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Contributed Paper

Micropsalliota brunneosquamata, a New Species from Thailand

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ABSTRACT

A new species of *Micropsalliota*, *M. brunneosquamata* is described and illustrated from northern Thailand based on both morphological and molecular data. The new species is characterized by brownish, erect scales at the centre of the pileus, a stipe covered by concolourous fibrils, and microscopically, by the shape of cheilocystidia which sometimes show obtuse mucro at apex. The new species is compared with morphologically similar species. Phylogenetic analyses of ITS sequences also support its novelty.

Keywords: Agaricaceae, Agaricales, ITS, phylogeny, taxonomy

1. INTRODUCTION

The genus *Micropsalliota* HÖhn. was established by von HÖhnel [1] and emended by Pegler and Rayner [2] and Heinemann [3]. *Micropsalliota* species are mainly distributed in tropic areas of Africa, Asia and the Americas [4-9]. Zhao et al. provided a monograph of *Micropsalliota* from Northern Thailand based on morphological and molecular data, and described 23 species, including 10 new taxa and 13 new records for Thailand. Phylogenetic relationships within the genus have been investigated using analysis of nrITS and nrLSU sequence datasets [10]. *Micropsalliota* comprises species with small to middle sized basidiomes with a membranous partial veil, basidiospores with an apically thickened endospore and lack of a germ spore, capitate or subcapitate cheilocystidia and incrusted pileipellis hyphae which often turn olive-green in ammonia solution [10].

The present paper is part of our continued research on macrofungi from Northern Thailand [10-15], which already revealed high biodiversity. *Micropsalliota brunneosquamata* is introduced here as a new species based on morphological and molecular data.

2. MATERIALS AND METHODS

2.1 Morphological Character Examination

Collections of marcofungi were made in Northern Thailand from 2004 to 2012 [14, 15]. Photographs were taken in the field, the odour and colour change on bruising were recorded at the same time. Specimens were gathered and wrapped in aluminum foil to avoid mixing or crushing. The study of macrocharacters, including chemical testing and further photography of fresh samples were carried out following the methodology described by Largent [16], once returned to the laboratory. Colour terms followed those of Kornerup and Wanscher [17]. Specimens were dried using a food drier, sealed in plastic bags, and deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU) and BIOTEC Bangkok Herbarium (BBH). Duplicate specimens are deposited in Herbarium Mycologicum Academiae Sinicae (HMAS), Beijing China.

Microscopic features, including of anatomy of lamellae, pileipellis, and partial veil, and features of basidiospores, basidia and cystidia, were examined from dried specimens following the protocols of Largent [16]. Measurements of microscopic features (spores, basidia and cheilocystidia) are 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.

2.2 Molecular Phylogenetic Research

2.2.1 Nucleic acid preparation and ITS amplification

DNA was extracted from dried fungal specimens with a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01, Omega Bio-Tek) according to manufaturer protocol. The PCR reactions were performed using primers ITS4/ITS5 following the protocol of White *et al.* [18] with some modification [10]. Amplification failed in collections ZRL3073 and ZRL4038. Sequencing was performed on an ABI 3730 XL DNA analyzer (Applied Biosystems) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China. The ITS sequence generated from this study is available in GenBank under the accession number of KP316210.

2.2.2 Sequence alignment and phylogenetic analysis

In order to investigate the phylogenetic position of the new species, representative sequences were selected based on the previous study of Zhao et al. [10]. The original sequence generated from this study plus those derived from GenBank were initially aligned using T-coffee ver 8.99 [19], then manually adjusted in BioEdit v. 7.0.4 [21].

A Maximum likelihood (ML) tree was constructed using PhyML ver 3.0 [22] on the Phylogeny. fr platform (http://www.phylogeny.fr/). The HKY85 substitution model was selected with an estimated proportion of invariable sites of 0.413 and assuming 4 gamma-distributed rate categories. The gamma shape parameter 0.547 was directly estimated from the data. Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 [23], by heuristic searches with unordered character states, gaps treated as missing data, random addition of sequences, and the tree bisection-reconnection (TBR) branch swapping. Bootstrap support values (BS) were obtained from 100 and 1000 replicates for ML or MP trees. Bayesian analysis was performed with MrBayes 3.1.2 [24]. Six Markov chains using a GTR+I+G model nucleotide substitution detected by MrModeltest 2.2 [25] were run for one million generations and sampled every 100th generation, which resulted in 10,000 trees.

Those trees sampled prior to Markov chains reaching an average standard deviation of split frequency value of 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 visualized in TreeView and exported to graphics programs [26].

3. RESULTS

3.1 Phylogenetic Analyses

The ITS dataset includes the 5.8S gene and contains 33 ITS sequences representing 21 *Micropsalliota* taxa, *Leucoagaricus meleagris* (Gray) Singer and *L. americanus* (Peck) Vellinga were included as outgroup taxa. The final alignment contained 655 characters, of which 463 were constant, 58 were parsimony-uninformative and 134 were parsimony-informative. The phylogenetic trees generated by ML, MP and Bayesian methods exhibited very similar topologies. Differences are only noted in the branching pattern of *M. allantoidea* and *M. alba*. The Maximum Likelihood (ML) tree is shown in Figure 1. In all analyses the outgroup taxa group at the base of the tree. Various species clades received moderate to strong support (76-100% BS), however, the deeper nodes indicating relationships amongst species are generally not well-resolved. The new species is sister to a well-supported clade (99/97/100) comprising *M. pusillissima*, *M. alboserica*, *M. gracilis*, *M. subalba*, *M. alba*, *M. allantoidea*, *M. bifida*, *M. subarginea*, *M. arginophaea* and *M. pseudoarginea.*

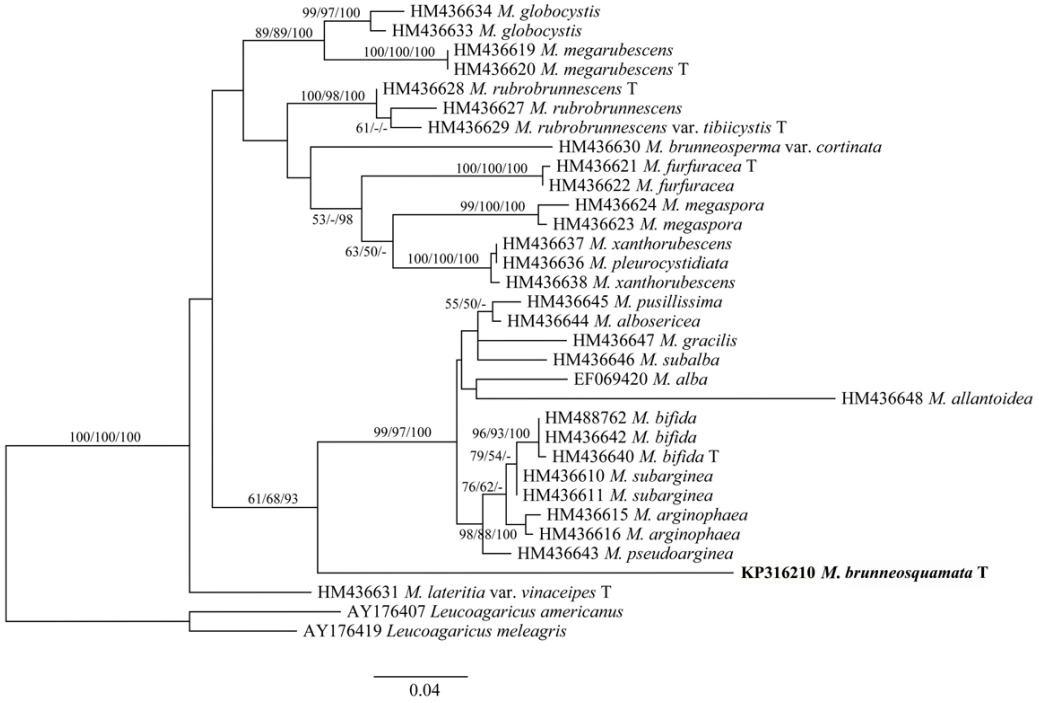

Figure 1. Maximum likelihood tree of *Micropsalliota* based on ITS sequence data. *Leucoagaricus meleagris* and *L. americanus* are outgroup taxa. Bootstrap support values (ML/MP) above 50% and Bayesian posterior probabilities (PP) values above 90% are shown at nodes. The new species *M. brunneosquamata* is indicated in bold type. "T" refers to sequence from type specimen.

3.2 Taxonomy

Micropsalliota brunneosquamata L.J. Chen, R.L. Zhao & K.D. Hyde, sp. nov. (Figure 2, 3)

MycoBank: MB811168

Etymology: refers to the brownish scales on the pileus.

Pileus 31-40 mm in diam., convex to applanate, subumbonate; cuticle exceeding the lamellae; surface dry, covered by erect scales at disc and appressed scales or fibrillose squamules elsewhere, reddish-brown (8E6) or dark brown, against whitish background. *Context* 2 mm broad, soft, white, unchanging when cut. *Lamellae* free, crowded, ventricose, lamellae with 4 series, 3 mm broad, reddish-grey (8C2) to light brown. *Stipe* 28-43 × 3-5 mm, cylindrical with slightly bulbous base, occasionally with rhizomorphs, hollow; surface above ring white, fibrillose, below ring heavily covered by brown fibrils. *Annulus* white, superous, fragile. *Odor* of anise or seaweed.

Macrochemical reactions: KOH reaction reddish brown on surface of pileus, then dark green.

Basidiospores 5-6 × 3-4 µm, [x = 5.4 ± 0.22 × 3.4 ± 0.21, Q = 1.37-1.83, Q_m = 1.61 ± 0.03, n = 20], amygdaliform to ellipsoid, apiculate at apex and base, without germ pore, light yellowish brown to light brown. *Basidia* 11-17 × 5.5-7 µm, clavate, hyaline, smooth, 4-spored, occasionally 2-spored. *Cheilocystidia* various in shape, 15-42 × 8-23 µm, napiform, ventricose-clavate, rarely clavate, sometimes mucronate to lageniform (obtuse mucro up to 6-18 µm long), hyaline. *Pleurocystidia* absent. *Pileipellis* a cutis composed of hyphae of 5-25 µm diam., smooth-walled, constricted at the septa, with brown, incrusted, membranous pigments. *Annulus* hyphae of (2.5)7.5-12.5 µm in diam, hyaline, smooth, similar to pileipellis. *Stipitipellis* composed of hyphae similar to pileipellis.

Habit: Solitary in soil of forest.

Material examined: THAILAND, Chiang Mai Prov., Mae Pong Nature Trail, 5 June 2012, collector Jie Chen, Philippe Callac, LD201236 (MFLU 120880, holotype), GenBank accession number: KP316210; Chiang Mai Prov., Tung Joaw, 30 June 2006, collector Le Huyen, ZRL3073 (BBH); Chiang Mai Prov., Mae Taeng Dist. Highway 1095 at 22 km marker, 30 June 2007, collector Else Vellinga, ZRL4038 (BBH).

Figure 2. Morphology of *M. brunneosquamata*. a. Brownish scales on pileus surface (LD201236 holotype). b. Annulus and stipe characters (LD201236 holotype). c. Context when cut (ZRL3073). d. Sporocarps in laboratory (ZRL3073). a and b photographed by P. Callac.

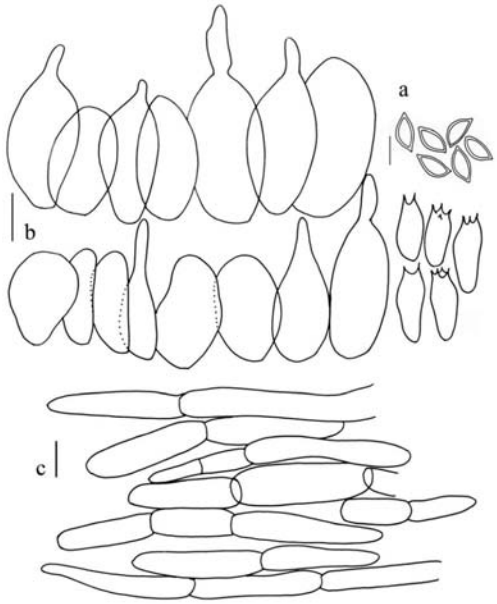

Figure 3. Microcharacters of *M. brunneosquamata*. a. Basidiospores. b. Basidia and Cheilocystidia. c. Pileipellis. Scale bars: a, b = 10 µm, c = 5 µm.

4. DISCUSSION

Micropsalliota brunneosquamata is characterized by brownish, erect scales at the centre of the pileus, the stipe with concolourous fibrils, and microscopically, by the particular shape of the cheilocystidia. *Micropsalliota brunneola* Heinem is the most phenotypically similar species because both of them have brownish erect squamules at the centre of the pileus and concolourous fibrils on the surface of the stipe below the annulus. However, the latter species differs in having smaller sporocarps (up to 30 mm in diam), smaller spores (4.1-4.9 × 2.9-3.3 µm) and brownish cheilocystidia of which the shape and size were not mentioned by Heinemann [4]. Several species exhibit colored fibrils on the stipe surface, for example: *M. lateritia* var. *vinaceipes* R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde, a variety newly described from Thailand, which is easily distinguished from the new species by its violet brown squamules of the pileus, violet tones on the stipe surface, and subcapitate cheilocystidia [10]; *M. megaspora* R.L. Zhao, Desjardin, K. Soytong & K. D. Hyde has much smaller sporocarps (5-12 mm in diam), larger basidiospore (6-8 × 3.8-4.5 µm), and ventricose-rostrate to pyriform cheilocystidia [10]; *M. pilicystis* Heinem. differs by purple squamules of the pileus and much larger cheilocystidia, which are clavate or cylindrical with an capitate apex [4]; *M. pleurocystidiata* Heinem. & Little Flower differs in forming more robust sporocarps up to 130 mm in diam., a pileus surface entirely covered with appressed fibrillose squamules, and the presence of pleurocystidia [3, 10]; *M. suthepensis* R.L. Zhao, Desjardin, K. Soytong & K.D. Hyde differs in having a reddish-brown pileus and clavate cheilocystidia [10]. Molecular phylogenetic analyses also support *M. brunneosquamata* as a distinct species.

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