Species clarification for the medicinally valuable ‘sanghuang’ mushroom

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ABSTRACT. A legendary and very valuable medicinal fungus first known in China 2000 years ago is recognized in this study as a new species. The sanghuang mushroom (sanghuang) is a popular medicinal polypore used throughout China, Japan, and Korea. While its medicinal properties were recognized in an early Tang Dynasty herbal written in the 7th century, modern scientific research has only recently confirmed its highly effective antitumor properties. Although Japanese and Korean mycologists have adopted Phellinus linteus or P. baumii as the scientific name for sanghuang that belongs to the Inonotus baumii-I. linteus group in the Hymenochaetales (Basidiomycota), its species identity has not been satisfactorily answered. This study delimited the species of the I. baumii-I. linteus group including sanghuang, based on an analysis of morphological characteristics and nrDNA ITS sequences. Both morphological and molecular features were useful in separating different fungal species in this monophyletic group that are generally specialized with their host tree species. The true sanghuang is a new and previously undescribed species that grows solely on Morus in China, Japan, Korea, and Taiwan. It is now rare and endangered in the wild. Six related species of the I. baumii-I. linteus group distributed in Asia have evolved to specific host tree species: I. baumii on Syringa, I. lonicericolor on Lonicera, I. lonicerinus comb. nov. on Lonicera, I. sanghuang on Morus, I. vaninii on Populus, and I. weigelae sp. nov. on Weigela; a key is provided to ease the determination of these taxa.

Keywords: Basidiomycota; Hymenochaetaceae; Inonotus sanghuang; Medicinal fungi; Morus; New species; Phellinus; Taxonomy.

INTRODUCTION

The sanghuang mushroom (sanghuang) is a popular and medicinally important polypore species famous in China, Japan, and Korea. On mainland China, the most valuable traditional medicinal fungus is the ‘Chinese caterpillar fungus’ (Ophiocordyceps sinensis (Berk.) G.H. Sung et al., syn.: Cordyceps sinensis (Berk.) Sacc.), while in Taiwan, it is ‘niu-chang-chih’ (Taiwanofungus camphoratus (M. Zang & C.H. Su) Sheng H. Wu et al.). In Japan and Korea, however, ‘sanghuang’ is considered the most valuable fungus. The Chinese character ‘桑’ (sang) means the tree genus Morus and ‘黄’ (huang) means yellow; accordingly, sanghuang is a yellow organism that grows on Morus.

Sanghuang is called ‘meshimakobu’ in Japan. ‘Meshima’ is a Japanese island in Nagasaki Prefecture, and ‘kobu’ means wart. Meshimakobu hence means ‘warts of Meshima Island’ since the sanghuang mushroom was once fairly common on Meshima Island and resembles warts growing on the trunks of Morus trees. Korea uses the term ‘sanghwang’ for this fungus (Park et al., 2002), which, due to its identical pronunciation, was likely introduced from China long ago. Medicinal usage of sanghuang was first appeared 2000 years ago in the oldest Chinese medicinal book Shennong’s compendium of materia medica by the name ‘sanger’ (ear of Morus). Medicinal application of sanghuang was provided in The Characters of Drugs written by Quan Zhen in the early Tang Dynasty (ca. AD 630), and was later (AD 659) recorded in the Newly Revised Materia Medica, the world’s earliest pharmacopoeia issued...
by the government. Sanghuang was also described in the famous Chinese Compendium of Materia Medica, written by Shi-Zhen Li in the Ming Dynasty (AD 1596) (Zeng et al., 2008). Early records of meshimakobu (sanghuang) in the Japanese scientific literature date from the beginning of the 20th century, and were based on specimens identified as Fomes yucatanensis (Murrill) Saccardo & D. Saccardo or P. rimosus (Berk.) Cooke, according to Ito (1955). This species was known as Phellinus yucatanensis (Murrill) Imazeki (synonym of Phellinus linteus (Berk. & M.A. Curtis) Teng) in Japan after 1943 (Imazeki, 1943), and in recent decades as P. linteus following Teng et al. (1963), because Teng et al. regarded F. yucatanensis as a synonym of P. linteus. Teng (1939) first reported F. yucatanensis (synonym of P. linteus) in China, but this polypore species was not mentioned by him to be associated with medicinal function or with the legendary sanghuang mushroom described in Chinese books on herbal medicines. All known scientific binomials for naming sanghuang pertain to the Hymenochaetales of the Basidiomycota.

Although medicinal applications of the ‘sanghuang’ can be traced back to 2000 years ago, its highly effective antitumor action was only recently detected and confirmed using modern scientific methods (Ikekawa et al., 1968; Shibata et al., 1968; Chung et al., 1993; Kim et al., 1996; Lee et al., 1996; Han et al., 1999; Shon, 2003; Kim et al., 2004; Hwang et al., 2005; Guo et al., 2007; Ohno et al., 2007; Zhu et al., 2007; Silva et al., 2008), who assigned their material to either P. linteus or P. baumii, both with the same species concept. The sanghuang mushroom was always said to grow on Morus in Japan (Ito, 1955; Imazeki and Hongo, 1989), and part of the specimens assigned P. baumii or P. linteus from East Asian collections studied were taken from Morus trees (Ito, 1955; Kim et al., 1999; Lim et al., 2003; this study). An association between P. igniarius and Morus, however, was never reported. In addition, Xie et al. (2010) identified the fungal strains of the so-called sanghuang by analyzing nrDNA ITS sequences, and concluded that most medicinally applied strains are ‘P. baumii’ (I. sanghuang in this study) and ‘P. linteus’ (I. vaninii in this study), but not P. igniarius. Consequently, this study focuses on the P. baumii-P. linteus group, not on the P. igniarius complex.

Several studies detecting the phylogenetic relationships within Phellinus s.l. have been presented in the last decade (Wagner and Fischer, 2002; Nam et al., 2003; Jeong et al., 2005; Larsson et al., 2006; Decock et al., 2007). We consulted those literature to find out which species and strains were sampled for analysis. Dai (1999) placed species of the P. baumii-P. linteus group in the subgenus Fulvipomes (Murrill) Y.C. Dai of Phellinus. However, phylogenetic studies based on analysis of nLSU sequence (Wagner and Fischer, 2002) and ITS and nLSU sequences (Larsson et al., 2006; Dai, 2010) showed that these species belong to a monophyletic group composed of Inonotus spp. The traditional concept of ‘typical’ Inonotus was the annual basidiocarp, monomitic hyphal system, and softer basidiocarps. Instead, species in P. baumii-P. linteus are dimitic and have fairly hard basidiocarps which are generally perennial; hence it can be considered a group morphologically resembling Phellinus while phylogenetically belonging to now because many species of Phellinus s.l. with pileate fruiting bodies were regarded as sanghuang. Thus the term sanghuang, recorded in ancient Chinese medical texts, used throughout Japan for a century, and well-known in Korea for it its medicinal properties was never fully defined. Accurate identification of many polypore fungi, including Phellinus s.l., is generally difficult and needs taxonomic experience. This study attempted to recognize and separate related taxa in the P. baumii-P. linteus species complex, based on analyses of morphological characteristics and nrDNA ITS sequences, and to clarify which species really represents the medicinally important sanghuang. Previous surveys (Dai, 1999; 2010; Fischer and Binder, 2004) showed that many species of Phellinus s.l., including the P. baumii-P. linteus group, have more or less specific relationships with tree host species. The potential correlation between P. baumii-P. linteus species and their host tree species proved significant in separating related fungal species, and is examined in this study.

Numerous medicinal and biochemical studies of sanghuang were conducted, mostly by Korean researchers (e.g., Ikekawa et al., 1968; Shibata et al., 1968; Chung et al., 1993; Kim et al., 1996; Lee et al., 1996; Han et al., 1999; Shon, 2003; Kim et al., 2004; Hwang et al., 2005; Guo et al., 2007; Ohno et al., 2007; Zhu et al., 2007; Silva et al., 2008), who assigned their material to either P. linteus or P. baumii, both with the same species concept. The sanghuang mushroom was always said to grow on Morus in Japan (Ito, 1955; Imazeki and Hongo, 1989), and part of the specimens assigned P. baumii or P. linteus from East Asian collections studied were taken from Morus trees (Ito, 1955; Kim et al., 1999; Lim et al., 2003; this study). An association between P. igniarius and Morus, however, was never reported. In addition, Xie et al. (2010) identified the fungal strains of the so-called sanghuang by analyzing nrDNA ITS sequences, and concluded that most medicinally applied strains are ‘P. baumii’ (I. sanghuang in this study) and ‘P. linteus’ (I. vaninii in this study), but not P. igniarius. Consequently, this study focuses on the P. baumii-P. linteus group, not on the P. igniarius complex.

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Inonotus. We trust the result derived from the phylogenetic analysis, and in the latter part of this paper, we place the *P. baumii-P. linteus* group in *Inonotus*.

Kim et al. (1999; 2001), Nam et al. (2002), Park et al. (2002), Wang et al. (2010), and Xie et al. (2010) conducted phylogenetic studies probing species separation within the *I. baumii-I. linteus* group, and/or tried to detect the ‘real’ sanghuang mushroom species, based on ITS sequence analyses. However, none of those studies succeeded in finding a new species representing the real sanghuang mushroom growing on *Morus*, from other allied species with similar morphological characteristics, and reasons for the failure are detailed below in the ‘Discussion’ section.

**MATERIALS AND METHODS**

**Sources of materials for the morphological and phylogenetic studies**

The studied specimens of the so-called sanghuang mushroom and related species were obtained from wild collections gathered by researchers, or purchased from vendors who sell sanghuang mushrooms. Researcher-conducted collections possess more reliable information than purchased ones, especially collection site and host tree species data. All specimens studied were collected from East and Central Asia, including China, Japan, Taiwan, Russia, Kazakhstan, Turkmenistan, and Uzbekistan. Specimens we studied are deposited in herbaria at the Institute of Applied Ecology, Chinese Academy of Sciences (IFP), Shenyang, China; Estonian University of Life Sciences Herbarium (TAA), Tartu, Estonia; Forestry and Forest Products Research Institute, Herbarium of Forest Mycology and Pathology (TFM), Inashiki, Japan; and the Herbarium of the National Museum of Natural Science (TNM), Taichung, Taiwan. The source of species and strains used for the phylogenetic study included scientifically collected specimens, purchased basidiocarps, commercial fungal cultures, and DNA sequences derived from GenBank (Table 1). Preliminary surveys based on morphological studies of the basidiocarps or phylogenetic analyses excluded some specimens or strains from the final analysis of this study.

**Methods of morphological study and culture isolation**

Observations and measurements were based on dried specimens. For observations and measurements of microscopic characters, thin-sections of basidiocarps were mounted in 5% KOH to ensure rehydration. Melzer’s reagent (IKI) was employed to detect amyloidity and dextrinoidity. Cotton blue (CB) was used as a mounting medium to determine cyanophily. Descriptions provided in this study were taken from dried basidiocarps. In the text, the following abbreviations were used: L, mean spore length with the standard deviation; W, mean spore width with the standard deviation; Q, mean L/W ratio; and n, number of spores measured from each specimen. Living mycelia were isolated from the context of basidiocarps or from the woody substratum beneath the basidiocarps, and grown on 1.5% malt extract agar.

**DNA extraction, PCR amplification, DNA cloning, and DNA sequencing**

DNA was isolated from basidiocarps or living cultures using the Plant Genomic DNA Extraction Miniprep System (Viogene, Taiwan) according to the manufacturer’s instructions. A pair of primers (ITS1 and ITS4) was used to amplify the nrDNA ITS region. For some samples, primers ITS5, 5.8SR, and LR1 were also used for successful amplification. The region bounded by the primer pair ITS1/ITS4 was subjected to the following phylogenetic analysis. Details of PCR amplification and DNA sequencing were previously described in Wu et al. (2007). For strains with intragenomic heterogeneity, DNA cloning was performed using a yT&A cloning vector and competent ECOSTM 9-5 cells (Yeastern Biotech, Taiwan). A single positive colony was selected for PCR amplification and DNA sequencing.

**Sequence alignment and phylogenetic analysis**

Sequence alignments were performed with Clustal X 1.83 (Thompson et al., 1997) and adjusted manually in BioEdit 7.0.4.1 (Hall, 1999). Sequences obtained from this study were deposited in GenBank (accession nos.: JN642565-JN642598, JN794061), and the aligned data matrix was deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S11836). The optimized sequence dataset of 60 taxa with 942 alignment sites was submitted for subsequent analysis with the exclusion of 264 ambiguous sites at both ends.

A phylogenetic analysis was performed with the maximum-parsimony (MP) method using the heuristic search algorithm of the phylogenetic analysis using parsimony (PAUP*) 4.0b10 (Swofford, 2002). Heuristic searches with 1000 random taxa stepwise addition sequences, TBR branch swapping, and MAXTREES set to autoincrease were performed. All transformations were considered unordered and equally weighted, with gaps treated as missing data. *Inonotus tropica* was used as an outgroup for rooting purposes. The relative robustness of the cladest was assessed by the bootstrap method using 1000 heuristic search replicates with random taxa stepwise addition sequences and TBR branch swapping with MAXTREES set to autoincrease.

**TAXONOMY**

**Key to six allied species of the *I. baumii-I. linteus* group distributed in Asia**

1. Pores of hymenial surface < 5/mm .......... *I. lonicericola*
2. On *Lonicera*. Basidiospores mostly < 4.1 µm long and mostly < 3.1 µm wide ..............................
3. Not on *Lonicera*. Basidiospores mostly > 4.1 µm long and mostly > 3.1 µm wide ..............................
Table 1. Taxa used in this study, along with their specimen /strain numbers, locality/host information and GenBank accession numbers.

<table>
<thead>
<tr>
<th>Accepted name</th>
<th>Name from specimen/ GenBank</th>
<th>Specimen/Strain no.</th>
<th>Locality/Host</th>
<th>GenBank accession no.</th>
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3. On *Populus*. Basidiocarp resupinate or pileate. Pileus surface indistinctly or moderately sulcate, with furrows < 3 cm. Young and aged pileus with a wide yellow marginal zone on pileus surface ................. *I. vaninii*

3. Not on *Populus*. Basidiocarp pileate. Pileus surface densely sulcate, with furrows > 3 cm. Aged pileus usually lacking a wide yellow marginal zone on pileus surface ........................................... *I. weigelae*

4. On *Weigela*. Basidiocarp sessile to effused-reflexed. Pileus planate, frequently becoming decurrent with more or less effused base; a thin and black crust on pileus surface present; pileus margin usually thin and acute ........................................... *I. weigelae*  

4. Not on *Weigela*. Basidiocarp always sessile. Pileus applanate or applanate with an umbo to convex, adaxially flat, slightly convex or slightly concave. Pore surface golden-yellow, brownish yellow, to yellowish brown. Pileus margin turning dark-red with KOH ................................................................. *I. baumii*

5. On *Morus*. Pileus applanate or applanate with an umbo to convex, adaxially flat, slightly convex or slightly concave. Pore surface golden-yellow, brownish yellow, to yellowish brown. Pileus margin turning dark-red with KOH .............................. *I. baumii*

5. On *Syringa*. Pileus subapplanate, adaxially more or less concave. Pore surface yellowish brown. Pileus margin not turning dark-red with KOH ................................................................. *I. baumii*

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**Table 1.** (Continued)

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<th>Locality/Host</th>
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*Taxa in bold indicate sequences from this study.
**The host information should be mistake.


Distribution. Middle Asia (Parmasto and Parmasto, 2001).

Remarks. Inonotus lonicericinus is distinguished from other species of the I. baumii-I. linteus group in having larger pores (4.5-5 mm). This species grows on several species of Lonicera including L. altmannii, L. korolkovi, and L. nummulariifolia (Parmasto and Parmasto, 2001). Inonotus lonicericinus was regarded by Lyubarski and Vasilyeva (1975), Dai and Xu (1998), Dai (1999), and Núñez and Ryvarden (2000) as a synonym of I. baumii, and regarded by Bondartseva (1986) as a synonym of I. linteus.

Inonotus sanghuang Sheng H. Wu, T. Hatt. & Y.C. Dai, sp. nov.

Etymology. From sanghuang, the name of this medicinal fungus in Chinese folklore.

Basidiocarps perennial, sessile, pileate; pilei apllanate, apllanate with an umbo to convex, broadly attached or semi-circular, projecting up to 20 cm, 35 cm wide and 10 cm thick at base, odor slightly sour, no distinct taste, woody hard when dry. Pileus surface yellowish brown, brown, grayish brown, or blackish brown when old, distinctly sulcate, radially rimose and cracking when old, uneven, indistinctly hirsute when young, becoming rough and glabrous with age; margin obtuse, lemon-yellow to golden-yellow becoming dark-red with KOH when young, yellowish brown when old; porous circular or angular, 6-8 per mm. Context yellow, brownish yellow, yellowish-brown when old; pores circular or semicircular, projecting up to 20 cm, 35 cm wide and 10 cm thick near pileus surface, 0.5-2 cm thick. Tube layer shiny woody-colored to woody-brown, stratified. Context and tube layer darkening in KOH.

Hyphal system dimitic; all septa lacking clamp connections; context and tubes darkening in KOH, dominated by skeletal hyphae; context generative hyphae occasionally branched, colorless to yellowish brown, 2-3.5 µm diam., thin-walled; context skeletal hyphae yellow to yellowish brown, unbranched, 2-3.5 µm diam., thick-walled; context and tube layer darkening in KOH.

Remarks. Inonotus lonicericinus is distinguished from other species of the I. baumii-I. linteus group in having larger pores (4.5-5 mm). This species grows on several species of Lonicera including L. altmannii, L. korolkovi, and L. nummulariifolia (Parmasto and Parmasto, 2001). Inonotus lonicericinus was regarded by Lyubarski and Vasilyeva (1975), Dai and Xu (1998), Dai (1999), and Núñez and Ryvarden (2000) as a synonym of I. baumii, and regarded by Bondartseva (1986) as a synonym of I. linteus.

Inonotus sanghuang Sheng H. Wu, T. Hatt. & Y.C. Dai, sp. nov.

Etymology. From sanghuang, the name of this medicinal fungus in Chinese folklore.
Figure 1. *Inonotus sanghuang* (holotype). A, Basidiocarp pileus surface; B, Pore surface. *Inonotus weigelae* (holotype); C, Basidiocarp pileus surface; D, Pore surface; E, Lateral section of basidiocarp, arrow indicates a thin black layer (crust) below tomentum.

Figure 2. *Inonotus sanghuang* (holotype). A, Contextual skeletal hyphae; B, Contextual generative hyphae; C, Tramal skeletal hyphae; D, Tramal generative hyphae; E, Hymenial setae; F, Basidia; G, Basidiospores.

Figure 3. *Inonotus weigelae* (holotype). A, Contextual skeletal hyphae; B, Contextual generative hyphae; C, Tramal skeletal hyphae. D, Tramal generative hyphae; E, Hymenial setae; F, Basidia. G, Basidiospores; H, Crystals in trama.
2-3 µm diam., thick-walled. Hymenial setae variably abundant, thick-walled, brown or dark-brown, ventricose or subulate, 18.35-7.14 µm. Basidia subclavate, 11.16 × 4.5 µm, 4-sterigmat. Basidiospores broadly ellipsoid, yellowish, brownish yellow, or brownish, smooth, slightly thick-walled or thick-walled, (3.8)-4.0-4.9(-5.1) × (3.0-)3.1-3.9(-4.1) µm (Wu 0903-I: L = 4.27 ± 0.16 µm, W = 3.36 ± 0.19 µm, Q = 1.27 (n = 30); FS656176: L = 4.41 ± 0.30 µm, W = 3.58 ± 0.23 µm, Q = 1.23 (n = 30); CA: L = 4.25 ± 0.20 µm, W = 3.41 ± 0.14, Q = 1.25 (n = 30); TFM F-21771: L = 4.31 ± 0.20 µm, W = 3.33 ± 0.20, Q = 1.29 (n = 30); TH: L = 4.37 ± 0.28 µm, W = 3.36 ± 0.22 µm, Q = 1.30 (n = 30)), IKI-, CB-.


**Remarks.** Inonotus sanghuang is a new species, proposed in this paper to represent the real sanghuang mushroom, known in China for more than 1000 years. This species was previously wrongly assigned to *I. linteus* or *I. baumii*, mainly due to their similar morphological characteristics. The basidiocarp of *I. sanghuang* fairly resembles those of *I. baumii* and *I. vaninii*. The young hymenial surface of *I. sanghuang* is golden-yellow, becoming brownish yellow to yellowish brown with age. The hymenial surface of *I. baumii* is pale-brown or brown. The adaxial part of the planate form of the *I. sanghuang* basidiocarp is generally flat, slightly convex or slightly concave, and occasionally tuberculate, while that of *I. baumii* is more or less concave. *Inonotus sanghuang* only grows on Morus, while *I. baumii* mainly grows on Syringa, and occasionally on other angiosperms. *Inonotus vaninii* shares a golden-yellow hymenial surface with *I. sanghuang*, especially in young basidiocarps. *Inonotus vaninii* may display resupinate or pileate basidiocarps, depending on different growth orientations, while *I. sanghuang* generally lacks the resupinate form. The pileus surface of *I. vaninii* is moderately sulcate (< 3 furrows/cm), and bears a wide yellowish brown marginal zone. The pileus surface of *I. sanghuang* is densely sulcate (> 3 furrows/cm), and the yellow marginal zone of its pileus is usually distinct when young; this zone grows much narrower or disappears with age. Moreover, *I. vaninii* only grows on *Populus*.

*Inonotus linteus* was commonly used for naming sanghuang mushroom, but is distributed in tropical America and Africa, not in E Asia, does not grow on *Morus*, and has subglobose and larger basidiospores (4.3-5.5 × 3.8-4.8 µm, according to Dai and Xu (1998)).


**Distribution.** North America, North China, Russian Far East, Japan (Núñez and Ryvarden, 2000), Korea (this study).

**Remarks.** Dai (2010) offered a comprehensive morphological description for *I. vaninii*. This species grows exclusively on *Populus* (Núñez and Ryvarden, 2000; Dai, 2010). Resupinate or pileate basidiocarps can be present in *I. vaninii* according to different growth orientations. The resupinate part develops on the underside below the woody substratum while the pileate part is formed from the lateral side of the woody substratum. Many strains of *I. vaninii* were previously assigned to *I. baumii* or *I. linteus* (Table 1) according to GenBank information, implying that *I. vaninii* was often regarded as conspecific with the real sanghuang (*I. sanghuang*), mainly due to their shared golden-yellow hymenial surfaces. Dai (2010) reported that the golden and lustrous pileus marginal zone of *I. vaninii* becomes bloody red with KOH. A similar color reaction also occurs in *I. sanghuang*. 

**Inonotus sanghuang**
Inonotus weigelae  T. Hatt. & Sheng H. Wu, sp. nov.

Figures 1C-E, 3

MycoBank: 563092.

Carpophorom perenne, pileatrum. Facies porosum brunneoae, brunnneaeterrae, rotundi vel angulati, 6-8 per mm. Systema hypharum dimitticum, hyphae generatioriae septatae sine fibulis, hyphae skeletales textuum 2.5-4.5 μm diam. Setae non abundae in hymenio, ventricoseae, brunneaes, 15-30 × 6-13 μm. Basidia subclavata, 10-14 × 4-5 μm, 4-sterigmatibus. Basidiosporae late ellipsoideae vel subglobosae, incoloratae, flavidae, vel pallidio-luteobrunneoae, (3.7-)3.8-4.2(-4.4) × (2.9-)3.0-3.6(-3.8) μm, IKI-, CB-.

Holotype. JAPAN. Nagano Pref., Chino, on Weigela coraeensis, 19 Sep 1993 (TFM F16899, voucher of the culture WD-1839; isotype: TNM F24806).

Etymology. Referring to the genus Weigela, the host of this new species.

Basidiocarps perennial, pileate, occasionally effused-reflexed, apllanate to slightly triquetrous, often decurrent with effused base, projecting up to 5 cm, 8 cm wide and 5 cm thick at base, without distinct odor or taste, very hard when dry. Pileus surface brownish, brownish black, or grayish black, densely sulcate, sometimes radially rimose and cracking, minutely tomentose when young, becoming glabrous from base, with a thin black layer (crust) below tomentum; thinning towards a thin, acute margin. Pore surface brownish-yellow, yellowish-brown to pale-brown, slightly lustrous; pores circular or angular, 6-8 per mm. Context woody-brown, thin. Tubes concolorous with pore surface, stratified.

Hyphal system dimitic; all septa lacking clamp connections; context and tubes darkening in KOH, dominated by skeletal hyphae; context generative hyphae occasionally branched, colorless to yellowish-brown, 2-3.5 μm diam., thin-walled; context skeletal hyphae yellow to yellowish-brown, unbranched, 2.5-4.5 μm diam., thick-walled; tramaal generative hyphae occasionally branched, colorless, 1.5-2 μm diam., thin-walled. Tramaal skeletal hyphae yellow to yellowish brown, unbranched, 2-3.5 μm diam., thick-walled; rectangular or amorphous crystals scattered in trama. Hymenial setae occasionally present, thick-walled, brown or dark-brown, mostly ventricose, 15-30 × 6-13 μm. Basidia subclavate, 10-14 × 4-5 μm, 4-sterigate. Basidiospores broadly ellipsoid or subglobose, colorless, yellowish, to pale yellowish brown, smooth, slightly thick-walled, (3.7-)3.8-4.2(-4.4) × (2.9-)3.0-3.6(-3.8) μm (TFM F24806 isotype: L = 4.10 ± 0.18 μm, W = 3.26 ± 0.14 μm, Q = 1.26 (n = 30); F16898: L = 3.96 ± 0.14 μm, W = 3.36 ± 0.20 μm, Q = 1.18 (n = 30)), IKI-, CB-.

Clade I consists of 19 materials labeled Inonotus baumii, I. linteus or I. sanghuang, and was assigned as the new species I. sanghuang. Changes across the sequences consisted of no to five steps. This clade had 83% support from the bootstrap analysis.

Clade II comprises 11 materials named I. baumii, I. linteus, or I. vaninii, and an unidentified species of Phellinus, which had 90% support from the bootstrap analysis. There are no to four step changes across the sequences. Clade III is composed of two materials of I. baumii or I. linteus with one step change. This clade received complete support (100%) from the bootstrap analysis. Clade IV contains only the single material named P. johnsonianus, which clustered with clades II and III with 61% bootstrap support. There are 19 and 37 step changes between clades IV and II, and between clades IV and III, respectively. Clade V consists of nine materials of I. baumii with no to 13 step changes. Clade VI is composed of four materials labeled as I. lonicericola or I. lonicerinus with 100% bootstrap support, and was assigned as I. lonicericola. There are one to three steps changes among this clade. This clade was grouped together with clade V with 83% bootstrap support.

Clade VII is composed of six materials labeled I.
baumii, I. lonicerinus, or I. lonicerinus (?)}, which received 98% bootstrap support. There are no to two step changes among them. This clade was designated the new species I. weigela. Clade VIII contains two material named I. lonicerinus or I. linteus with 98% bootstrap support. There are six step changes between them. Clades VIII and VII were clustered together with 97% bootstrap support.

Clade IX is composed of only one material of I. weirianus, which served as the sister taxon to the clades I to VIII with < 50% bootstrap support. Clade X consists of two strains of I. linteus with 100% bootstrap support. There is one step change between the sequences. Clade XI contains only a single strain of I. repandus, which was clustered together with clade X with 97% bootstrap support. There are 55 step changes between clades XI and X.

Two strains, respectively assigned to P. johnsonianus (Murrill) Ryvarden and P. repandus (Overh.) Gilb. from GenBank, are shown to be members of the I. baumii-I. linteus group in this study (Figure 4). According to our analysis the strains for these sequences should belong to Inonotus. Further studies based on basidiocarps together with their ITS sequences are necessary to examine generic placement of these two species.

**Figure 4.** One of the 1968 most parsimonious trees derived from the ITS sequence data. The upper and lower numerals at the nodes denote the number of estimated substitutions and proportions of bootstrap replicates, respectively. Only bootstrap values ≥ 50% are shown.
DISCUSSION

Results showed that morphological characteristics coupled with nrDNA ITS sequences were useful in separating fungal species around the sanghuang mushroom. All of them pertain to the *I. baumii-I. linteus* group of species and are generally specialized to a host tree species. Results of this study, together with those of Jeong et al. (2005) and Wagner and Fischer (2002), indicate that these species belonging to the *I. baumii-I. linteus* group are *I. baumii*, *I. linteus*, *I. lonicericola*, *I. lonicerinus*, *I. sanghuang*, *I. vaninii*, *I. weigelae*, *I. weirianus*, two strains respectively assigned to *P. johnsonianus* and *P. repandus*, and one unknown species of *Inonotus* (*Inonotus* sp. in Figure 4). Two strains of the *Inonotus* sp. in Figure 4 (*I. baumii* SFC20001106-4 (AF534066), Lim et al., 2003; *I. linteus* SFCC10208 (AY558627), Jeong et al., 2005) were collected from Korea, and formed a highly supported monophyletic group (Figure 4), probably representing an undescribed species in the *I. baumii-I. linteus* group.

*Inonotus linteus* has long been erroneously accepted as the binomial for the sanghuang mushroom known in East Asia. Dai and Xu (1998), Dai (1999), Lim et al. (2003), and Dai (2010) studied authentic specimens of *I. baumii* and *I. linteus* including the holotypes, and reached the conclusion that *P. linteus* is distributed in tropical America and Africa, while the so-called *P. linteus*, occurring in temperate East Asia, is *P. baumii*. This opinion is correct, except that their concept of *I. baumii* does not represent a single species, but several allied ones such as *I. lonicericola*, *I. sanghuang*, *I. weigelae* and *I. vaninii*. These species share more or less similar morphological features, but grow on different host species. The impossibility that *I. linteus* represents the real sanghuang is further confirmed by the nrDNA ITS sequence analysis of this study (Figure 4). Moreover, *I. linteus* has larger basidiospores than other species of the *I. baumii-I. linteus* group. It is also distinguished from *I. sanghuang* by a different distribution, and its host is not *Morus*. Parmasto and Parmasto (2001) successfully separated four morphologically similar species (*I. baumii*, *I. linteus*, *I. lonicericola*, and *I. lonicerinus*) of the *I. baumii-I. linteus* group by analyzing spore length, spore width, and the number of pores/mm. They emphasized the specific correlation of these fungal species with host tree species. Deviation of basidiospore measurements of some species within the *I. baumii-I. linteus* group among several studies (Dai and Xu, 1998; Dai, 1999; Lim et al., 2003; Dai, 2010; Parmasto and Parmasto, 2001; this study) is remarkable, mainly due to previous problems with species recognition. In addition, it is necessary to use good-quality microscopes and special caution when measuring basidiospores of the *I. baumii-I. linteus* group, as their spores are fairly small.

Xie et al. (2010) identified ‘sanghuang’ by analyzing nrDNA ITS sequences of many strains, and concluded that most medicinally-applied strains are ‘*P. baumii*’ (*I. sanghuang* in this study) and ‘*P. linteus*’ (*I. vaninii* in this study). They successfully detected the real sanghuang species by molecular methods, but failed to designate correct binomials for the studied culture strains, due to a lack of study of their basidiocarps and the taxonomy of related species. Combined results from this study and those of Xie et al. (2010) show that the commercially important strains of sanghuang mushroom are mainly *I. sanghuang* on *Morus* and *I. vaninii* on *Populus*. *Phellinus igniarius* and some other species of *Phellinus* s.l. such as *P. laevigatus* (Fr.) Bourdot & Galzin [in E. Asia this is *P. betulinus* (Murrill) Parmasto ssp. orienticus Parmasto], *P. larixis* (Jacq. ex Pilát) Pilát, *P. lundellii* Niemelä, *P. pini* (Brot.) A. Ames, *P. tremulae* (Bondartsev) Bondartsev & P.N. Borisov, *P. tuberculosus* (Baumg.) Niemelä, and *P. yamanoi* (Imazeki) Parmasto are also recognized by Chinese people as sanghuang mushrooms; however, these mushrooms do not belong to the core group.

Japanese mycologists correctly regarded meshimakobu and *I. baumii* as different species a half century ago. In the *Mycological Flora of Japan* (Ito, 1955), meshimakobu (as *P. yucatanensis*, synonym *I. linteus*) was described as distributed in central and southern Japan (Honsu, Kyushu, and the Bonin Islands) and growing on *Morus*. In the same book, *P. baumii* was described as another species distributed in northern Japan (Hokkaido) and growing on *Syringa*. For a long period afterward, many scholars regarded these two species as conspecific. This study proves that the identity of specimens from *Syringa* is *I. baumii*, and that specimens collected from *Morus* with similar morphological characteristics and bearing high traditional medicinal value, represent a previously scientifically undescribed species, *I. sanghuang*, that is neither *I. baumii* nor *I. linteus*.

So far, all examined Chinese specimens reported to be *I. linteus* or *I. baumii* (Zhao and Zhang, 1992; Teng, 1996; Dai, 1999; Zhang and Dai, 2005; Dai, 2010) were not collected on *Morus*, but on *Acer, Alnus, Coriaria, Crataegus, Cyclobalanopsis, Ligustrina, Lonicera, Populus, Prunus, Quercus, Rhus, Sambucus, Syringa, Xylosma*, etc. All of them may represent several closely related species with similar morphological features, but not *I. sanghuang*. So far, no evidence shows that any specimen of *I. sanghuang* has ever been described in the taxonomic literature of China; however, several strains of *I. sanghuang* were included in a study by Xie et al. (2010), but those strains were from edible mushroom institutes or agricultural culture collections in China, lacking information concerning their original collection localities. In this study, several specimens of *I. sanghuang* were collected from different provinces in China, representing the first scientific record of this species in China. This study proves that the *I. sanghuang* reported 1000 years ago in China, known as the legendary medicinal organism ‘sanghuang’ on *Morus* trees, still grows wild in the country, but is rare. Several decades ago, *Inonotus sanghuang* was fairly abundant on *Morus* in southern and central Japan, but is now also becoming rare due to over-collection (Ito, 1955). The distribution of
I. sanghuang in Japan was confirmed in this study based on specific collection information on some of the examined specimens. This study (Figure 4) proved that two strains collected from Korea and assigned to I. baumii (SFC20001106-1 and SFC20001106-2) by Lim et al. (2003) were the species I. sanghuang. These I. sanghuang collections from Korea were described as growing on Morus bombycis (a synonym of M. australis). In this study, we examined specimens of I. sanghuang collected in Taiwan and confirmed its distribution on this island for the first time. Inonotus sanghuang is known by Taiwanese as a ‘mushroom on Morus’, and is said to grow on M. australis. The question of which or how many species of Morus are associated with I. sanghuang should be further studied. Morus australis is widely distributed in many provinces of China, and also distributed in Taiwan, Korea, and Japan; its distribution range corresponds to that of I. sanghuang.

The ITS proved to be a good region for separating species of the I. baumii-I. linteus group in this study. ITS is a tool that has been adopted by many scholars in the last decade to conduct more or less similar work (Kim et al., 2001; Nam et al., 2003; Park et al., 2002; Wang et al., 2010; Xie et al., 2010). The first reason for failure to distinguish different species of the I. baumii-I. linteus group in previous studies was a lack of intensive morphological study of basidiocarps of the analyzed strains. Morphological differences among closely related species (I. baumii, I. sanghuang and I. vaninii) are generally not distinct. The second reason was inappropriate arrangement of the species of both the outgroup and the ingroup in their analyses. The outgroup chosen in each previous analysis was phylogenetically remotely related to the ingroup, and was consequently inappropriate to separate closely related species. Moreover, sampling of the analyzed species and strains in each previous analysis was insufficient or lacking: additionally, most analyzed strains in previous studies were from mushroom collection cultures or sequence data from GenBank, so it was difficult to know their collection localities, basidiocarp morphologies, and host species.

Sanghuang is a rare East Asian, economically valuable traditional medicinal fungus. Local collectors usually keep their gathering sites secret, so there is a paucity of collection data on sanghuang from GenBank, tissue culture, or research samples collected for scientific publications. The pharmaceutical industry has collected and evaluated data on the different medicinal properties of different sanghuang mushrooms, but has not shared these findings with taxonomic experts. These practices have all hindered the taxonomic study of sanghuang. The strains/specimens included in this analysis were from GenBank, culture collections, purchased fruiting bodies, and collected specimens with collection information that were especially important.

This study proves that the specific species referred to in the early Chinese folklore as sanghuang that grew on Morus still exists. Does I. sanghuang possess the best medicinal functions among the several related species of the so-called sanghuang? This question cannot be soon definitively answered because species delimitations in the I. baumii-I. linteus group were not clear before this study. In addition, published papers of medicinal, biochemical, and clinical studies of sanghuang usually did not offer information on the strains studied. According to Song et al. (2005) and Qi et al. (2010), the ‘sanghuang mushroom’ on Morus has the best medicinally effective functions compared to the ones on different host species. A comprehensive evaluation and comparison of the healthcare effects and/or medicinal function among so many fungal related species assigned to sanghuang can be further conducted after clarification of their species delimitations.

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LITERATURE CITED


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珍貴藥用真菌「桑黃」蕈之種類釐清

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一種流傳中國千年以上，富於傳奇性而珍貴的藥用真菌，本研究發現是個新種。桑黃，在中國、日本及韓國是具有高經濟價值的藥用真菌。桑黃醫藥使用記載於西元七世紀的藥典，於其高抗癌功效卻是晚近才由現代科學研究所證實。近數十年來，日本及韓國真菌學者採用 Phellinus linteus 或 P. baumii 當做桑黃的學名。桑黃屬於 Inonotus baumii-I. linteus 群，然而民間傳說的桑黃究竟是哪一種？過去未有令人滿意的答案。本研究藉由分析形態特徵以及核酸體內轉錄間隔區 (ITS region)，以釐清 I. baumii-I. linteus 群內種間界定，並由此辨識出傳說中的桑黃。本研究結果顯示形態及分子特徵對於界定這類真菌的種間區分是可行的，並且多數這些真菌種類與其寄主樹種間具有專一性。真正的桑黃是一種未曾發表過的新種 I. sanghuang，野外僅長在活的桑屬樹幹，分布於中國、日本、韓國以及台灣，在野外為稀有及瀕危狀態。本研究顯示已知屬於 I. baumii-I. linteus 群的真菌種類有 I. baumii, I. linteus, I. lonicericola, I. lonicerinus (新組合), I. sanghuang (新種), I. vaninii, I. weigelae (新種), I. weirianus，一個 Inonotus 未知種, P. johnsonianus 以及 P. repandus。 Inonotus baumii-I. linteus 群內分布於亞洲的六種演化出與其寄主樹木的專一性：I. baumii 長在丁香屬 (Syringa), I. lonicericola 長在忍冬屬 (Lonicera), I. lonicerinus 長在忍冬屬 (Lonicera), I. sanghuang 長在桑屬 (Morus), I. vaninii 長在楊屬 (Populus), I. weigelae 長在錦帶花屬 (Weigela)。本文並提供這六種的形態特徵檢索表。

關鍵詞：擔子菌；刺革菌科 (Hymenochaetaceae)；桑黃 (Inonotus sanghuang)；藥用真菌；桑屬 (Morus)；木層孔菌屬 (Phellinus)；新種；分類學。