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FULL PAPER

Macrolepiota subcitrophylla sp. nov., a new species with yellowish lamellae from southwest China

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Abstract *Macrolepiota subcitrophylla* sp. nov. is described from Yunnan and Hunan Provinces, China. Morphologically, it is characterized by its yellowish lamellae, relatively small, ovoid to subamygdaliform-ellipsoid, dextrinoid basidiospores with distinct germ pore, narrowly clavate, clavate to subfusiform cheilocystidia, and a pileus covering composed of a trichoderm of subcylindrical hyphae. Sequences from the internal transcribed spacer region show that *M. subcitrophylla* is distinct from all other *Macrolepiota* species tested, suggesting it is a taxon close to *M. clelandii*, a species originally described from Australia.

Keywords Agaricaceae · Agaricales · Lepiotaceous fungi · New taxon · Taxonomy

Introduction

Macrolepiota Singer (Agaricaceae, Agaricales, Basidiomycota) is a genus distributed worldwide and containing three sections: sect. *Macrolepiota* Singer, sect. *Macrosporae* Singer, and sect. *Volvatae* Z.W. Ge, Zhu L. Yang & Vellinga (Singer 1986; Vellinga et al. 2003; Ge et al. 2010). Recent study based on the internal transcribed spacer region (ITS), as well as morphological data, showed that there are six species occurring in China (Ge et al. 2010). As the field expeditions were carried out, three interesting *Macrolepiota* collections with yellowish lamellae were encountered. Subsequent morphological examination and molecular phylogenetic analyses based on ITS sequences confirmed that these collections are distinct from the other species of *Macrolepiota*; thus, they are described here as a new species.

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Materials and methods

Morphological observations

The examined materials were collected in Hunan Province, central China, and Yunnan Province, southwestern China, and are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Additional materials also used in this study include *M. dolichaula* (Berk. & Broome) Pegler & R.W. Rayner (HKAS 59721) collected from Hainan Province, *M. velosa* Vellinga & Zhu L. Yang (HKAS 59720) from Yunnan Province, and *M. detersa* Z.W. Ge, Zhu L. Yang & Vellinga (HKAS 58245 and HKAS 58252) from Hunan Province. Color notations indicated in the description are from Kornerup and Wanscher (1978). In the description, macromorphology is based on the field notes and color slides of the material; micromorphology is based on observations of the material under a light microscope at 1,000× magnification. Tissue was sectioned by hand and mounted in 5% KOH, and pileal structure, cheilocystidia, spores, and basidia were observed. KOH mounts were then stained in Congo red for the preparation of line drawings. Melzer's reagent was used to test the amyloidity of spores. Spore wall reaction to Cresyl blue and Congo red were also checked. In the descriptions of basidiospores, the

abbreviation "*n*/*m*/*p*" indicates *n* basidiospores measured from *m* fruit bodies of *p* collections. Dimensions for basidiospores are given using notation of the form (a)b–c(d). The range b–c contains a minimum of 90% of the measured values. Extreme values (a and d) are given in parentheses. *Q* indicates length/width ratio of a spore in side view; avQ means average *Q* of all basidiospores ± sample standard deviation.

DNA isolation and amplification

Tissues were ground in 1.5-ml Eppendorf tubes using a plastic pestle, and genomic DNA was extracted from dried material with a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Gardes and Bruns 1993). ITS/5.8 S rDNA were amplified using primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993), and the polymerase chain reaction (PCR) parameters follow that used in Ge et al. (2010). PCR products were purified using a QIAquick PCR purification kit (Qiagen Science, USA). Sequencing primers for the ITS regions were ITS1F and ITS4. PCR products were sent to Shanghai Sangon Biological Engineering Technology and Service Co., Ltd. for sequencing. Sequence chromatograms were compiled with Seqman (DNA STAR Package; DNAStar, Madison, WI, USA). The sequences produced in this study have been deposited in Genbank with accession numbers JN180320–JN180325.

Phylogenetic analyses

Sequences obtained from this study were aligned with ITS sequences of those taxa in fig. 1 of Ge et al. (2010) using CLUSTAL X 1.81 (Thompson et al. 1997), followed by manual inspection and correction. The final alignment was deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11720). The resulting ITS data set was evaluated using two tree-building methodologies: the maximum parsimony (MP) criterion in PAUP* and the Bayesian criterion. Gaps were treated as missing data in all analyses.

Maximum parsimony analysis was performed using PAUP* 4.0b10 (Swofford 2004). One hundred heuristic searches were conducted with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithms, collapsing zero-length branches and saving all minimal length trees (MulTrees), and MaxTrees were set to 2,000. To measure relative support for the resulting clades, 100 bootstrap replications were performed with the same parameters as for the parsimony analyses (Felsenstein 1985).

To test alternative phylogenetic relationships, the Bayesian analysis was performed using the Metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm with Mr. Bayes V3.1.2 (Ronquist and Huelsenbeck 2003). TVM + G was determined as the best-fit evolutionary model by comparing different evolutionary models via the Akaike information criterion (AIC) in Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004). Bayesian analyses with the selected evolutionary model were repeated for 3 million generations by running one cold and three heated chains in two parallel analyses, and sampled every 100th generation. The burn-in was determined by checking the average deviation of split frequencies that were less than 0.01 (Ronquist and Huelsenbeck 2003), and inspecting the log-likelihood by generation plot generated with Tracer v1.5 (Rambaut and Drummond 2007). The first 7,500 trees were discarded as the burn-in, and the remaining trees were summarized in a 50% majority-rule consensus tree, yielding the Bayesian posterior probability (PP) of each clade being monophyletic.

Results

The aligned data set included 757 base pairs, of which 26 bases were ambiguous and were excluded in the analyses. Among the analyzed 731 base pairs, 490 are constant, 53 are variable parsimony-uninformative characters, and 188 variable parsimony-informative characters were used to reconstruct the phylogeny. Maximum parsimony analysis resulted in three equally parsimonious trees with a tree length of 427 steps (CI = 0.719, RI = 0.947). Figure 1 shows one of the most parsimonious trees. Bayesian analysis produced in similar results, except that the clade formed by *M. eucharis* Vellinga & Halling and *M. velosa* changed to be the sister group of the/macrosporae clade composed by *M. excoriata* (Schaeff.) Wasser, *M. mastoidea* (Fr.) Singer and their allies, but this result did not achieve statistical support.

As shown in Fig. 1, within *Macrolepiota*, three major clades, namely/volvatae clade,/macrosporae clade, and/macrolepiota clade, were recovered, and this is in accordance with results from previous studies (Vellinga et al. 2003; Ge et al. 2010). Within the/macrolepiota clade, the two ITS sequences of *M. subcitrophylla* form a well-supported monophyletic group and have strong bootstrap (100%) and Bayesian PP support (1.00). A species described from Australia, *M. clelandii* Grgur., forms a sister clade of *M. subcitrophylla* in the strict consensus tree (figure not shown), and this clade had 50% bootstrap support.

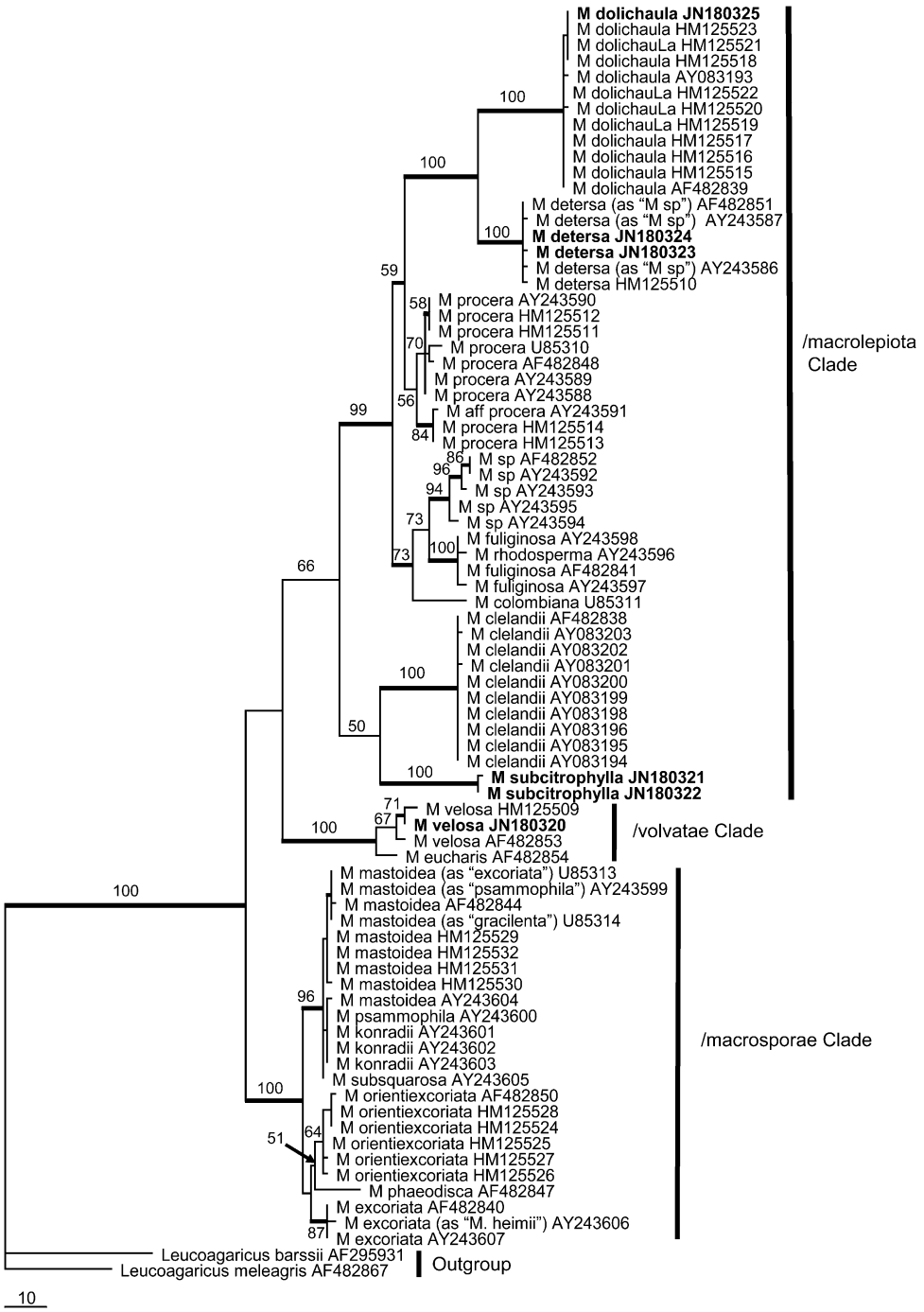


Fig. 1 One of three equally parsimonious trees ($L = 427$, CI = 0.719, RI = 0.947) obtained in parsimony analysis of internal transcribed spacer (ITS) sequence data. Terminal taxa represent individual specimens with GenBank accession number, and branch lengths are proportional to the number of steps (character changes) along the branch. Bootstrap support (≥50%) is shown *above* or *below* the branches; clades with posterior probabilities greater than 0.95 are indicated with *thick branches*. New sequences generated for this article are in *bold*

Taxonomy

Macrolepiota subcitrophylla Z.W. Ge, sp. nov. Fig. 1

MycoBank: MB 561826

Pileus 10.5–12.0 cm diametro, initio ovoideus vel hemisphaericus, dein convexus vel plano-convexus, albus vel albidus, squamulis pallide brunneis vel brunneo-aurantiacis vel pallide rubro-brunneis. Lamellae liberae, flavae, luteolae, luteae vel sulfureae, confertae. Stipes albidus, 12.0–14.0 × 1.0–1.6 cm, subcylindricus, minutus sursum, basim incrassatus. Annulus superus, albidus, membranaceus. Caro alba vel cremea; sapor mitis. Basidia 28–36 × 11–12 μm, clavata, hyalina, 4-sporigera, rarius 2-sporigera. Basidiosporae (9.0)9.5–11.5(12.0) × 6.5–7.5 (8.0) μm, ovoideae vel subamygdaliformes-ellipsoideae, glabrae, hyalinae, dextrinoideae. Pleurocystidia absentia.

Cheilocystidia anguste clavata, interdum clavata vel subfusiformia, hyalina, 18–38 × 7–15 µm. Squamulae pilei trichoderma, apicalibus hyphis erectibus, luteis vel luteobrunneis, subcylindricis compositae. Fibulae praesentes. Habitatio: terrestris.

Holotypus: China, Yunnan Province, Chuxiong Yi Autonomous Prefecture, Zixi Mountain, 2,200 m a.s.l., solitary with pine, terrestrial, 4 September 2010, Z.W. Ge 2701 (HKAS 61624); rDNA sequence ex holotype: JN 180321.

Etymology: "*subcitrophylla*" refers to the yellowish lamellae of this species.

Additional material examined. China, Hunan Province, Shimen county, Hupingshan National Nature Reserve, 750 m a.s.l., 18 September 2009, Z.H. Chen 30520 (HKAS 58248). Yunnan Province, Chuxiong Yi Autonomous Prefecture, Zixi Mountain, 2,200 m a.s.l., solitary with pine, terrestrial, 9 September 2011, Z.W. Ge 3003 (HKAS 70508).

Basidiomata (Fig. 2) medium-sized to large. Pileus 10.5–12.0 cm in diameter, ovoid to hemispherical when young, becoming convex to plano-convex with age, white to whitish, covered with scattered, brownish-yellow (5C8), pale brown (5E7–5E8), light brown (6C7–6D7), to brownish orange (6C8) to reddish brown (8–9E3–4) patch-like squamules; disc smooth, reddish brown (8E4). Lamellae free, crowded, yellowish white (2A2) when young, yellowish (3A4), sulfur yellow (1A5), pastel yellow (2A4), to butter yellow (4A5) when mature, up to 1 cm in height, thin, with 1–2 tiers of lamellulae, edge finely fimbriate. Stipe whitish, 12.0–14.0 × 1.0–1.6 cm, subcylindrical, attenuating upward; base enlarged to subglobose; with tiny pale yellowish (3A4) to apricot yellow (5B6) farinose squamules, hollow. Annulus ascending, whitish, membranous, complex, big, with brownish patchy squamules on the underside, movable when mature. Context white to cream, spongy, unchanging when cut. Spore print not determined, but probably white to cream. Taste mild or indistinct. Smell indistinct.

Basidiospores (Fig. 3) [50/2/2] (9.0)9.5–11.5(12.0) × 6.5–7.5(8.0) µm, $Q = (1.33)\ 1.40–1.62\ (1.77)$, avQ = 1.49 ± 0.08, ovoid to subamygdaliform-ellipsoid in side view, ellipsoid in front view, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic in Cresyl blue, with a germ pore caused by an interruption in the episporium on the rounded apex, covered with a hyalinous cap in KOH; apiculus about 1 µm long. Basidia (Fig. 4) 28–36 × 11–12 µm, clavate, thin-walled, hyaline, 4-spored, rarely 2-spored. Cheilocystidia (Fig. 5) 18–38 × 7–15 µm, narrowly clavate, sometimes clavate to subfusiform, colorless and hyaline, thin-walled, forming a sterile lamella edge. Pleurocystidia absent. Squamules on the pileus (Fig. 6) a palisade of vertically arranged subcylindrical, clampless hyphae measuring (40)70–105(120) µm in length, (6.5)8–10.0(13) µm in diameter, septate, moderately branched, with terminal elements slightly attenuate toward the tip, with yellowish to brownish, slightly thickened wall; hyphae with pale yellowish vacuolar pigment; some occasionally with obvious brown vacuolar pigment. Clamp connections occasionally observed at the base of basidia.

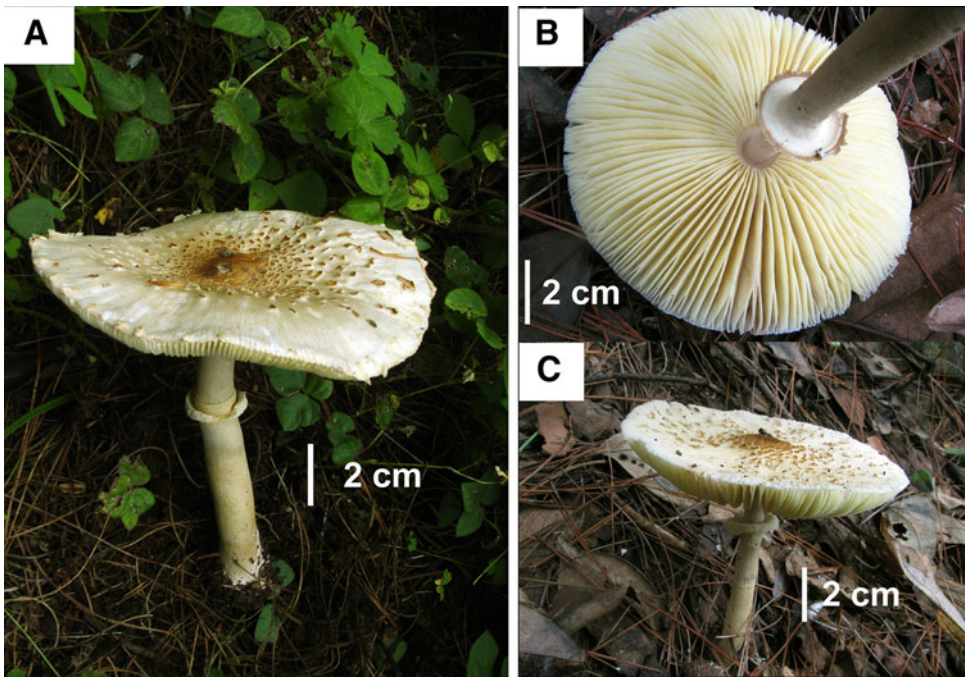
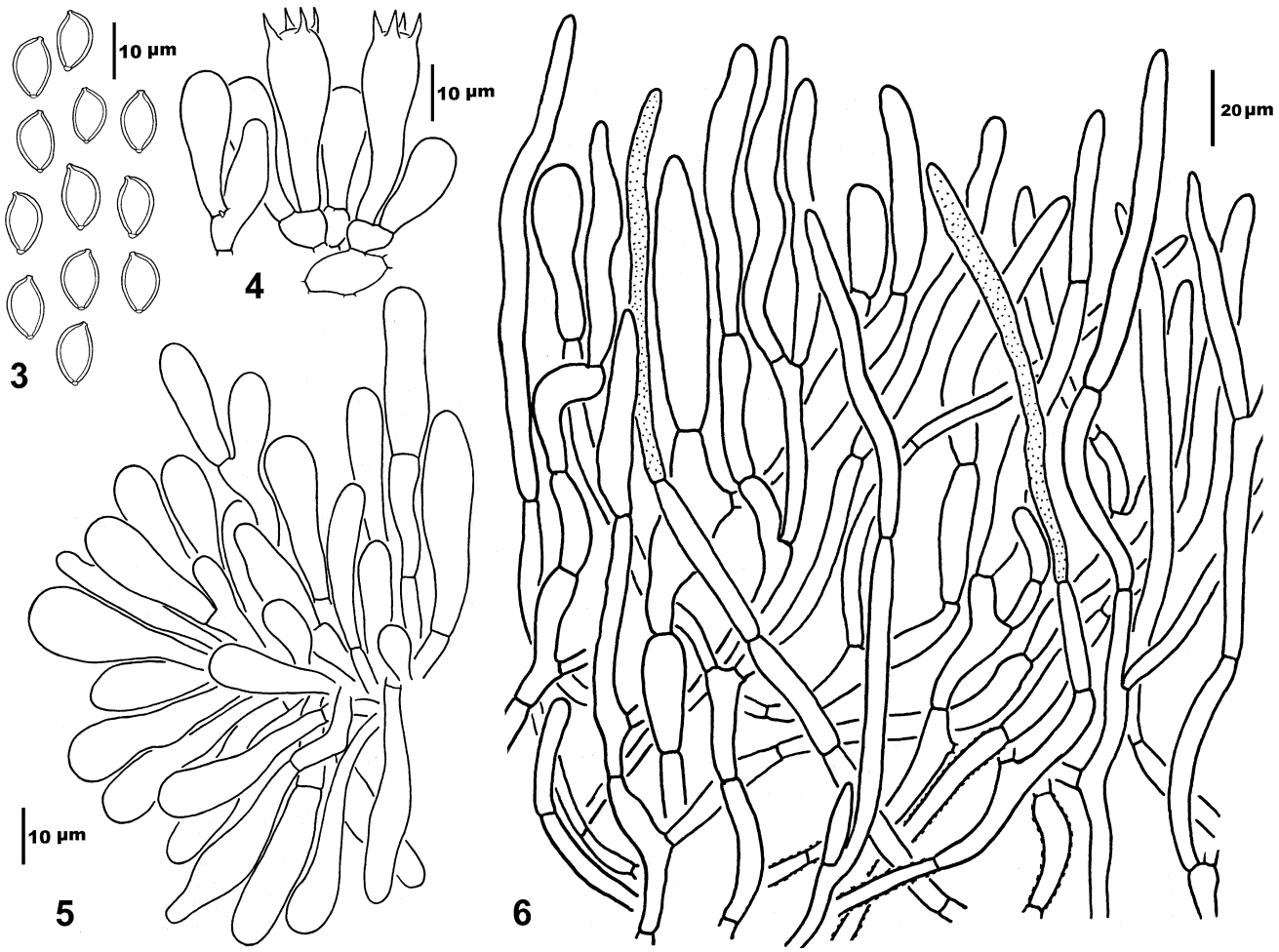


Fig. 2 Basidiomata of *Macrolepiota subcitrophylla*: **a** from holotype HKAS 61624; **b**, **c** from HKAS 58248



Figs. 3–6 *Macrolepiota subcitrophylla* (all from holotype HKAS 61624). **3** Basidiospores. **4** Basidia. **5** Cheilocystidia. **6** Pileipellis

Discussion

Macrolepiota subcitrophylla clearly differs from the other known species of *Macrolepiota* by a combination of characters including the yellowish lamellae, relatively small, ovoid to subamygdaliform-ellipsoid spores, narrowly clavate cheilocystidia, and a pileus covering composed of a trichoderm of subcylindrical hyphae that branch only infrequently. Phylogenetic analyses of the ITS region place *M. subcitrophylla* in the/macrolepiota clade, and closely related to *M. clelandii*, a species originally described from Australia. However, the lamellae of *M. clelandii* are white, its basidia are generally two-spored, and its basidiospores are much larger, measuring 13.5–28.5 × 9–16 µm (Grgurinovic 1997; Vellinga 2003).

Macrolepiota subcitrophylla resembles *M. dolichaula* because both species bear a whitish to white pileus with brownish squamules. *Macrolepiota dolichaula* was originally described from Sri Lanka and also found in southern and southwestern China, including Yunnan Province. However, *M. dolichaula* has white lamellae and larger basidiospores (12.5–16.0 × 8.0–10.5 µm). In addition, squamules of *M. dolichaula* are composed of short frequently branched filamentous hyphae (Pegler 1986; Ge et al. 2010). The ITS tree (Fig. 1) clearly indicates that *M. dolichaula* is sister to *M. detersa*, distantly related to *M. subcitrophylla*.

Macrolepiota orientiexcoriata Z.W. Ge, Zhu L. Yang & Vellinga and *M. mastoidea* (Fr.: Fr.) Singer also appear to be similar in appearance, but the yellowish lamellae, narrowly clavate cheilocysitidia, and smaller basidiospores of *M. subcitrophylla* clearly separate it from *M. orientiexcoriata* and *M. mastoidea* (Ge et al. 2010).

Macrolepiota detersa, which was described from Anhui Province, and also found in Hunan Province in this study, can be separated from *M. subcitrophylla* by forming plate-like squamules, white lamellae, a large annulus, and larger basidiospores measuring 14.0–16.0 × 9.5–10.5 µm.

Macrolepiota velosa bears similar small basidiospores (9.0–11.0 × 6.0–7.5) and was also described from Yunnan Province and known from Hainan Province (Ge et al. 2010). However, *M. velosa* usually has a volva, the pileus is covered with dark brown squamules on a brownish background, and its cheilocystidia are cylindrical (Vellinga and Yang 2003).

The detection of *M. subcitrophylla* only during recent field trips may seem unusual as this species produces striking medium- to large-sized basidiomata. The lack of observed fruiting could be because the species has been confused with *M. dolichaula* and simply overlooked. Records of this species from other parts of southern China could be expected.

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