ITS barcoding-based species identification for *Sanghuangporus* (Basidiomycota), a genus of medicinal mushrooms

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Research

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Abstract

“Sanghuang” is a kind of important medicinal mushrooms and taxonomically represented by members in the fungal genus *Sanghuangporus*. Species of *Sanghuangporus* referred to medicinal studies and industry are discriminated mainly by BLAST search of GenBank with ITS barcoding region as a query. However, the inappropriately labeled ITS sequences related to “Sanghuang” in GenBank restrict accurate species identification and, to some extent, the utilization of these medicinal resources. Here, we examined all available 271 ITS sequences related to “Sanghuang” from GenBank including 31 newly submitted sequences for this study. Of these sequences, more than half were mislabeled and the corresponding species names are corrected. The mislabeled sequences mainly came from strains by non-taxonomists. Based on the analyses of ITS sequences submitted by taxonomists, we treat *Sanghuangporus toxicodendri* as a later synonym of *S. quercicola*, and the intraspecific and interspecific differences are below 1.50% (but *S. weirianus*) and above 1.50%, respectively. Moreover, ten potential diagnostic sequences are provided for hyperbranched rolling circle amplification to rapidly detect three common commercial species, viz. *S. baumii*, *S. sanghuang* and *S. vaninii*. Generally, the current results provide a practical method for ITS barcoding-based species identification of *Sanghuangporus*, and will promote medicinal studies and industrial development from the taxonomic perspective.

Introduction

Macrofungi are a group of fungi producing fruiting bodies visible by naked eyes. Many macrofungi are famous medicinal mushrooms and possess diverse medicinal functions (Wu et al. 2019a). Of them, “Sanghuang”, a kind of important wood-inhabiting medicinal mushrooms, has been utilized as folk medicines for the past two thousand years in China and adjacent countries (Zhou et al. 2020). After that modern scientific studies did reveal some medicinal functions from “Sanghuang”, including antitumor, antioxidant, anti-inflammation, immunomodulation and so on (Zhou et al. 2020), this kind of fungal resources attracts the attentions from European fungal chemists and pharmacologists (Chepkirui et al. 2018; Cheng et al. 2019). Secondary metabolites, such as, polysaccharides, polyphenols, pyrones and terpenes are in charge of these medicinal functions of “Sanghuang” (Zhou et al. 2020). Nowadays, “Sanghuang” are mainly consumed in a tea form of chips and pieces of cultivated basidiocarps and occasionally in an oral form of mycelial powders.

Like other precious wood-inhabiting medicinal mushrooms, such as “Lingzhi” (Cao et al. 2012; Wang et al. 2012; Yao et al. 2013, 2020; Dai et al. 2017), “Niuchangchih” (Wu et al. 2012b, c) and “Fuhling” (Redhead and Ginns 2006), there was a hot debate about what the taxonomic identity of “Sanghuang” is. For now, most of fungal taxonomists have agreed that “Sanghuang” is represented by species in *Sanghuangporus* Sheng H. Wu, L.W. Zhou & Y.C. Dai (Zhou et al. 2020). A total of 14 species have been described and accepted as members of *Sanghuangporus*: 11 species are distributed in Asia, one in Africa, one in North America and one in Europe (Zhou et al. 2020). In addition, more new species of *Sanghuangporus* await to be described from Africa (Chepkirui et al. 2018; Cheng et al. 2019) and maybe also from other parts of the world. Besides morphological and ecological characters, ITS barcoding region provides the most powerful evidence for discriminating species of *Sanghuangporus* (Zhou et al. 2020).

As a hot topic, transdisciplinary studies on *Sanghuangporus* have been performed to promote the utilization of these medicinal resources (Zhou et al. 2016; Cai et al. 2019; Zhu et al. 2019; Shao et al. 2020). Most of this kind of medicinal studies try to identify their materials via BLAST search of GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with ITS barcoding region as a query. However, even though each of 14 species of *Sanghuangporus* was given a reliable accession number of ITS sequence (Zhou et al. 2020), sometimes it is not easy to determine which species a material represents by the simple ITS-based BLAST search. This is because some redundant and even incorrectly labeled ITS sequences are present in GenBank. With these obstacle sequences as references, it is undoubtful that certain collections will be inaccurately identified to a species level and the corresponding ITS sequences generated from these inaccurately identified collections will be submitted to GenBank as new obstacles for later species identification. In this situation, some medicinal results will attribute to inappropriately identified species names. Meanwhile, before the erection of the genus *Sanghuangporus* published online in 2015 (Zhou et al. 2016), the ITS sequences generated from “Sanghuang” were labeled under other generic names, such as *Inonotus* P. Karst. and *Phellinus* Quél., even though with correct epithets. This phenomenon confuses certain fungal chemists and pharmacologists who are lack of taxonomic knowledge, and also results in a misapplication of species names to certain medicinal functions. This kind of misapplications has a negative effect on obtaining permissions from government for industrial development (Zhou 2020).

As stated by Zhou (2020), the use of correct Latin names for fungal species is crucial for the traditional Chinese medicinal studies and industry of macrofungi. To facilitate the medicinal utilization of *Sanghuangporus*, all ITS sequences related to “Sanghuang” in GenBank should be examined for assisting species identification. Given the above, the aim of the current study is to correct previously mislabeled ITS sequences for species of *Sanghuangporus* in GenBank, to re-delimit species boundary of *Sanghuangporus* on the basis of ITS barcoding region, and to provide candidates of diagnostic ITS sequences for rapid species identification of *Sanghuangporus* using Hyperbranched Rolling Circle Amplification (HRCA).

Materials And Methods

Molecular sequencing

A small piece of specimens or strains was taken for DNA extraction using CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing). The crude DNA was used as templates for PCR amplifications of ITS region. The primer pairs ITS1F/ITS4 and ITS5/ITS4 (White et al. 1990; Gardes and Bruns 1993) were selected for amplification and subsequent sequencing at the Beijing Genomics Institute, Beijing, China. The PCR procedure was as follow: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, 57.2 °C for 45 s and 72 °C for 1 min, and a final extension at 72 °C for 10 min. All newly generated sequences are deposited in GenBank (Table 1).

Table 1
Information of analyzed ITS sequences of *Sanghuangporus*

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
1.	*Sanghuangporus alpinus*	*Inonotus alpinus*	Cui 9646	JQ860313*	Angiosperm	Tibet, China	Specimen	Tian XM et al.
2.		*Inonotus alpinus*	Cui 9652	JQ860309*	Angiosperm	Tibet, China	Specimen	Tian XM et al.
3.		*Inonotus alpinus*	Cui 9658	JQ860310*	Angiosperm	Tibet, China	Specimen	Tian XM et al.
4.		*Inonotus alpinus*	Cui 9666	JQ860311*	Angiosperm	Tibet, China	Specimen	Tian XM et al.
5.		*Sanghuangporus alpinus*	Cui 12444	MF772782*	*Lonicera*	Sichuan, China	Specimen	Zhu L & Cui BK
6.		*Sanghuangporus alpinus*	Cui 12474	MF772783*	*Lonicera*	Sichuan, China	Specimen	Zhu L & Cui BK
7.		*Sanghuangporus alpinus*	Cui 12485	MF772781*	*Lonicera*	Sichuan, China	Specimen	Zhu L & Cui BK
8.		*Inonotus alpinus*	Yu 35	JQ860312*	*Lonicera*	Tibet, China	Specimen	Tian XM et al.
9.		Inonotus alpinus	**Yuan 6396**	**MT348577***	Lonicera	**Qinghai, China**	**Specimen**	**This study**
10.		Inonotus alpinus	**Yuan 6405**	**MT348578***	Lonicera	**Qinghai, China**	**Specimen**	**This study**
11.		Inonotus alpinus	**Yuan 6438**	**MT343579***	**Angiosperm**	**Qinghai, China**	**Specimen**	**This study**
12.	*S. baumii*	*Tropicoporus linteus*	ASI 26030	KT862142		South Korea	Strain	Han JG et al.
13.		*Tropicoporus linteus*	ASI 26086	KT862157		Samchoek, South Korea	Strain	Han JG et al.
14.		*Tropicoporus linteus*	ASI 26087	KT862158		Mokpo, South Korea	Strain	Han JG et al.
15.		*Sanghuangporus baumii*	ASI 26108	KT862162		Inje, South Korea	Strain	Han JG et al.
16.		*Inonotus baumii*	BZ-2029	JN642565	Pruchased	China	Strain	Wu SH et al.
17.		*Inonotus baumii*	BZ-2030	JN642566	Pruchased	China	Strain	Wu SH et al.
18.		*Inonotus baumii*	Cui 3573	JQ860307*	*Syringa*	Jilin, China	Specimen	Tian XM et al.
19.		*Sanghuangporus baumii*	Cui 11769	MF772784*	Angiosperm	Heilongjiang, China	Specimen	Zhu L & Cui BK
20.		*Sanghuangporus baumii*	Cui 11903	KY328305*	*Alnus*	Heilongjiang, China	Specimen	Zhu L & Cui BK
21.		*Phellinus baumii*	Dai 2340	AF534069			Strain	Lim YW et al.
22.		*Inonotus baumii*	Dai 3683	JN642567*	*Syringa*	Heilongjiang, China	Strain	Wu SH et al.
23.		*Inonotus baumii*	Dai 3684	JN642568*	*Syringa*	Heilongjiang, China	Strain	Wu SH et al.
24.		*Inonotus baumii*	Dai 3694	JN642569*	*Syringa*	Heilongjiang, China	Strain	Wu SH et al.
25.		Inonotus baumii	**Dai 13360**	**MT343580***	Prunus	**Shanxi, China**	**Specimen**	**This study**
26.		*Sanghuangporus baumii*	Dai 16900	MF772785*	*Syringa*	Heilongjiang, China	Specimen	Zhu L & Cui BK
27.		*Inonotus baumii*	FS 656165	HM584807			Strain	Yu TW
28.		*Inonotus baumii*	FS 656164	GU903007			Strain	Yu TW
29.		*Inonotus baumii*	HLJU	KC312696			Strain	Liu Y et al.
30.		*Sanghuangporus baumii*	KUC 10644	MH168100			Strain	Heo YM et al.

new sequenced specimens and strains are in bold

* sequences considered to be reliable for further analysis

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
31.		*Inonotus baumii*	KUC 20130809-20	KJ668511		South Korea	Specimen	Jang Y & Kim JJ
32.		Inonotus baumii	**LWZ 20190722-18**	**MT348581***	**Angiosperm**	**Beijing, China**	**Specimen**	**This study**
33.		*Inonotus baumii*	MDJCBS 84	DQ103887			Strain	Jiang J et al.
34.		*Inonotus baumii*	SFC 050511-32	AY972811			Strain	Jung HS & Lee JS
35.		*Inonotus baumii*	SFC 050527-67	AY972812			Strain	Jung HS & Lee JS
36.		*Phellinus baumii*	SFC 960405-4	AF534068			Strain	Lim YW et al.
37.		*Phellinus linteus*	SFC 970527-1	AF534073			Strain	Lim YW et al.
38.		*Sanghuangporus baumii*	SFCC 50029	AY558608			Strain	Jeong WJ et al.
39.		*Inonotus baumii*	SH 3	FJ190412			Strain	Zou L et al.
40.		*Inonotus baumii*	Wu 0910 – 54	JN642570*	*Syringa*	Beijing, China	Strain	Wu SH et al.
41.		*Inonotus baumii*	Yuan 2444	JX069836*	Angiosperm	Shanxi, China	Specimen	Tian XM et al.
42.		*Sanghuangporus baumii*	Yuan 4909	KY328310*	Angiosperm	Heilongjiang, China	Specimen	Zhu L & Cui BK
43.		*Sanghuangporus baumii*	Yuan 4929	KY328306*	*Alnus*	Heilongjiang, China	Specimen	Zhu L & Cui BK
44.	*S. ligneus*	*Sanghuangporus ligneus*	MG 12	KR073081*	*Lonicera caucasica*	Iran	Strain	Ghobad-Nejhad M
45.		*Sanghuangporus ligneus*	MG 13	KR073082*	*Lonicera caucasica*	Iran	Strain	Ghobad-Nejhad M
46.	*S. lonicericola*	*Inonotus baumii*	BM-3753	HQ845063		China	Strain	Hu W & Deng X
47.		*Inonotus baumii*	BM-8335	HQ845064		China	Strain	Hu W & Deng X
48.		*Sanghuangporus lonicericola*	Cui 10994	MF772786*		China	Specimen	Zhu L & Cui BK
49.		*Inonotus lonicericola*	Dai 8322	JN642571*	*Lonicera*	Heilongjiang, China	Specimen	Wu SH et al.
50.		*Inonotus lonicericola*	Dai 8335	JN642573*	*Lonicera*	Heilongjiang, China	Specimen	Wu SH et al.
51.		*Inonotus lonicericola*	Dai 8340	JN642574*	*Lonicera*	Heilongjiang, China	Specimen	Wu SH et al.
52.		*Inonotus lonicericola*	Dai 8376	JQ860308*	*Lonicera*	Heilongjiang, China	Specimen	Tian XM et al.
53.		Sanghuangporus lonicericola	**Dai 17304**	**MT348582***	Lonicera	**Liaoning, China**	**Strain**	**This study**
54.		*Phellinus* sp.	HN100K9	KF589300		South Korea	Strain	Kang HW & Kim JK
55.		*Phellinus ribis*	SFCC 50032	AY558643			Strain	Jeong WJ et al.
56.		*Inonotus lonicericola*	TAA 105317	JN642572*	*Lonicera ruprechtiana*	Russian Far East	Specimen	Wu SH et al.
57.	*S. lonicerinus*	*Sanghuangporus lonicerinus*	Dai 17093	MF772788*	*Lonicera*	Uzbekistan	Specimen	Zhu L & Cui BK
58.		*Sanghuangporus lonicerinus*	Dai 17095	MF772787*	*Lonicera*	Uzbekistan	Specimen	Zhu L & Cui BK
59.		*Sanghuangporus lonicerinus*	MG 280	KU213573*			Specimen	Langer EJ & Ghobad-Nejhad M

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No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
60.		*Sanghuangporus lonicerinus*	MG 281	KU213574*			Specimen	Langer EJ & Ghobad-Nejhad M
61.		*Inonotus* sp.	TAA 55428	JN642575*	*Lonicera*	Turkmenistan	Strain	Wu SH et al.
62.		Inonotus lonicerinus	**TAA 55696**	**MT348583***	Lonicera	**Turkmenistan**	**Specimen**	**This study**
63.		*Phellinus linteus*	TAA-104264	AF534074			Strain	Lim YW et al.
64.	*S. microcystideus*	*Sanghuangporus microcystideus*	O 915609	KP030787*	*Olea africana*	Tanzania	Specimen	Zhou LW et al.
65.	*S. pilatii*	*Phellinus pilatii*	BRNM 771989	KT428764*	*Populus alba*	Czech Republic	Specimen	Tomšovský M
66.	*S. quercicola*	*Phellinus rhabarbarinus*	CBS 282.77	AY558642			Strain	Jeong WJ et al.
67.		*Sanghuangporus quercicola*	Dai 13947	KY328309*		Chongqing, China	Specimen	Zhu L & Cui BK
68.		*Sanghuangporus quercicola*	Li 445	KY328311*	Angiosperm	Henan, China	Specimen	Zhu L & Cui BK
69.		*Sanghuangporus quercicola*	Li 1149	KY328312*	*Quercus*	Henan, China	Specimen	Zhu L & Cui BK
70.		Sanghuangporus quercicola	**LWZ 20170821-13**	**MT348584***	**Angiosperm**	**Hubei, China**	**Specimen**	**This study**
71.		Sanghuangporus quercicola	**LWZ 20170821-14**	**MT348585***	**Angiosperm**	**Hubei, China**	**Specimen**	**This study**
72.		Sanghuangporus quercicola	**LWZ 20170821-18**	**MT348586***	**Angiosperm**	**Hubei, China**	**Specimen**	**This study**
73.		Sanghuangporus quercicola	**Wei 7575**	**MT348587***	Quercus	**Henan, China**	**Strain**	**This study**
74.		*Sanghuangporus* sp.	Wu 1805-2	MK400422*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
75.		*Sanghuangporus* sp.	Wu 1805-3	MK400423*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
76.		*Sanghuangporus* sp.	Wu 1805-5	MK400424*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
77.		*Sanghuangporus* sp.	Wu 1807-2	MK729538*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
78.		*Sanghuangporus* sp.	Wu 1807-3	MK729540*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
79.		*Sanghuangporus* sp.	Wu 1807-4	MK729539*	*Toxicodendron*	Hubei, China	Specimen	Wu SH et al.
80.	*S. sanghuang*	*Inonotus baumii*		KM385537		Viet Nam	Strain	Hanh VV & Nguyet NT
81.		S. sanghuang	**AH1**	**MT421899***	**Cultivated**	**Anhui, China**	**Strain**	**This study**
82.		S. sanghuang	**AH2**	**MT421900***	**Cultivated**	**Anhui, China**	**Strain**	**This study**
83.		S. sanghuang	**AH3**	**MT421901***	**Cultivated**	**Anhui, China**	**Strain**	**This study**
84.		S. sanghuang	**AH4**	**MT421902***	**Cultivated**	**Anhui, China**	**Strain**	**This study**
85.		S. sanghuang	**AH5**	**MT421903***	**Cultivated**	**Anhui, China**	**Strain**	**This study**
86.		*Phellinus igniarius*	ASI 26010	KT862134		Jeongseon, South Korea	Strain	Han JG et al.
87.		*Tropicoporus linteus*	ASI 26011	KT862135		India	Strain	Han JG et al.
88.		*Tropicoporus linteus*	ASI 26016	KT862136		South Korea	Strain	Han JG et al.
89.		*Tropicoporus linteus*	ASI 26021	KT862138		Hongcheon, South Korea	Strain	Han JG et al.
90.		*Tropicoporus linteus*	ASI 26022	KT862139		Hongcheon, South Korea	Strain	Han JG et al.

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* sequences considered to be reliable for further analysis

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
91.		*Tropicoporus linteus*	ASI 26025	KT862140		Wonju, South Korea	Strain	Han JG et al.
92.		*Tropicoporus linteus*	ASI 26026	KT862141		Wonju, South Korea	Strain	Han JG et al.
93.		*Tropicoporus linteus*	ASI 26039	KT862143		Pyeongchang, South Korea	Strain	Han JG et al.
94.		*Tropicoporus linteus*	ASI 26046	KT862144		Hongcheon, South Korea	Strain	Han JG et al.
95.		*Tropicoporus linteus*	ASI 26049	KT862145		Hongcheon, South Korea	Strain	Han JG et al.
96.		*Tropicoporus linteus*	ASI 26054	KT862147		Hongcheon, South Korea	Strain	Han JG et al.
97.		*Tropicoporus linteus*	ASI 26062	KT862148		Hwacheon, South Korea	Strain	Han JG et al.
98.		*Tropicoporus linteus*	ASI 26063	KT862149		Jeongseon, South Korea	Strain	Han JG et al.
99.		*Tropicoporus linteus*	ASI 26066	KT862150		Inje, South Korea	Strain	Han JG et al.
100.		*Tropicoporus linteus*	ASI 26067	KT862151		Inje, South Korea	Strain	Han JG et al.
101.		*Tropicoporus linteus*	ASI 26070	KT862152			Strain	Han JG et al.
102.		*Tropicoporus linteus*	ASI 26071	KT862153			Strain	Han JG et al.
103.		*Tropicoporus linteus*	ASI 26073	KT862154		South Korea	Strain	Han JG et al.
104.		*Tropicoporus linteus*	ASI 26074	KT862155		Seongnam, South Korea	Strain	Han JG et al.
105.		*Tropicoporus linteus*	ASI 26082	KT862156		Mokpo, South Korea	Strain	Han JG et al.
106.		*Tropicoporus linteus*	ASI 26088	KT862159		Sancheong, South Korea	Strain	Han JG et al.
107.		*Tropicoporus linteus*	ASI 26114	KT862164		South Korea	Strain	Han JG et al.
108.		*Tropicoporus linteus*	ASI 26115	KT862165		South Korea	Strain	Han JG et al.
109.		*Phellinus linteus*	ATCC 26710	AF153010		South Korea	Strain	Kim GY et al.
110.		*Sanghuangporus sanghuang*	Batch 1-12192170-1	KT693244	Purchased	USA	Strain	Raja HA et al.
111.		*Sanghuangporus sanghuang*	Batch 2-10221252-2	KT693275	Purchased	USA	Strain	Raja HA et al.
112.		*Sanghuangporus sanghuang*	Batch 2-12192170-1	KT693246	Purchased	USA	Strain	Raja HA et al.
113.		S. sanghuang	**BJ**	**MT421904***	**Cultivated**	**Beijing, China**	**Strain**	**This study**
114.		*Inonotus* sp.	BZ-A	JN642589*	*Morus*	Hunan, China	Strain	Wu SH et al.
115.		*Inonotus* sp.	BZ-C	JN642587*	*Morus*	Hunan, China	Strain	Wu SH et al.
116.		*Inonotus* sp.	CA	JN642579*	*Morus*	Jiangxi, China	Strain	Wu SH et al.
117.		*Inonotus* sp.	CB	JN642580*	*Morus*	Jiangxi, China	Strain	Wu SH et al.
118.		*Inonotus* sp.	CC	JN642581*	*Morus*	Jiangxi, China	Strain	Wu SH et al.
119.		*Sanghuangporus sanghuang*	Cui 14419	MF772789*	*Morus*	Shaanxi, China	Specimen	Zhu L & Cui BK
120.		*Sanghuangporus sanghuang*	Cui 14420	MF772790*	*Morus*	Shaanxi, China	Specimen	Zhu L & Cui BK
121.		*Inonotus sanghuang*	Dai 12723	JQ860316*	*Morus*	Sichuan, China	Specimen	Tian XM et al.

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No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
122.		S. sanghuang	**DB1**	**MT421905***	**Cultivated**	**Northeast China**	**Strain**	**This study**
123.		*Phellinus linteus*	DGUM25003	AF082102			Strain	Chung JW et al.
124.		*Phellinus linteus*	DGUM25004	AF080458			Strain	Chung JW et al.
125.		*Inonotus linteus*	FS 656160	GU903004			Strain	Yu TW
126.		*Inonotus linteus*	FS 656161	HM584806			Strain	Yu TW
127.		*Tropicoporus linteus*	FS 656179	KU867779			Strain	Yu TW
128.		*Tropicoporus linteus*	FS 656180	KU867780			Strain	Yu TW
129.		S. sanghuang	**HB**	**MT421907***	**Cultivated**	**Hubei, China**	**Strain**	**This study**
130.		*Phellinus linteus*	IFO 6980	AF200226			Strain	Kim GY & Lee JD
131.		*Inonotus linteus*	IFO 6989	AY640937			Strain	Lee JS & Jung HS
132.		*Phellinus linteus*	IMSNU 31014	AF082101			Strain	Chung JW et al.
133.		*Sanghuangporus sanghuang*	JL-01	MG062789			Strain	Xu X
134.		S. sanghuang	**JS1**	**MT421908***	**Cultivated**	**Jiangsu, China**	**Strain**	**This study**
135.		*Inonotus linteus*	KAB-PL-01	DQ462333		Taiwan, China	Strain	Chiou SJ & Yen JH
136.		*Phellinus linteus*	KCTC 6190	AF077678			Strain	Chung JW et al.
137.		*Phellinus igniarius*	KCTC 16890	AY189708			Strain	Nam BH et al.
138.		*Inonotus linteus*	KFDA 016	AY436626			Strain	Yun JC et al.
139.		*Inonotus linteus*	KFDA P38	AY513234			Strain	Jin CY et al.
140.		*Inonotus linteus*	KSSW01	EF506943			Strain	Park SY et al.
141.		*Inonotus linteus*	LT-0802	HQ845059		South Korea	Strain	Hu W & Deng X
142.		*Inonotus linteus*	LT-CBS83	HQ845060		South Korea	Strain	Hu W & Deng X
143.		Sanghuangporus sanghuang	**LWZ 20180927-3**	**MT348588***	Morus	**Yunnan, China**	**Specimen**	**This study**
144.		*Phellinus linteus*	MPNU 7016	AF153009			Strain	Kim GY et al.
145.		*Inonotus linteus*	MUCL 47139	GU461973		Cuba	Strain	Amalfi M et al.
146.		*Inonotus linteus*	NAAS00002	JN043317			Strain	Seok SJ et al.
147.		*Phellinus linteus*	Namsan No1	AF080457			Strain	Chung JW et al.
148.		*Inonotus linteus*	PL 0801	FJ940906			Strain	Xie LY et al.
149.		*Inonotus linteus*	PL 5	EF095712			Strain	Park BW et al.
150.		*Inonotus* sp.	PL 10	JN642588*		China	Strain	Wu SH et al.
151.		*Sanghuangporus sanghuang*	S3	MN153568			Strain	Song JI et al.
152.		*Phellinus* sp.	SA 01	EF694971			Strain	Zeng NK et al.
153.		*Phellinus baumii*	SFC 20001106-1	AF534064			Strain	Lim YW et al.
154.		*Phellinus baumii*	SFC 20010212-1	AF534062			Strain	Lim YW et al.

new sequenced specimens and strains are in bold

* sequences considered to be reliable for further analysis

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
155.		*Sanghuangporus sanghuang*	SS	MG209821			Strain	Cai C & Zhao G
156.		*Inonotus* sp.	T004	JN642586*	*Morus*	Taiwan, China	Strain	Wu SH et al.
157.		*Inonotus* sp.	TH	JN642582*	*Morus*	Taiwan, China	Strain	Wu SH et al.
158.		*Inonotus* sp.	TJ	JN642585*	*Morus*	Taiwan, China	Strain	Wu SH et al.
159.		*Inonotus* sp.	TM	JN642583*	*Morus*	Taiwan, China	Strain	Wu SH et al.
160.		*Inonotus* sp.	TN	JN642584*	*Morus*	Taiwan, China	Strain	Wu SH et al.
161.		*Inonotus* sp.	WD 1222	JN642576*	*Morus*	Japan	Strain	Wu SH et al.
162.		*Inonotus* sp.	WD 2261	JN642577*	*Morus*	Japan	Strain	Wu SH et al.
163.		*Inonotus* sp.	WD 2300	JN642578*	*Morus*	Japan	Strain	Wu SH et al.
164.		*Inonotus* sp.	Wu 0903-1	JN794061*	*Morus*	Jilin, China	Strain	Wu SH et al.
165.		*Inonotus* sp.	ZhangjiaJie	MN242716	Cultivated		Strain	Wang Y
166.		S. sanghuang	**ZJ1**	**MT421910***	**Cultivated**	**Zhejiang, China**	**Strain**	**This study**
167.		S. sanghuang	**ZJ2**	**MT421911***	**Cultivated**	**Zhejiang, China**	**Strain**	**This study**
168.		S. sanghuang	**ZJ4**	**MT421913***	**Cultivated**	**Zhejiang, China**	**Strain**	**This study**
169.		S. sanghuang	**ZJ5**	**MT421914***	**Cultivated**	**Zhejiang, China**	**Strain**	**This study**
170.	*S. vaninii*	*Inonotus vaninii*		HQ845058		China	Strain	Hu W & Deng X
171.		*Inonotus* sp.	BeiJing	MN242720	Cultivated	China	Strain	Wang Y
172.		*Inonotus vaninii*	BZ-2031	JN642593*	*Populus*	China	Strain	Wu SH et al.
173.		*Inonotus vaninii*	CJC 01	JN642592*	Cultivated	Taiwan, China	Strain	Wu SH et al.
174.		*Sanghuangporus vaninii*	Cui 9939	MF772792*		Jilin, China	Specimen	Zhu L & Cui BK
175.		*Sanghuangporus vaninii*	Cui 14082	MF772793*	*Populus*	Jilin, China	Specimen	Zhu L & Cui BK
176.		*Inonotus vaninii*	Dai 3624	JN642590*	*Populus*	China	Strain	Wu SH et al.
177.		*Inonotus vaninii*	Dai 7011	JN642591*	*Populus davidiana*	Jilin, China	Strain	Wu SH et al.
178.		*Sanghuangporus vaninii*	Dai 8236	MF772791*	*Populus*	Jilin, China	Specimen	Zhu L & Cui BK
179.		S. vaninii	**DB2**	**MT421906***	**Cultivated**	**Northeast China**	**Strain**	**This study**
180.		*Inonotus baumii*	FS 656170	GU903008			Strain	Yu TW
181.		*Fuscoporia gilva*	FS 656175	HM584811			Strain	Yu TW
182.		*Sanghuangporus vaninii*	HZ-01	MG062791			Strain	Xu X
183.		*Inonotus* sp.	JinZhai	MN242717	Cultivated	China	Strain	Wang Y
184.		S. vaninii	**JS2**	**MT421909***	**Cultivated**	**Jiangsu, China**	**Strain**	**This study**
185.		*Inonotus* sp.	KangNeng	MN242721	Cultivated	China	Strain	Wang Y
186.		*Inonotus baumii*	KFDA 015	AY436623			Strain	Yun JC et al.
187.		*Inonotus baumii*	KFDA 022	AY436624			Strain	Yun JC et al.
188.		*Inonotus linteus*	KFDA 024	AY436627			Strain	Yun JC et al.

new sequenced specimens and strains are in bold

* sequences considered to be reliable for further analysis

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
189.		*Inonotus baumii*	KFDA 029	AY436625			Strain	Yun JC et al.
190.		*Inonotus baumii*	KFDA P36	AY509198			Strain	Jin CY et al.
191.		*Inonotus baumii*	KFDA P40	AY509199			Strain	Jin CY et al.
192.		*Inonotus baumii*	KFDA P45	AY509201			Strain	Jin CY et al.
193.		*Inonotus* sp.	Korea	MN242719	Cultivated	China	Strain	Wang Y
194.		*Sanghuangporus baumii*	LC 6686	MK818502			Strain	Li ZN
195.		*Inonotus linteus*	LT-HG	HQ845061			Strain	Hu W & Deng X
196.		*Fuscoporia gilva*	MDJCBS87	DQ103884			Strain	Jiang J et al.
197.		*Phellinus baumi*	MPNU 7004	AF200229			Strain	Kim GY & Lee JD
198.		*Phellinus baumi*	MPNU 7005	AF200230			Strain	Kim GY & Lee JD
199.		*Phellinus baumi*	MPNU 7006	AF200231			Strain	Kim GY & Lee JD
200.		*Phellinus* sp.	MPNU 7007	AF200235			Strain	Kim GY & Lee JD
201.		*Phellinus* sp.	MPNU 7010	AF153007		South Korea	Strain	Kim GY et al.
202.		*Phellinus* sp.	MPNU 7012	AF153008		South Korea	Strain	Kim GY et al.
203.		*Phellinus* sp.	MPNU 7013	AF153011		South Korea	Strain	Kim GY et al.
204.		*Inonotus baumii*	PB 0802	FJ940907			Strain	Xie LY et al.
205.		*Inonotus baumii*	PB 0803	FJ940908			Strain	Xie LY et al.
206.		*Inonotus baumii*	PB 0806	FJ940911			Strain	Xie LY et al.
207.		*Inonotus baumii*	PB 0808	FJ940913			Strain	Xie LY et al.
208.		*Inonotus baumii*	PB 0809	FJ940914			Strain	Xie LY et al.
209.		*Inonotus* sp.	QianDaoHu	MN242718	Cultivated	China	Strain	Wang Y
210.		*Sanghuangporus vaninii*	S1	MN153566			Strain	Song JL et al.
211.		*Sanghuangporus baumii*	S2	MN153567			Strain	Song JL et al.
212.		*Fuscoporia gilva*	S12	MT275660			Strain	Li Y & Huo J
213.		*Phellinus* sp.	SA 02	EF694972			Strain	Zeng NK et al.
214.		*Phellinus* sp.	SA 03	EF694973			Strain	Zeng NK et al.
215.		*Phellinus* sp.	SA 04	EF694974			Strain	Zeng NK et al.
216.		*Inonotus baumii*	SA 05	EF694975			Strain	Zeng NK et al.
217.		*Phellinus* sp.	SA 06	EF694976			Strain	Zeng NK et al.
218.		*Phellinus* sp.	SA 07	EF694977			Strain	Zeng NK et al.
219.		*Phellinus linteus*	SFC 970605	AF534071			Strain	Lim YW et al.
220.		*Phellinus linteus*	SFC 20001106-7	AF534070			Strain	Lim YW et al.
221.		*Phellinus baumii*	SFC 20010212-2	AF534063			Strain	Lim YW et al.
222.		*Tropicoporus linteus*	SFCC 10209	AY558628			Strain	Jeong WJ et al.
223.		*Fuscoporia gilva*	SH 1	FJ190410			Strain	Zou L et al.

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No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
224.		*Inonotus baumii*	SJ	JN887691			Strain	Shin KS
225.		*Inonotus vaninii*	Wei 3382	JN169788*		Jilin, China	Specimen	Zhou LW & Qin WM
226.		*Inonotus vaninii*	WN 0801	HQ845054		China	Strain	Hu W & Deng X
227.		*Inonotus vaninii*	WN-1	HQ845055		China	Strain	Hu W & Deng X
228.		*Inonotus vaninii*	WN-2	HQ845056		China	Strain	Hu W & Deng X
229.		*Inonotus vaninii*	WN-4	HQ845065		China	Strain	Hu W & Deng X
230.		*Inonotus vaninii*	WN 8213	HQ845052		China	Strain	Hu W & Deng X
231.		*Inonotus vaninii*	WN 8824	HQ845051		China	Strain	Hu W & Deng X
232.		*Inonotus vaninii*	WN 3624	HQ845050		China	Strain	Hu W & Deng X
233.		*Sanghuangporus baumii*	XZ-01	MG062790			Strain	Xu X
234.		*Inonotus baumii*	YC	JN887692			Strain	Shin KS
235.		*Sanghuangporus vaninii*	Yuan 2764	KY328308*	*Quercus*	Shaanxi, China	Specimen	Zhu L & Cui BK
236.		*Sanghuangporus vaninii*	Yuan 5604	KY328307*	*Quercus*	Jilin, China	Specimen	Zhu L & Cui BK
237.		S. vaninii	**ZJ3**	**MT421912***	**Cultivated**	**Zhejiang, China**	**Strain**	**This study**
238.	*S. weigelae*	*Sanghuangporus weigelae*	420526MF0201	MH142013		Hubei, China	Specimen	Wang R et al.
239.		*Inonotus weigelae*	Cui 6010	JQ860318*	*Lonicera*	Jiangxi, China	Specimen	Tian XM et al.
240.		*Inonotus weigelae*	Cui 6012	JQ860319*	*Lonicera*	Jiangxi, China	Specimen	Tian XM et al.
241.		*Inonotus weigelae*	Cui 7176	JQ860320*	*Syringa*	Hebei, China	Specimen	Tian XM et al.
242.		*Inonotus weigelae*	Dai 6352	JQ860317*		Zhejiang, China	Specimen	Tian XM et al.
243.		*Inonotus weigelae*	Dai 11694	JQ860315*		Hunan, China	Specimen	Tian XM et al.
244.		*Sanghuangporus weigelae*	Dai 15770	MF772795*	*Weigela*	Chongqing, China	Specimen	Zhu L & Cui BK
245.		Sanghuangporus weigelae	**Dai 16072**	**MT348589***	Weigela	**Inner Mongolia, China**	**Specimen**	**This study**
246.		*Sanghuangporus weigelae*	Dai 16077	MF772794*	*Weigela*	Inner Mongolia, China	Specimen	Zhu L & Cui BK
247.		Sanghuangporus weigelae	**LWZ 20150802-3**	**MT348590***	Weigela	**Jiangxi, China**	**Specimen**	**This study**
248.		Sanghuangporus weigelae	**LWZ 20150802-5**	**MT348591***	Weigela	**Jiangxi, China**	**Specimen**	**This study**
249.		*Phellinus baumii*	SFC 20000111-10	AF534067			Strain	Lim YW et al.
250.		*Inonotus* sp.	WD 1186	JN642597*	*Weigela*	Japan	Strain	Tian XM et al.
251.		*Inonotus* sp.	WD 1187	JN642598*	*Weigela*	Japan	Strain	Tian XM et al.
252.		*Inonotus* sp.	WD 1667	JN642594*	*Weigela cordeenis*	Japan	Strain	Wu SH et al.
253.		*Inonotus* sp.	WD 1837	JN642595*	*Weigela cordeenis*	Japan	Strain	Wu SH et al.
254.		*Inonotus* sp.	WD 1838	JN642596*	*Weigela cordeenis*	Japan	Strain	Wu SH et al.

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* sequences considered to be reliable for further analysis

No.	Species name accepted here	Species name in GenBank	Voucher No.	GenBank No.	Host plant	Geographic origin	Type of material	Identifier
255.		*Inonotus weigelae*	Wei 2120	JQ860314*	*Coriaria*	Hubei, China	Specimen	Tian XM et al.
256.		*Inonotus weigelae*	Wei 2267	JX069835*	Angiosperm	Hubei, China	Specimen	Tian XM et al.
257.		*Inonotus tenuicontextus*	Yuan 5526	JN169786*	Angiosperm	Guizhou, China	Specimen	Zhou LW & Qin WM
258.	*S. weirianus*	*Sanghuangporus weirianus*	CBS 618.89	AY558654*			Strain	Jeong WJ et al.
259.		*Phellinus weirianus*	IMSNU 32021	AF110989*			Strain	Chung JW et al.
260.	*S. zonatus*	*Inonotus zonatus*	Cui 6631	JQ860305*	Angiosperm	Hainan, China	Specimen	Tian XM et al.
261.		*Inonotus zonatus*	Cui 8327	JX069837*	Angiosperm	Yunnan, China	Specimen	Tian XM et al.
262.		*Inonotus zonatus*	Dai 10841	JQ860306*	Angiosperm	Hainan, China	Specimen	Tian XM et al.
263.	*S.* sp. 1	*Inonotus* sp.	AM-08	JF895464		Ethiopia	Specimen	Assefa A et al.
264.		*Inonotus* sp.	AM-19	JF895465		Ethiopia	Specimen	Assefa A et al.
265.		*Inonotus linteus*	F915611	JX985739		Ethiopia	Specimen	Assefa A et al.
266.		*Inonotus linteus*	Teng 3279	JX985738	*Xylosoma*	China	Specimen	Assefa A et al.
267.	*S.* sp. 2	*Phellinus* sp.	DLL 2010 – 102	JQ673184	*Populus tremuloides*	USA	Strain	Brazee NJ et al.
268.		*Sanghuangporus vaninii*	DLL 2010 – 102	KU139197	*Populus tremuloides*	USA	Strain	Brazee NJ
269.	*S.* sp. 3	*Phellinus baumii*	SFC 20001106-4	AF534066		South Korea	Strain	Lim YW et al.
270.	not *Sanghuangporus*	*Sanghuangporus baumii*	DL 101	KP974834		China	Strain	Sun T et al.
271.	not *Sanghuangporus*	*Inonotus vaninii*	WN-3	HQ845057		China	Strain	Hu W & Deng X
new sequenced specimens and strains are in bold								
* sequences considered to be reliable for further analysis								

Downloading sequences from GenBank

The genus name *Sanghuangporus* and the epithets of 14 *Sanghuangporus* species were firstly used as queries to search GenBank. Meanwhile, the reliable sequences of 14 *Sanghuangporus* species (Zhou et al. 2020) were used as queries to perform BLAST search in GenBank. The cut-off value of similarity for the resulting sequences was set as 95%. All these ITS sequences by April 30, 2020 were retrieved from GenBank (Table 1). In addition, the recently published papers related to the taxonomy of *Sanghuangporus* were checked for supplementing sequence information (Wu et al. 2012a, 2019b; Zhou and Qin 2012; Tian et al. 2013; Ghobad-Nejhad 2015; Tomšovský 2015; Han et al. 2016; Zhou et al. 2016; Zhu et al 2019; Shao et al. 2020).

Phylogenetic analyses

The datasets of ITS sequences were separately aligned using MAFFT 7.110 (Katoh and Standley 2013) under the G-INI-i option (Katoh et al. 2005). All resulting alignments are deposited in TreeBASE (http://www.treebase.org; accession number S26272; Reviewer access URL: http://purl.org/phylo/treebase/phylows/study/TB2:S26272?x-access-code=cb4ee00b60c33d03f7496ee08038e86d&format=html). jModelTest (Guindon and Gascuel 2003; Posada, 2008) was used to estimate the best-fit evolutionary model for each alignment with calculation under corrected Akaike information criterion. Following the estimated models, maximum likelihood (ML) and Bayesian inference (BI) algorithms were used to construct midpoint-rooted trees for the alignments. The ML algorithm was performed using raxmlGUI 2.0 (Stamatakis, 2014; Edler et al., 2019), and the bootstrap (BS) replicates were calculated under the auto FC option (Pattengale et al. 2010). The BI algorithm was performed using MrBayes 3.2 (Ronquist et al. 2012), which employed two independent runs each with four chains and starting from random trees. Trees were sampled every 1000th generation, of which the first 25% were removed as burn-in and the other 75% were retained for constructing a 50% majority consensus tree and calculating Bayesian posterior probabilities (BPPs). Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) was used to judge the convergence of chains.

Evaluation of genetic distances of ITS sequences

The genetic distances of an alignment of ITS sequences was estimated using MEGA X (Kumar et al. 2018; Stecher et al. 2020). For genetic distances between and within species of *Sanghuangporus*, the parameters were both set as follows: a BS method of variance estimation with 1000 BS replications, a *p*-distance substitution model including transitions and transversions, the uniform rates among sites, and a pairwise deletion treatment of gaps and missing data.

Identification of diagnostic ITS sequences

According to the alignment of ITS sequences generated using MAFFT 7.110 (Katoh and Standley 2013) under the G-INI-i option (Katoh et al. 2005), if a more than one-nucleotide-long fragment was unique for one species and not variant within this species, this fragment was identified as a potential diagnostic sequence for this species.

Results

A total of 13 specimens and 18 strains were newly sequenced, and the resulting ITS sequences were submitted to GenBank (Table 1). According to our criterion, 240 ITS sequences were downloaded from GenBank, but two sequences (HQ845057 and KP974834) showed unexpectedly large differences from other sequences of *Sanghuangporus* by BLAST search and thus excluded from subsequent phylogenetic analyses (Table 1). Eventually, a dataset of all available 269 ITS sequences (31 newly sequenced and 238 downloaded from GenBank) from *Sanghuangporus* species was employed to construct a preliminary phylogenetic frame of this genus. An alignment of 941 characters was resulted from this dataset, and HKY + G was estimated as the best-fit evolutionary model for phylogenetic analysis. The ML search stopped after 850 bootstrap replicates. All chains in BI converged after ten million generations, which is indicated by the estimated sample sizes (ESSs) of all parameters above 500 and the potential scale reduction factors (PSRFs) close to 1.000. The ML and BI algorithms generated nearly congruent topology in main lineages (Additional file 1: Tree S1, Additional file 2: Tree S2). Therefore, only the topology from the ML algorithm is visualized in a circle form; the midpoint-rooted tree recovered 13 species and three undescribed lineages of *Sanghuangporus* (Fig. 1). The one species gap comparing with the 14 accepted species is caused by that collections previously identified as *S. quercicola* Lin Zhu & B.K. Cui and *S. toxicodendri* Sheng H. Wu, B.K. Cui & Guo Z. Jiang were nested within a single clade (Fig. 1). Of the 13 recovered species of *Sanghuangporus*, the clades of *S. lonicericola* (Parmasto) L.W. Zhou & Y.C. Dai and *S. sanghuang* (Sheng H. Wu, T. Hatt. & Y.C. Dai) Sheng H. Wu, L.W. Zhou & Y.C. Dai did not receive well statistical supports, and the clade of *S. alpinus* (Y.C. Dai & X.M. Tian) L.W. Zhou & Y.C. Dai was strongly supported just by the BI algorithm, while other species were all strongly supported by both the ML and the BI algorithms (Additional file 1: Tree S1, Additional file 2: Tree S2). *Sanghuangporus microcystideus* (Har. & Pat.) L.W. Zhou & Y.C. Dai was merged together with *S.* sp. 1 in the tree inferred from the ML algorithm (Fig. 1, Additional file 1: Tree S1), but was separated from *S.* sp. 1 in the BI tree (Additional file 2: Tree S2). The relationship between *S. microcystideus* and *S.* sp. 1 is still not clear, so we tentatively treat the specimen O 915609 as the single representative of *S. microcystideus*.

In GenBank, species names from nine out of 77 phylogenetically analyzed specimens were misapplied (tips labeled in green color in Fig. 1), while those from 131 out of 192 phylogenetically analyzed strains were wrongly identified to a species level (tips labeled in red color in Fig. 1). Besides, two ITS sequences of strains (HQ845057 and KP974834) labeled as members of *Sanghuangporus* were extremely deviated and maybe came from inappropriate readings of Sanger sequencing chromatograms (Table 1). Most of these errors came from submitters of non-taxonomists. Therefore, to delimit species boundary of *Sanghuangporus*, we selected the ITS sequences submitted to GenBank by taxonomists for a new round of phylogenetic analysis (Table 1). The new dataset included 122 ITS sequences and resulted in an alignment of 871 characters with HKY + I + G as the best-fit evolutionary model. The ML search stopped after 450 bootstrap replicates. All chains in BI converged after four million generations, which is indicated by the ESSs of all parameters above 1000 and the PSRFs close to 1.000. The ML and BI algorithms generated nearly congruent topology in main lineages, and only the midpoint-rooted ML tree is presented along with the BPPs at the nodes (Fig. 2). Similar to Fig. 1, this tree also recovered 13 species of *Sanghuangporus* with *S. quercicola* and *S. toxicodendri* nested within a single clade (Fig. 2). Among these 13 species, *S. lonicericola* was still not strongly supported as a monophyletic lineage, and *S. alpinus* and *S. sanghuang* were moderately supported from the ML algorithm and fully supported from the BI algorithm, while all other species received strong statistical supports from both the ML and the BI algorithms (Fig. 2).

To further explore the species relationships among *Sanghuangporus*, the alignment with 122 selected ITS sequences was conducted a genetic distance analysis. In addition to *Sanghuangporus microcystideus* and *S. pilatii* (Černý) Tomšovský each referring to a single collection, the genetic distances of ITS sequences within species of *Sanghuangporus* was mostly below 1.00% (even 0.00% within *S. ligneus* Ghob.-Nejh.), whereas those within *S. baumii* (Pilát) L.W. Zhou & Y.C. Dai, *S. weirianus* (Bres.) L.W. Zhou & Y.C. Dai and *S. zonatus* (Y.C. Dai & X.M. Tian) L.W. Zhou & Y.C. Dai were 1.29%, 2.68% and 1.14%, respectively (Table 2). Regarding the genetic distances between species, all were above 2.00% (mostly above 4.00%) but those between *Sanghuangporus alpinus*, *S. lonicerinus* (Bondartsev) Sheng H. Wu, L.W. Zhou & Y.C. Dai and *S. weigelae* (T. Hatt. & Sheng H. Wu) Sheng H. Wu, L.W. Zhou & Y.C. Dai (1.56–1.83%); moreover, those between *Sanghuangporus microcystideus* and all other species were more than 10.00% (Table 2).

Table 2
Genetic distances of ITS sequences between and within species of *Sanghuangporus*

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*S. alpinus*	*0.0049 ± 0.0016*												
2	*S. baumii*	0.0445 ± 0.0073	*0.0129 ± 0.0026*											
3	*S. ligneus*	0.0529 ± 0.0097	0.0439 ± 0.0084	*0*										
4	*S. lonicericola*	0.0417 ± 0.0070	0.0315 ± 0.0059	0.0249 ± 0.0066	*0.0045 ± 0.0016*									
5	*S. lonicerinus*	0.0156 ± 0.0042	0.0502 ± 0.0082	0.0600 ± 0.0102	0.0498 ± 0.0082	*0.0046 ± 0.0017*								
6	*S. microcystideus*	0.1083 ± 0.0118	0.1166 ± 0.0119	0.1173 ± 0.0135	0.1104 ± 0.0121	0.1083 ± 0.0121	*n.a.*							
7	*S. pilatii*	0.0476 ± 0.0079	0.0576 ± 0.0086	0.0532 ± 0.0097	0.0493 ± 0.0079	0.0508 ± 0.0085	0.1191 ± 0.0127	*n.a.*						
8	*S. quercicola*	0.0610 ± 0.0087	0.0654 ± 0.0087	0.0657 ± 0.0103	0.0662 ± 0.0090	0.0711 ± 0.0096	0.1313 ± 0.0126	0.0490 ± 0.0079	*0.0044 ± 0.0014*					
9	*S. sanghuang*	0.0390 ± 0.0069	0.0479 ± 0.0076	0.0581 ± 0.0100	0.0485 ± 0.0077	0.0391 ± 0.0074	0.1046 ± 0.0118	0.0370 ± 0.0071	0.0524 ± 0.0080	*0.0010 ± 0.0003*				
10	*S. vaninii*	0.0592 ± 0.0089	0.0686 ± 0.0096	0.0622 ± 0.0103	0.0628 ± 0.0092	0.0663 ± 0.0096	0.1210 ± 0.0126	0.0304 ± 0.0065	0.0590 ± 0.0084	0.0480 ± 0.0079	*0.0049 ± 0.0012*			
11	*S. weigelae*	0.0172 ± 0.0045	0.0474 ± 0.0078	0.0507 ± 0.0095	0.0438 ± 0.0075	0.0183 ± 0.0049	0.1064 ± 0.0119	0.0501 ± 0.0085	0.0696 ± 0.0094	0.0391 ± 0.0072	0.0667 ± 0.0095	*0.0031 ± 0.0012*		
12	*S. weirianus*	0.0605 ± 0.0086	0.0658 ± 0.0085	0.0631 ± 0.0102	0.0630 ± 0.0088	0.0622 ± 0.0090	0.1271 ± 0.0124	0.0540 ± 0.0081	0.0755 ± 0.0093	0.0416 ± 0.0069	0.0724 ± 0.0095	0.0585 ± 0.0086	*0.0268 ± 0.0061*	
13	*S. zonatus*	0.0695 ± 0.0091	0.0629 ± 0.0088	0.0672 ± 0.0105	0.0495 ± 0.0078	0.0769 ± 0.0101	0.1333 ± 0.0131	0.0803 ± 0.0101	0.0902 ± 0.0106	0.0763 ± 0.0097	0.0836 ± 0.0103	0.0712 ± 0.0094	0.0983 ± 0.0108	*0.[illegible] ± 0.[illegible]*

The genetic distances between species are shown down the diagonal, and those within species are shown in italic along the diagonal.

Fifty-eight ITS sequences of *S. baumii*, *S. sanghuang* and *S. vaninii* (Ljub.) L.W. Zhou & Y.C. Dai that are the most common species in medicinal studies and products (Zhou et al., 2020) were further retrieved from the dataset with 122 selected sequences. These 58 ITS sequences were realigned and the alignment is presented with shadows (Fig. 3). From this alignment, 10 potential diagnostic sequences with two to six nucleotide differences were identified for HRCA to discriminate species: two for *S. baumii*, two for *S. sanghuang* and six for *S. vaninii* (Fig. 3, Table 3).

Table 3
Diagnostic sequences adopted from Fig. 3 potential for discriminating species of *Sanghuangporus baumii*, *S. sanghuang* and *S. vaninii* using hyperbranched rolling circle amplification

Label in Fig. 3	Differentiated species	Diagnostic sequence	Position in the alignment of Fig. 3	Length of differences (nt)
A	*S. sanghuang*	AWYTY	41–45	5
B	*S. vaninii*	TCA	85–87	3
C	*S. vaninii*	CTG	143–145	3
D	*S. baumii*	CGGTAGGAA	159–167	4
E	*S. vaninii*	GAGCGG	221–226	6
F	*S. vaninii*	CCCCC	266–270	4
G	*S. vaninii*	AG	561–562	2
H	*S. baumii*	AGG	655–657	2
I	*S. vaninii*	ACG	669–671	2
J	*S. sanghuang*	TT	695–696	2

Discussion

In this study, we summarized all available ITS barcoding sequences of “Sanghuang” from GenBank. A total of 271 ITS sequences related to “Sanghuang” including 31 newly generated sequences for this study were analyzed. More than half of these sequences, or say 142, were mislabeled. So many errors undoubtfully raised chaos when BLAST search, especially for non-taxonomists.

Comparing with specimens, much more mislabeled sequences came from strains. Most of these sequences were submitted by non-taxonomists. One typical case is a recently published paper on genome sequencing of “Sanghuang” that meanwhile submitted six ITS sequences to GenBank (Shao et al. 2020). In GenBank, all these six sequences were labeled as *Inonotus* sp. rather than certain species of *Sanghuangporus* (MN242716–MN242721), while the six strains generating these sequences were named as *Sanghuangporus sanghuang* in the paper submitting these sequences (Shao et al., 2020). However, five of the six strains including that subject to genome sequencing are actually *Sanghuangporus vaninii* (Fig. 1, Zhou et al., 2020). That is to say, five out of six strains were wrongly identified to a species level. Therefore, this incorrected species identification makes the whole genome sequence of “Sanghuang” misapplied to an inappropriate species. Even worse, Shao et al. (2020) stated that these six strains are commercially cultivated, which further results in the name chaos for commercial products of “Sanghuang”. Another case is a paper specially on the species identity of “Sanghuang” strains (Han et al. 2016). Thirty strains deposited in the Agricultural Sciences Institute culture collection (Mushroom Research Division, Rural Development Administration, Republic of Korea) were correctly identified as *Sanghuangporus vaninii* and *S. sanghuang* according to an ITS-based phylogenetic analysis; however, unfortunately, most of these ITS sequences were mislabeled when being submitted to GenBank.

Nine mislabeled sequences came from specimens. These errors were caused mainly by the update of taxonomic recognition. Six sequences of specimens originally labeled as *Sanghuangporus* sp. are accepted to represent *S. quercicola* (Table 1). In the paper submitting these six sequences, the specimens generating them were newly described as *Sanghuangporus toxicodendri* (Wu et al. 2019b). However, in that paper the separation of *S. toxicodendri* and *S. quercicola* was actually not supported from a phylogenetic perspective, and moreover, the morphological differences between these two species are not on the basis of stable characters (Wu et al. 2019b). In the current phylogenetic analyses, the six specimens of *S. toxicodendri*, three specimens of *S. quercicola* and additional four collections merged together in a fully supported clade (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). Therefore, *S. toxicodendri* and *S. quercicola* are considered to be conspecific, and *S. quercicola* has priority over *S. toxicodendri*. Another mislabeled sequence was generated from a specimen originally described as *Inonotus tenuicontextus* L.W. Zhou & W.M. Qin (Zhou and Qin 2012). Although this species was online published earlier than *Inonotus weigelae* T. Hatt. & Sheng H. Wu, the basionym of *Sanghuangporus weigelae* (Wu et al. 2012a), its online date is before January 1st, 2012 and thus not effective. Soon, *I. tenuicontextus* was treated as a later synonym of *I. weigelae* (Tian et al. 2013). Therefore, this mislabeled sequence is accepted to represent *S. weigelae* (Table 1).

The independence of *Sanghuangporus lonicericola* was not well supported in the current phylogenetic analyses (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). Similarly, *Sanghuangporus alpinus* and *S. sanghuang* were not strongly supported as monophyletic species by the ML algorithm (Fig. 2). However, the intraspecific difference of ITS sequences in each of the three species was quite low (0.10–0.49%, Table 2). So, we still accept *S. alpinus*, *S. lonicericola* and *S. sanghuang* as three independent species. Maybe a phylogenetic analysis employing more loci will improve the resolution. On the contrary, *Sanghuangporus baumii*, *S. weirianus* and *S. zonatus* are the only three species with more than 1.00% of intraspecific ITS differences (Table 2). However, these three species all received strong supports as independent lineages (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). Noteworthily, Chinese collections of *Sanghuangporus baumii* formed three strongly supported subclades corresponding to geographic origins, viz. nine from Northeast China, two from Beijing and two from Shanxi; regarding *S. zonatus*, two collections of from Hainan, China grouped together with full statistical support, and then formed a fully supported clade with the collection from Yunnan, China (Table 1, Fig. 2). Moreover, branch lengths of the only two available collections of *S. weirianus* were extremely different (Fig. 2). A more comprehensive sampling of these three species in phylogenetic analyses will further clarify their intraspecific relationships. For now, we tentatively accept them as monophyletic species.

Although intact mature specimens of “Sanghuang” are not difficult to be morphologically identified to a species level in a short time, most of commercial products are chips and pieces or even powders. Normally, it is impossible to rapidly determine which species such kind of commercial products really represents. Like other medicinal mushrooms (Raja et al. 2017), species names of *Sanghuangporus* are sometimes misapplied to certain products of “Sanghuang” (Shao et al. 2020). This confused situation to some extent restricts the industrial development of “Sanghuang” (Zhou 2020). Therefore, to standardize the industry of “Sanghuang”, ten candidate sequences were provided for HRCA based on the accurate boundaries among three commonly studied and cultivated species, viz. *Sanghuangporus baumii*, *S. sanghuang* and *S. vaninii* (Lin et al. 2017; Zhou et al. 2020). HRCA is an isothermal amplification approach and thus provides a rapid, simple and low-cost detection of specific nucleic acid sequences (Nilsson et al. 1994; Lizardi et al. 1998). This approach has been widely used for clinic detection of human-pathogenic microfungi (Zhou et al. 2008; Trilles et al. 2014; Rodrigues et al. 2015), and recently, was also reported for rapid detection of poisonous macrofungi (He et al. 2019a, 2019b). Regarding lethal *Amanita* species, a more than two-nucleotide-long difference was evidenced to be valid for identification of *α-amanitin* gene (He et al. 2019a). Here, to provide more candidates, two and more nucleotide differences are given, because it was reported that this approach could reveal single nucleotide differences (Nilsson et al. 1997). Hopefully, certain candidates will work well in future experiments.

Conclusion

Generally, to promote medicinal studies and industrial development, the ITS barcoding region of *Sanghuangporus* is comprehensively analyzed for accurate species identification. Firstly, the names of all available ITS sequences in GenBank related to “Sanghuang” are carefully corrected. Secondly, the intraspecific ITS difference for each species of *Sanghuangporus* but *S. weirianus* is evaluated to be below 1.50%, while the interspecific ITS difference is always above 1.50%. This provides a practical cut-off value for BLAST search-based species identification. Finally, ten potential diagnostic sequences are provided for HRCA assay to rapidly discriminate three commonly studied and cultivated species, viz. *Sanghuangporus baumii*, *S. sanghuang* and *S. vaninii*.

Abbreviations

BI: Bayesian inference; BPP: Bayesian posterior probability; CTAB: cetyltrimethylammonium bromide; ML: Maximum likelihood; ITS: nuclear ribosomal internal transcribed spacer; PCR: polymerase chain reaction.

Declarations

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Adherence to national and international regulations

Not applicable.

Authors’ contributions

S-LL, SS and L-WZ retrieved and analyzed all data. J-HJ prepared fungal samples and performed molecular sequencing. L-WZ conceived the work and wrote the manuscript. All authors approved the manuscript.

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Availability of data and materials

The materials are available as Additional files 1 and 2. All sequence data generated for this study can be accessed via GenBank: https://www.ncbi.nlm.nih.gov/genbank/. Alignments are available at TreeBase (ID: 26272).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests

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Supplementary Information

Additional file 1: Tree S1. The phylogenetic tree inferred from 269 ITS sequences. The topology was generated from the maximum likelihood algorithm and bootstrap values are presented at the nodes.

Additional file 2: Tree S2. The phylogenetic tree inferred from 269 ITS sequences. The topology was generated from the Bayesian inference algorithm and Bayesian posterior probabilities are presented at the nodes.

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Figures

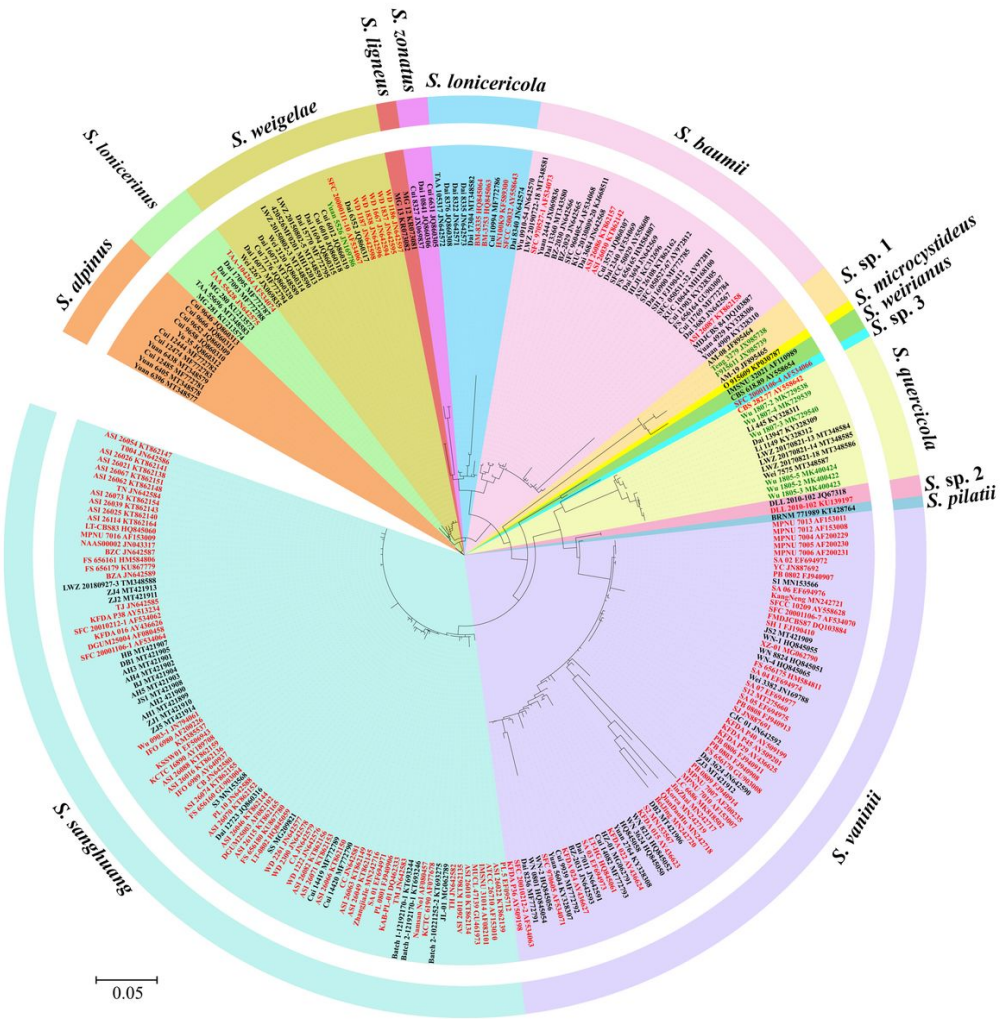

Figure 1

The phylogenetic tree inferred from 269 ITS sequences. The topology was generated from the maximum likelihood algorithm. The tips in blue color represent name-mislabeled specimens, while those in red color represent name-mislabeled strains.

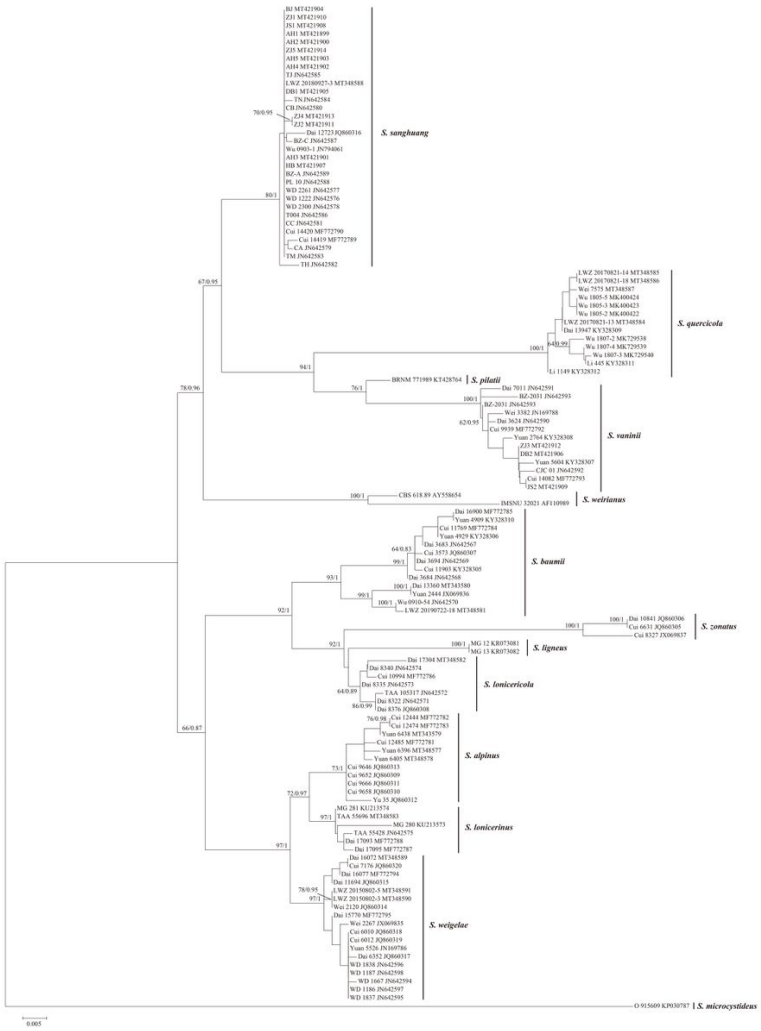

Figure 2

The phylogenetic tree inferred from ITS sequences submitted by taxonomists. The topology was generated from the maximum likelihood algorithm, and bootstrap values and Bayesian posterior probabilities simultaneously above 50% and 0.8, respectively, are presented at the nodes.

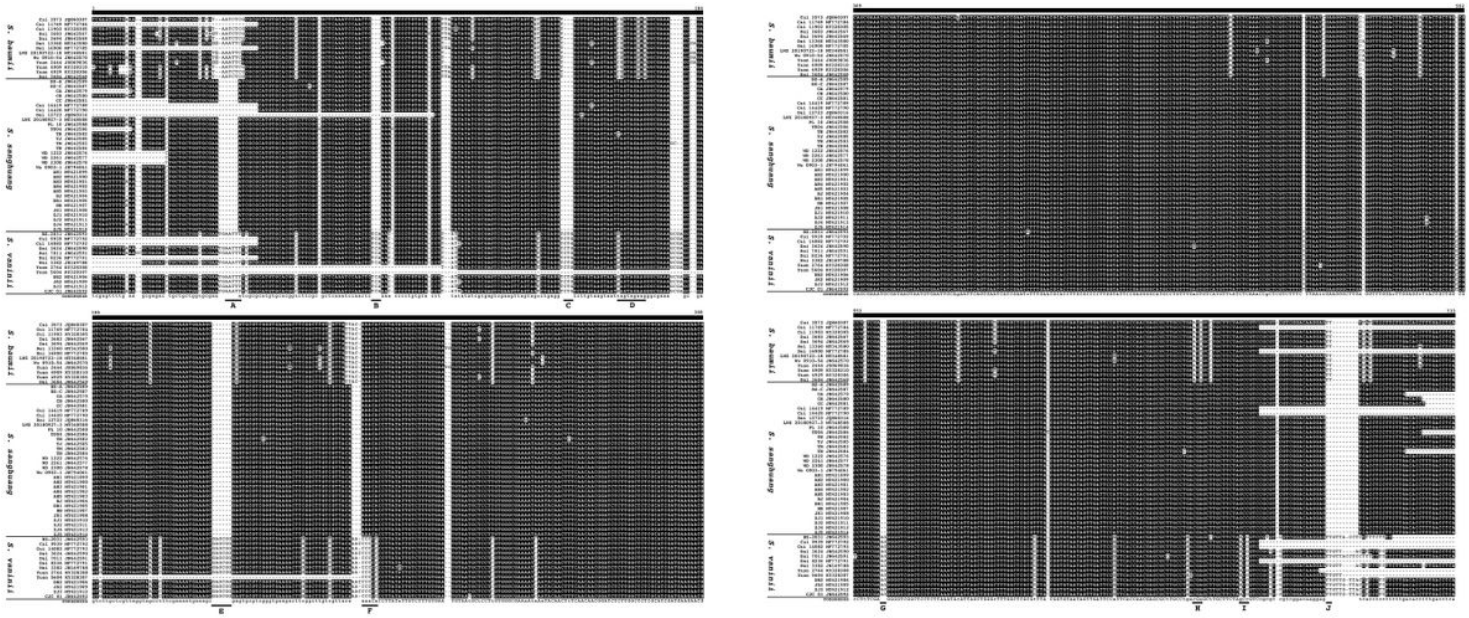

Figure 3

The alignment of Sanghuangporus baumii, S. sanghuang and S. vaninii generated from ITS sequences submitted by taxonomists. Ten potential diagnostic sequences for hyperbranched rolling circle amplification are labeled in capital letters.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalFile2TreeS2.tre
- AdditionalFile1TreeS1.tre