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# A new species of *Fuscoporia* (Hymenochaetales, Basidiomycota) from southern China

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**Abstract**

A new polypore species, *Fuscoporia subferrea*, is described from southern China based on morphological characters and phylogenetic analysis. The new species is characterized by annual, resupinate basidiocarp, hyaline, thin-walled, smooth, cylindrical basidiospores measured as 4.2–5.8 × 2.2–2.6 µm. It resembles *F. ferrea*, but differs by smaller pores (7–10/mm vs. 5–7/mm) and narrower basidiospores (4.2–6.2 × 2.0–2.6 µm vs. 4.2–5.2 × 2.8–3.5 µm). Phylogenetic analyses inferred from the ITS and nLSU sequences indicate that the new species forms a distinct lineage with strong support and is closely related to *F. ferrea*.

**Key words** – Hymenochaetaceae – phylogeny – taxonomy – wood-rotting fungi

## Introduction

*Fuscoporia* Murrill was proposed by Murrill (1907) and typified by *F. ferruginosa* (Schrad.) Murrill. Most mycologists (Overholts 1953, Lowe 1966, Ryvarden & Johansen 1980, Larsen & Cobb-Poulle 1990, Ryvarden & Gilbertson 1994) treated this genus as a synonym of *Phellinus* Quél. Nevertheless, Fiasson & Niemelä (1984) recognized *Fuscoporia* as a monophyletic genus characterized by annual to perennial and resupinate to pileate basidiomata, a dimitic hyphal system with the encrustations on generative hyphae, the presence of hymenial setae, hyaline, thin-walled and smooth basidiospores, and the genus was confirmed by Wagner & Fischer (2001, 2002) through their nuclear large subunit (nLSU) ribosomal RNA-based phylogeny.

Recently, some new species were reported in the genus mostly by molecular analyses (Niemelä et al. 2001, Groposo et al. 2007, Baltazar et al. 2009, Baltazar & Gibertoni 2010, Dai 2010, Raymundo et al. 2013, Spirin et al. 2014, Pires et al. 2015).

During the survey of wood-rotting fungi in China, three unknown specimens were collected in Hainan Province, southern China, they are rather similar to *Fuscoporia ferrea* (Pers.) G. Cunn.but with distinct smaller pores and narrower basidiospres, and we propose a new species for these collections. To support our proposal, phylogenetic analyses on the position of the new species and related taxa were done based on internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

## Materials & Methods

### Morphological studies

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The studied specimens are deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions are based on field notes and dry herbarium specimens. Color terms follow Petersen (1996). Microscopic measurements and drawings were made from slide preparations of dried specimens stained with Cotton Blue and Melzer's reagent, by light microscopy following Dai (2010). Sections were studied at ultimate magnification ×1000 using Nikon Eclipse 80i microscopy and phase contrast illumination. Drawings were made with the aid of drawing tube. Spores were measured in tube sections. In presenting spore size variation, 5% of measurements were excluded from each end of the range and given in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, 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 specimens studied, n (a/b) = number of spores (a) measured from given number of specimens (b).

**DNA extraction and sequencing**

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to extract total genomic DNA from dried specimens following the manufacturer's instructions with some modifications (Chen et al. 2015, 2016). ITS regions were amplified with primers ITS4 and ITS5 (White et al. 1990), and the nLSU with primers LR0R and LR7. The PCR procedure for ITS was as follows: 6v initial denaturation at 95°C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 1min, 50 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers. All newly generated sequences were deposited at GenBank and listed in Table 1.

Besides the sequences generated from this study, other reference taxa for our phylogenetic analysis were selected from GenBank, and the original publications of the phylogenetic analyses contributing the sequences were referenced in Table 1. Sequences were aligned in MAFFT 6 (Katoh & Toh 2008) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999).

**Phylogenetic analyses**

Maximum Likelihood (ML) analysis was applied to the combined dataset. It was conducted with RAxML-HPC2 on Abe through the Cipres Science Gateway involved 1000 replicates under the GTRGAMMA model, with all model parameters estimated by the program. In addition 1000 rapid bootstrap replicates were run with the GTRCAT model.

Maximum parsimony (MP) analysis was also applied to the combined dataset as in Zhou et al. (2016b). Tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated.

Bayesian inference (BI) was applied to the combined dataset too. Substitution models suitable for each partition in the dataset were determined using Akaike Information Criterion (AIC) implemented in MrModeltest2.3 (Posada & Crandall 1998, Nylander 2004). The GTR+I+G model was estimated as the best-fit evolution models for all partition in the combined dataset. BI was calculated with MrBayes3.1.2 (Ronquist & Huelsenbeck 2003) with GTR+I+G model of DNA substitution and an invgamma distribution rate variation across sites. Four Markov chains were run for 2 runs from random starting trees for 2 million generations of the combined ITS and nLSU dataset and sampled every 100 generations. The burn-in was set to discard the first 25% of the

trees. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum parsimony (MP), maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP/BS) and 0.95 (BPP) were considered as significantly supported, respectively.

## Results

### Phylogenetic analyses

The combined ITS+nLSU dataset included sequences from 39 fungal specimens representing 15 species. The dataset had an aligned length of 2253 characters, of which 1722 are constant, 40 are variable but parsimony-uninformative, and 491 are parsimony-informative. Maximum Likelihood analysis resulted in a best tree, which is shown in Fig 1. Maximum parsimony analysis yielded 4 similar topologies (TL = 1177, CI = 0.650, RI = 0.873, RC = 0.568, HI = 0.350). Bayesian also resulted in a similar consensus tree with 1 million generation and an average standard deviation of split frequencies = 0.007745. The outgroup selected for ITS+nLSU analysis was *Coniferiporia weirii* (Murrill) L.W. Zhou & Y.C. Dai, and *Coniferiporia sulphurascens* (Pilát) L.W. Zhou & Y.C. Dai because *Coniferiporia* was a sister group of *Fuscoporia* (Zhou et al. 2016a).

In the phylogeny (Fig. 1) inferred from the combined ITS+nLSU sequences shows that the three newly sequenced specimens are clustered into a lineage with high support (100/100/1.00), which is closely related to *Fuscoporia ferrea.*

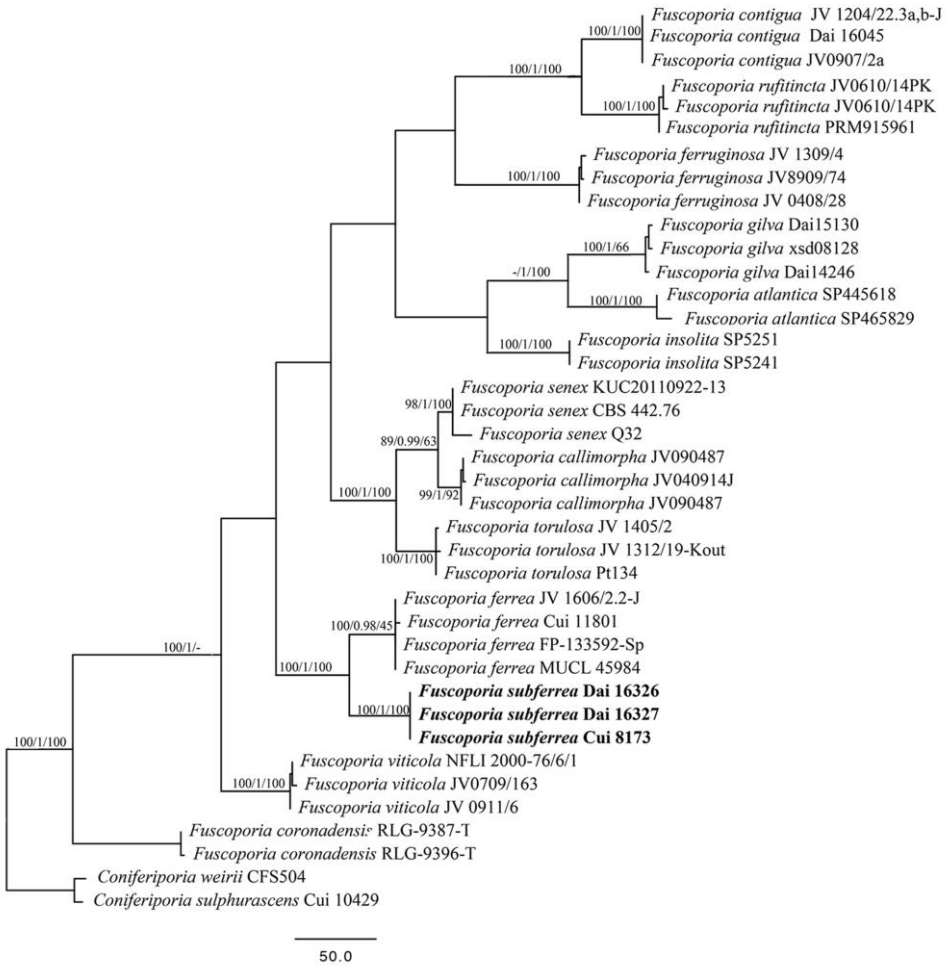


**Figure 1** – Phylogeny of *Fuscoporia subferrea* and related species generated by maximum likelihood analysis based on combined ITS and nLSU sequences. Branches are labeled with maximum likelihood bootstrap value higher than 50%, parsimony bootstrap value higher than 50%, and Bayesian posterior probabilities more than 0.95.

## Taxonomy

***Fuscoporia subferrea*** Q. Chen & Yuan Yuan , sp. nov. Figs 2–3

MycoBank – MB 819479

Holotype – China, Hainan Province, Baisha County, Yinggeling Nature Reserve, on fallen angiosperm branch, 17 November 2015, Dai 16327 (BJFC020414, holotype).

Etymology – *Subferrea* (Lat.) – referring to the morphological resemblance to *Fuscoporia ferrea.*

Fruiting body – Basidiocarps annual, resupinate, inseparable, without odour or taste and corky when fresh, light-weight and hard corky when dry, up to 26 cm long, 3 cm wide and 2 mm thick at centre. Pore surface mouse-grey to ash-grey when fresh, more or less fawn, cracked with age; sterile margin distinct, dark reddish brown when dry, up to 1.5 mm wide; pores circular, 7–10 per mm; dissepiments thin, entire, abundant setae seen in tube cavities (under lens). Subiculum dull brown, corky, about 0.4 mm thick. Tubes yellowish brown, paler contrasting with pores and subiculum, hard corky, up to 1.6 mm long.

Hyphal structure – Hyphal system dimitic; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH.

Subiculum – Generative hyphae infrequent, hyaline, thin- to slightly thick-walled, occasionally branched, frequently simple septate, 2.0–2.6 μm in diam; skeletal hyphae dominant, rust-brown, thick-walled with a narrow to medium lumen, unbranched, flexuous, interwoven, 2.4–3.2 μm in diam.

Tubes – Generative hyphae infrequent, mostly present at subhymenium, hyaline, thin-walled, frequently branched and simple septate, 1.8–2.4 μm in diam, some of them at dissepiment edges and in hymenium encrusted; skeletal hyphae dominant, yellowish brown, thick-walled with a narrow to medium lumen, unbranched, more or less straight, interwoven, 2.2–3.0 μm in diam. Setae frequent, mostly originating from hymenium, subulate, dark brown, thick-walled, 18–34 × 4–7 μm; fusoid cystidioles frequent, hyaline and thin-walled, sometimes covered with crystals; basidia barrel-shaped, with four sterigmata and a simple septum at the base, 9.5–11 × 4.8–6.2 μm; basidioles dominating in hymenium, in shape similar to basidia, but slightly smaller.

Spores – Basidiospores cylindric, hyaline, thin-walled, smooth, usually glued in tetrads, IKI–, CB–, sometimes with one or two guttules, (4.0–)4.2–6.2(–6.4) × (1.8–)2.0–2.6(–2.8) μm, L = 5.11 μm, W = 2.28 μm, Q = 2.15–2.27 (n = 60/2).

Additional specimens examined (paratypes) – China, Hainan Province, Baisha County, Yinggeling Nature Reserve, on fallen angiosperm branch, 17 November 2015, Dai 16326 (BJFC020413); Yunnan Province, Baoshan County, Gaoligong Mountain Nature Reserve, 25 October 2009, Cui 8173 (BJFC006662).

## Discussion

According to the phylogenetic analysis (Fig. 1), four specimens of *F. ferrea* from USA (JV 1606/2.2–J, FP-133592–Sp), France (MUCL 45984) and northern China (Cui 11801) are closely related to the *F. subferra*, but the two species are in two distinct lineages in our phylogeny.

*Fuscoporia subferrea* is morphologically similar to *F. ferrea* in sharing annual, resupinate basidiocarps and cylindric basidiospores, but the latter has distinctly wider basidiospores (4.2–5.2 × 2.8–3.5 μm) and larger pores (5–7 per mm). In addition, these two species have different distributions in China: *F. subferrea* mainly distributes in southern China, while *F. ferrea* has a distribution in northern China. *F. suferrea* sometimes is confused with *F. ferruginosa*, but the latter has ellipsoid basidiospores and abundant mycelial setae at the sterile margin. *F. chrysea* (Lév.) Baltazar & Gibertoni is similar to *F. subferrea* by resupinate fruiting bodies and tiny pores (9–10 per mm), but it has shorter basidiospores (3.5–4 × 2.5–3 μm, Baltazar & Gibertoni 2010).

**Table 1** Taxa and GenBank accession numbers for ITS and nLSU sequences used in the phylogenetic analyses.

| Species | Sample no. | Locality | GenBank accession no. | |
|---|---|---|---|---|
| | | | ITS | nLSU |
| *Fuscoporia atlantica* | SP445618 | Brazil | KP058515 | KP058517 |
| *Fuscoporia atlantica* | SP465829 | Brazil | KP058514 | KP058516 |
| *Fuscoporia callimorpha* | JV090487 | USA | JF692190 | – |
| *Fuscoporia callimorpha* | JV090487 | USA | JF692191 | – |
| *Fuscoporia callimorpha* | JV040914J | USA | JF692193 | – |
| *Fuscoporia contigua* | JV0907/2a | USA | JQ794547 | – |
| *Fuscoporia contigua* | Dai 16045 | USA | KX961105 [a] | KY189105 [a] |
| *Fuscoporia contigua* | JV1204/22.3a,b-J | USA | KX961104 [a] | KY189104 [a] |
| *Fuscoporia coronadensis* | RLG-9387-T | USA | JX110073 | JX110117 |
| *Fuscoporia coronadensis* | RLG-9396-T | USA | JX110074 | JX110118 |
| *Fuscoporia ferrea* | JV 1606/2.2-J | USA | KX961100 [a] | KY189100 [a] |
| *Fuscoporia ferrea* | Cui 11801 | China | KX961101 [a] | KY189101 [a] |
| *Fuscoporia ferrea* | MUCL 45984 | France | KX961112 [a] | KY189112 [a] |
| *Fuscoporia ferrea* | FP-133592-Sp | USA | KU139189 | KU139259 |
| *Fuscoporia ferruginosa* | JV1309/4 | Slovakia | KX961102 [a] | KY189102 [a] |
| *Fuscoporia ferruginosa* | JV0408/28 | Czech | KX961103 [a] | KY189103 [a] |
| *Fuscoporia ferruginosa* | JV8909/74 | Germany | JQ794573 | – |
| *Fuscoporia gilva* | Dai14246 | China | KX961108 [a] | KY189109 [a] |
| *Fuscoporia gilva* | Dai15130 | China | KX961109 [a] | KY189108 [a] |
| *Fuscoporia gilva* | xsd08128 | China | FJ481039 | – |
| *Fuscoporia insolita* | SP5251 | Russia | KJ677113 | – |
| *Fuscoporia insolita* | SP5241 | Russia | KJ677114 | – |
| *Fuscoporia rutincta* | PRM915961 | USA | GU594160 | – |
| *Fuscoporia rutincta* | JV0610/14PK | Belize | JQ794579 | – |
| *Fuscoporia rutincta* | JV0610/14PK | Belize | JQ794580 | – |
| *Fuscoporia senex* | CBS 442.76 | Korea | AY558647 | – |
| *Fuscoporia senex* | KUC20110922-13 | Korea | JX463658 | JX463652 |
| *Fuscoporia senex* | Q32 | China | KC414230 | – |
| *Fuscoporia subferrea* | Dai 16326 | China | KX961097 [a] | KY053472 [a] |
| *Fuscoporia subferrea* | Dai 16327 | China | KX961098 [a] | KY053473 [a] |
| *Fuscoporia subferrea* | Cui 8173 | China | KX961099 [a] | KY189111 [a] |
| *Fuscoporia torulosa* | JV 1405/2 | Czech | KX961106 [a] | KY189106 [a] |
| *Fuscoporia torulosa* | JV 1312/19-Kout | Spain | KX961107 [a] | KY189107 [a] |
| *Fuscoporia torulosa* | Pt134 | Italy | EF068139 | – |
| *Fuscoporia viticola* | JV0911/6 | Czech | KX961110 [a] | KY189110 [a] |
| *Fuscoporia viticola* | NFLI 2000-76/6/1 | – | JQ358814 | – |
| *Fuscoporia viticola* | JV0709/163 | USA | JQ794583 | – |
| *Coniferiporia sulphurascens* | Cui 10429 | China | KR350565 | KR350555 |
| *Coniferiporia weirii* | CFS504 | Canada | AY829341 | AY829345 |

[a]Sequences newly generated in this study

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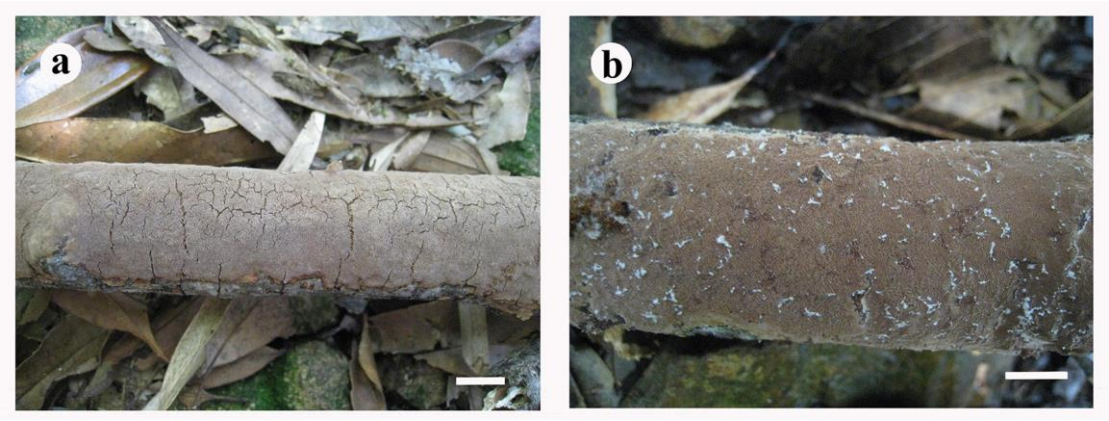


**Figure 2** – Basidiocarps of *Fuscoporia subferrea*. a: holotype, Dai 16327; b: Dai 17159. Scale bars = 10 mm.

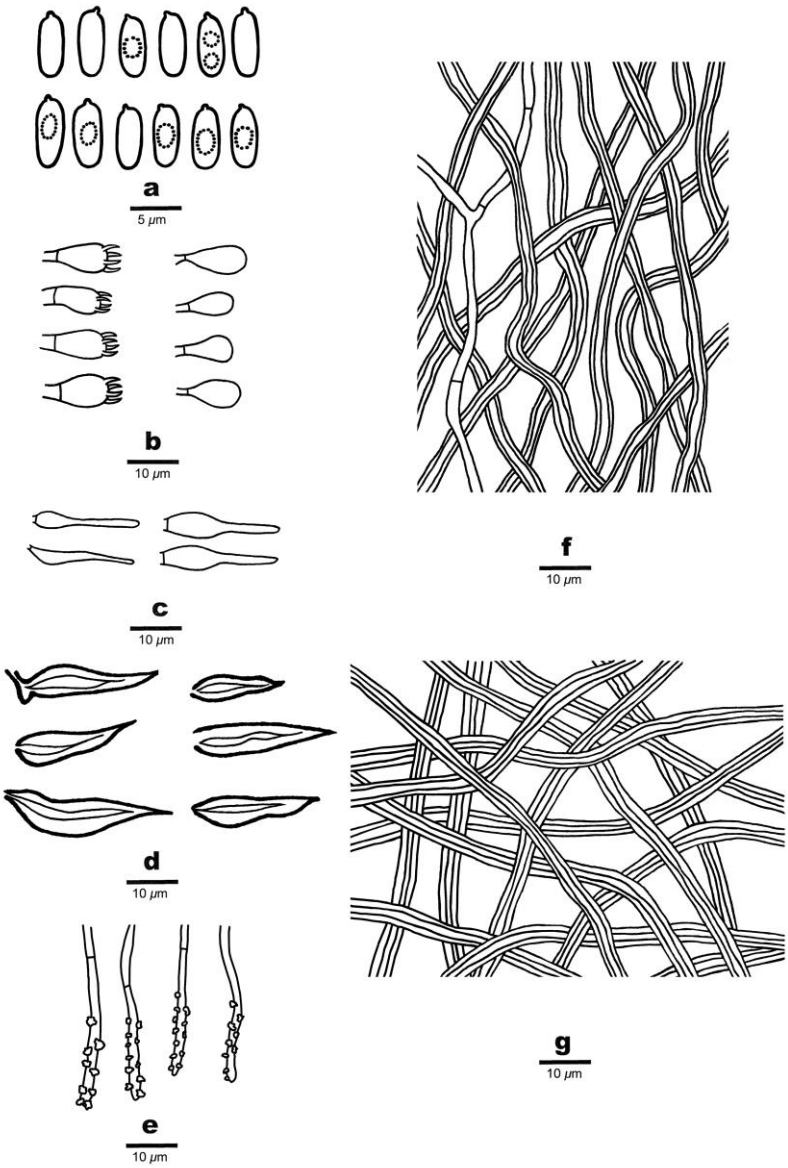


**Figure 3** – Microscopic structures of *Fuscoporia subferrea*. (the holotype, Dai 16327). a: basidiospores; b: basidia and basidioles; c: cystidioles; d: setae; e: generative hyphae at dissepiment edge; f: hyphae from trama; g: hyphae from subiculum.

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