylindrical stipe and the stipe surface also is smooth.

The brownish-orange or light brown, grain-like squamules and pink discoloration on the pileus of *A. flocculosipes* easily distinguish it from the north temperate species *A. arvensis* (type of subsect. *Arvenses* with white, smooth, yellow-staining pileus) and *A. augustus* (type of subsect. *Augusti* mostly with ochraceous, brown-squamulose, yellow-staining pileus). In addition, these latter species form larger basidiospores (6.5–9 × 4–6 μm) than does *A. flocculosipes* (av$X = 5.91 \pm 0.2 \times 3.72 \pm 0.4$ μm). Our molecular data also indicate that *A. flocculosipes* is distinct. Compared with other species in section *Arvenses*, which have mainly been described from temperate areas of Europe and America (Cappelli 1984; Kerrigan 1986; Nauta 2001), *A. flocculosipes* can be distinguished by its small basidiospores and with scattered erect floccose squamules on the stipe.

A number of species in section *Arvenses* have been described from tropical regions (Heinemann 1956a, b, c, 1974a, b, 1980, 1986a, b, 1990a, b, 1993; Pegler 1986). *Agaricus flocculosipes* is remarkable amongst tropical *Agaricus* species, because of its relatively large-sized basidiocarps that often grow gregariously, while most tropical species of *Agaricus* have solitary, small-sized basidiocarps. The erect floccose squamules on the stipe of *A. flocculosipes* distinguish it from known tropical members of sect. *Arvenses*, such as *A. agrocyboides* Heinem. & Gooss.-Font., *A. bambusae* Beeli, *A. brunneolus* (J.E. Lange) Pilát, *A. croceolutescens* Heinem. & Gooss.-Font., *A. heterocystis* Heinem. & Gooss.-Font., *A. kivuensis* Heinem. & Gooss.-Font., *A. meijeri* Heinem., *A. ochrascens* Heinem. & Gooss.-Font., *A. pseudoaugustus* Raithelh., and *A. xanthosarcus* Heinem. & Gooss.-Font. Exceptions include *A. albidoperonatus* Heinem. and *A. tornocephalus* Berk. & Broome. *Agaricus albidoperonatus*, described from Malaysia by Heinemann (1980), differs in forming yellow, clavate, non-catenulate cheilocystidia. *Agaricus tornocephalus*, reported from Sri Lanka (Pegler 1986), differs in forming smaller basidiocarps with very pale lemon yellow, radially fibrillose pileus 3–8 cm in diameter. Data on cheilocystidia morphology are unfortunately lacking for the latter species. *Agaricus ochraceosquamulosus* Heinem., which has been accepted as a species in subgenus *Lanagaricus* (Heinemann 1961) (but Heinemann thought it is close to section *Arvenses*), is similar to *A. flocculosipes* generally in morphology. The main difference between them is that *A. ochraceosquamulosus* forms short lanceolate, non-catenulate cheilocystidia and a much smaller cap

(60 mm in diameter), whereas *A. flocculosipes* forms clavate or subspherical, catenulate cheilocystidia and larger caps (110–180 mm in diameter).

We introduce *Agaricus flocculosipes* as a new species in section *Arvenses* distinguished by erect floccose squamules on the surface of the stipe, relatively small basidiospores (less than 6.5 μm long), and relatively large basidiocarps with pilei often 100 mm or more in diameter.

Other members of sect. *Arvenses* are often collected from the wild for the table, including *A. arvensis*, *A. augustus*, *A. albolutescens*, and *A. subrufescens*. Moreover, *A. subrufescens* (also named *A. brasiliensis*, an illegitimate name, already used by Fries 1830) has been commercially cultivated in North America and today mainly in South America (Kerrigan 2005), whereas *A. arvensis* or allied species are commercially cultivated in Europe. We postulate that *A. flocculosipes* is also a good edible species that may have potential commercial value as a new cultivatable species.

In the genus *Agaricus*, the major problems limiting species domestication are low infraspecific variability, a homothallic (haploid) life cycle in which outcrossing is not possible, or a low mycelium growth rate that makes cultivation not feasible. The variability observed between samples of *A. flocculosipes* collected in different sites in Thailand and in Mayotte suggests the existence of a large infraspecific variability. Inter-site genetic variability appears to be much higher than intra-site variability. The observed variability at 14 polymorphic loci in ITS1 + 2 agrees with an allelic polymorphism with homo- and heteroallelic genotypes and some evidence of recombination. The studied sample is strongly structured in two geographic populations that share a single allele at each locus while the other alleles are private. These data strongly suggest that the sporophores are heterokaryotic, that outcrossing and recombination occurs in this species, and that gene flow between the two geographic populations would be null or very low. The mycelium growth rate seems acceptable for substrate colonization. The present data indicate that *A. flocculosipes* is favorable for further investigation of the life cycle, genetic improvement of strains, and cultivation on appropriate substrates. However, the interfertility between the populations of Thailand and Mayotte requires verification.

Acknowledgments We are grateful to Amy Honan, Chan Hong Twu, Dirk Stubbe, Edward Grand, Jennifer Kerekes, Jiankui Liu, Nilam Wulandari, Maria Alice Neves, Nopparat Wannathes, Thitiya Boonpratuang (Pop), Phongeun Sysouphanthong, Todd Osmundson, Thanh Huyen Le, and Tran Thi My Hanh for their help in sample collection. We thank the National Natural Science Foundation of China (Project ID: 31000013 to Ruilin Zhao), the European Distributed Institute of Taxonomy (WP2-2010 integrating research grant, Callac Principal Investigator), and the National Science Foundation (USA; Grant DEB-0118776 to Desjardin) for providing partial financial support to this research. BRN grant (BRN049/2553) is acknowledged for support to study the taxonomy of potentially edible mushrooms in Thailand. The Global Research Network for Fungal Biology and King Saud University are also thanked for supporting this project. Gaston Heuclin and Luis Parra are thanked for advice on the Latin.

References

Alberto E (1998) *Agaricus santacatalinensis* a new species from Argentina. Mycotaxon 66:205–213

Bohus G (1975) *Agaricus* studies, V. (Basidiomycetes, Agaricaceae). Annales Historico Naturales Musei Nationalis Hungarici 67:37–40

Bohus G (1990) *Agaricus* studies, X. (Basidiomycetes, Agaricaceae). Annales Historico Naturales Musei Nationalis Hungarici 81:37–44

Callac P (1993) Morphological, genetic and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran desert of California. Mycologia 85:835–851

Callac P, Guinberteau J (2005) Morphological and molecular characterization of two novel species of *Agaricus* section *Xanthodermatei*. Mycologia 97:416–424

Callac P, Billette C, Imbernon M, Kerrigan RW (1993) Morphological, genetic, and interfertility analyses reveal a novel, tetrasporic variety of *Agaricus bisporus* from the Sonoran Desert of California. Mycologia 85:335–351

Callac P, Jacobe de Haut I, Imbernon M, Guinberteau J, Theochari I (2003) A novel homothallic variety of *Agaricus bisporus* comprises rare tetrasporic isolates from Europe. Mycologia 95:222–231

Cappelli A (1984) *Agaricus* L.: Fr. (*Psalliota* Fr.). Libreria Editrice Biella Giovanna, Saronno

Challen MP, Kerrigan RW, Callac P (2003) A phylogenetic reconstruction and emendation of *Agaricus* section *Duploannulatae*. Mycologia 95(1):61–73

Desjardin DE, Flegel T, Boonpratuang T (2004) Basidiomycetes. In: Jones EBG, Tabticharoen M, Hyde KD (eds) Thai fungal diversity. BIOTEC, Thailand

Desjardin DE, Binder M, Roekring S, Flegel T (2009) *Spongiforma*, a new genus of gastroid Boletes from Thailand. Fungal Divers 37:1–8

Esteve-Raventós F (1998) *Agaricus heinemannianus*, a new species of section *Minores*. Belg J Bot 131(2):163–168

Freeman AEH (1979a) *Agaricus* in North America: type studies. Mycotaxon 8:1–49

Freeman AEH (1979b) *Agaricus* in the southeastern United States. Mycotaxon 8:50–118

Fries E (1830) Eclogae fungorum. Praecipue ex Herbariis Germanorum de Scriptorum. Linnaea 5:509

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: application to identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Geml J, Laursen GA, Nusbaum HC, Taylor DL (2007) Two new species of *Agaricus* from the Subantarctic. Mycotaxon 100:193–208

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Series 41:95–98. http://www.mbio.ncsu.edu/bioedit/bioedit.html

Heinemann P (1953) Etat actuel de la systématique de genre *Agaricus*. Mushroom Sci 2:8–11

Heinemann P (1956a) Champignons récoltés au Congo belge par Mme M. Goossens-Fontana, II. *Agaricus* Fr. s.s. Bull Jard Bot l'Etat Bruxelles 26:1–127

Heinemann P (1956b) Champignons récoltés au Congo belge par Mme M. Goossens-Fontana, II. *Agaricus*. Note complémentaire. Flore Iconographique des Champignons du Congo 5:325–333

Heinemann P (1956c) Sur divers facteurs agissant sur la formation des primordiums de *Agaricus edulis* en culture pure. VIlle Congrès de Bot., C R des Séances et Rapports et Comm. Déposées lors du Congrès. dans les sect. 18, 19, 20:48–50

Heinemann P (1957a) *Agaricus* (suite) et *Pilosace*. Flore Iconographique des Champignons du Congo 6:121–130

Heinemann P (1957b) *Agaricus robynsianus* sp. nov. Bull Jard Bot l'Etat Bruxelles 27:449–452

Heinemann P (1961) Agarici Austroamericani I. Agarics of Trinidad. Kew Bull 15:231–248 (pl. 1)

Heinemann P (1962a) Agarci Austroamericani IV. Quatre *Agaricus* du Venezuela. Bull Jard Bot l'Etat Bruxelles 32:155–161

Heinemann P (1962b) Agarici Austroamericani II. *Agaricus* de Bolivie. Bull Jard Bot l'Etat Bruxelles 32:1–21

Heinemann P (1962c) Agarici Austroamericani III. Trois *Agaricus* de la Jamaïque. Bull Jard Bot l'Etat Bruxelles 32:23–29

Heinemann P (1962d) Agarici Austroamericani, V. Etude des types de C. Spegazzini. Bulletin de l'Institut Agronomique et des Stations de Recherches de Gembloux 30:273–282

Heinemann P (1965) Notes sur les Psalliotes (*Agaricus*) du Maroc. Bull Soc Mycol Fr 81:372–401

Heinemann P (1971) Quelques Psalliotes du Congo-Brazzaville. Cahier Makobé 9:5–10

Heinemann P (1974a) *Agaricus kuehnerianus* sp. nov., la Psalliote géante des Alpes de Haute Provence. Bulletin Mensuel de la Société Linnéenne de Lyon No. sp: 181–187

Heinemann P (1974b) Quelques *Agaricus* de Nouvelle-Zélande. Bull Jard Bot Natl Belg 44:355–366

Heinemann P (1977) Les Psalliotes. Nat Belges 58:145–165

Heinemann P (1978) Essai d'une clé de determination des genres *Agaricus* et *Micropsalliota*. Sydowia 30:6–37

Heinemann P (1980) Les genres *Agaricus* et *Micropsalliota* en Malaisie et en Indonésie. Bull Jard Bot Natl Belg 50:3–68

Heinemann P (1982) Quelques Psalliotes de Nouvelle Guinée (Papua New Guinea). Bull Jard Bot Natl Belg 52:405–413

Heinemann P (1986a) Agarici austroamericani VI. Apercu sur les *Agaricus* de Patagonie et de la terre de Feu. Bull Jard Bot Natl Belg 56:417–446

Heinemann P (1986b) *Agaricus geesteranii* sp. nov. A very remarkable agaric discovered in the Netherlands (en collaboration avec C. Bas). Persoonia 13:113–121

Heinemann P (1987) Clave para la determinacion de las especies de *Agaricus* (Agaricales) de la Patagonia y Tierro del Fuego. Darwiniana 28:283–291

Heinemann P (1988) Novitates generis *Micropsalliota* (Agaricaceae). Bull Jard Bot Natl Belg 58:540–542

Heinemann P (1990a) Agarici austroamericani VII: Agariceae des zones tempérées de 1'Argentine et du Chili. Bull Jard Bot Natl Belg 60:331–370

Heinemann P (1990b) *Agaricus singaporensis* sp. nov. Bull Jard Bot Natl Belg 60:417–419

Heinemann P (1993) Agarici Austroamericani VIII. Agariceae des regions intertropicales d'Amérique du Sud. Bull Jard Bot Natl Belg 62:355–384

Høiland K, Schumacher T (1982) Agaric, clavarioid and some heterobasidiomycetous fungi from north Thailand. Nordic J Bot 2:265–271

Hotson JW, Stuntz DE (1938) The genus *Agaricus* in western Washington. Mycologia 30:204–234

Huelsenbeck JP, Ronquist F (2001) MrBayesian: Bayesian inference of phylogeny. Biometrics 17:754–755

Huelsenbeck JP, Ronquist F, Bollback JP (2001) Bayesian inference of phylogeny and in its impact on evolutionary biology. Science 294:2310–2314

Huijsman HSC (1960) Notes sur le genre *Agaricus*. Persoonia 1(3):321–324

Jin L, Chakraborty R (1993) Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. Mol Biol Evol 11:120–127

Karunarathna SC, Yang ZL, Zhao RL, Vellinga EC, Bahkali Chukeatirote AHE, Hyde KD (2010) Three new species of *Lentinus* from northern Thailand. Mycol Prog. doi:10.1007/s11557-010-0701-6

Kerekes JF, Desjardin DE (2009) A monograph of the genera *Crinipellis* and *Moniliophthora* from Southeast Asia including a molecular phylogeny of the nrITS region. Fungal Divers 37:101–152

Kerrigan RW (1985) Studies in *Agaricus* III: new species from California. Mycotaxon 20(2):419–434

Kerrigan RW (1986) Agaricales of California. Agaricaceae, vol 6. Mad River Press, Eureka

Kerrigan RW (1989) Studies in *Agaricus* IV: new species from Colorado. Mycotaxon 34:119–128

Kerrigan RW (2005) *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. Mycologia 97:12–24

Kerrigan RW, Callac P, Guinberteau J, Challen MP, Parra LA (2006("2005")) *Agaricus* section *Xanthodermatei*: a phylogenetic reconstruction with commentary on taxa. Mycologia 97: 1292–1315

Kornerp A, Wanscher JH (1978) Methuen handbook of colour, 3rd edn. Methuen, London

Lacheva MN, Stoichev GT (2004) New species (records) of the genus *Agaricus* (Agaricaceae) for Bulgaria. Mycol Balcan 1:35–40

Lanconelli L (2002) *Agaricus padanus* sp. nov. Riv Microl 1:29–37

Langella O (1999–2010) POPULATIONS 1.2.32. http://www.bioinformatics.org/project/?group_id=84

Largent DL (1986) How to identify mushrooms to genus, vols 1–5. Mad River Press, Eureka

Mitchell AD (1999) Phylogenetic relationships of *Agaricus* species based on ITS-2 and 28S ribosomal DNA sequences. Mycologia 91:811–819

Mitchell AD, Walter M (1999) Species of *Agaricus* occurring in New Zealand. N Z J Bot 37:715–725

Möller FH (1950) Danish *Psalliota* species, I–II. Friesia IV:1–60

Murrill WA (1912) The Agaricaceae of the Pacific Coast III. Mycologia 4:294–300

Murrill WA (1918) The Agaricaceae of tropical North America, VIII. Mycologia 10:62–85

Murrill WA (1941) More Florida novelties. Mycologia 33:434–448

Natarajan K, Kumaresan V, Narayanan K (2005) A checklist of Indian Agarics and Boletes (1984–2002). Kavaka 33:61–128

Nauta MM (1999) Notulae ad floram Agaricinam neerlandicam–XXXIII: notes on *Agaricus* section *Spissicaules*. Persoonia 17(2):221–233

Nauta MM (2000) Notulae ad floram Agaricinam neerlandicam–XXXVII: notes on *Agaricus* section *Arvenses*. Persoonia 17(3):457–463

Nauta MM (2001) *Agaricus* L. and *Allopsalliota Nanta* et Bas. In: Noordeloos ME, Kyuper TW, Vellinga EC (eds) Flora Agaricina Neerlandica: Critical monographs on families of Agarics and Boleti occurring in the Netherlands, vol 5. Family Agaricaceae. Balkema, Lisse, pp 23–62

Nylander JAA (2004) MrModeltest 2.2 Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Orton PD (1960) New check list of British Agarics and Boleti: part III. Notes on genera and species in the list. Trans Br Mycol Soc 43:159–439

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computer. Comput Appl Biosci 12:357–358

Parra LA (2003) Contribution to the knowledge of the genus *Agaricus* [Fingi Delineati. Pars XXIV]. Edizioni Candusso, Alassio

Parra LA (2008) *Agaricus* L. *Allopsalliota* Nauta & Bas. Edizioni Candusso, Alassio

Pegler DN (1977) A preliminary Agaric flora of East Africa. Kew Bull Addit Ser 6:1–615

Pegler DN (1983) Agaric flora of the lesser Antilles. Kew Bull Addit Ser 9:1–667

Pegler DN (1986) Agaric flora of Sri Lanka. Kew Bull Addit Ser 12:1–519

Pegler DN (1990) Agaricales of Brazil described by J.P.F.C. Montagne. Kew Bull 45:161–177

Pegler DN, Rayner RW (1969) A contribution to the Agaric flora of Kenya. Kew Bull 23:347–412

Peterson KR, Desjardin ED, Hemmes Don E (2000) Agaricales of Hawaiian Islands. 6. Agaricaceae L. Agaricaceae: *Agaricus* and *Melanophyllum*. Sydowia 52(2):204–257

Robinson MM, Chiang B, Horgen PA (2001) A phylogeny of the genus *Agaricus* based on mitochondrial atp6 sequences. Mycologia 93:30–37

Ronquist F, Heulsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Ruksawong P, Flegel TW (2001) Thai Mushrooms and other Fungi. National Centre for Genetic Engineering and Biotechnology (BIOTEC). National Science and Technology Development Agency, Bangkok

Saini SS, Atri NS, Gupta AK (1997) Studies on the genus *Agaricus* L.: Fr.: the subgenus *Agaricus* section *Sanguinolenti* Schaeff et Motter from northwest India. Mushroom Res 6(2):53–58

Singer R (1986) The Agaricales in modern taxonomy, 4th ed. Koeltz Scientific Books, Koenigsternm, Germany, p981

Smith AH (1944) New North American Agarics. Mycologia 36:242–262

Swofford DL (2004) PAUP*: Phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Sunderland

Thomson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4875–4882

Valenzuela E, Heinemann P, Esteve-raventos FS, Polette GM, Castells W (1997) A new contribution to the knowledge of the genus *Agaricus* in Chile. *Agaricus wrightii* sp. nov. Mycotaxon 63:71–76

Wannathes N, Desjardin DE, Hyde KD, Perry BA, Lumyong S (2009) A monograph of *Marasmius* (Basidiomycota) from Northern Thailand based on morphological and molecular (ITS sequences) data. Fungal Divers 37:209–306

White TJ, Bruns TL, Lee S, Taylor JW (1990) Application and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gefand DH, Sninsky JJ, White JT (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego

Zhao RL, Desjardin DE, Soytong K, Perry BA, Hyde KD (2010) A monograph of *Micropsalliota* in Northern Thailand based on morphological and molecular data. Fungal Divers 45:33–79

Zhao RL, Karunarathna S, Raspé O, Parra LA, Guinberteau J, Moinard M, De Kesel A, Barroso G, Courtecuisse R, Hyde KD, Guelly AK, Desjardin DE, Callac P (2011) Major clades in tropical *Agaricus*. Fungal Divers (in press)