



Lactifluus dinghuensis sp. nov. from southern China

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With 3 figures

Abstract: *Lactifluus dinghuensis*, a novel species of *Lactifluus* subgenus *Gerardii*, is described from the Dinghushan Biosphere Reserve, Guangdong Province, P. R. of China based on both morphological and molecular analysis. The new species is characterized macroscopically by its pale yellowish brown pileus, the blackish brown and faintly longitudinally wrinkled stipe, and the distant and cream colored lamellae. A molecular phylogenetic analysis based on both rDNA ITS and a fragment of the 28S rDNA sequences fully supports the establishment of the new species. A detailed morphological comparison is given with the closely related species: *L. cfr. sepiaceus* and *L. cf. gerardii* var. *agicola*.

Key words: *Lactifluus* subgenus *Gerardii*, taxonomy, morphology.

Introduction

Lactifluus was proposed as a genus by Roussel in 1806 with *Lactifluus piperatus* as type species. The genus name *Lactifluus* was later considered a synonym of *Lactarius* Persoon until 2008, when a molecular phylogenetic analysis showed that *Lactarius* was not a monophyletic group (Buyck et al. 2008), and had to be split in *Lactarius*, *Lactifluus* and *Multifurca* [MV1] Buyck & V. Hofst. New combinations have since been proposed for several species formerly classified in *Lactarius* (Verbeken et al. 2011, 2012, Stubbe et al. 2012).

Lactifluus currently contains 6 subgenera: *L. subg. Edules*, *L. subg. Lactariopsis*, *L. subg. Russulopsis*, *L. subg. Lactifluus*, *L. subg. Piperati* and *L. subg. Gerardii* (Stubbe et al. 2012; Verbeken et al. 2011; Verbeken et al. 2012). *L. subgenus Gerardii* is characterized by the often dark brown pigments in pileus and stipe, often distant lamellae, white spore print, reticulate spore ornamentation with interconnecting ridges and palisade-like pileipellis (Stubbe et al. 2010). So far, *L. subgenus Gerardii* comprises 21 described species, all from America, Asia and Australian region (Montoya et al. 2012; Morozova et al. 2013; Stubbe et al. 2012; Wang et al. 2012). In China, 5 species

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and 2 varieties of *L.* subgenus *Gerardii* have been reported, which are *L. atrovelutinus* J.Z.Ying, *L. gerardii* Peck, *L. parvigerardii* X.H.Wang & Stubbe, *L. subgerardii* (Hesler & A.H.Smith) Stubbe, *L. ochrogalatus* (Hashiya) X.H.Wang, *L. gerardii* var. *fagicola* (A.H.Sm. & Hesler) Hesler & A.H.Sm. and *L. gerardii* var. *subrubescens* (A.H.Sm. & Hesler) Hesler & A.H.Sm. (Wang et al. 2006; Wang et al. 2012; Ying 1991; Zhou & Wen 2005). Stubbe et al. (2010) have demonstrated the existence of several cryptic species and species complexes in this subgenus, and the total number of species will likely increase after more data are gathered.

The Dinghushan Biosphere Reserve, located in Zhaoqing city, China, is considered a region highly diverse in macro-fungi but no species in *L.* subg. *Gerardii* has been recorded up to now (Bi et al. 1994; Zheng GY et al. 1985). During a foray in the Dinghushan Biosphere Reserve, specimens superficially reminding representatives of *L.* subg. *Gerardii* have been found. A macro- and micro-morphological study together with molecular analyses showed that they represent a novel species of *L.* subgenus *Gerardii*.

Materials and methods

SAMPLING: Random sampling was conducted in September 2013 and May 2014 in Dinghushan Biosphere Reserve, Guangdong Province, China (112°33'E, 23°10'N). The collections are deposited in Guangdong Institute of Microbiology (No. GDGM 44602). The description is deposited in MycoBank (<http://www.mycobank.org/>).

MORPHOLOGICAL STUDY: Fresh basidiomes were photographed using a Canon ixus 220hs digital camera, and macroscopic characteristics of only the intact fresh fruit bodies were recorded under the daylight conditions in the field. Microscopic characters were also studied from fresh specimen. Paraffin section of the pileipellis was taken at approximately pileus mid-radius. Drawings were made with a drawing tube at 100x magnification for pellis structure. Basidiospores were observed in Melzer's reagent and measured in side view, excluding ornamentation and apiculus which were observed by SEM. The protocol to determine spore sizes followed that of Das & Verbeken (2012) and thirty basidiospores were recorded. Colour codes are from HTML Color Codes (<http://www.htmlcolorcode.org/>).

DNA EXTRACTION, PCR AND SEQUENCING: DNA was extracted from fresh fruitbody using the method described by Xu et al. (2010). Two nuclear loci were amplified and sequenced: (1) the ITS region of the nuc rDNA, comprising ITS1, 5.8S and ITS2 (Gardes & Bruns 1993; White et al. 1990); (2) a part of the LSU nuc rDNA, using primers LR0R and LR5 (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>). The protocols for PCR amplification consisted of a 5 min activation at 94°C, followed by 32 cycles of 30 S at 94°C, 30 S at 52°C (for LSU at 54°C) and 1 min at 72, and a final 12 min extension at 72°C. Direct sequencing of PCR products was performed using the PCR primers as sequencing primers. Sanger dideoxy sequencing were performed with ABI 3730xl DNA analyzer (IGE, Guangzhou, China). Sequences obtained were submitted to GenBank (*L. dinghuensis* MEBF07 accession numbers: KP1694969 and KP1694970, *L. dinghuensis* MF004 accession numbers: KR149425 and KR149426).

PHYLOGENETIC ANALYSIS: To establish its taxonomic position within *L.* subg. *Gerardii*, the sequences of 16 described species of the subgenus were added to the dataset of ITS-LSU. Sequences were aligned with Clustal X and manually modulated when necessary. Some ambiguously aligned terminal regions were excluded. The final alignment covered 1399 bases (contains gaps) and was submitted to TreeBASE (ID 16720). Neighbor-Joining analyses of the phylogenetic relationships among the taxa and the genetic distances (compute pairwise distances) were performed using MEGA 5.05 with the Kimura-2-parameter model and gaps in alignment were treated as missing data. Bootstrap analysis was conducted with 1000 replicates. The outgroup chosen here is *Lactifluus volemus* (Fries) Kuntze, *Lactifluus glaucescens* (Crossland) Verbeken and *Lactifluus piperatus*.

Results

Taxonomy

Lactifluus dinghuensis J.B.Zhang sp. nov.

Figs 1–2

MYCOBANK: MB810918

ETYMOLOGY: Referring to the locality (Dinghushan Biosphere Reserve) of the type specimens.

HOLOTYPE: CHINA, Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, 3 May 2014, J.B.Zhang MEBF07 (GDGM 44602)

Pileus 30–55 mm diam., convex with depressed centre or irregularly concave when young, shallow infundibuliform at maturity; margin somewhat waving; surface radially wrinkled, grooved and reticulately veined towards margin, dry, sheeny, light ash brown to pale yellowish brown (#CC9900). Lamellae 2.5–5 mm broad, decurrent, distant, pale yellowish white (#FFFFFF4) when young, cream-colored (#FFFDD0) at maturity, with abundant lamellulae (1–2 lamellulae between two lamellae); edge smooth and pale yellowish brown, concolorous with stipe apex. Stipe 25–35 × 6–11 mm, more or less cylindrical and equal or tapering downwards, drumstick-like at the base; surface dry, faintly longitudinally wrinkled, blackish brown (#2D0000). Context mostly solid, occasionally somewhat hollow in stipe, white, unchanging when exposed; smell fragrant when drying. Latex moderately abundant, white; colour unchanging after exposure on air. Spore print white.

Basidiospores 6.0–7.3–9.0 × 5.0–6.6–8.0 μm, Q = 1.0–1.11–1.27, globose to subglobose or broadly ellipsoid; ornamentation amyloid, reticulate, with rounded ridges of 0.5–1 μm high. Basidia 25–35 × 3–4 μm, narrowly clavate, 4-spored with some 2- and 3-spored basidia present; sterigmata 2–5 × 0.5–2 μm, firm and relatively long. Pleuropseudocystidia 3–5 μm diam., moderately scarce, subcylindrical or subclavate, with dense particle contents, projecting above hymenium level. Lamellar edge sterile and mostly composed of cheileptocystidia of 10–25 × 3–5 μm, broadly clavate or subcylindrical, occasionally septate. Lamellar trama almost entirely cellular; lactifers 5–7 μm diam., not abundant. Pileipellis a palisade, 50–75 μm thick, completely composed of thin-walled elements, upper layers containing intracellular, diffuse or agglutinated brown pigmentation; suprapellis composed of erect and oblique terminal elements, 10–25 × 2–4 μm, subcylindrical, sometimes subclavate or conical, sometimes 2-celled; subpellis composed of 2–4 layers of subglobose to broadly ellipsoid cells, 11–30 × 15–18 μm; underlying layer composed of densely packed, parallel hyphae.

HABITAT: Gregarious in monsoon evergreen broadleaf forest

SPECIMENS EXAMINED: CHINA, Guangdong Province, Zhaoqing City-3 May 2014, J.B.Zhang MEBF07 (GDGM 44602, holotypus); 2 September 2013, J.B.Zhang MF004.

Phylogenetic analysis

The phylogenetic analysis based on the combined ITS and a part of LSU sequences (Fig. 3) showed that *Lactifluus dinghuensis* sp. nov. forms a subclade together with *L. cf. gerardii* var. *fagicola* and *L. cf. sepiaceus* within the group of other described species



Fig. 1. *Lactifluus dinghuensis*. a, b: MEBF07 (holotype); c, d: MF004. Bar: a, b, c, d = 1 cm.

of *Lactifluus* subgenus *gerardii*, which forms a monophyletic group within *Lactifluus* (Stubbe et al. 2012). The genetic distances (number of nucleotide substitutions per

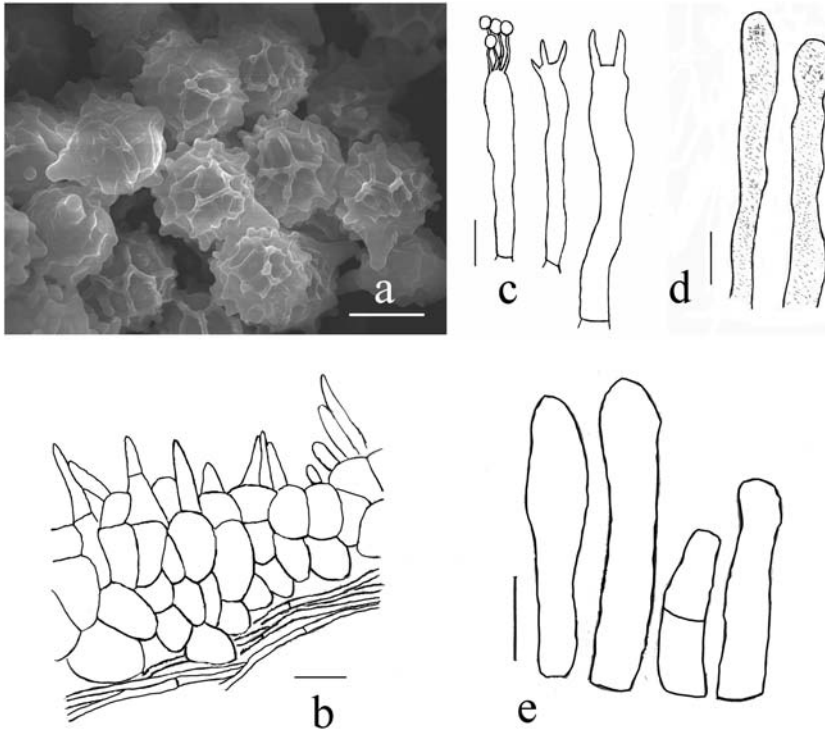


Fig. 2. *Lactifluus dinghuensis* MEBF07(holotype). a. Basidiospores. b. Pileipellis. c. Basidia. d. pleurospseudocystidia. e. cheiloleptocystidia. Bars: a, b, c, d, e = 5 μ m.

site) between *L. dinghuensis* sp. nov. and its closely related species *L. cf. gerardii* var. *fagicola* and *L. cfr. sepiaceus* are 0.047 and 0.044, respectively, which is greater than many of the closely related species of the group, such as *L. coniculus* and *L. cf. wirrabara* (0.021), *L. reticulatovenosus* and *L. subgerardii* (0.023), and *L. petersenii* and *L. leonardii* (0.021), indicating that *L. dinghuensis* sp. nov. is clearly distinct from previously described species of the group.

Discussion

The combination of the general habit of the basidiocarps, the reticulate spore ornamentation, the palisade structure of the pileipellis, and white spore print places this new species in *Lactifluus* subgenus *gerardii*. The new species is morphologically and genetically distinct from other species in the subgenus. *Lactifluus dinghuensis* is characterized by the pale yellowish brown pileus and cream colored lamellae in combination with the faintly longitudinally wrinkled stipe. Microscopically the size of basidia, the size and shape of terminal elements of the suprapellis, and the short

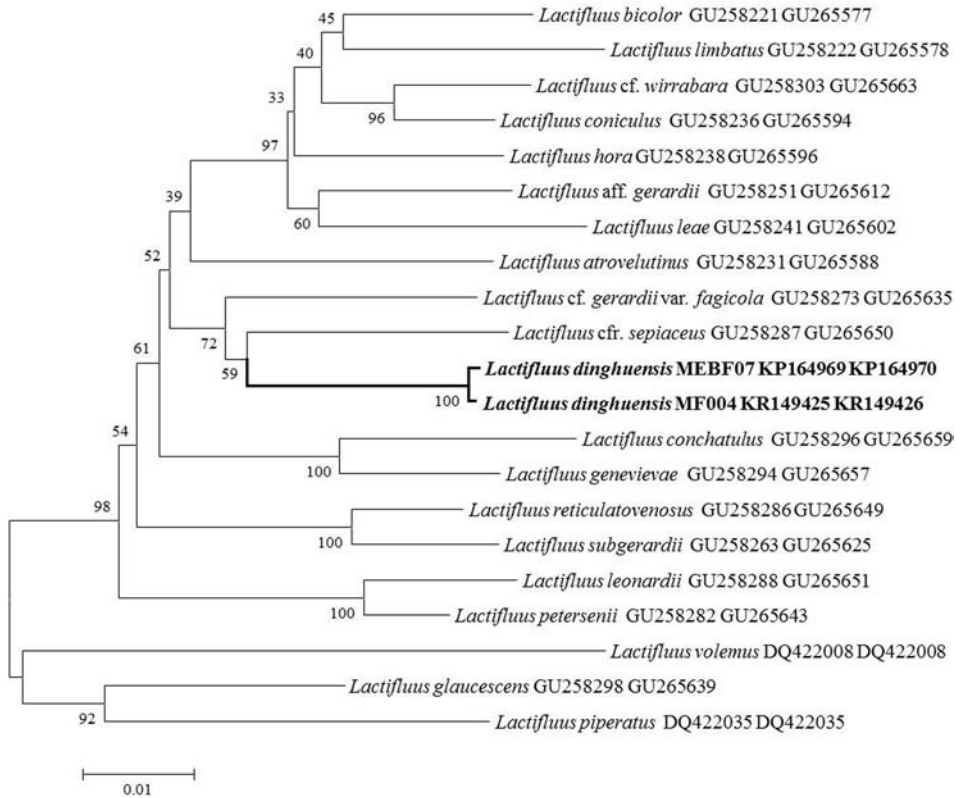


Fig. 3. NJ tree of *Lactifluus dinghuensis* sp. nov. based on analysis of the combined ITS and a part of LSU sequences (681 bp) with *Lactifluus volemus*, *Lactifluus piperatus* and *Lactifluus glaucescens* as outgroup. Bootstrap values resulted from a 1000 replicates rapid boot strapping algorithm are incicated before the branches.

and subcylindrical cheiloleptocystidia are good characters to recognize the species. In comparison with the closely related species: *L. cfr. gerardii* var. *fagicola* (Hesler & Smith 1979) and *L. cfr. sepiaceus* (McNabb 1971), the differences are as follows: The new species (basidia: 25–35 × 3–4 μm, basidiospores: 6–9 × 5–8 μm) shows smaller size in basidia and basidiospores than *L. cf. gerardii* var. *fagicola* (basidia: 40–62 × 9–13 μm, basidiospores: 8–11 × 7–10 μm) and *L. cfr. sepiaceus* (basidia: 55–80 × 7.5–10.5 μm, basidiospore: 7.5–11 × 6.5–9.5 μm). Futher, *L. dinghuensis* do not occur color change on the context when exposed to air, whereas *L. cf. gerardii* var. *fagicola* will change to brown and *L. cfr. sepiaceus* will have very faint salmon pink tints on the context. What's more, some cheiloleptocystidia of *L. dinghuensis* had septum, which was not found in *L. cf. gerardii* var. *fagicola*. And also, *L. cfr. sepiaceus* conspicuously differs from *L. dinghuensis* by its dark brown pruinose to subvelutinate pileus and stipe, longer sterigmata ("up to 11" vs 2–5 μm) and longer filamentous terminal elements of pileipellis ("up to 80" vs. 10–25 μm).

Other taxa exhibiting similarities with *L. dinghuensis* are *L. atrovelutinus* from China (Ying 1991), *L. leae* Stubbe & Verbeken from Australia (Stubbe et al. 2012), *L. aff. gerardii* Peck from Thailand (Le et al. 2007) and *L. bicolor* Masee from Singapore. *L. atrovelutinus* differs from *L. dinghuensis* by having the really small pileus (0.7–1.5 cm), the branching marginal cells of lamellae, the subpellis composed of more layers cells and the pink or brownish pink color reaction of the latex; *L. leae* differs by the short cheiloleptocystidia with inflated basal cells and the ornamentation of basidiospores with large meshes on lateral basidiospore sides and few isolated warts; *L. aff. gerardii* differs by the subuniform cheiloleptocystidia, larger basidia ($42\text{--}72 \times 9.5\text{--}11.5 \mu\text{m}$) and larger Pleuroseuodocystidia ($8\text{--}10 \mu\text{m}$). *L. bicolor* is distinguished by its dark brown color, the distinctly coloured lamellar edges and a little larger basidiospores ($8.1\text{--}9.1\text{--}10.1 \times 6.7\text{--}8.1\text{--}9.5 \mu\text{m}$).

The phylogenetic tree presented here is almost in coincidence with the result of Stubbe et al. (2012). Five species of the subgenus, which are *L. ochrogalactus*, *L. uyedae* Singer, *L. venosus* Verbeken & E. Horak, *L. fuscomarginatus* Montoya, Bandala & Haug and *L. parvigerardii*, were not added in phylogenetic analysis of *L. subg. Gerardii* for their incomplete ITS-LSU dataset. According to the morphological-phylogenetic analysis by Stubbe et al. (2010), the subgenus is divided into three major clades: *L. gerardii* clade with dark-coloured basidiocarps, *L. uyedae* clade with white pleurotoid basidiomata and *L. ochrogalactus*-*L. petersenii* clade with yellow-brown latex. Based on the research of Verbeken & Horak (2000) and Montoya et al. (2012), *L. venosus* and *L. fuscomarginatus* are closely related with *L. reticulatovenosus* and at the basis of *L. gerardii* clade. *L. uyedae* and *L. parvigerardii* (Wang et al. 2012) are at *L. uyedae* clade. *L. ochrogalactus* are at the *L. ochrogalactus*-*L. petersenii* clade. Both of them show distant phylogenetic relationships and substantial morphological difference with *L. dinghuensis*.

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