Systematic revisit of *Sparsitubus* (*Basidiomycota, Aphyllophorales*), an unusual cyphelloid polypore from China

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Sparsitubus is a monotypic genus found in East Asia that has a bizarre cyphelloid hymenophore. The position of *Sparsitubus* in the *Polyporaceae* has been suggested previously based on morphological characters. To study the relationships of *Sparsitubus* among other Homobasidiomycetes, rDNA sequences of *Sparsitubus* and polypores representatives were generated or downloaded from GenBank. Parsimony analyses using rDNA data suggest that *Sparsitubus* is in a clade of polypores producing a white rot and maybe closely related to the *Ganodermataceae*. Characters of fruiting body, hyphal systems, and basidiospore were compared and discussed between *S. nelumbiformis* and related polypores or similar cyphelloid fungi.

Key words: phylogeny, taxonomy, wood-inhabiting fungi

Introduction

Traditional taxonomy of major fungal groups is mainly based on gross morphology of the fruiting body. There has been convergent evolution in fruiting body (basidiocarp) morphology in mushroom forming fungi indicated with anatomical and molecular evidence (Oberwinkler, 1985; Hibbett and Thorn, 2001; Kirk *et al*., 2001; Hibbett and Binder, 2002), and recognition of form groups sometimes does not reflect the real evolutionary relationships among these fungi (e.g. Bodensteiner *et al*., 2004). Polypores are characterized by producing basidiospores on inner surface of pipe-like structures, which open as many pores on the lower surface of the basidiocarp. Polypores have been intensively studied as major wood decomposers on earth, and two principal modes of wood decay are recognized in the polypores: white rot and brown rot (Rayner and Boddy, 1988). Recent molecular phylogenies

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suggested that polypores are morphologically diverse and include fungi with resupinate, teeth- or leaf-like basidiocarps in addition to poroid fruiting bodies, while some poroid fungi, such as *Fistulina* and *Lentinula* species, do not belong to polypores as they were traditionally treated (Hibbett and Thorn, 2001; Bodensteiner *et al*., 2004).

Sparsitubus nelumbiformis was first reported from southern China on exposed root of hard woods with unusual morphology (Xu and Zhao, 1980). Due to its poroid and woody basidiocarp, it was classified as a member of polypores by the authors. Tubes are coherent in polypores which is somehow analogous to the contest of a honey comb. However, tubes and hyphae structures surrounding the tube openings in *S. nelumbiformis* are so well developed that pores in this fungus look like aggregated mini-volcanoes in a shallow basin (Figs 1, 2.). Tubes of *Fistulina* species, formerly accommodated in the *Polyporales*, are isolated from each other as well, and molecular data suggest that *Fistulina* species are closely related to species of *Schizophyllum*, mushroom-forming fungi (Binder *et al*., 2006). To understand how this bizarre morphology of *Sparsitubus nelumbiformis* evolved, a phylogeny inferred from molecular data is required.

Materials and methods

Morphological studies

The studied materials are deposited at the Herbarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS). The microscopic procedures used in this study are as presented by Dai (1999). The following abbreviations are used: L = mean spore length (arithmetical mean of all spores), W = mean spore width (arithmetical mean of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width), n = number of spores measured. In presenting the variation in the size of spores, 5% of the measurements were excluded from each end of the range, and are given in parentheses; IKI stands for Melzer's reagent and KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue. CB+ means cyanophilous and IKI– means both inamyloid and indextrinoid.

Molecular techniques

DNA was extracted from dried herbarium materials following standard protocols as in Wang et al., (2004). Sequences of LSU-rDNA were generated

using primer LR0R and LR5 (Vilgalys and Hester, 1990). PCR reaction mixes (Promega Corp., Madison, Wisconsin) contained 2.5 µL 10x PCR buffer, 5µM dNTP, 12.5 pM of primers LR0R and LR5, and 5µL DNA in 25 µL. The PCR program included 35 cycles of 94°C for 30s, 53°C for 1 min, and 72°C for 1 min. PCR products were purified using GeneClean (Bio 101, Carlsbad City, California) and sequenced using the ABI Prism BigDye-terminator cycle sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's protocols. Sequencing reactions were purified using Pellet Paint (Novagen, Madison, Wisconsin) and were run on an Applied Biosystems 377XL automated DNA sequencer. Sequences were edited with Sequencher version 3.1 (GeneCodes Corporation, Ann Arbor, Michigan). Sequences generated and downloaded from GenBank in this study were *Sparsitubus nelumbiformis* DQ887631, *Albatrellus syringae* (Parmasto) Pouzar AF393045, *Antrodia carbonica* (Overh.) Ryvarden & Gilb. AF287844, *Auriporia aurea* (Peck) Ryvarden AF287846, *Ceriporia purpurea* (Fr.) Donk AF287852, *Climacodon septentrionalis* (Fr.) P. Karst. DQ250684, *Coriolopsis aspera* (Jungh.) Teng AY351956, *C. sanguinaria* (Klotzsch) Teng AY351950, *Cryptoporus volvatus* (Peck) Shear AF393050, *Daedaleopsis confragosa* (Bolton) J. Schröt AF261542, *Dentocorticium sulphurellum* (Peck) M.J. Larsen & Gilb. AF393055, *Dichomitus gunnii* D.A. Reid AJ487513, *D. squalens* (P. Karst.) D.A. Reid AJ487514, *Earliella scabrosa* (Pers.) Gilb. & Ryvarden AY351946, *Fomes fomentarius* (L.) J.J. Kickx AF261538, *Fomitopsis pinicola* (Sw.:Fr.) P. Karst. DQ250685, *Ganoderma applanatum* (Pers.) Pat. AJ406526, G. *australe* (Fr.) Pat. AY333807, *G. lucidum* (Curtis) P. Karst. X78776, *G. microsporum* R.S. Hseu X78779, *G. tsugae* Murrill X78778, *Grammothele fuligo* (Berk. & Broome) Ryvarden AJ406506, *Grifola frondosa* (Dicks.:Fr.) S.F. Gray DQ250686, *Hexagonia tenuis* J.M. Hook AY351936, *Laetiporus sulphureus* (Bull.:Fr.) Murrill DQ250687, *Lentinus tigrinus* (Fr.) Fr. DQ250688, *Lenzites betulinus* (L.) Fr. AF393063, *Meripilus giganteus* (Pers.) P. Karst. AF287874, *Microporus affinis* (Blume & T. Nees) Kuntze AY351931, *M. vernicipes* (Berk.) Kuntze AY351930, *Neolentiporus maculatissimus* (Lloyd) Rajchenb. AF518632, *Oligoporus rennyi* (Berk.& Broome) Donk DQ250689, *Phaeolus schweinitzii* (Fr.) Pat. DQ250690, *Piptoporus betulinus* (Bull.:Fr.) P. Karst DQ250694, *Polyporus melanopus* (Pers.) Fr. AF393068, *P. squamosus* Huds.:Fr. DQ250692, *P. varius* (Pers.) Fr. AF393071, *Postia lactea* (Fr.) P. Karst. DQ250693, *Pycnoporellus fulgens* (Fr.) Donk DQ250696, *Pycnoporus cinnabarinus* (Jacq.) Fr. AF518643, *Sparassis latifolia* Y.C. Dai & Z. Wang, DQ250697, *S. spathulata* Schwein. :Fr. DQ250708, *Spongipellis pachyodon* (Pers.) Kotl. & Pouzar AY629322.

Phylogenetic analyses

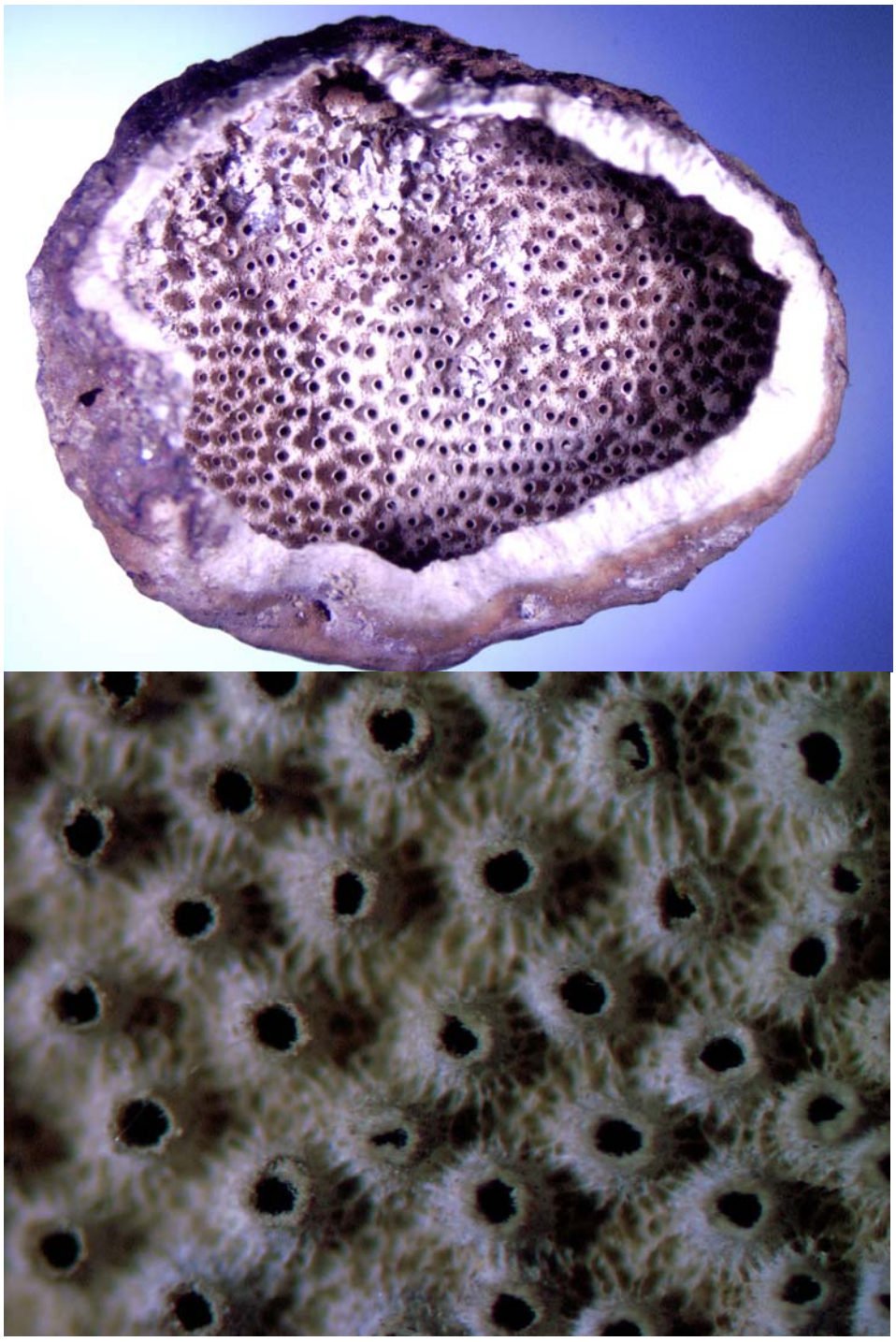
A data set was composed of LSU-rDNA sequences. Sequences were aligned by eye in the data editor of PAUP* 4.0b (Swofford, 1999). The data set was analyzed in PAUP* 4.0b, with gaps treated as missing data, and ambiguous or unalignable positions excluded. LUS-rDNA sequences of three *Dichomitus* species were about 300bp shorter than the others. The data set was rooted using *Meripilus giganteus* (Hibbett and Donoghue, 2001). Parsimony analyses were performed using equal weighting of characters and transformations. Heuristic searches were performed with one thousand replicate searches, each with a random taxon addition sequence. MAXTREES was set to autoincrease, and TBR branch swapping was employed. A bootstrap analysis was performed with 500 replicates, each with 20 random taxon addition sequences, MAXTREES was set to autoincrease, and TBR branch swapping was employed.

Results

Sparsitubus nelumbiformis L.W. Xu & J.D. Zhao, Acta Microbiol. Sinica 20: 237, (1980) (Figs 1-3)

Fruitbody. — Basidiocarps annual to biennial, effused-reflexed to pileate, hard corky when dry. Pileus projecting up to 1.5 cm, 3 cm wide, and 2 cm thick at base. Pileal surface dark vinaceous grey, indistinctly concentrically zonate, glabrous; sterile margin wide, cream, distinctly paler than other part, acute, wavy and curved down when dry, even to incised. Pore surface ash-grey brown when dry; margin distinct, cream, up to 4 mm wide; pores developed by the development of an apical pore which isolated and separated each other by a distinct distance, circular, 2–4 per mm, dissepiments thin, entire; hymenophore among tubes pale grey, subtomentose. Context pinkish buff, hard corky to woody hard when dry, up to 10 mm thick, concentrically zonate; a distinct black zone present between two layers of context when biennial; a distinct black cuticle present on the pileal surface. Tube layer mouse grey, darker than hymenophore among tubes, hard corky when dry, up to 1 mm long.

Hyphal structure. — Hyphal system dimitic, generative hyphae infrequent, mostly with clamp connections, sometimes with simple septa, hyaline, thin-walled; skeleto-binding hyphae dominant, thick-walled, with a wide to narrow lumen or subsolid, dendritically branched and tapering in the end, dextrinoid in Melzer’s reagent, cyanophilous in Cotton Blue; contextual tissue unchanged in KOH; tubes and upper surface darkening in KOH.



Figs 1, 2. *Sparsitubus nelumbiformis* (from holotype). **1.** Basidiocarp. **2.** Tubes.

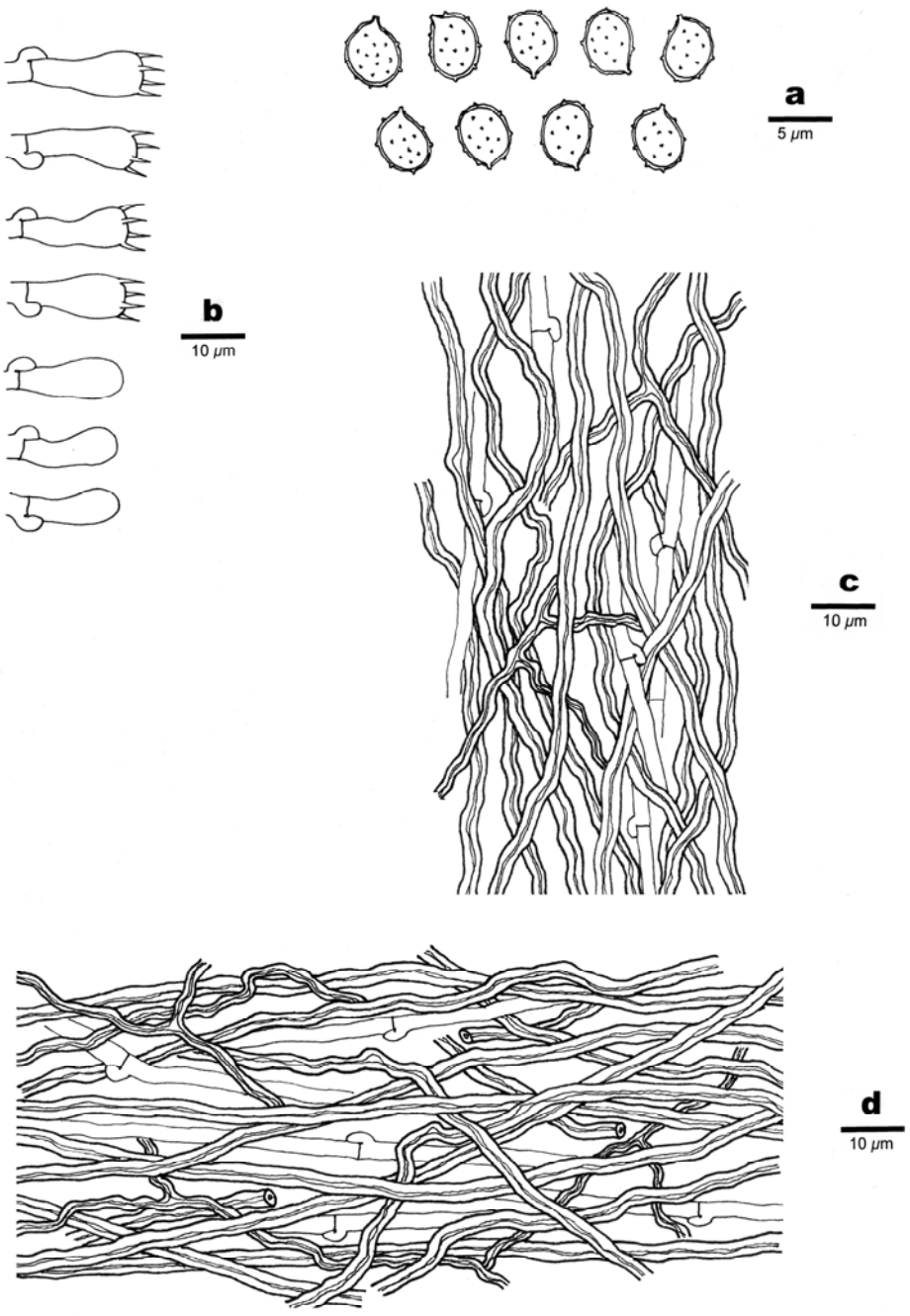


Fig. 3. Microscopic structures of *Sparsitubus nelumbiformis* (from holotype). **a.** Basidiospores. **b.** Basidia and basidioles. **c.** Hyphae from trama. **d.** Hyphae from context.

Context. — Generative hyphae hyaline, thin-walled, occasionally with clamp connections, very rarely with simple septa, rarely branched, 2–3 µm in diam; skeleto-binding hyphae thick-walled, flexuous, interwoven, 2.5–3.7 µm in diam. Generative hyphae in upper cuticle mostly with simple septa; skeletals in upper cuticle golden yellow, thick-walled with a distinct lumen, flexuous, strongly gelatinized, 2.5–4 µm in diam.

Tubes. — Generative hyphae hyaline, thin-walled, occasionally with clamp connections, unbranched, 1.5–2.5 µm in diam; skeleto-binding hyphae thick-walled with a narrow lumen, skeletal part subparallel along the tubes, 2–3 µm in diam. Cystidia and cystidioles absent; basidia barrel-shaped, with a basal clamp connection and four sterigmata, 15–17 × 6.5–7.5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores. — Basidiospores broadly ellipsoid to subglobose, yellowish, fairly thick- to thick-walled, asperulate, mostly collapsed when mature, IKI–, CB+, (4.3–)4.5–5.4(–5.8) × (3.6–)3.8–4.4(–5) µm, L = 4.99 µm, W = 4.07 µm, Q = 1.19–1.25 (n = 90/3).

Type of rot. — Unknown.

Distribution. — Hainan and Yunnan Province of China.

Specimens examined. — CHINA, Hainan Province, Baisha County, Bawangling Nature Reserve, on rotten angiosperm wood, 17 April 1977, J.S. Han 655 (HMAS 41036); Wangning County, 6 April 1993, C.L. Fu 1774 (HMAS 61421). Yunnan Province, Simao County, on fallen angiosperm trunk, 13 April 1957, L.W. Xu 623 (HMAS 41035, **holotype**).

Phylogenetic relationships based on LSU-rDNA. The data set of LSU-rDNA had an aligned length of 920 base pairs with 111 uninformative variable positions and 175 parsimony-informative positions. Parsimony analysis based on LSU-rDNA generated 20 equally parsimonious trees of 890 steps and consistency index (CI) = 0.433 (Fig. 4). Core polypore (followed Binder et al., 2005) clade including both white rot and brown rot polypores was strongly supported (bootstrap = 88%). *Sparasitubus nelumbiformis* and many polypores producing a white rot, such as species of *Ganoderma* and *Polyporus*, formed a clade (bootstrap = 51%), within which *S. nelumbiformis* shared a clade (bootstrap<50%) with *Dichomitus squalens* and *Ganoderma* species without support. Relationships among white rot polypores were not well resolved. Polypores producing a brown rot were paraphyletic in our tree, and there were clades receiving support from 62% to 99% in bootstrap value.

Discussion

Sparsitubus nelumbiformis was originally described from subtropical China (Xu and Zhao, 1980), and a few discussions were made on its generic relationship after its publication (Jülich, 1981; Ryvarden, 1991; Zhao, 1988).

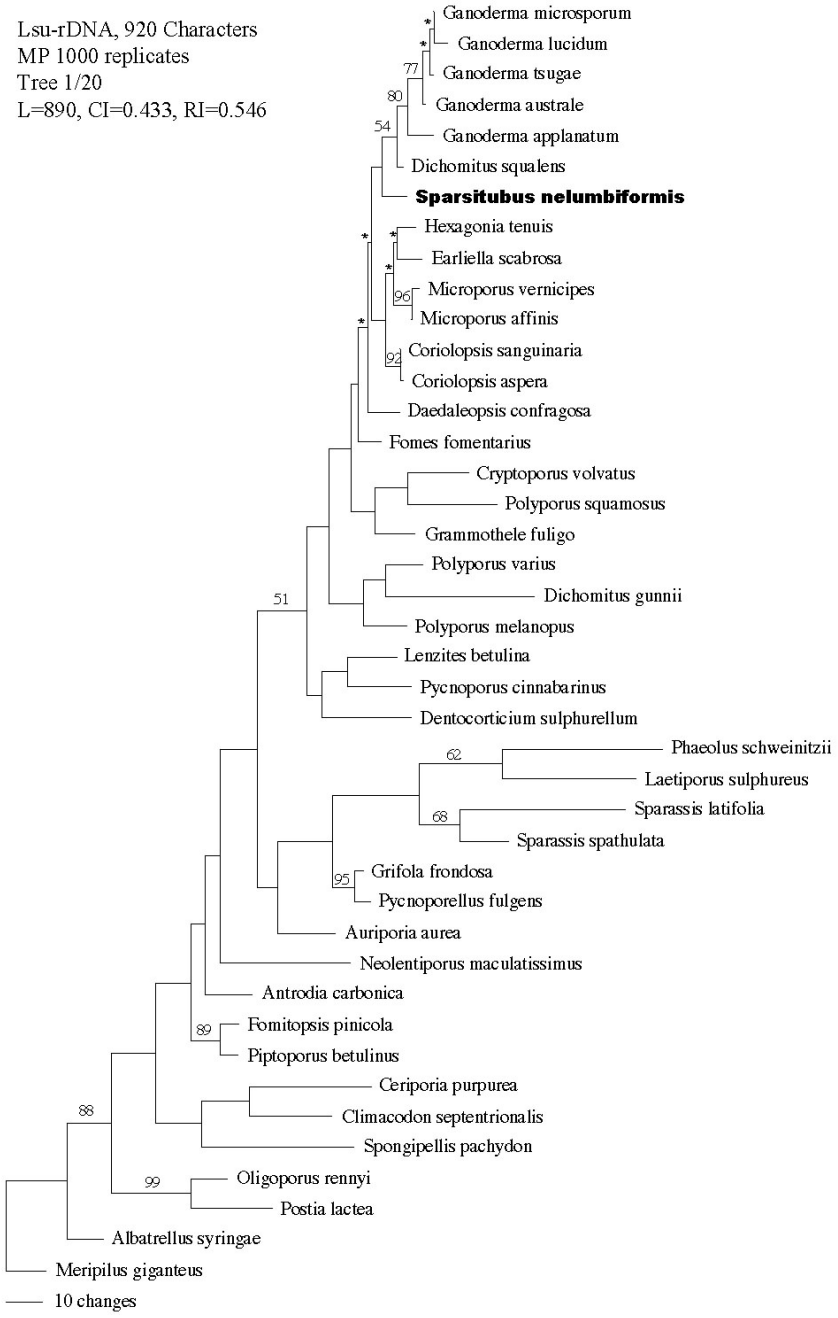


Fig. 4. Phylogenetic tree of *Sparsitubus nelumbiformis* and the related species based on LSU-rDNA.

The basidiospores of the species in the original description was mentioned as hyaline, 5.5–6.2 × 4.5–5 µm, but after the careful reexamination of the holotype and recent collections, we found its basidiospores are thick-walled, yellowish, 4.5–5.4 × 3.8–4.4 µm, so they are distinctly smaller than the data in its original description.

The tube morphology of *Sparsitubus nelumbiformis* is somewhat between *Fistulina hepatica* (Schaeff.:Fr.) With. and *Stromatoscypha fimbriata* (Pers.:Fr.) Donk, and this is one reason for *Sparsitubus* to be classified within *Polyporaceae* by its' finders. However *F. hepatica* has a monomitic hyphal structure, and its basidiospores are hyaline, thin-walled and smooth. Phylogenetic affinity of *Fistulina* to the Agaricales rather than polypores was suggested by previous studies based on rDNA data (Bodensteiner *et al*., 2004; Binder *et al*., 2005), and our tree confirmed *Sparsitubus nelumbiformis* a member of polypores *Stromatoscypha fimbriata* (≡ *Hydnopolyporus fimbriatus* (Fr.) D.A. Reid) also has a dimitic hyphal system and its skeletals are cyanophilous, but its basidiospores are hyaline, thin-walled, smooth, and negative in Cotton Blue. *Stromatoscypha fimbriata* is traditionally regarded as closely related to *Meripilus* species (Kirk *et al*., 2001), a lineage phylogenetically distant related to the core clade of polypores (Binder *et al*., 2005). But, no rDNA data is hitherto available for *S. fimbriata*.

Sparsitubus nelumbiformis, *Cryptoporus volvatus*, and *Ganoderma* species share the same hyphal structure, especially they all have cyanophilous skeleto-binding hyphae, and cyanophilous basidiospores. However, macroscopically tubes are separate in *S. nelumbiformis* while they are coherent in *Ganoderma* and *Cryptoporus*. In addition, basidiospores of *S. nelumbiformis* are thin, single-walled and asperulate, while basidiospores are thick-walled in *C. volvatus* and double-walled in *Ganoderma* species.

The hyphal structure of *Sparsitubus nelumbiformis* is close to *Polyporus* P. Micheli ex Adans.:Fr. and *Dichomitus* D.A. Reid by sharing so-called skeleto-binding hyphae, in which the proximal and central parts are straight, unbranched and regular, and the apex is divided into numerous whip-like branches. However, basidiospores of *Polyporus* and *Dichomitus* are cylindrical, hyaline, thin-walled, smooth, and negative in Cotton Blue, and its skeleto-binding hyphae are indextrinoid. *Megasporoporia* Ryvarden & J.E. Wright has very similar hyphal characters with *Sparsitubus*, but the former has hyaline, thin-walled, smooth, and acyanophilous basidiospores. Basidiospores of *Sparsitubus nelumbiformis* are similar to those in *Haploporus odorus* (Sommerf.) Bondartsev & Singer by sharing the asperulate ornamentation and cyanophilous skeletal hyphae, but basidiospores of the latter species are

hyaline, and its hyphal structure is normally dimitic, in which generative and skeletal hyphae are present, and no binding hyphae are existed.

Sparsitubus nelumbiformis has very unique combination of following characters: tubes separated each other, a dimitic hyphal structure with skeleto-binding hypahe, skeleto-binding hypahe strongly dextrinoid and cyanophilous, basidiospores yellowish, thick-walled, asperulate and cyanophilous.

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