



***Hymenochaete* (Hymenochaetales, Basidiomycota) in China 7. *H. cana* sp. nov. and *H. denticulata* New to China**

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ABSTRACT

Hymenochaete cana, collected from Guangxi Autonomous Region, southern China, is described as new to science, based on morphological and molecular evidence from combined ITS and nLSU rDNA sequence data. It is similar to *H. nanospora*, *H. unicolor* and *H. indica*, but they can be easily distinguished. *Hymenochaete denticulata* collected from the same region is newly recorded in China, and this is the first report outside its type locality, Réunion Island in the Indian Ocean. Illustrations, descriptions and notes on the new species are provided, while *Hymenochaete denticulata* is illustrated and discussed.

Keywords: Hymenochaetaceae, phylogeny, taxonomy, wood-decaying fungi

1. INTRODUCTION

In recent years, the taxonomy and phylogeny of the poroid genera of Hymenochaetaceae in China have been intensively studied [1-6]. However, the study of the corticioid *Hymenochaete*, which is the second largest genus in the Hymenochaetaceae, is far behind those poroid genera, despite recent publications [7-10]. Many species of this group are still undescribed in subtropical and tropical areas of China because of the extremely high diversity of plants and wood-inhabiting fungi.

Two intensive surveys of wood-inhabiting fungi were carried out in Guangxi Autonomous Region in the summer of 2011 and 2012, and about 300 specimens of *Hymenochaete* were collected. These materials have been carefully studied through both

morphological and phylogenetic methods, and a new species and a new Chinese record were identified. We describe and illustrate these two species in the present paper.

2. MATERIALS AND METHODS

2.1 Morphological Studies

Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure follows [7]. In presenting the size range of spores and setae, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetical average of all spores), W = mean spore width (arithmetical average 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 of each specimen), n = the number of spores measured from given number of specimens. IKI stands for Melzer's reagent, KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue in lactic acid. IKI- = inamyloid and nondextrinoid, CB- = acyanophilous. Special color terms follow [11].

2.2 DNA Extraction and Sequencing

A CTAB Plant genome rapid extraction kit (Aidlab Biotechnologies Co., Ltd) was employed for DNA extraction from herbarium specimens according to the manufacturer's instructions. Approximately 700 base pairs of the ITS region were amplified with primers ITS5 and ITS4, using the following procedure: initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 40 s, 58°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. About 1,300 base pairs of the nLSU rDNA region were amplified with primers LR0R and LR7, using the following procedure: initial denaturation at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. DNA sequencing was performed at Beijing Genomics Institute using the same primers as PCR procedures, and all the newly generated sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>).

2.3 Phylogenetic Analyses

The molecular phylogeny was inferred from the combined ITS and nLSU rDNA sequences of 34 species of *Hymenochaete* and 10 species of *Pseudochaete* T. Wagner & M. Fisch. Except for sequences of the

new species and the Chinese material of *Hymenochaete denticulata* J.C. Léger & Lanq., all other sequences were downloaded from GenBank. *Fomitiporia bannaensis* Y.C. Dai and *F. punctata* (P. Karst.) Murrill were selected as outgroups [10]. These sequences were aligned using the ClustalX 1.83 [12]. The alignment was optimized manually in BioEdit 7.0.5.3 [13].

Maximum parsimony analysis was performed using PAUP* 4.0b10 [14]. Gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm, with all characters equally weighted. Branch supports for all parsimony analysis were estimated by performing 1,000 bootstrap replicates [15] with a heuristic search of 10 random-addition replicates for each bootstrap replicate. The tree length (TL), consistency indices (CI), retention indices (RI), rescaled consistency indices (RC) and homoplasy index (HI) were calculated for each tree generated. Trees were figured in TreeView 1.6.6 [16].

3. RESULTS

3.1 Phylogeny

Three ITS and three nLSU rDNA sequences were newly obtained in this study. The alignment of combined ITS and nLSU rDNA sequences of 46 taxa resulted in 2235 sites with 630 parsimony informative characters. Six strict consensus trees were generated from the analysis with TL = 2558, CI = 0.500, RI = 0.771, RC = 0.386 and HI = 0.500. The strict consensus tree showed that the new species nested within the *Hymenochaete* clade, and it formed a distinct sister group of *H. nanospora* J.C. Léger with a high bootstrap value (94%; Figure 1). This indicated that

H. cana is phylogenetically different from any sampled species. Meanwhile, sequences from the Chinese and French specimens of

Hymenochaete denticulata clustered together to form a strongly support lineage (bootstrap value = 100%; Figure 1).

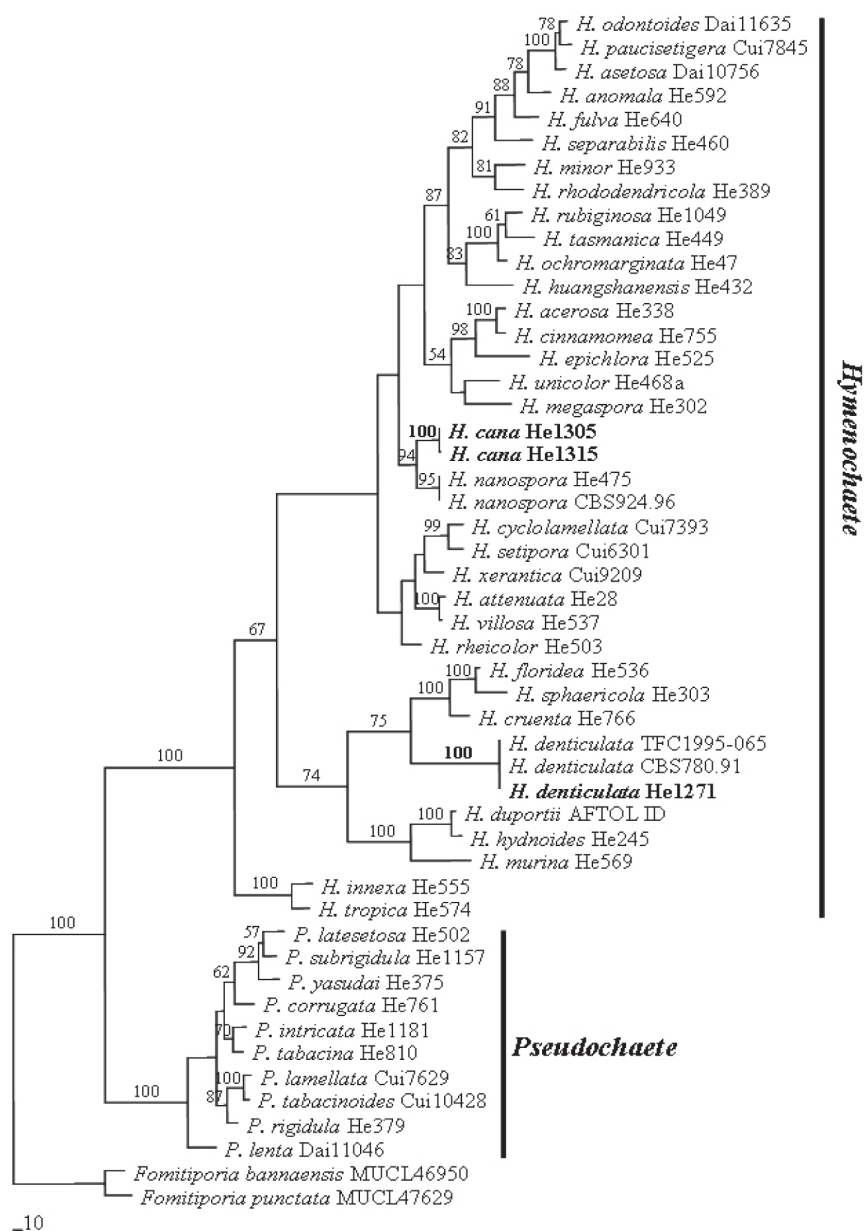


Figure 1. The strict consensus tree obtained from Maximum Parsimony analysis of combined ITS and nLSU rDNA sequences of *Hymenochaete* and *Pseudochaete*. *Fomitiporia bannaensis* and *F. punctata* were used as outgroups. The new species and new record are in bold. Parsimony bootstrap values (> 50%) are shown.

3.2 Taxonomy

Hymenochaete cana S.H. He & Hai J. Li, sp. nov. Figures. 2–3

MycoBank MB 805071

Type: CHINA – Guangxi Autonomous Region, Shangsi County, Shiwandashan National Forestry Park, on fallen angiosperm trunk, 26 July 2012, SH He 1305 (holotype, BJFC 014156; GenBank no. ITS: KF438169, LSU: KF438172).

Etymology – the epithet “*cana*” refers to the grayish or ash-colored hymenophore.

Fruitbody – Basidiocarps annual, effuse, closely adnate, corky or woody hard when dry, first as small colonies, later confluent up to 20 cm or more in the longest dimension, 100–500 μm thick in section. Hymenophore smooth, mouse-gray to ash-gray, usually densely and minutely cracked; margin thinning out, distinct or indistinct, white when juvenile, becoming vinaceous gray or concolorous with hymenophore when mature.

Hyphal structure – Hyphal system

monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum – Tomentum, cortex and hyphal layer absent.

Stratified hymenium – Generative hyphae hyaline to yellowish brown, thin-to thick-walled, more or less agglutinated, interwoven, 1.8–2.8 μm in diam. Setal layer thickening, composed of several to many rows of overlapping setae. Setae very numerous, subulate, reddish brown, with acute tips, some enmeshed with a thin hyphal sheath, without encrustations, projecting up to 15 μm above the hymenium, 20–40 $\times$ 5–8 μm . Cystidia and hyphidia absent. Basidia clavate, with four sterigmata and a simple septum at base, 10–18 $\times$ 2.8–3 μm ; basidioles in shape similar to basidia, but distinctly smaller.

Spores – Basidiospores cylindrical to oblong ellipsoid, slightly curved, hyaline, thin-walled, smooth, IKI-, CB-, (2.8–)3–3.4(–3.6) $\times$ (1.5–)1.6–1.9(–2) μm , L = 3.17 μm , W =



Figure 2. Basidiocarps of *Hymenochaete cana* (from holotype).

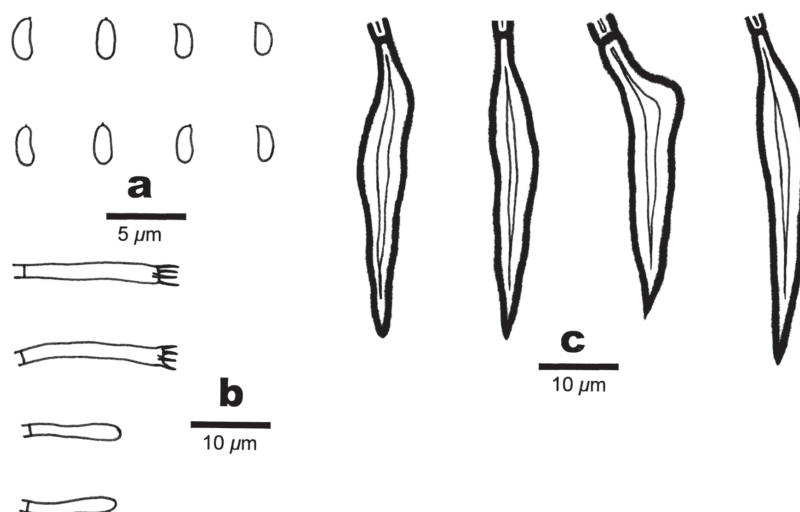


Figure 3. Microscopic structures of *Hymenochaete cana* (drawn from holotype). **a** Basidiospores; **b** Basidia and basidioles; **c** Setae.

1.76 μm , $Q = 1.8$ ($n = 30/1$).

Additional specimen examined: CHINA – Guangxi Autonomous Region, Shangsi County, Shiwandashan National Forestry Park, fallen trunk of angiosperm, 26 July 2012, SH He 1315 (BJFC 014166; GenBank no. ITS: KF438170, LSU: KF438173).

Type of rot. – Causes a white pocket rot of angiosperm wood.

Hymenochaete cana is characterized by its grayish basidiocarps, anatomical structure in section (without a cortex and hyphal layer), relatively short setae and small basidiospores. In both morphological and phylogenetic aspects, the new species seems to be very close to *H. nanospora* (bootstrap value = 94%; Figure 1), which has both similar setae and basidiospores. However, *H. nanospora* differs from *H. cana* by having brown to fuscous basidiocarps, presence of a cortex and smaller basidiospores ($2.5\text{--}3 \times 0.8\text{--}1 \mu\text{m}$) [17].

Microscopically, *Hymenochaete cana* is also similar to *H. unicolor* Berk. & M.A.

Curtis and *H. indica* Parmasto, all of which have the same anatomical structure in section, relatively short setae and small basidiospores. However, *H. unicolor* has umber or brown basidiocarps, longer setae ($35\text{--}60 \mu\text{m}$) and larger basidiospores ($4\text{--}5 \times 2.4\text{--}3.2 \mu\text{m}$) [18], and it is distantly related to the clade of *H. cana* in the phylogram (Figure 1). *H. indica* differs from *H. cana* by having vinaceous buff basidiocarps and broadly ellipsoid basidiospores ($3.5\text{--}4.3 \times 2.4\text{--}3.1 \mu\text{m}$) [19]. Another species, *H. legeri* Parmasto, which has similar basidiocarp appearance with *H. cana*, can be easily distinguished from the new species by its large and encrusted setae ($75\text{--}100 \times 10\text{--}15 \mu\text{m}$) and distinctly cylindrical basidiospores ($6\text{--}8 \times 2.2\text{--}3.2 \mu\text{m}$) [20].

Previously, *Hymenochaete denticulata* was only reported from Réunion Island in the Indian Ocean, and it is characteristic for its anatomical structure in section (without a cortex and hyphal layer) and two types of setae with several to many conical teeth [17]. A specimen collected from Nonggang Natural Reserve, Guangxi

Autonomous Region, was morphologically and phylogenetically identified as *H. denticulata* (He 1217, BJFC 014122; GenBank no. ITS: KF438171, LSU: KF438174; Figures. 1, 4, 5). It is new to China.



Figure 4. Basidiocarps of *Hymenochaete denticulata* (from He 1271).

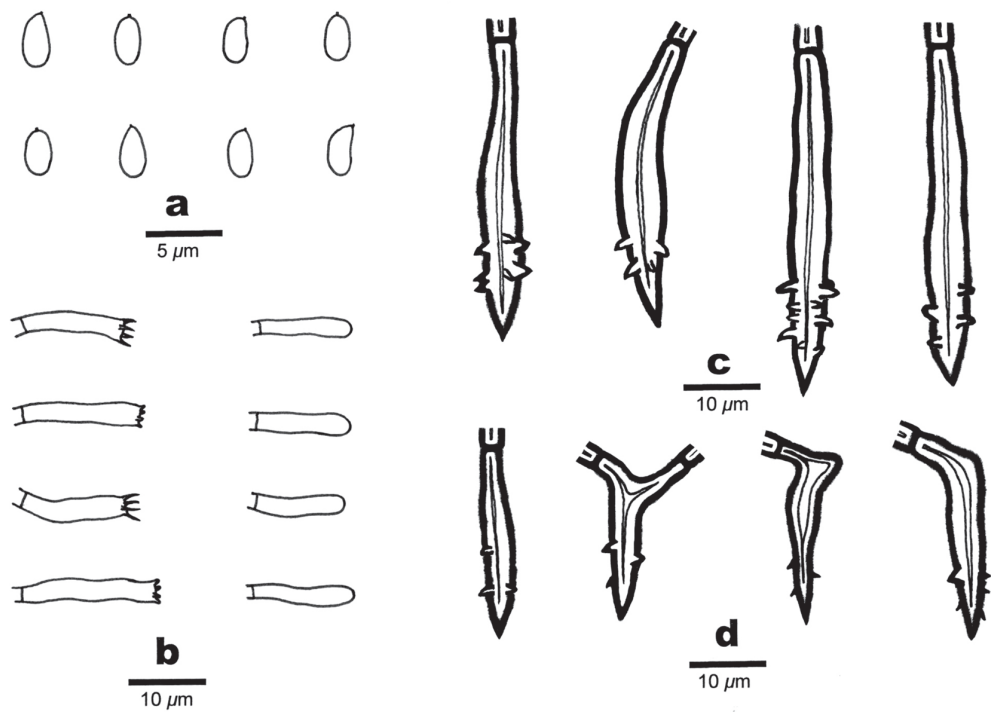


Figure 5. Microscopic structures of *Hymenochaete denticulata* (drawn from He 1271). a Basidiospores; b Basidia and basidioles; c,d Setae.

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