

Morphological and molecular evidences for a new species of *Lignosus* (Polyporales, Basidiomycota) from tropical China

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Abstract *Lignosus hainanensis* sp. nov., is described and illustrated on the basis of collections originating from tropical forest in Hainan Province, southern China. Both the morphology and phylogeny support this new species. It is characterized by its centrally stipitate basidiocarps arising from a distinct sclerotium, yellowish brown to cinnamon-brown pileal surface, cream to cream-buff pore surface, trimitic hyphal system with clamped generative hyphae, and oblong ellipsoid to cylindrical basidiospores. *Lignosus hainanensis* may be confused with *L. rhinocerus*, also occurring in southern China, but *L. rhinocerus* can be distinguished by its smaller pores (6–8 per mm) and smaller and distinctly broadly ellipsoid basidiospores (4–5×2.8–3 µm).

Keywords Molecular phylogeny · Polypore · Sclerotium · Taxonomy

Introduction

Lignosus Lloyd ex Torrend is characterized by centrally stipitate basidiocarps arising from a distinct sclerotium buried in the ground, a trimitic hyphal structure with clamped generative hyphae, hyaline, cylindrical to ellipsoid

basidiospores. The genus occurred in tropical Asia and Africa (Ryvarden and Johansen 1980; Ryvarden 1991).

During the survey of wood-rotting fungi in the tropical forests of Jianfengling Nature Reserve, Hainan Province, southern China, a species of *Lignosus* was found with characters fitting the genus *Lignosus*, but not matching any described species. To confirm the affinity of the new taxon and infer its evolutionary relationships among the similar species of *Lignosus*, phylogenetic analyses were carried out based on ITS and nLSU sequences data.

Materials and methods

Morphological studies

The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Sections were studied at magnification up to ×1,000 by using a Nikon Eclipse E 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Microscopic features, measurements and drawings were made from slide preparations stained with Cotton Blue or Melzer's reagent. Spores were measured from sections cut from the tubes (Cui et al. 2008). In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. In the text, the following abbreviations are used: MLZ = Melzer's reagent, MLZ- = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n = number of spores measured from

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given number of specimens. Special color terms are from Anonymous (1969) and Petersen (1996).

Molecular procedures and phylogenetic analyses

Total DNA was extracted from the fruitbody and the sclerotium of the holotype specimen of *Lignosus hainanensis* using a CTAB procedure of Doyle and Doyle (1987) with some modifications. In the study, nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5, and the large subunit (nLSU) with the primers LROR and LR5. All sequences were deposited in GenBank, with the following accession codes. ITS: GU580883 (fruitbody), GU580884 (sclerotium); nLSU: GU580885 (fruitbody), GU580886 (sclerotium); these sequences were used in phylogenetic analyses along with the following retrieved from GenBank: *L. sacer* (Afzel. ex Fr.) Ryvarden GU001675, GU001674; *L. rhinocerus* (Cooke) Ryvarden FJ899143; *L. rhinocerus* FJ380871; *Daedaleopsis confragosa* (Bolton) J. Schröt. FJ81017; and *D. sinensis* (Lloyd) Y.C. Dai FJ627256.

Maximum parsimony and MrBayesian analysis were applied to the ITS and nLSU dataset. All characters were treated as unordered and equal weight. Maximum parsimony analysis (PAUP* version 4.0) was used (Swofford 2002), based on strict heuristic searches with a tree-bisection reconnected (TBR) branch swapping algorithm, stepwise addition and collapse of branch if maximum length is zero. Bootstrap values were calculated from 1,000 replicates. Bayesian analysis with MrBayes3.1.2 (Ronquist and Huerlenbeck 2003) implementing the Markov Chain Monte Carlo (MCMC) technique and parameters predetermined with MrMODELTEST2.3 was performed. When four simultaneous Markov chains were run with 1,000,000 generations, the average standard deviation of split frequencies was 0.000283.

Results

Taxonomy

Lignosus hainanensis B.K. Cui, sp. nov. (Fig. 1)

Mycobank no.: MB 518072

Carpophorum annum, stipitatum. Facies pororum cremea vel cremea bubalinua; pori rotundi vel angulati, 3–4 per mm. Sclerotinum irregularis, elongatus, 6×5 cm. Systema hypharum trimiticum, hyphae generatiae fibulatae, hyphae skeletales contexti 2.6–5.5 μm in diam. Sporae hyalinae, oblongae ellipsoideae vel cylindricae, MLZ–, CB–, 4.9–6×2.2–2.9 μm.

Type. CHINA. Hainan Province, Ledong County, Jianfengling Nature Reserve, on ground of angiosperm forest, 12.XII.2008 Dai 10670 (holotype in BJFC).

Etymology. *hainanensis* (Lat.): referring to the locality of the type specimen.

Basidiomata. Basidiocarps annual, terrestrial, centrally stipitate, solitary, the stipe arising from a distinct sclerotium in the ground, without odour or taste when fresh, hard corky to woody hard when dry. Pileus more or less circular, up to 10 cm in diam., and 5 mm thick at centre. Pileal surface yellowish brown to cinnamon-brown, glabrous, concentrically zonate; margin sharp, wrinkled, cinnamon-brown. Pore surface cream to cream-buff when dry, slightly shining; pores round to angular, 3–4 per mm, dissepiments thin, entire. Context cream, corky upon drying, up to 1 mm thick. Tubes cream, hard corky when dry, up to 4 mm long. Stipe usually branched at the base, pale mouse-grey to pale greyish brown, hard corky upon drying, up to 8 cm long and 0.8 cm in diam.; context from stipe cream, soft corky. Sclerotium irregular, elongated, up to 6 cm long, 5 cm wide, wrinkled, very light-weighted, the surface dirty brown to fawn-brown, the context cream, loose or soft.

Hyphal structure. Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal hyphae MLZ–, CB–; tissues unchanged in KOH.

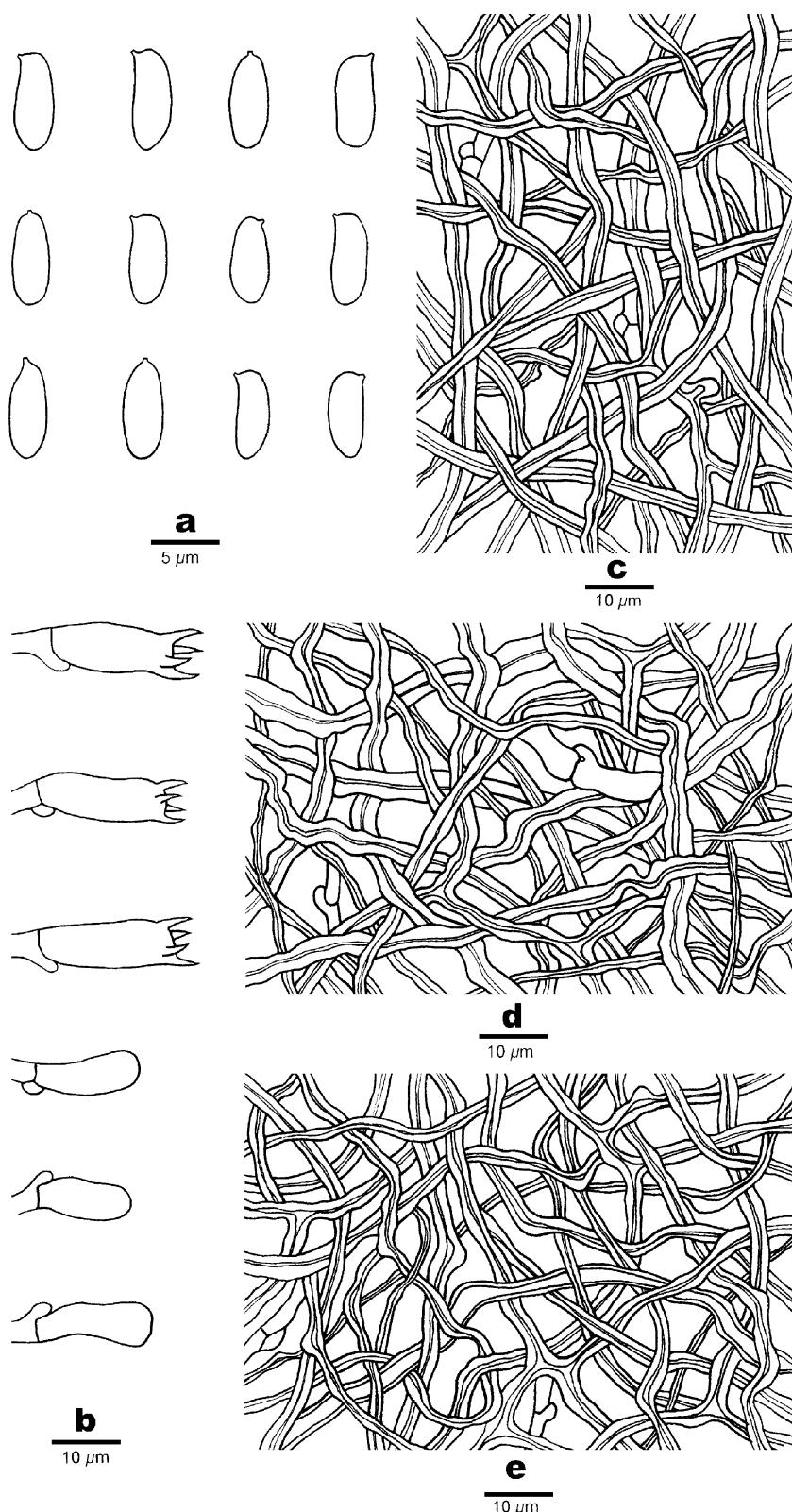
Context. Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2–3.8 μm in diam.; skeletal hyphae dominant, thick-walled with a narrow lumen, often branched, interwoven, 2.6–5.5 μm in diam. Binding hyphae occasionally present, branched, flexuous, interwoven, 1.5–2.5 μm in diam.

Sclerotium. Sclerids (Fig. 2) common in sclerotium, variable in shape from globose, pear-shape, ellipsoid to irregular, inflated and thick-walled, with a rather narrow lumen, 15–40×30–55 μm. Inflated and thick-walled sclerids connected to hyphae, as observed in Glycerol aqueous solution or water, but appearing stratified or layered in KOH. Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 2–3.4 μm in diam; skeletal hyphae dominant, thick-walled with a narrow lumen, often branched, interwoven, 2.4–5 μm in diam; binding hyphae present, branched, flexuous, interwoven, 1.3–2.4 μm in diam.

Tubes. Generative hyphae infrequent, hyaline, thin-walled, usually unbranched, 1.8–3.5 μm in diam.; skeletal hyphae dominant, thick-walled with a narrow lumen, frequently branched, flexuous, interwoven, 2.5–5 μm in diam. Cystidia and cystidioles absent. Basidia clavate, with a basal clamp connection and four sterigmata, 10–16×5–7 μm; basidioles in shape similar to basidia, but distinctly smaller.

Spores. Basidiospores oblong ellipsoid to cylindrical, hyaline, thin-walled, smooth, MLZ–, CB–, (4.6–)4.9–6 (–6.4) × (2.1–)2.2–2.9(–3) μm, L=5.54 μm, W=2.6 μm, Q=2.13 (n=50/1).

Fig. 1 Microscopic structures of *Lignosus hainanensis* B.K. Cui (drawn from the holotype). **a** Basidiospores. **b** Basidia and basidioles. **c** Hyphae from trama. **d** Hyphae from context. **e** Hyphae from sclerotium



Molecular phylogeny

The ITS dataset contained 761 characters of which 623 characters were constant, 71 were parsimony-uninformative

characters, and 117 parsimony-informative characters. The parsimony analyses yielded a tree of length of 156 steps, a consistency index (CI) of 0.919, a retention index (RI) of 0.947.

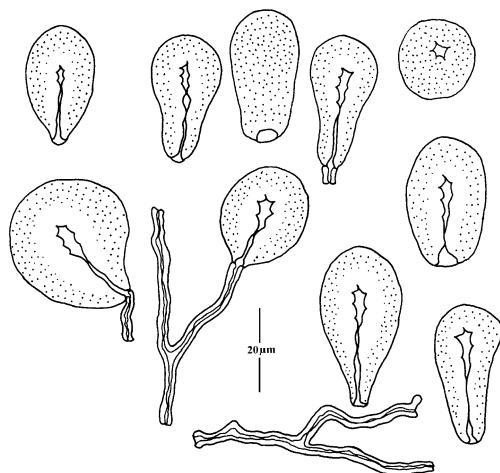


Fig. 2 Microscopic structure of sclerids from the sclerotium of *Lignosus hainanensis* B.K. Cui

In phylogenetic reconstruction, the genus *Daedaleopsis* J. Schröt. was chosen as outgroup according to Sotome et al. (2008). The ITS strict consensus tree (Fig. 3) generated by Bayesian analysis (Ronquist and Huelsenbeck 2003) and Maximum Parsimony (Swofford 2002) showed sequences of *Lignosus hainanensis* were grouped together with other species of the genus *Lignosus* as a monophyletic cluster with strong support. The same topology was observed whatever the methodology parsimony or Bayesian.

This analysis confirmed *L. hainanensis* is a distinct species, and grouped with *L. rhinocerus* in the same subclade suggesting their closely phylogenetic relationship.

Discussion

Phylogenetic analysis showed that the Hainan collection clusters within *Lignosus*, and is so far closely related to *L. rhinocerus* belonging to the genus *Lignosus*. Both morphology and rDNA data confirmed that the Hainan collection currently should be recognized as a new species in the genus *Lignosus*.

Lignosus hainanensis is characterized by its centrally stipitate basidiocarps arising from a sclerotium, yellowish brown to cinnamon-brown pileal surface, cream to cream-buff pore surface with 3–4 pores per mm, a trimitic hyphal system with clamped generative hyphae, and oblong ellipsoid to cylindrical basidiospores. This species is a fairly rare species in the investigated area, and only one fruiting body was found; it occurs on the ground of a mixed evergreen forest.

Lignosus ekombitii Douanla-Meli, recently described from Cameroon (Douanla-Meli and Langer 2003), resembles *L. hainanensis* in having similar pores, but differs by having thin basidiocarps (up to 1.5 mm thick), larger

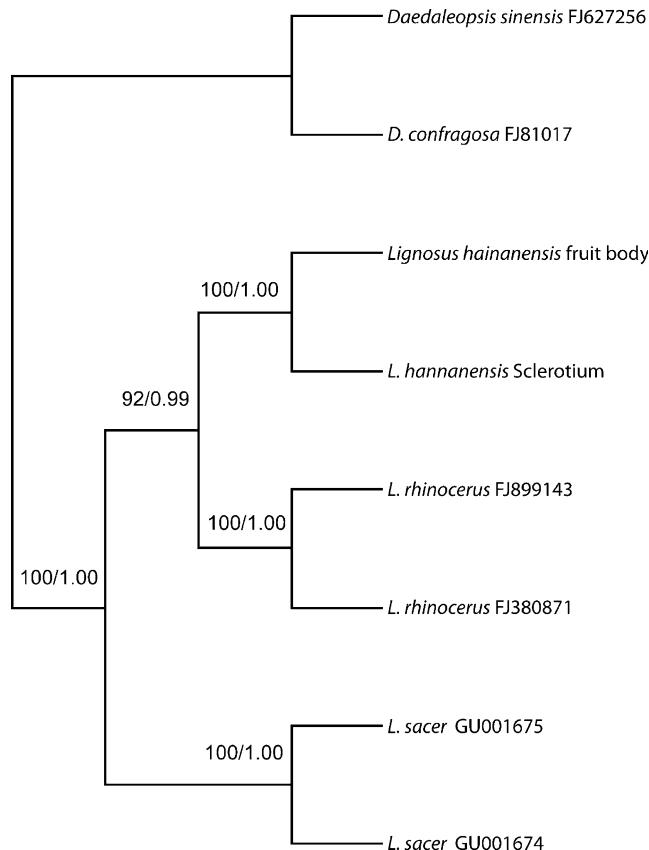


Fig. 3 Strict consensus tree illustrating the phylogeny of *Lignosus hainanensis* B.K. Cui generated by Bayesian Analysis and Maximum Parsimony based on ITS. Posterior Probabilities and Bootstrap values are shown above the lines. (Bayesian Analysis: Generations = 1,000,000, Average standard deviation of split frequencies = 0.000283). (Maximum Parsimony: Tree length = 156, CI = 0.919, RI = 0.947)

basidiospores ($6\text{--}8.2 \times 2.8\text{--}3.2 \mu\text{m}$), and abundant dendrophidia (type specimen studied: *Lignosus ekombitii*: Cameroon, Mbalmayo-Ekombitie, on ground, DMC 136 (HYUI).

Lignosus rhinocerus also occurs in tropical China (Núñez and Ryvarden 2001), and has centrally stipitate basidiocarps arising from a distinct sclerotium, cinnamon-brown and concentrically zonate pileal surface, glabrous, cream to cream-buff pore surface. However, it differs from *L. hainanensis* by its smaller pores (6–8 per mm), smaller and distinctly broadly ellipsoid basidiospores ($4\text{--}5 \times 2.8\text{--}3 \mu\text{m}$).

Five species have been transferred to or described in the genus *Lignosus* previously (Douanla-Meli and Langer 2003; Núñez and Ryvarden 2001; Ryvarden and Johansen 1980): *L. dimiticus* Ryvarden, *L. ekombitii*, *L. goetzi* (Henn.) Ryvarden, *L. rhinocerus*, and *L. sacer*. Literature and some specimens of these species were studied (see below), and a key to the accepted species in *Lignosus* is provided in the following.

Key to known species of *Lignosus* in the world

1. Pileus white to ochraceous 2
1. Pileus light brown to brown 3
2. Pores smaller, 6–8 per mm *L. dimiticus* Ryvarden
Basidiospores $3\text{--}4.5 \times 2.5\text{--}3 \mu\text{m}$ (Ryvarden & Johansen 1980)
2. Pores larger, 0.5–2 per mm *L. goetpii* (Henn.) Ryvarden
Basidiospores unknown (Ryvarden & Johansen 1980)
3. Pores smaller, 5–8 per mm
..... *L. rhinocerus* (Cooke) Ryvarden
Basidiospores $4\text{--}5(5.1) \times 2.8\text{--}3 (-3.5) \mu\text{m}$, L=4.56 μm ,
W=2.93 μm , Q=1.56 ($n=30/1$)
3. Pores larger, 1–4 per mm 4
4. Basidiospores broadly ellipsoid; pores 1–3 per mm
..... *L. sacer* (Afzel. ex Fr.) Ryvarden
Basidiospores $5\text{--}7 \times 3\text{--}4.5 \mu\text{m}$ (Ryvarden & Johansen 1980)
4. Basidiospores oblong ellipsoid to cylindrical; pores 3–4 per mm 5
5. Basidiospores $>6 \mu\text{m}$ in length, dendrohyphidia present *L. ekombitii* Douanla-Meli
Basidiospores $6\text{--}8.2(-8.5) \times (2.7\text{--})2.8\text{--}3.2(-3.4) \mu\text{m}$,
L=7.42 μm , W=2.97 μm , Q=2.5 ($n=30/1$)
5. Basidiospores $<6 \mu\text{m}$ in length, dendrohyphidia absent ..
..... *L. hainanensis* B.K. Cui
Basidiospores $(4.6\text{--})4.9\text{--}6(-6.4) \times (2.1\text{--})2.2\text{--}2.9(-3) \mu\text{m}$,
L=5.54 μm , W=2.6 μm , Q=2.13 ($n=50/1$)

Other specimens studied *Lignosus ekombitii*: Cameroon, Mbalmayo-Ekombitie, on ground, DMC 136 (HYUI). *L. rhinoceros*: China, Hainan Province, Baoting County, on ground of angiosperm forest, 15.X.1958 HMAS 30036 (HMAS); New Guinea, Lae, H.F. Winters, on ground, 2.

IX.1970 Ryvarden 11325 (O). *L. sacer*: Ethiopia, Arsi, in Lepis forest, on ground, VIII.1987 Ryvarden 15568 (O).

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