

# Species clarification for the medicinally valuable ‘sanghuang’ mushroom

Sheng-Hua WU<sup>1,8,\*</sup>, Yu-Cheng DAI<sup>2</sup>, Tsutomu HATTORI<sup>3</sup>, Tu-Wen YU<sup>4</sup>, Dong-Mei WANG<sup>5</sup>, Erast PARMAS<sup>6</sup>, Heng-Yuan CHANG<sup>7</sup>, and Siang-Yun SHIH<sup>8</sup>

<sup>1</sup>Department of Botany, National Museum of Natural Science, Taichung 404, Taiwan

<sup>2</sup>Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, P.R. China

<sup>3</sup>Forestry and Forest Products Research Institute, Kansai Research Center, Kyoto 612-0855, Japan

<sup>4</sup>Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan 717, Taiwan

<sup>5</sup>Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, P.R. China

<sup>6</sup>Natural History Museum, Tartu University, Tartu EE51014, Estonia

<sup>7</sup>Department of Pharmacy, Tsaotun Psychiatric Center, Nantou 542, Taiwan

<sup>8</sup>Department of Plant Pathology, National Chungshing University, Taichung 402, Taiwan

(Received October 6, 2011; Accepted November 18, 2011)

**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 (桑黃, 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 7<sup>th</sup> 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 delimits 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. lonicericola* on *Lonicera*, *I. lonicerinus* comb. nov. on *Lonicera*, *I. sanghuang* on *Morus*, *I. vaninii* on *Populus*, and *I. weigela* sp. nov. on *Weigela*; a key is provided to ease the determination of these taxa.

**Keywords:** Basidiomycota; Hymenochaetales; *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 ‘黃’ (*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

\*Corresponding author: E-mail: shwu@mail.nmns.edu.tw.

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 20<sup>th</sup> century, and were based on specimens identified as *Fomes yucatanensis* (Murrill) Saccardo & D. Saccardo or *F. 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 *P. 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). For the past 20 years, its medicinal and economic value have gained sanghuang a high reputation in East Asia, especially in Korea and Japan. Dai et al. (2009, 2010) reviewed the current literature on traditional medicines and summarized the purported role of sanghuang (as ‘*Phellinus baumii* Pilát’) in septic shock alleviation, anti-inflammation, antioxidation, antitumor, lowering serum lipids, prevention and treatment of autoimmune joint inflammation, and treatment of diabetes and pneumonia.

In China, sanghuang mushroom and its purported medicinal functions were described by Liu (1978) in *Medicinal Fungi of China* as being both *Phellinus igniarius* (L.) Quél. and *Pyropolyporus yucatanensis* Murrill (synonym of *P. linteus*), and that the latter was indicated by Liu as the real sanghuang. Both Japanese and Korean mycologists adopted *P. linteus* as the scientific binomial for sanghuang more than a half century ago. Dai and Xu (1998), Dai (1999), Lim et al. (2003), and Dai (2010) studied authentic specimens including holotypes of both *P. baumii* and *P. linteus*. Those reports concluded that *P. linteus* is distributed in tropical America and Africa, while the so-called *P. linteus* in temperate Asia is *P. baumii*. This opinion is partially correct, and will be detailed below in the discussion of this paper. Japanese and Korean colleagues currently use *P. baumii* and *P. linteus* interchangeably when referring to the sanghuang mushroom. Different scholars in China, however, respectively support *P. baumii* (or *P. linteus*) and *P. igniarius* as sanghuang. *Phellinus igniarius*, a species complex that is widely distributed in temperate regions of the world, including at least 12 species in Europe, grows on many angiosperm tree species (Sell, 2008).

Species identity of sanghuang remained unclear until

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 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; Sliva 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 *Fulvifomes* (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

*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  $\lambda$ T&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/phyloids/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 clades 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. lonicerinus*
1. Pores of hymenial surface > 5/mm ..... 2
2. On *Lonicera*. Basidiospores mostly < 4.1  $\mu$ m long and mostly < 3.1  $\mu$ m wide ..... *I. lonicericola*
2. Not on *Lonicera*. Basidiospores mostly > 4.1  $\mu$ m long and mostly > 3.1  $\mu$ m wide ..... 3

**Table 1.** Taxa used in this study, along with their specimen /strain numbers, locality/host information and GenBank accession numbers.

Accepted name	Name from specimen/ GenBank	Specimen/Strain no.	Locality/Host	GenBank accession no.
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	BZ-2029	China/?	JN642565
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	BZ-2030-	China/?	JN642566
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	Dai3683	China/ <i>Syringa</i> sp.	JN642567
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	Dai3684	China/ <i>Syringa</i> sp.	JN642568
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	Dai3694	China/ <i>Syringa</i> sp.	JN642569
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	SFC960405-4	Korea/ <i>Morus australis</i> **	AF534068
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	SFCC50029	China (Jeong et al., 2005)	AY558608
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	Wu0910-54	China/ <i>Syringa</i> sp.	JN642570
<i>Inonotus baumii</i>	<i>Inonotus baumii</i>	FS656165	China/ <i>Syringa</i> sp.	HM584807
<i>Inonotus linteus</i>	<i>Inonotus linteus</i>	SFC99052002	Costa Rica/angiosperm	AY558629
<i>Inonotus linteus</i>	<i>Inonotus linteus</i>	SFC970520-1	Mexico/angiosperm	AF534075
<i>Inonotus lonicericola</i>	<i>Inonotus lonicericola</i>	Dai8322	China/ <i>Lonicera</i> sp.	JN642571
<i>Inonotus lonicericola</i>	<i>Inonotus lonicericola</i>	TAA105317	Russian Far East/ <i>L. ruprechtiana</i>	JN642572
<i>Inonotus lonicericola</i>	<i>Inonotus lonicericola</i>	Dai8335	China/ <i>Lonicera</i> sp.	JN642573
<i>Inonotus lonicericola</i>	<i>Inonotus lonicericola</i>	Dai8340	China/ <i>Lonicera</i> sp.	JN642574
<i>Inonotus lonicerinus</i>	<i>Inonotus linteus</i>	TAA104264	Russian Far East/ <i>Lonicera</i> sp.	AF534074
<i>Inonotus lonicerinus</i>	<i>Inonotus lonicerinus</i>	TAA55428	Turkmenia/ <i>Lonicera</i> sp.	JN642575
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	WD-1222	Japan/ <i>Morus</i> sp.	JN642576
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	WD-2261	Japan/ <i>Morus</i> sp.	JN642577
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	WD-2300	Japan/ <i>Morus</i> sp.	JN642578
<i>Inonotus sanghuang</i>	<i>Inonotus baumii</i>	SFC20001106-2	Korea/ <i>Morus australis</i>	AF534065
<i>Inonotus sanghuang</i>	<i>Inonotus baumii</i>	SFC20001106-1	Korea/ <i>Morus australis</i>	AF534064
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	Wu0903-1	China/ <i>Morus</i> sp.	JN794061
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	CA	China/ <i>Morus</i> sp.	JN642579
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	CB	China/ <i>Morus</i> sp.	JN642580
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	CC	China/ <i>Morus</i> sp.	JN642581
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	TH	Taiwan/ <i>Morus</i> sp.	JN642582
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	TM	Taiwan/ <i>Morus</i> sp.	JN642583
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	TN	Taiwan/ <i>Morus</i> sp.	JN642584
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	TJ	Taiwan/ <i>Morus</i> sp.	JN642585
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	T004	Taiwan/ <i>Morus</i> sp.	JN642586
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	BZ-C	China/ <i>Morus</i> sp.	JN642587
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	PL10	China???	JN642588
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	FS656166	China/ <i>Morus</i> sp.	HM584808
<i>Inonotus sanghuang</i>	<i>Inonotus sanghuang</i>	BZ-A	China/ <i>Morus</i> sp.	JN642589
<i>Inonotus sanghuang</i>	<i>Inonotus linteus</i>	SH2	???	FJ190411
<i>Inonotus tropicalis</i>	<i>Inonotus tropicalis</i>	CBS617.89	Costa Rica/deciduous wood	AF534077
<i>Inonotus vaninii</i>	<i>Inonotus baumii</i>	SFC20001106-7	Korea/ <i>Quercus</i> sp.**	AF534070
<i>Inonotus vaninii</i>	<i>Inonotus linteus</i>	SFCC10209	Korea (Jeong et al., 2005)	AY558628
<i>Inonotus vaninii</i>	<i>Inonotus vaninii</i>	Dai3624	China/ <i>Populus</i> sp.	JN642590
<i>Inonotus vaninii</i>	<i>Inonotus vaninii</i>	Dai7011	China/ <i>Populus</i> sp.	JN642591
<i>Inonotus vaninii</i>	<i>Inonotus baumii</i>	PB0806	???	FJ940911

**Table 1.** (Continued)

Accepted name	Name from specimen/ GenBank	Specimen/Strain no.	Locality/Host	GenBank accession no.
<i>Inonotus vaninii</i>	<i>Inonotus baumii</i>	PB0809	?/?	FJ940914
<i>Inonotus vaninii</i>	<i>Phellinus</i> sp.	SA04	?/?	EF694974
<i>Inonotus vaninii</i>	<i>Inonotus vaninii</i>	FS656175	China/ <i>Populus</i> sp.	HM584811
<i>Inonotus vaninii</i>	<i>Inonotus baumii</i>	FS656170	China/ <i>Betula</i> sp.**	GU903008
<i>Inonotus vaninii</i>	<i>Inonotus baumii</i>	MPNU 7005	?/?	AF200230
<b><i>Inonotus vaninii</i></b>	<b><i>Inonotus linteus</i></b>	<b>CJC01</b>	<b>?/?</b>	<b>JN642592</b>
<b><i>Inonotus vaninii</i></b>	<b><i>Inonotus vaninii</i></b>	<b>BZ-2031</b>	<b>China/<i>Populus</i> sp.</b>	<b>JN642593</b>
<i>Inonotus weigelae</i>	<i>Inonotus lonicerinus</i>	WD-1667	Japan/ <i>weigela cordeenis</i>	JN642594
<i>Inonotus weigelae</i>	<i>Inonotus lonicerinus</i>	WD-1837	Japan/ <i>weigela cordeenis</i>	JN642595
<i>Inonotus weigelae</i>	<i>Inonotus lonicerinus</i>	WD-1838	Japan/ <i>weigela cordeenis</i>	JN642596
<i>Inonotus weigelae</i>	<i>Inonotus lonicerinus?</i>	WD-1186	Japan/ <i>Weigela</i> sp.	JN642597
<i>Inonotus weigelae</i>	<i>Inonotus lonicerinus?</i>	WD-1187	Japan/ <i>Weigela</i> sp.	JN642598
<i>Inonotus weigelae</i>	<i>Inonotus baumii</i>	SFC20000111-10	Korea/ <i>Lonicera</i> sp.**	AF534067
<i>Inonotus weirianus</i>	<i>Inonotus weirianus</i>	IMSNU32021	USA/ <i>Juglans major</i>	AF110989
<i>Inonotus</i> sp.	<i>Inonotus baumii</i>	SFC20001106-4	Korea/ <i>Quercus</i> sp.	AF534066
<i>Inonotus</i> sp.	<i>Inonotus linteus</i>	SFCC10208	Korea (Jeong et al. 2005)/?	AY558627
<i>Phellinus johnsonianus</i>	<i>Phellinus johnsonianus</i>	ATCC60051	USA/ <i>Fraxinus</i> sp.	AF250931
<i>Phellinus repandus</i>	<i>Phellinus repandus</i>	CBS 616.89	USA, Montana/ <i>Picea engelmannii</i>	AF534076

\*Taxa in bold indicate sequences from this study.

\*\*The host information should be mistake.

3. On *Populus*. Basidiocarp resupinate or pileate. Pileus surface indistinctly or moderately sulcate, with furrows < 3 per 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 per cm. Aged pileus usually lacking a wide yellow marginal zone on pileus surface ..... 4
4. On *Weigela*. Basidiocarp sessile to effused-reflexed. Pileus appanate, 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 appanate to concave or subungulate; a thin crust absent, although pileus surface may become black and crustose in aged specimens; pileus margin acute or blunt ..... 5
5. On *Morus*. Pileus appanate or appanate 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. sanghuang*
5. On *Syringa*. Pileus appanate to subungulate, adaxially more or less concave. Pore surface yellowish brown. Pileus margin not turning dark-red with KOH ..... *I. baumii*

***Inonotus baumii*** (Pilát) T. Wagner & M. Fisch., *Mycologia* 94: 1009 (2002).

≡ *Phellinus baumii* Pilát, *Bull. Trimest. Soc. Mycol. Fr.* 48(1): 25 (1932).

*Specimens examined.* **CHINA.** Jilin Prov., Antu Co., Baoma, on living tree of *Syringa*, 7 Sep 1993, *Dai 1137* (IFP, TNM F6145); Wangqing Co., Lanjia, on dead tree of *Syringa*, *Dai 1215* (IFP, TNM F6146); Huadian Co., Dongxing, on living tree of *Syringa*, 19 Oct 1993, *Dai 1743* (IFP, TNM F6149); Yanbian Autonomous State, Dunhua City, on *Syringa* sp., 8 Jun 2009, *FS656165* (TNM F24783). Heilongjiang Prov., Yichun, Fenglin Nature Reserve, on living trunk of *Syringa* sp., 8 Sep 2002, *Dai 3683* (IFP, TNM F24784), *Dai 3684* (IFP), *Dai 3694* (IFP, TNM F24785). Beijing, Xiangshan Park, 39.59N, 116.11E, elev. 70 m, on trunk of living *Syringa* sp., 14 Oct 2009, *Wu 0910-52* (TNM F23844), *Wu 0910-54* (TNM F23846). Purchased basidiocarp without collection information, *BZ-2029* (TNM F24787), *BZ-2030* (TNM F24786).

*Distribution.* China (Dai, 2010), Japan (Ito, 1955), Korea (Lim et al., 2003), Russian Far East (Parmasto and Parmasto, 2001).

*Remarks.* Dai (2010) provided a detailed description and discussion of *I. baumii*. Former identity and descriptions of Chinese *I. baumii* by Dai and Xu (1998), Dai (1999) and Zhang and Dai (2005) also comprised specimens of *I. lonicericola*. Typical *I. baumii* grows on living

*Syringa* sp. and is distributed in temperate NE Asia. The identity of specimens assigned to *I. baumii* by Dai (1999), Zhang and Dai (2005) and Dai (2010) not collected from *Syringa* sp., and/or those from tropical-subtropical Asia should be further clarified.

**Inonotus lonicericola** (Parmasto) Y.C. Dai, Fungal Diversity 45: 276 (2010).

≡ *Phellinus lonicericola* Parmasto, Folia Cryptog. Estonica 38: 59 (2001).

*Specimens examined.* **RUSSIA.** Distr. Ussurisk, Kamenushka, on dead trunk of *L. maackii*, 2 Jun 1976 (TAA 100080, TNM F6154). Distr. Khasansk, Kedrovaya Pad' Nature Reserve, at Rivulet 1 Zolotoi, on living trunk of *L. maackii* in a nemoral forest, 22 Jun 1984 (TAA 105739); Kedrovaya Pad' Nature Reserve, on *Lonicera* sp., 7 Aug 1979 (TAA 125081). Region Primorye, Distr. Lazovsk, Petrov Is., on living trunk of *L. ruprechtii*, 2 Sep 1961 (TAA 13933, holotype). Distr. Ussurisk, Kamenushka, on *L. ruprechtiana*, 22 May 1983 (TAA 105317, TNM F15790). Distr. Partisansk, Mountain Pass, on a dead branch of *Lonicera* sp., 16 Aug 1986 (TAA 107676). **CHINA.** Jilin Prov., Huadian Co., Dongxing, on living tree of *Lonicera*, 16 Oct 1993, *Dai 1611* (IFP, TNM F6148). Heilongjiang Prov., Ningan Co., Jingbo Lake, on living tree of *Lonicera* sp., 8 Sep 2007, *Dai 8322* (IFP, TNM F24788), *Dai 8335* (IFP, TNM F24789), *Dai 8336* (IFP, TNM F24790), *Dai 8377* (IFP, TNM F24791), *Dai 8340* (IFP, TNM F24792).

*Distribution.* Russian Far East (Parmasto and Parmasto, 2001), China (Dai, 2010; 2011).

*Remarks.* See Dai (2010) for a detailed description and discussion of this species, which is separated from *I. baumii* by exclusively living on *Lonicera* and bearing smaller basidiospores (Parmasto and Parmasto, 2001; Dai, 2010). Specimens of *I. lonicericola* collected in China were named *I. baumii* before 2010 (cfr. Dai, 2010).

**Inonotus lonicerinus** (Bondartsev) Sheng H. Wu, Y.C. Dai & T. Hattori, comb. nov.

Basionym: *Fomes lonicerinus* Bondartsev, Trudy Bot. Inst. Akad. Nauk. SSR, II 2: 500 (1935).

≡ *Porodaedalea lonicerina* (Bondartsev) Imazeki, Colored Illustrations of Mushrooms of Japan 2: 191 (1989).

≡ *Phellinus lonicerinus* (Bondartsev) Bondartsev & Singer, Annales Mycologici 39 (1): 56 (1941).

≡ *Cryptoderma lonicerinum* (Bondartsev) Imazeki, Bulletin of the Tokyo Science Museum 6: 107 (1943).

*Specimens examined.* **TURKMENIA.** Distr. Bakharden, Montes Kopet-dagh, Bakharden, Arvaz., elev. 1,800 m, on *Lonicera* sp., 17 Oct 1971 (TAA 55428). **UZBEKISTAN.** Distr. Bostanlyksk, Yubileinyi, alt. 2,100 m, on living trunk of *Lonicera altmannii*, 22 Apr 1982 (TAA 104277). **KAZAKHSTAN.** Turgen Valley near Alma-Ata, on living trunk of *Lonicera* sp., 2 May 1984 (TAA 105677,

TNM F15791).

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

*Remarks.* *Inonotus lonicerinus* is distinguished from other species of the *I. baumii*-*I. linteus* group in having larger pores (4-5/mm). This species grows on several species of *Lonicera* including *L. altmannii*, *L. korolkovii*, and *L. nummulariifolia* (Parmasto and Parmasto, 2001). *Inonotus lonicerinus* was regarded by Lyubarski and Vasilyeva (1975), Dai and Xu (1998), Dai (1999), and Núñez and Ryvardeen (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. Figures 1A-B, 3

Mycobank: 563091

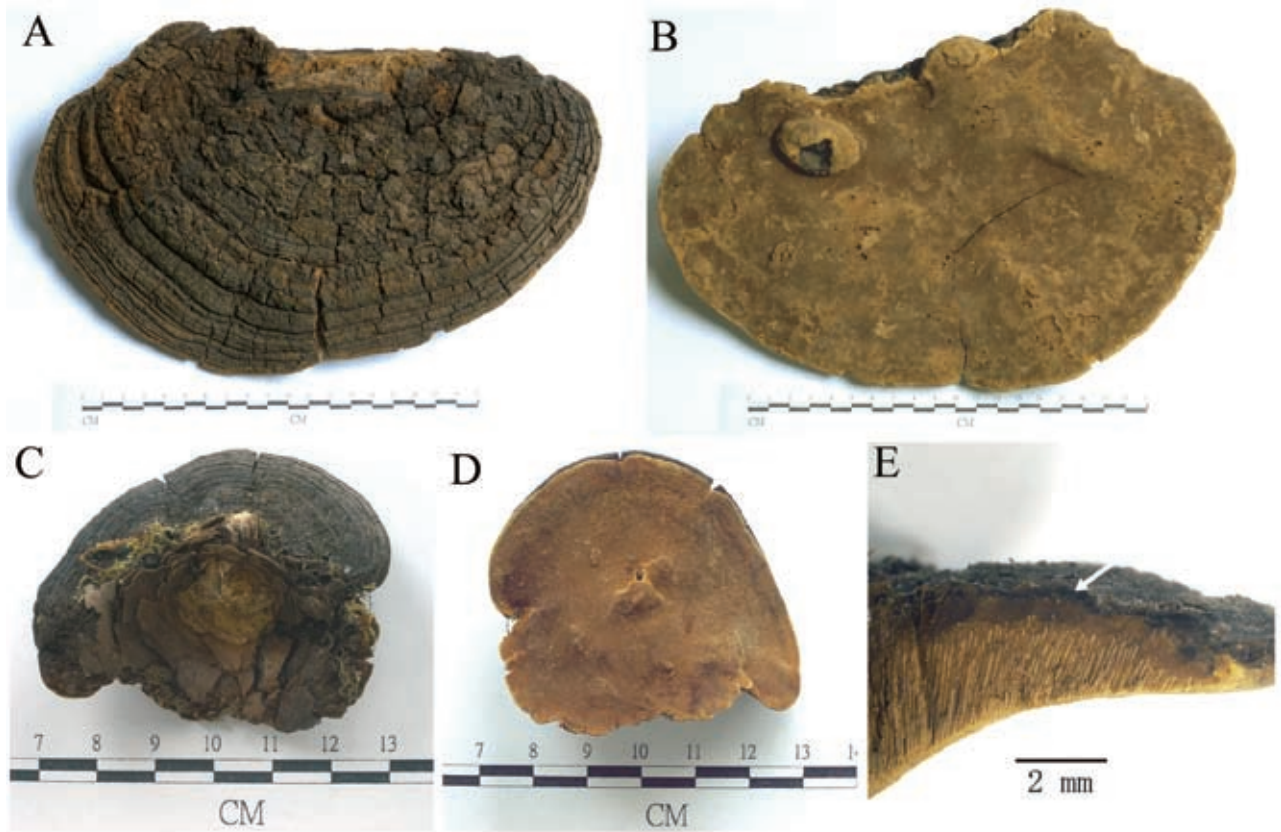
Carpophorum perenne, pileatum. Facies porosum aurea vel flavobrunnea, rotundi vel angulati, 6-8 per mm. Systema hypharum dimiticum, hyphae generatoriae septatae sine fibulis, hyphae skeletales contextum 2.5-5 µm diam. Setae in hymenio, ventricosae vel subulatae, brunneae, 18-35 × 7-14 µm. Basidia subclavata, 11-16 × 4-5 µm, 4-sterigmatibus. Basidiosporae late ellipsoideae, flavidae, brunneoluteae vel brunneolae, (3.8-4.0-4.9(-5.1) × (3.0-) 3.1-3.9(-4.1) µm, IKI-, CB-.

*Holotype.* **CHINA.** Jilin Prov., Baishan City, on *Morus* sp., Mar 2009, *Wu 0903-1* (TNM F24793).

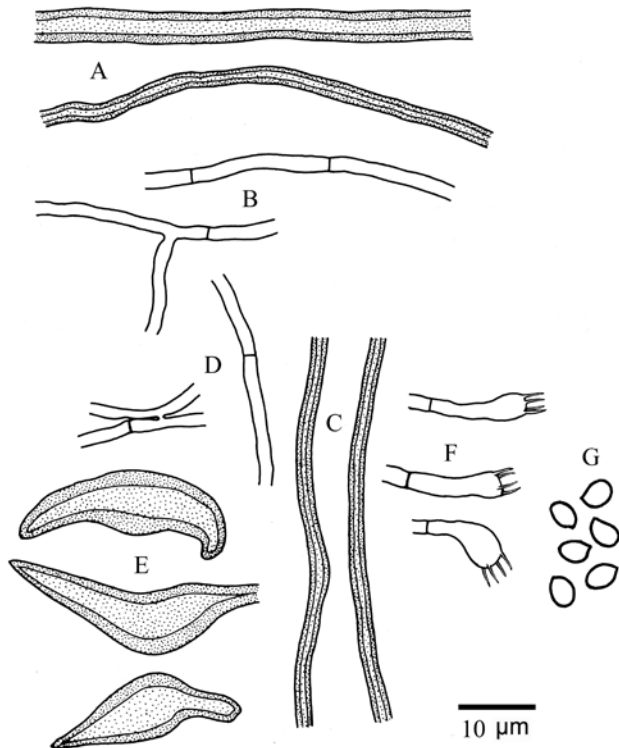
*Etymology.* From sanghuang, the name of this medicinal fungus in Chinese folklore.

Basidiocarps perennial, sessile, pileate; pilei appanate, appanate with an umbo to convex, broadly attached or semicircular, 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; poroid surface golden-yellow or brownish yellow, yellowish-brown when old; pores circular or angular, 6-8 per mm. Context yellow, brownish yellow, yellowish brown or shiny woody color, often darker near pileus surface, 0.5-2 cm thick. Tube layer shiny woody-colored to woody-brown, stratified. Context and tube layer darkening in KOH.

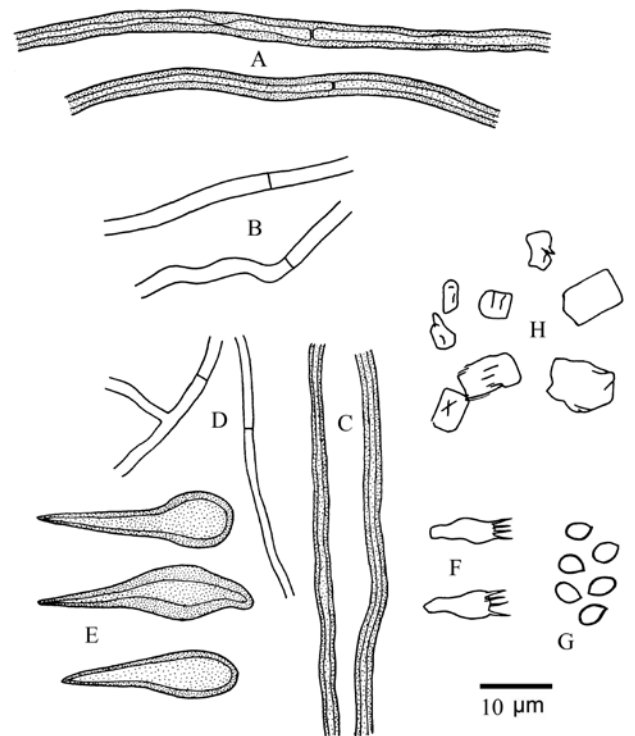
Hyphal system dimitic; all septa lacking clamp connections; context and tubes darkening in KOH, dominated by skeletal hyphae; context generative hyphae occasionally branched, colorless to yellowish brown, 2-3.5 µm diam., thin-walled; context skeletal hyphae yellow to yellowish-brown, unbranched, 2.5-5 µm diam., thick-walled, with a distinct lumen; tramal generative hyphae occasionally branched, colorless, 1.5-2.5 µm diam., thin-walled; tramal skeletal hyphae yellow to yellowish brown, unbranched,



**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  $\mu\text{m}$  diam., thick-walled. Hymenial setae variably abundant, thick-walled, brown or dark-brown, ventricose or subulate, 18-35  $\times$  7-14  $\mu\text{m}$ . Basidia subclavate, 11-16  $\times$  4-5  $\mu\text{m}$ , 4-sterigmate. Basidiospores broadly ellipsoid, yellowish, brownish yellow, or brownish, smooth, slightly thick-walled or thick-walled, (3.8-)4.0-4.9(-5.1)  $\times$  (3.0-)3.1-3.9(-4.1)  $\mu\text{m}$  (*Wu 0903-I*: L = 4.27  $\pm$  0.16  $\mu\text{m}$ , W = 3.36  $\pm$  0.19  $\mu\text{m}$ , Q = 1.27 (n = 30); *FS656176*: L = 4.41  $\pm$  0.30  $\mu\text{m}$ , W = 3.58  $\pm$  0.23  $\mu\text{m}$ , Q = 1.23 (n = 30); *CA*: L = 4.25  $\pm$  0.20  $\mu\text{m}$ , W = 3.41  $\pm$  0.14, Q = 1.25 (n = 30); *TFM F-21771*: L = 4.31  $\pm$  0.20  $\mu\text{m}$ , W = 3.33  $\pm$  0.20, Q = 1.29 (n = 30); *TH*: L = 4.37  $\pm$  0.28  $\mu\text{m}$ , W = 3.36  $\pm$  0.22  $\mu\text{m}$ , Q = 1.30 (n = 30)), IKI-, CB-.

*Additional specimens examined.* **CHINA.** Jilin Prov., Baishan City, on *Morus* sp., 7 Jun 2009, *FS656176* (TNM F24926), *FS656177* (TNM F24927), *FS656178* (TNM F24928). Hunan Prov., Shuanfeng Co., on *Morus* sp., OCT 2006, *BZ-A* (TNM F24794), *BZ-C* (TNM F24795). Jiangxi Prov., Nanchang, on *Morus* sp., 2004, purchased basidiocarp, *CA* (TNM F24796), *CB* (TNM F24797), *CC* (TNM F24798). **JAPAN.** Miyazaki Pref., Aya, Kawanaka, on *Morus* sp., 3 Oct 2003 (TFM F21475, voucher of the culture WD-2261), Suki-mura, on *M. bombycis* (= *M. australis*), 2-3 Aug 1938 (TFM F1324, ex TNS F206786). Tokyo Pref., Hachiyojima Is., 18 Aug 2005 (TFM F21771, voucher of the culture WD-2300), Hachijo Is., on *Morus* sp., 22 Apr 1950 (TFM F1995, possibly the voucher of the culture WD-1222), Mikura Is., Apr 2002 (TFM F20053), Toshima Is., on *M. alba*, 16 May 1951 (TFM F23284). Tottori Pref., Kawahara-cho, on *Morus* sp., 8 Jul 1972 (TFM F23285). Ishikawa Pref., Tsurugi-cho, on *Morus* sp., 3 Aug 1983 (TFM F20059). Gumma Pref., Kiryu, on *Morus* sp., 6 Dec 2004 (TFM F21303). Hyogo Pref., Sayo-cho, on *Morus* sp., 15 Jul 1961 (TFM F23282). **TAIWAN.** Nantou Co., Hsingyi Hsiang, Hoshe, on *Morus* sp., 2005, purchased basidiocarp, *TH* (TNM F24799), *TJ* (TNM F24800), *TM* (TNM F24801), *TN* (TNM F24802). Taitung Co., Yienping Hsiang, on *Morus* sp., May 2011, purchased basidiocarp, *Wu 1105-4* (TNM F24925).

*Distribution.* China, Japan, Korea (Lim et al., 2003), Taiwan.

*Remarks.* *Inonotus sanghuang* is a new species, proposed in this paper to represent the real sanhuang 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 applanate 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 yellow 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  $\times$  3.8-4.8  $\mu\text{m}$ , according to Dai and Xu (1998)).

*Inonotus vaninii* (Ljub.) T. Wagner & M. Fisch., Mycologia 94: 1009 (2002).

$\equiv$  *Phellinus vaninii* Ljub., Botanicheskie Materialy 15: 115 (1962).

*Specimens examined.* **CHINA.** Jilin Prov., Antu Co., Changbaishan Forest Reserve, on living tree of *Populus davidiana*, 28 Jul 1993, *Dai 812* (IFP, TNM F6269); on living tree of *P. davidiana*, 8 Sep 1995, *Dai 1978* (IFP, TNM F6272); on fallen trunk of *P. davidiana*, 8 Sep 1995, *Dai 1980* (IFP, TNM F6273); on living tree of *P. davidiana*, 14 Sep 1995, *Dai 2102* (IFP, TNM F6275); on fallen trunk of *P. davidiana*, 2005, *Dai 7011* (IFP); Yanbian Autonomous State, Helong Co., on *P. davidiana*, 3 Aug 2010, *FS656175* (TNM F24803). Locality in China unknown, on *Populus*, *BZ-2031* (TNM F24804). **RUSSIA.** Reservatum Bolshechechtshirski apud urbem Chabarovsk, pr. ostium fluminis Tshirka, on *P. davidiana*, 24 Aug 1982 (TAA 105094, TNM F15789). Commercially cultivated immature basidiome with no collection information, *CJC01* (TNM F24805).

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

Figures 1C-E, 3

Mycobank: 563092.

Carpophorum perenne, pileatum. Facies porosum brunneolae, brunnealterae vel canoalterae, rotundi vel angulati, 6-8 per mm. Systema hypharum dimiticum, hyphae generatoriae septatae sine fibulis, hyphae skeletales contextum 2.5-4.5  $\mu\text{m}$  diam. Setae non abundae in hymenio, ventricosae, brunneae, 15-30  $\times$  6-13  $\mu\text{m}$ . Basidia subclavata, 10-14  $\times$  4-5  $\mu\text{m}$ , 4-sterigmatibus. Basidiosporae late ellipsoideae vel subgloboseae, incoloratae, flavidae, vel pallido-luteobrunneolae, (3.7-)3.8-4.2(-4.4)  $\times$  (2.9-)3.0-3.6(-3.8)  $\mu\text{m}$ , IKI-, CB-.

**Holotype.** JAPAN. Nagano Pref., Chino, on *Weigela coraeensis*, 19 Sep 1993 (TFM F16899, voucher of the culture WD-1838; isotype: TNM F24806).

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

Basidiocarps perennial, pileate, occasionally effused-reflexed, applanate 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. Poroid 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  $\mu\text{m}$  diam., thin-walled; context skeletal hyphae yellow to yellowish-brown, unbranched, 2.5-4.5  $\mu\text{m}$  diam., thick-walled; tramal generative hyphae occasionally branched, colorless, 1.5-2  $\mu\text{m}$  diam., thin-walled; tramal skeletal hyphae yellow to yellowish brown, unbranched, 2-3.5  $\mu\text{m}$  diam., thick-walled; rectangular or amorphous crystals scattered in trama. Hymenial setae occasionally present, thick-walled, brown or dark-brown, mostly ventricose, 15-30  $\times$  6-13  $\mu\text{m}$ . Basidia subclavate, 10-14  $\times$  4-5  $\mu\text{m}$ , 4-sterigmate. Basidiospores broadly ellipsoid or subglobose, colorless, yellowish, to pale yellowish brown, smooth, slightly thick-walled, (3.7-)3.8-4.2(-4.4)  $\times$  (2.9-)3.0-3.6(-3.8)  $\mu\text{m}$  (TFM F24806 (isotype): L = 4.10  $\pm$  0.18  $\mu\text{m}$ , W = 3.26  $\pm$  0.14  $\mu\text{m}$ , Q = 1.26 (n = 30); F16898: L = 3.96  $\pm$  0.14  $\mu\text{m}$ , W = 3.36  $\pm$  0.20  $\mu\text{m}$ , Q = 1.18 (n = 30)), IKI-, CB-.

**Additional specimens examined.** JAPAN. Nagano Pref., Chino, on *W. cordeenis*, 19 Sep 1993 (TFM F16898, voucher of the culture WD-1837); Nagano Pref., Chiisagata, 1 Aug 1965 (TFM F10998, voucher of the culture WD-1186). Gumma Pref., Usui, on *Weigela* sp., 2 Oct 1968 (TFM F11136, voucher of the culture WD-1187). Ibaraki

Pref., Mt. Tsukuba, on *W. cordeenis*, 7 Nov 1991 (TFM F16412, voucher of the culture WD-1667).

**Distribution.** Japan, Korea (strain SFC20000111-10 (Lim et al., 2003) was proven in this study to belong to *I. weigelae*).

**Remarks.** *Inonotus weigelae* resembles *I. lonicericola* in general macro-morphology, while it is distinguished from the latter in having larger basidiospores. General microscopic characteristics of *I. weigelae* are similar to those of *I. baumii*. In *I. weigelae*, its pileus is applanate, frequently becomes decurrent with a more or less effused base, a thin and black crust on pileus surface is present; pileus margin is thin and acute. The pileus of *I. baumii* is applanate to subungulate and always sessile, a thin and black crust on pileus surface is absent; the pileus margin is obtuse, and is fairly thick except for the very margin. *Inonotus weigelae* specializes by growing on *Weigela*.

## RESULTS

### Phylogenetic analysis

Amplification of the nrDNA LSU region yielded fragments of approximately 600 bp long. Among the final 942 alignment sites, 678 were used for phylogenetic analyses. MP analysis revealed 1968 most parsimonious trees (369 steps, with a consistency index (CI) of 0.789 and a retention index (RI) of 0.916). Of the 678 sites included, 450 were constant, 71 were variable but parsimoniously uninformative, and 157 were parsimoniously informative.

One of the most parsimonious trees is presented in Figure 4. In this tree, the ingroup taxa were separated into 11 distinct clades. These clades are respectively labeled I-XI in Figure 4.

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 step changes among them. This clade was grouped together with clade V with 83% bootstrap support.

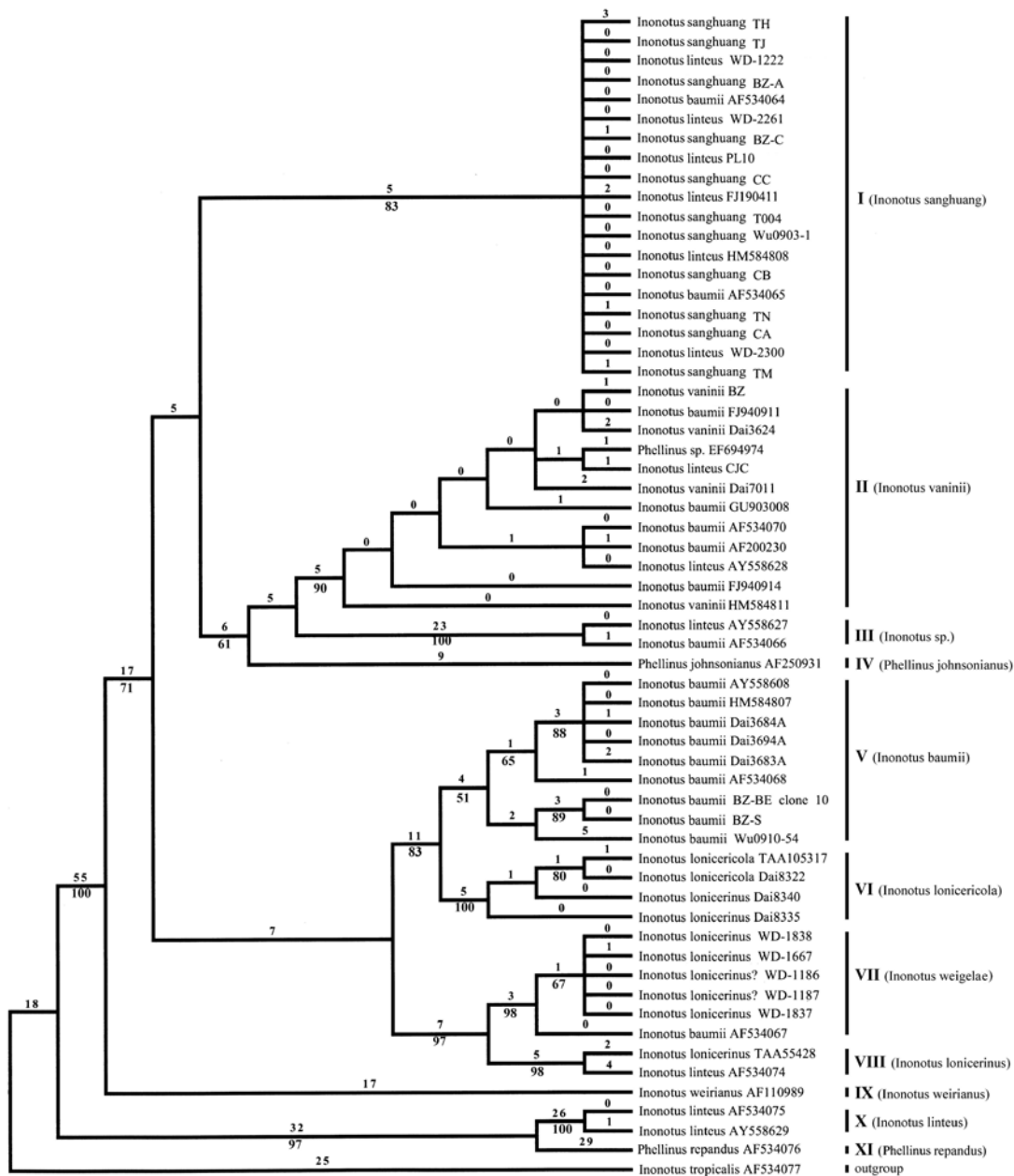
Clade VII is composed of six materials labeled *I.*

*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. weigelae*. 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) Ryvardeen 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  $\geq 50\%$  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. weigela*, *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) included 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. weigela* 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. laricis* (Jacz. ex Pilát) Pilát, *P. lundellii* Niemelä, *P. pini* (Brot.) A. Ames, *P. tremulae* (Bondartsev) Bondartsev & P.N. Borisov, *P. tuberculatus* (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.

**Acknowledgements.** This study was supported by a grant (NSC100-1201-05-5-06) from the National Science Council of Taiwan to S.H. Wu, by a grant (30910103907) from the National Natural Science Foundation of China to Y.C. Dai, and by the Foundation of Guangdong Academy of Sciences, China, for 2008 Outstanding Young Science and Technology Talents, and Science and Technology Planning Project of Guangdong Province, China (2009B020304003) to D.M. Wang. We thank Yu-Ping Chen, Kelly Shih, Shu-Wen Chou and Tsai-Jie Hsieh for their assistance in obtaining DNA sequences. We are grateful to X.Q. Zhang for sharing knowledge about sanghuang mushroom at the early stage of this study, to Mr. Jung-Kun Su for donating some specimens. We are obliged to Prof. Nils Hallenberg and an anonymous reviewer for reviewing this article.

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## 珍貴藥用真菌「桑黃」蕈之種類釐清

吳聲華<sup>1,8</sup> 戴玉成<sup>2</sup> Tsutomu HATTORI<sup>3</sup> 俞篤文<sup>4</sup> 王冬梅<sup>5</sup>  
Erast PARMASTO<sup>6</sup> 張恆源<sup>7</sup> 史香芸<sup>8</sup>

<sup>1</sup> 國立自然科學博物館 植物學組

<sup>2</sup> 中國科學院 瀋陽應用生態研究所

<sup>3</sup> 日本京都林業與林產品研究所 關西研究中心

<sup>4</sup> 嘉南藥理科技大學 藥學系

<sup>5</sup> 中國廣東省微生物研究所 廣東省菌種保藏與應用重點實驗室

<sup>6</sup> 塔爾圖大學 自然歷史博物館

<sup>7</sup> 署立草屯療養院 藥劑科

<sup>8</sup> 國立中興大學 植病系

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中藥古籍，然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糖體內轉錄間隔區 (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. weigela* (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. weigela* 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。