Addressing widespread misidentifications of traditional medicinal mushrooms in *Sanghuangporus* (*Basidiomycota*) through ITS barcoding and designation of reference sequences

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**Abstract**

"Sanghuang" refers to a group of important traditionally-used medicinal mushrooms belonging to the genus *Sanghuangporus*. In practice, species of *Sanghuangporus* referred to in medicinal studies and industry are now differentiated mainly by a BLAST search of GenBank with the ITS barcoding region as a query. However, inappropriately labeled ITS sequences of "Sanghuang" in GenBank restrict accurate species identification and, to some extent, the utilization of these species as medicinal resources. We examined all available 271 ITS sequences related to "Sanghuang" in GenBank including 31 newly submitted sequences from this study. Of these sequences, more than half were mislabeled so we have now corrected the corresponding species names. The mislabeled sequences mainly came from strains utilized by non-taxonomists. Based on the analyses of ITS sequences submitted by taxonomists as well as morphological characters, we separate the newly described *Sanghuangporus subbaumii* from *S. baumii* and treat *S. toxicodendri* as a later synonym of *S. quercicola*. Fourteen species of *Sanghuangporus* are accepted, with intraspecific distances up to 1.30% (except in *S. vaninii*, *S. weirianus* and *S. zonatus*) and interspecific distances above 1.30% (except between *S. alpinus* and *S. lonicerinus*, and *S. baumii* and *S. subbaumii*). To stabilize the concept of these 14 species of *Sanghuangporus*, their taxonomic information and reliable ITS reference sequences are provided. Moreover, ten potential diagnostic sequences are provided for Hyperbranched Rolling Circle Amplification to rapidly confirm three common commercial species, viz. *S. baumii*, *S. sanghuang*, and *S. vaninii*. Our results provide a practical method for ITS barcoding-based species identification of *Sanghuangporus* and will promote medicinal studies and commercial development from taxonomically correct material.

**Keywords:** Hymenochaetaceae, Phylogeny, Species boundary, Taxonomy, Wood-inhabiting fungi, One new taxon
INTRODUCTION

Many macrofungi are established in traditional medicine and possess diverse properties (Wu et al. 2019a). “Sanghuang” comprises an important group of wood-inhabiting mushrooms that have been utilized in traditional medicine in China and adjacent countries for 2000 years (Zhou et al. 2020). Modern scientific studies have revealed several medicinal attributes of “Sanghuang”, including antitumor, antioxidant, anti-inflammatory, and immunomodulation activities (Zhou et al. 2020). This fungal resource has also attracted the attentions of fungal chemists and pharmacologists outside Asia (Chepkirui et al. 2018; Cheng et al. 2019). Natural products, such as polysaccharides, polyphenols, pyrones and terpenes are the bioactive compounds responsible for the medicinal properties of “Sanghuang” (Zhou et al. 2020). Today, “Sanghuang” is mainly consumed in a brewed tea made from small pieces of cultivated basidiomes or occasionally powdered mycelia.

Like other wood-inhabiting traditional 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, 2012c) and “Fuhling” (Redhead and Ginns 2006), there has been much debate about the taxonomic identity of “Sanghuang”. Most fungal taxonomists now agree that “Sanghuang” is represented by species of Sanghuangporus (Zhou et al. 2020). Fourteen species have been described and accepted as members of Sanghuangporus: 11 species in Asia, and one in each of Africa, Europe, and North America (Zhou et al. 2020). In addition, more new species await to be described from Africa (Chepkirui et al. 2018; Cheng et al. 2019) and perhaps other parts of the world. Besides morphological and ecological (host preference) characters, the ITS barcoding region provides the most powerful tool for differentiating species of the genus. For example, more than half of the known species of Sanghuangporus were discovered with the aid of the ITS region alone (Wu et al. 2012a, 2019b; Tian et al. 2013; Ghabad-Nejhad 2015; Tomšovský 2015; Zhu et al. 2017). Moreover, the reliability of the ITS region for species differentiation in the genus has been substantiated by a multilocus-based phylogenetic analysis (Zhu et al. 2019). Consequently, Zhou et al. (2020) reported ITS sequences from reliably identified voucher collections of the known species in the genus.

Transdisciplinary studies on Sanghuangporus have been performed to promote the utilization of this medicinal resource (Zhou et al. 2016; Cai et al. 2019; Zhu et al. 2019; Shao et al. 2020). Most of these studies aimed to identify their materials via a BLAST search of GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using the ITS barcoding region as the query. However, even though each of the 14 species of Sanghuangporus has a reliable ITS sequence accession number (Zhou et al. 2020), it is not always easy to determine material in hand by a simple ITS-based BLAST search. This is a consequence of redundant and even incorrectly labeled ITS sequences in GenBank (Nilsson et al. 2006; Hofstetter et al. 2019). With inaccurately identified sequences emerging as potential matches, more collections will inevitably be inaccurately identified and the ITS sequences generated from the inaccurately identified collections will be submitted to GenBank compounding the issue and presenting new obstacles for later accurate identification. This means that there is high likelihood of medicinal and other attributes being attributed to incorrectly named species of “Sanghuang”. Meanwhile, before the erection of the genus Sanghuangporus (Zhou et al. 2016), ITS sequences generated from “Sanghuang” were labeled under other generic names, such as Inonotus and Phellinus, even though with the correct epithets. This phenomenon confuses researchers who lack taxonomic knowledge, and results in a misapplication of species names to medicinal properties, which then has a negative effect on obtaining permissions from regulatory authorities for commercial development (Zhou 2020).

As stated by Zhou (2020), the use of correct scientific names for fungal species is crucial to studies of traditional Chinese medicine and their commercial exploitation. To facilitate the rational medicinal utilization of Sanghuangporus, all ITS sequences related to “Sanghuang” in GenBank should be re-examined to assist species identification. The aim of the current study is therefore to assess the utility of the ITS region for species discrimination in Sanghuangporus, and reset the species circumscriptions on the basis of the ITS barcoding region, in order to facilitate the correction of previously mislabeled ITS sequences in GenBank, and to provide candidate diagnostic ITS sequences for use in rapid species identification of Sanghuangporus using Hyper-branched Rolling Circle Amplification (HRCA).

MATERIALS AND METHODS

Morphological examination

The newly sequenced specimens and strains are deposited in HMAS, IFP and BJFC. The specimens were observed with an Olympus BX43 light microscope (Tokyo, Japan) at magnifications up to 1000×. Microscopic procedure followed Zhou et al. (2016). Specimen sections were prepared in Cotton blue (CB), Melzer’s reagent (IKI), and 5% potassium hydroxide (KOH). All measurements were made from material mounted in heated CB. When presenting the variation of basidiospore sizes, 5% of the measurements were excluded from each end of the range and are given in parentheses. Drawings were made with the aid of a drawing tube. In the text, L = mean basidiospore length (arithmetic average of all measured basidiospores), W = mean basidiospore width (arithmetic average
of all measured basidiospores), \( Q \) = variation in the \( L/W \) ratios between the studied specimens, and \((a/b) = \) number of basidiospores \((a)\) measured from given number \((b)\) of specimens.

Molecular sequencing
A small piece of the basidiome or culture was taken for DNA extraction, which was performed using a CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies, Beijing). The crude DNA was used as templates for the PCR amplifications of the 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. The PCR procedure was as follows: 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).

Downloading sequences from GenBank
The genus name Sanghuangporus and the epithets of 14 Sanghuangporus species were used first as queries to search GenBank. Meanwhile, the reliable sequences of 14 Sanghuangporus species (Zhao et al. 2020) were used as queries to perform BLAST searches in GenBank. The cut-off value of similarity for the resulting sequences was set as 95%. All the ITS sequences matching these queries that had been deposited until 30 April 2020 were retrieved from GenBank (Table 1). In addition, recently published papers related to the taxonomy of Sanghuangporus were checked for supplementary information on collections generating these sequences (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; Huo et al. 2020; Shao et al. 2020).

Phylogenetic analyses
Two datasets of ITS sequences were assembled, one consisting of all sequences recovered from searches of GenBank and newly generated sequences, and the other consisting of the subset of sequences originating from material identified by taxonomists. The datasets were separately aligned using MAFFT 7.110 (Katoh and Standley 2013) under the G-INS-i option (Katoh et al. 2005). All resulting alignments are deposited in TreeBASE (http://www.treebase.org; accession number S26272), jModelTest (Guindon and Gascuel 2003; Posada 2008) was used to estimate the best-fit evolutionary model for each alignment with calculations made under the 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. 2021), 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 (BPs). Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) was used to judge the convergence of the chains.

Evaluation of molecular species delimitation
Molecular species delimitation was estimated using multi-rate Poisson Tree Processes (mPTP) method (Kapli et al. 2017). The Newick tree file generated from the ML algorithm was directly uploaded to the web-service version (https://mptp.h-its.org/#/tree) with no outgroup taxon.

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

Identification of diagnostic ITS sequences
Identification of diagnostic ITS sequences was according to the alignment of the ITS sequences generated using MAFFT 7.110 (Katoh and Standley 2013) under the G-INS-i option (Katoh et al. 2005); if a fragment was more than one nucleotide long and was unique for one species and not variant within this species then 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 criteria, 240 ITS sequences were downloaded from GenBank, but two sequences (HQ845057 and KP974834, originally identified as Inonotus vaninii and Sanghuangporus baumii, respectively) showed unexpectedly large differences from other sequences of Sanghuangporus by BLAST search, and thus were considered not to belong to the genus and were excluded from subsequent phylogenetic analyses (Table 1). Eventually, a dataset of all available
| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|---------------------------|-------------------------|-------------|-------------|------------|------------------|------------------|------------------------|
| 1.  | S. alpinus                | I. alpinus              | Cui 9646    | JQ860313*   | Angiosperm | Tibet, China     | Specimen         | Tian XM et al.         |
| 2.  | S. alpinus                | I. alpinus              | Cui 9652    | JQ860309*   | Angiosperm | Tibet, China     | Specimen         | Tian XM et al.         |
| 3.  | S. alpinus                | I. alpinus              | Cui 9658    | JQ860310*   | Angiosperm | Tibet, China     | Specimen         | Tian XM et al.         |
| 4.  | S. alpinus                | I. alpinus              | Cui 9666    | JQ860311*   | Angiosperm | Tibet, China     | Specimen         | Tian XM et al.         |
| 5.  | S. alpinus                | I. alpinus              | Cui 12444   | MF772782*   | Lonicera   | Sichuan, China  | Specimen         | Zhu L & Cui BK         |
| 6.  | S. alpinus                | I. alpinus              | Cui 12474   | MF772783*   | Lonicera   | Sichuan, China  | Specimen         | Zhu L & Cui BK         |
| 7.  | S. alpinus                | I. alpinus              | Cui 12485   | MF772781*   | Lonicera   | Sichuan, China  | Specimen         | Zhu L & Cui BK         |
| 8.  | I. alpinus                | Cui 35                 | JQ860312*   | Angiosperm  | Tibet, China | Specimen         | Tian XM et al.    |                       |
| 9.  | S. alpinus                | Yuan 6396 (IFP)        | MT348577*   | Lonicera    | Qinghai, China | Specimen    | This study    |                       |
| 10. | S. alpinus                | Yuan 6405 (IFP)        | MT348578*   | Lonicera    | Qinghai, China | Specimen    | This study    |                       |
| 11. | S. alpinus                | Yuan 6438 (IFP)        | MT343579*   | Angiosperm  | Qinghai, China | Specimen    | This study    |                       |
| 12. | S. baumii                 | T. linteus             | ASI 26030   | KT862142    | South Korea | Strain    | Han JG et al. |                       |
| 13. | S. baumii                 | T. linteus             | ASI 26086   | KT862157    | South Korea | Strain    | Han JG et al. |                       |
| 14. | S. baumii                 | ASI 26087              | KT862158    | Mokpo, South Korea | Strain    | Han JG et al. |                       |
| 15. | S. baumii                 | ASI 26108              | KT862162    | Inje, South Korea | Strain    | Han JG et al. |                       |
| 16. | I. baumii                 | Cui 3573               | JQ860307*   | Syringa     | Jilin, China | Specimen    | Tian XM et al. |                       |
| 17. | S. baumii                 | Cui 11769              | MF772784*   | Angiosperm  | Heilongjiang, China | Specimen    | Zhu L & Cui BK |                       |
| 18. | S. baumii                 | Cui 11903              | KY328305*   | Alnus       | Heilongjiang, China | Specimen    | Zhu L & Cui BK |                       |
| 19. | S. baumii                 | Dai 2340               | AF534069    | Strain      | Lim YW et al. | Steering    |                       |                       |
| 20. | I. baumii                 | Dai 3683               | JN642567*   | Syringa     | Heilongjiang, China | Strain    | Wu SH et al. |                       |
| 21. | I. baumii                 | Dai 3684               | JN642568*   | Syringa     | Heilongjiang, China | Strain    | Wu SH et al. |                       |
| 22. | I. baumii                 | Dai 3694               | JN642569*   | Syringa     | Heilongjiang, China | Strain    | Wu SH et al. |                       |
| 23. | S. baumii                 | Dai 16900              | MF772785*   | Syringa     | Heilongjiang, China | Specimen    | Zhu L & Cui BK |                       |
| 24. | I. baumii                 | FS 656165              | HMS84807    | Strain      | Yu TW |                       |                       |                       |
| 25. | I. baumii                 | FS 656164              | GU903007    | Strain      | Yu TW |                       |                       |                       |
| 26. | I. baumii                 | HLJU                   | KC312696    | Strain      | Liu Y et al. |                       |                       |                       |
| 27. | S. baumii                 | KUC 10644              | MH168100    | Strain      | Heo YM et al. |                       |                       |                       |
| 28. | I. baumii                 | KUC 2570809–20         | KJ668511    | South Korea | Specimen    | Jang Y & Kim JJ |                       |                       |
| 29. | I. baumii                 | MDJCBS 84              | DQ103887    | Strain      | Jian J et al. |                       |                       |                       |
| 30. | I. baumii                 | SFC 050511–32          | AY972811    | Strain      | Jung HS & Lee JS |                       |                       |                       |
| 31. | I. baumii                 | SFC 050527–67          | AY972812    | Strain      | Jung HS & Lee JS |                       |                       |                       |
| 32. | P. baumii                 | SFC 960405–4           | AF534068    | Strain      | Lim YW et al. |                       |                       |                       |
| 33. | S. baumii                 | SFCC 50029             | AYS98608    | Strain      | Jeong WI et al. |                       |                       |                       |
| 34. | I. baumii                 | SH 3                   | FJI90412    | Strain      | Zou L et al. |                       |                       |                       |
| 35. | S. baumii                 | Yuan 4909              | KY328310*   | Angiosperm  | Heilongjiang, China | Specimen    | Zhu L & Cui BK |                       |
| 36. | S. baumii                 | Yuan 4929              | KY328306*   | Angiosperm  | Heilongjiang, China | Specimen    | Zhu L & Cui BK |                       |
| 37. | S. ligneus                | MG 12                  | KR073081*   | Lonicera caucasica | Iran | Strain    | Ghobad-Nejhad M |                       |
| 38. | S. ligneus                | MG 13                  | KR073082*   | Lonicera caucasica | Iran | Strain    | Ghobad-Nejhad M |                       |
| 39. | S. lonicericola           | I. baumii              | BM-3753     | HQ845063    | China | Strain    | Hu W & Deng X |                       |
| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|---------------------------|-------------------------|-------------|-------------|------------|-------------------|------------------|------------------------|
| 40. | *I. baumii*               |                         | BM-8335     | HQ845064    |            | China             | Strain           | Hu W & Deng X         |
| 41. | *S. lonicericola*         | Cui 10994               |             | MF772786*   |            | China             | Specimen         | Zhu L & Cui BK        |
| 42. | *I. lonicericola*         | Dai 8322                |             | JN642571*   | *Lonicera* | Heilongjiang, China | Specimen | Wu SH et al.           |
| 43. | *I. lonicericola*         | Dai 8335                |             | JN642573*   | *Lonicera* | Heilongjiang, China | Specimen | Wu SH et al.           |
| 44. | *I. lonicericola*         | Dai 8340                |             | JN642574*   | *Lonicera* | Heilongjiang, China | Specimen | Wu SH et al.           |
| 45. | *I. lonicericola*         | Dai 8376                |             | JQ660308*   | *Lonicera* | Heilongjiang, China | Specimen | Tian XM et al.         |
| 46. | *S. lonicericola*         | Dai 17304 (BJFC)        | MT348582*   |             | *Lonicera* | Liaoning, China   | Strain           | This study             |
| 47. | *P. sp.*                  | HN100K9                 |             | KF589300    |            | South Korea       | Strain           | Kang HW & Kim JK     |
| 48. | *P. ribis*                | SFCC 50032              |             | AY558643    |            | Russia            | Strain           | Jeong Wi et al.       |
| 49. | *I. lonicericola*         | TAA 105317              |             | JN642572*   | *Lonicera* | Russian Far East  | Specimen         | Wu SH et al.           |
| 50. | *S. lonicinclus*          | Dai 17093               |             | MF772788*   | *Lonicera* | Uzbekistan        | Specimen         | Zhu L & Cui BK        |
| 51. | *S. lonicinclus*          | Dai 17095               |             | MF772787*   | *Lonicera* | Uzbekistan        | Specimen         | Zhu L & Cui BK        |
| 52. | *S. lonicinclus*          | MG 280                  |             | KU213573*   |            |                   | Specimen         | Langer EJ & Ghabad-Nejhad M |
| 53. | *S. lonicinclus*          | MG 281                  |             | KU213574*   |            |                   | Specimen         | Langer EJ & Ghabad-Nejhad M |
| 54. | *I. sp.*                  | TAA 55428               |             | JN642575*   | *Lonicera* | Turkmenistan      | Strain           | Wu SH et al.           |
| 55. | *S. lonicinclus*          | TAA 55696               | MT348583*   |             | *Lonicera* | Turkmenistan      | Specimen         | This study             |
| 56. | *P. linneus*              | TAA-104264              |             | AF534074    |            |                   | Strain           | Lim YW et al.         |
| 57. | *S. microcystideus*       | O 915609                |             | KP030787*   | *Olea africana* | Tanzania | Specimen         | Zhou LW et al.       |
| 58. | *S. pilatii*              | BRNM 771989             | KT428764*   |             | *Populus alba* | Czech Republic  | Specimen         | Tomšovský M           |
| 59. | *S. quercicola*           | CBS 282.77              |             | AY558642    |            |                   | Strain           | Jeong Wi et al.       |
| 60. | *S. quercicola*           | Dai 13947               |             | KY328309*   |            | Chongqing, China  | Specimen         | Zhu L & Cui BK        |
| 61. | *S. quercicola*           | Li 445                  |             | KY328311*   | *Angiosperm* | Henan, China     | Specimen         | Zhu L & Cui BK        |
| 62. | *S. quercicola*           | Li 1149                 |             | KY328312*   | *Quercus*  | Henan, China     | Specimen         | Zhu L & Cui BK        |
| 63. | *S. quercicola*           | LWZ 20170821–13 (IFP)   | MT348584*   |             | *Angiosperm* | Hubei, China    | Specimen         | This study             |
| 64. | *S. quercicola*           | LWZ 20170821–14 (IFP)   | MT348585*   |             | *Angiosperm* | Hubei, China    | Specimen         | This study             |
| 65. | *S. quercicola*           | LWZ 20170821–18 (IFP)   | MT348586*   |             | *Angiosperm* | Hubei, China    | Specimen         | This study             |
| 66. | *S. quercicola*           | Wei 7575 (IFP)          | MT348587*   |             | *Quercus*  | Henan, China     | Specimen         | This study             |
| 67. | *S. sp.*                  | Wu 1805–2               |             | MK010422*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 68. | *S. sp.*                  | Wu 1805–3               |             | MK010423*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 69. | *S. sp.*                  | Wu 1805–5               |             | MK010424*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 70. | *S. sp.*                  | Wu 1807–2               |             | MK729538*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 71. | *S. sp.*                  | Wu 1807–3               |             | MK729540*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 72. | *S. sp.*                  | Wu 1807–4               |             | MK729539*   | *Taxodiumendron* | Hubei, China | Specimen         | Wu SH et al.           |
| 73. | *S. sanghuang*            | *I. baumii*             | KM385537    |             |            | Viet Nam         | Strain           | Hahn VV & Nguyet NT   |
| 74. | *S. sanghuang*            | AH1 (HMAS)              | MT421899*   |             | *Cultivated* | Anhui, China     | Strain           | This study             |
| 75. | *S. sanghuang*            | AH2 (HMAS)              | MT421900*   |             | *Cultivated* | Anhui, China     | Strain           | This study             |
| 76. | *S. sanghuang*            | AH3 (HMAS)              | MT421901*   |             | *Cultivated* | Anhui, China     | Strain           | This study             |
| 77. | *S. sanghuang*            | AH4 (HMAS)              | MT421902*   |             | *Cultivated* | Anhui, China     | Strain           | This study             |
| 78. | *S. sanghuang*            | AHS (HMAS)              | MT421903*   |             | *Cultivated* | Anhui, China     | Strain           | This study             |
| 79. | *P. igniarius*            | ASI 26010               | KT862134    |             |            | Jeongseon, South Korea | Strain | Han JG et al.         |
| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|--------------------------|------------------------|-------------|-------------|------------|------------------|------------------|------------------------|
| 80. | T. linteus               | ASI 26011              | KT862135    | India       | Strain Han JG et al. |
| 81. | T. linteus               | ASI 26016              | KT862136    | South Korea | Strain Han JG et al. |
| 82. | T. linteus               | ASI 26021              | KT862138    | Hongcheon, South Korea | Strain Han JG et al. |
| 83. | T. linteus               | ASI 26022              | KT862139    | Hongcheon, South Korea | Strain Han JG et al. |
| 84. | T. linteus               | ASI 26025              | KT862140    | Wonju, South Korea | Strain Han JG et al. |
| 85. | T. linteus               | ASI 26026              | KT862141    | Wonju, South Korea | Strain Han JG et al. |
| 86. | T. linteus               | ASI 26039              | KT862143    | Pyeongchang, South Korea | Strain Han JG et al. |
| 87. | T. linteus               | ASI 26046              | KT862144    | Hongcheon, South Korea | Strain Han JG et al. |
| 88. | T. linteus               | ASI 26049              | KT862145    | Hongcheon, South Korea | Strain Han JG et al. |
| 89. | T. linteus               | ASI 26054              | KT862147    | Hongcheon, South Korea | Strain Han JG et al. |
| 90. | T. linteus               | ASI 26052              | KT862148    | Hwacheon, South Korea | Strain Han JG et al. |
| 91. | T. linteus               | ASI 26063              | KT862149    | Jeongseon, South Korea | Strain Han JG et al. |
| 92. | T. linteus               | ASI 26066              | KT862150    | Inje, South Korea | Strain Han JG et al. |
| 93. | T. linteus               | ASI 26067              | KT862151    | Inje, South Korea | Strain Han JG et al. |
| 94. | T. linteus               | ASI 26070              | KT862152    | Strain Han JG et al. |
| 95. | T. linteus               | ASI 26071              | KT862153    | Strain Han JG et al. |
| 96. | T. linteus               | ASI 26073              | KT862154    | South Korea | Strain Han JG et al. |
| 97. | T. linteus               | ASI 26074              | KT862155    | Seongnam, South Korea | Strain Han JG et al. |
| 98. | T. linteus               | ASI 26082              | KT862156    | Mokpo, South Korea | Strain Han JG et al. |
| 99. | T. linteus               | ASI 26088              | KT862159    | Sancheong, South Korea | Strain Han JG et al. |
| 100. | T. linteus              | ASI 26114              | KT862164    | South Korea | Strain Han JG et al. |
| 101. | T. linteus              | ASI 26115              | KT862165    | South Korea | Strain Han JG et al. |
| 102. | P. linteus               | ATCC 26710             | AF153010    | South Korea | Strain Kim GY et al. |
| 103. | S. sanghuang             | Batch 1-12192170-1     | KT693244    | Purchased | USA | Strain Raja HA et al. |
| 104. | S. sanghuang             | Batch 2-10221252-2     | KT693275    | Purchased | USA | Strain Raja HA et al. |
| 105. | S. sanghuang             | Batch 2-12192170-1     | KT693246    | Purchased | USA | Strain Raja HA et al. |
| 106. | S. sanghuang             | BJ (HMAS)              | MT421904*   | Cultivated | Beijing, China | Strain | This study |
| 107. | I. sp.                   | BZ-A                   | JN642589*   | Morus | Hunan, China | Strain | Wu SH et al. |
| 108. | I. sp.                   | BZ-C                   | JN642587*   | Morus | Hunan, China | Strain | Wu SH et al. |
| 109. | I. sp.                   | CA                     | JN642579*   | Morus | Jiangxi, China | Strain | Wu SH et al. |
| 110. | I. sp.                   | CB                     | JN642580*   | Morus | Jiangxi, China | Strain | Wu SH et al. |
| 111. | I. sp.                   | CC                     | JN642581*   | Morus | Jiangxi, China | Strain | Wu SH et al. |
| 112. | S. sanghuang             | Cui 14419              | MF772789*   | Morus | Shaanxi, China | Specimen | Zhu L & Cui BK |
| 113. | S. sanghuang             | Cui 14420              | MF772790*   | Morus | Shaanxi, China | Specimen | Zhu L & Cui BK |
| 114. | I. sanghuang             | Dai 12723              | QJ603616*   | Morus | Sichuan, China | Specimen | Tian XM et al. |
| 115. | S. sanghuang             | DB1 (HMAS)             | MT421905*   | Cultivated | Northeast China | Strain | This study |
| 116. | P. linteus               | DGUM25003              | AF082102    | | | Strain | Chung JW et al. |
| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|----------------------------|-------------------------|-------------|-------------|-------------|-------------------|------------------|------------------------|
| 117. | *P. linteus*             | DGUM25004                |             | AF080458    |             |                   | Strain            | Chung JW et al.       |
| 118. | *I. linteus*             | FS 656160                |             | GU903004    |             |                   | Strain            | Yu TW                 |
| 119. | *I. linteus*             | FS 656161                |             | HMS84806    |             |                   | Strain            | Yu TW                 |
| 120. | *T. linteus*             | FS 656179                |             | KU867779    |             |                   | Strain            | Yu TW                 |
| 121. | *T. linteus*             | FS 656180                |             | KU867780    |             |                   | Strain            | Yu TW                 |
| 122. | *S. sanghuang*           | HB (HMAS)                | MT421907\*  |             | Cultivated   | Hubei, China      | Strain            | This study            |
| 123. | *P. linteus*             | IFO 6980                 |             | AF200226    |             |                   | Strain            | Kim GY & Lee JD      |
| 124. | *I. linteus*             | IFO 6989                 |             | AY604937    |             |                   | Strain            | Lee JS & Jung HS     |
| 125. | *P. linteus*             | IMSNU 31014              |             | AF082101    |             |                   | Strain            | Chung JW et al.       |
| 126. | *S. sanghuang*           | JL-01                    |             | G062389     |             |                   | Strain            | Xu X                  |
| 127. | *S. sanghuang*           | JS1 (HMAS)               | MT421908\*  |             | Cultivated   | Jiangsu, China    | Strain            | This study            |
| 128. | *I. linteus*             | KAB-PL-01                |             | DQ462333    | Taiwan, China |                   | Strain            | Chioo SJ & Yen JH    |
| 129. | *P. linteus*             | KCTC 6190                |             | AF077678    |             |                   | Strain            | Chung JW et al.       |
| 130. | *P. igniarius*           | KCTC 16890               |             | AY189708    |             |                   | Strain            | Nam BH et al.         |
| 131. | *I. linteus*             | KFDA 016                 |             | YA436266    |             |                   | Strain            | Yun JC et al.         |
| 132. | *I. linteus*             | KFDA P38                 |             | AY51324     |             |                   | Strain            | Jin CY et al.         |
| 133. | *I. linteus*             | KSSW01                   |             | EF06943     |             |                   | Strain            | Park SY et al.        |
| 134. | *I. linteus*             | LT-0802                  |             | HQ845059    | South Korea  |                   | Strain            | Hu W & Deng X         |
| 135. | *I. linteus*             | LT-CBS83                 |             | HQ845060    | South Korea  |                   | Strain            | Hu W & Deng X         |
| 136. | *S. sanghuang*           | LWZ 20180927–3 (HMAS)    | MT348588\*  | Morus       | Yunnan, China | Specimen          | Strain            | This study            |
| 137. | *P. linteus*             | MPNU 7016                |             | AF153009    |             |                   | Strain            | Kim GY et al.         |
| 138. | *I. linteus*             | MUCL 47139               |             | GA461973    | Cuba        |                   | Strain            | Amalfi M et al.       |
| 139. | *I. linteus*             | NAAS00002                |             | JNO43317    |             |                   | Strain            | Seok SJ et al.        |
| 140. | *P. linteus*             | Namsan No1               |             | AF080457    |             |                   | Strain            | Chung JW et al.       |
| 141. | *I. linteus*             | PL 0801                  |             | FJ940906    |             |                   | Strain            | Xie LY et al.         |
| 142. | *I. linteus*             | PL 5                     |             | EF059712    |             |                   | Strain            | Park BW et al.        |
| 143. | *I. sp.*                 | PL 10                    |             | JN642588\*  | China        |                   | Strain            | Wu SH et al.          |
| 144. | *S. sanghuang*           | S3                       |             | MN153568    |             |                   | Strain            | Song JL et al.        |
| 145. | *P. sp.*                 | SA 01                    |             | EF694971    |             |                   | Strain            | Zeng NK et al.        |
| 146. | *P. baumii*              | SFC 20001106–1           |             | AF534064    |             |                   | Strain            | Lim YW et al.         |
| 147. | *P. baumii*              | SFC 20010212–1           |             | AF534062    |             |                   | Strain            | Lim YW et al.         |
| 148. | *S. sanghuang*           | SS                       |             | MG200921    |             |                   | Strain            | Cai C & Zhao G        |
| 149. | *I. sp.*                 | T004                     |             | JN642586\*  | Morus       | Taiwan, China     | Strain            | Wu SH et al.          |
| 150. | *I. sp.*                 | TH                       |             | JN642582\*  | Morus       | Taiwan, China     | Strain            | Wu SH et al.          |
| 151. | *I. sp.*                 | TJ                       |             | JN642585\*  | Morus       | Taiwan, China     | Strain            | Wu SH et al.          |
| 152. | *I. sp.*                 | TM                       |             | JN642583\*  | Morus       | Taiwan, China     | Strain            | Wu SH et al.          |
| 153. | *I. sp.*                 | TN                       |             | JN642584\*  | Morus       | Taiwan, China     | Strain            | Wu SH et al.          |
| 154. | *I. sp.*                 | WD 1222                  |             | JN642576\*  | Morus       | Japan             | Strain            | Wu SH et al.          |
| 155. | *I. sp.*                 | WD 2261                  |             | JN642577\*  | Morus       | Japan             | Strain            | Wu SH et al.          |
| 156. | *I. sp.*                 | WD 2300                  |             | JN642578\*  | Morus       | Japan             | Strain            | Wu SH et al.          |
| 157. | *I. sp.*                 | Wu 0903–1                |             | JN740601*   | Morus       | Jilin, China      | Strain            | Wu SH et al.          |
| 158. | *I. sp.*                 | Zhangjiajie              |             | MN242716    | Cultivated   |                   | Strain            | Wang Y                |
| 159. | *S. sanghuang*           | ZJ1 (HMAS)               | MT421910\*  | Cultivated  | Zhejiang, China | Strain            | This study          |
| 160. | *S. sanghuang*           | ZJ2 (HMAS)               | MT421911\*  | Cultivated  | Zhejiang, China | Strain            | This study          |
Table 1 Information of analyzed ITS sequences of Sanghuangporus (Continued)

| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|---------------------------|-------------------------|-------------|-------------|-------------|-------------------|-------------------|------------------------|
| 161 | S. sanghuang              | ZJ4 (HAMAS)             | MT421913    | Cultivated  | Zhejiang, China | Strain            | This study        |                        |
| 162 | S. sanghuang              | ZJ5 (HAMAS)             | MT421914    | Cultivated  | Zhejiang, China | Strain            | This study        |                        |
| 163 | S. subbaumii              | I. baumii BZ-2029       | JNE642565   | Purchased   | China        | Strain            | Wu SH et al.      |                        |
| 164 | S. subbaumii              | I. baumii BZ-2030       | JNE642566   | Purchased   | China        | Strain            | Wu SH et al.      |                        |
| 165 | S. subbaumii              | Dai 13360 (BJFC)        | MT343580    | Prunus      | Shanxi, China | Specimen          | This study        |                        |
| 166 | S. subbaumii              | LWZ 20190722–18 (HAMAS) | MT348581    | Angiosperm  | Beijing, China | Specimen          | This study        |                        |
| 167 | I. baumii                 | Wu 0910-54              | JNE642570   | Syringa     | Beijing, China | Strain            | Wu SH et al.      |                        |
| 168 | I. baumii                 | Yuan 2444               | JNE642592   | Populus     | China        | Strain            | Wu SH et al.      |                        |
| 169 | I. baumii                 | CJC 01                  | JNE642592   | Populus     | China        | Strain            | Wu SH et al.      |                        |
| 170 | I. baumii                 | Cui 9939                | MF772792    | Jilin, China | Specimen      | Zhu L & Cui BK    |                  |                        |
| 171 | I. vaninii                | SFC 970527–1            | AF534073    | Angiosperm  | China        | Strain            | Liu YW et al.     |                        |
| 172 | I. vaninii                | Wu 0910-54              | JNE642570   | Syringa     | Beijing, China | Strain            | Wu SH et al.      |                        |
| 173 | I. vaninii                | Yuan 2444               | JNE642592   | Populus     | China        | Strain            | Wu SH et al.      |                        |
| 174 | I. vaninii                | Cui 14082               | MF772793    | Jilin, China | Specimen      | Zhu L & Cui BK    |                  |                        |
| 175 | I. vaninii                | Dai 3624                | JNE642590   | Populus     | China        | Strain            | Wu SH et al.      |                        |
| 176 | I. vaninii                | Dai 7011                | JNE642591   | Populus davidiana | Jilin, China | Specimen            | Wu SH et al.      |                        |
| 177 | I. vaninii                | Dai 8236                | MF772791    | Jilin, China | Specimen      | Zhu L & Cui BK    |                  |                        |
| 178 | I. vaninii                | DB2 (HAMAS)             | MT421906    | Cultivated  | Northeast China | Strain          | This study        |                        |
| 179 | I. vaninii                | FS 656170               | GUJ903008   | Strain      | Ya TW        | Strain            | Wu SH et al.      |                        |
| 180 | F. gilva                  | FS 656175               | HMR84811    | Strain      | You TW       | Strain            | Wu SH et al.      |                        |
| 181 | S. vaninii                | HZ-01                   | MG062791    | Strain      | Xue et al.   | Strain            | Wu SH et al.      |                        |
| 182 | I. vaninii                | JinZhai                 | MN242717    | Cultivated  | China        | Strain            | Wang Y            |                        |
| 183 | I. vaninii                | JS2 (HAMAS)             | MT421909    | Cultivated  | Jiangsu, China | Strain          | This study        |                        |
| 184 | I. vaninii                | JS2 (HAMAS)             | MT421909    | Cultivated  | Northeast China | Strain          | This study        |                        |
| 185 | I. vaninii                | KangNeng                | MN242721    | Cultivated  | China        | Strain            | Wang Y            |                        |
| 186 | I. baumii                 | KFDA 015                | AY436623    | Strain      | Yun JC et al. | Strain            | Wu SH et al.      |                        |
| 187 | I. baumii                 | KFDA 022                | AY436624    | Strain      | Yun JC et al. | Strain            | Wu SH et al.      |                        |
| 188 | I. baumii                 | KFDA 024                | AY436627    | Strain      | Yun JC et al. | Strain            | Wu SH et al.      |                        |
| 189 | I. baumii                 | KFDA 029                | AY436625    | Strain      | Yun JC et al. | Strain            | Wu SH et al.      |                        |
| 190 | I. baumii                 | KFDA P36                | AY509198    | Strain      | Jin CY et al. | Strain            | Wu SH et al.      |                        |
| 191 | I. baumii                 | KFDA P40                | AY509199    | Strain      | Jin CY et al. | Strain            | Wu SH et al.      |                        |
| 192 | I. baumii                 | KFDA P45                | AY509201    | Strain      | Jin CY et al. | Strain            | Wu SH et al.      |                        |
| 193 | I. baumii                 | Korea                   | MN242719    | Cultivated  | China        | Strain            | Wang Y            |                        |
| 194 | S. baumii                 | LC 6686                 | MK818502    | Strain      | Li ZN        | Strain            | Wu SH et al.      |                        |
| 195 | I. linteus                | LT-HG                   | HQR845061   | Strain      | Hu W & Deng X | Strain            | Wu SH et al.      |                        |
| 196 | F. gilva                  | MDJCS87                 | DQ108884    | Strain      | Jiang J et al. | Strain            | Wu SH et al.      |                        |
| 197 | P. baumii                 | MPNU 7004               | AF200229    | Strain      | Kim GY & Lee JD | Strain            | Wu SH et al.      |                        |
| 198 | P. baumii                 | MPNU 7005               | AF200230    | Strain      | Kim GY & Lee JD | Strain            | Wu SH et al.      |                        |
| 199 | P. baumii                 | MPNU 7006               | AF200231    | Strain      | Kim GY & Lee JD | Strain            | Wu SH et al.      |                        |
| 200 | P. sp.                    | MPNU 7007               | AF200235    | Strain      | Kim GY & Lee JD | Strain            | Wu SH et al.      |                        |
| 201 | P. sp.                    | MPNU 7010               | AF153007    | South Korea | Strain        | Kim GY et al.     | South Korea       |                        |
| 202 | P. sp.                    | MPNU 7012               | AF153008    | South Korea | Strain        | Kim GY et al.     | South Korea       |                        |
| 203 | P. sp.                    | MPNU 7013               | AF153011    | South Korea | Strain        | Kim GY et al.     | South Korea       |                        |
| 204 | I. baumii                 | PB 0802                 | FJ940907    | Strain      | Xie LY et al. | Strain            | Wu SH et al.      |                        |
Table 1 Information of analyzed ITS sequences of *Sanghuangporus* (Continued)

| No. | Species name accepted here | Species name in GenBank | Voucher No. | GenBank No. | Host plant | Geographic origin | Type of material | Identifier of material |
|-----|---------------------------|-------------------------|-------------|-------------|------------|------------------|------------------|------------------------|
| 205 | *I. baumii*               | PB 0803                 | FJ940908    |             |            |                  |                  | Xie LY et al.          |
| 206 | *I. baumii*               | PB 0806                 | FJ940911    |             |            |                  |                  | Xie LY et al.          |
| 207 | *I. baumii*               | PB 0808                 | FJ940913    |             |            |                  |                  | Xie LY et al.          |
| 208 | *I. baumii*               | PB 0809                 | FJ940914    |             |            |                  |                  | Xie LY et al.          |
| 209 | *I. sp. QianDaoHu*        | QianDaoHu               | MN242718    | Cultivated  | China      |                  |                  | Wang Y                |
| 210 | *S. vaninii*              | S1                      | MN153566    |             | China      |                  |                  | Song JL et al.        |
| 211 | *S. baumii*               | S2                      | MN153567    |             | China      |                  |                  | Song JL et al.        |
| 212 | *F. gilva*                | S12                     | MT275660    | Morus       | Zhejiang, China | Strain |                  | Li Y & Huo J          |
| 213 | *P. sp.*                  | SA 02                   | EF694972    |             |            |                  |                  | Zeng NK et al.        |
| 214 | *P. sp.*                  | SA 03                   | EF694973    |             |            |                  |                  | Zeng NK et al.        |
| 215 | *P. sp.*                  | SA 04                   | EF694974    |             |            |                  |                  | Zeng NK et al.        |
| 216 | *I. baumii*               | SA 05                   | EF694975    |             |            |                  |                  | Zeng NK et al.        |
| 217 | *P. sp.*                  | SA 06                   | EF694976    |             |            |                  |                  | Zeng NK et al.        |
| 218 | *P. sp.*                  | SA 07                   | EF694977    |             |            |                  |                  | Zeng NK et al.        |
| 219 | *P. linteus*              | SFC 970605              | AF534071    |             |            |                  |                  | Lim YW et al.          |
| 220 | *P. linteus*              | SFC 2001106–7           | AF534070    |             |            |                  |                  | Lim YW et al.          |
| 221 | *P. baumii*               | SFC 20010212–2          | AF534063    |             |            |                  |                  | Lim YW et al.          |
| 222 | *T. linteus*              | SFC 10209               | AY558628    |             |            |                  |                  | Jeong WJ et al.        |
| 223 | *F. gilva*                | SH 1                    | FJ190410    |             |            |                  |                  | Zou L et al.           |
| 224 | *I. baumii*               | SJ                      | JN169788a   |             | Jilin, China | Specimen |                | Zhou LW & Qin WM      |
| 225 | *I. vaninii*              | Wei 3382                | JN169788    |             |            |                  |                  | Jilin, China           |
| 226 | *I. vaninii*              | WN 0801                | HX845054    |             | China      |                  |                  | Hu W & Deng X         |
| 227 | *I. vaninii*              | WN-1                   | HX845055    |             | China      |                  |                  | Hu W & Deng X         |
| 228 | *I. vaninii*              | WN-2                   | HX845056    |             | China      |                  |                  | Hu W & Deng X         |
| 229 | *I. vaninii*              | WN-4                   | HX845065    |             | China      |                  |                  | Hu W & Deng X         |
| 230 | *I. vaninii*              | WN 8213                | HX845052    |             | China      |                  |                  | Hu W & Deng X         |
| 231 | *I. vaninii*              | WN 8824                | HX845051    |             | China      |                  |                  | Hu W & Deng X         |
| 232 | *I. vaninii*              | WN 3624                | HX845050    |             | China      |                  |                  | Hu W & Deng X         |
| 233 | *S. baumii*               | XZ-01                  | MG062790    |             | Strain     |                  |                  | Xue X                 |
| 234 | *I. baumii*               | YC                     | JN168792    |             | Strain     |                  |                  | Shin KS               |
| 235 | *S. vaninii*              | Yuan 2764              | KY328308a   | Quercus     | Shaanxi, China | Specimen |                | Zhu L & Cui BK        |
| 236 | *S. vaninii*              | Yuan 5604              | KY328307a   | Quercus     | Jilin, China | Specimen |                | Zhu L & Cui BK        |
| 237 | *S. vaninii*              | ZJ3 (HMAS)             | MT421912a   | Cultivated  | Zhejiang, China | Strain | This study     |                     |
| 238 | *S. weigelae*             | 420526MF0201            | MH142013    |             | Hubei, China | Specimen |                | Wang R et al.         |
| 239 | *I. weigelae*             | Cui 6010               | JQ860318a   | Lonicera     | Jiangxi, China | Specimen |                | Tian XM et al.        |
| 240 | *I. weigelae*             | Cui 6012               | JQ860319a   | Lonicera     | Jiangxi, China | Specimen |                | Tian XM et al.        |
| 241 | *I. weigelae*             | Cui 7176               | JQ860320a   | Syringa      | Hebei, China | Specimen |                | Tian XM et al.        |
| 242 | *I. weigelae*             | Dai 6352               | JQ860317a   |             | Zhejiang, China | Specimen |                | Tian XM et al.        |
| 243 | *I. weigelae*             | Dai 11694              | JQ860315a   |             | Hunan, China | Specimen |                | Tian XM et al.        |
| 244 | *S. weigelae*             | Dai 15770              | MF772795a   | Weigela      | Chongqing, China | Specimen |                | Zhu L & Cui BK        |
| 245 | *S. weigelae*             | Dai 16072 (BJFC)       | MT348589a   | Weigela      | Inner Mongolia, China | Specimen | This study     |                     |
| 246 | *S. weigelae*             | Dai 16077              | MF772794a   | Weigela      | Inner Mongolia, China | Specimen |                | Zhu L & Cui BK        |
| 247 | *S. weigelae*             | LWZ 20150802–3 (IFP)   | MT348590a   | Weigela      | Jiangxi, China | Specimen | This study     |                     |
269 ITS sequences (31 newly sequenced and 238 downloaded from GenBank) from *Sanghuangporus* species was used to construct a preliminary phylogenetic framework for this genus. An alignment of 941 characters 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 topologies in the 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 here; the midpoint-rooted tree recovered 13 species and four undescribed lineages of *Sanghuangporus* (Fig. 1). The one species gap compared with the 14 accepted species is a result of collections previously identified as *S. quercicola* and *S. toxicodendri* (this species is represented by collections Wu 1805–2, Wu 1805–3, Wu 1805–5, Wu 1807–2, Wu 1807–3 and Wu 1807–4) nesting within a single clade (Fig. 1). Of the 13 recovered species of *Sanghuangporus*, the clades of *S. lonicerciola* and *S. sanghuang* did not receive good statistical support, the clade of *S. alpina* was strongly supported just by the BI algorithm, and the 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* merged with *S. sp. 1 in the tree inferred from the ML algorithm (Fig. 1, Additional file 1: Tree S1, 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. One undescribed lineage including seven collections BZ-2029, BZ-2030, Dai 13360, LWZ 20190722–18, SFC 970527–1, Wu 0910–54 and Yuan 2444 showed a close relationship with S. baumii (Fig. 1).

In GenBank, species names from 10 out of 77 phylogenetically analyzed specimens were misapplied (tips labeled in green in Fig. 1), while those from 134 out of 192 phylogenetically analyzed strains were wrongly identified to species level (tips labeled in red in Fig. 1). Furthermore, two ITS sequences (HQ845057 and KP974834) of strains labeled as species of Sanghuangporus were extremely deviant and did not belong to the genus (Table 1). Most of these errors came from submissions by non-taxonomists. Therefore, to circumscribe species in Sanghuangporus, we selected the ITS sequences submitted to GenBank by

**Fig. 1** The phylogenetic tree inferred from 269 ITS sequences. The topology was generated from the maximum likelihood algorithm. The tips in green represent mislabeled specimens, while those in red represent mislabeled strains.
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+1+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 topologies in the main lineages, and so only the midpoint-rooted ML tree is presented along with the BPPs at the nodes (Fig. 2). As in 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, the clade of S. lonicericola was still not strongly supported, and the clades of S. alpinus and S. sanghuang were moderately supported from the ML algorithm and fully supported from the BI algorithm, while the clades of all other species received strong statistical support from both the ML and the BI algorithms (Fig. 2). Moreover, in the seven collections of the undescribed lineage close to S. baumii in Fig. 1, four were sampled in the new dataset, and the independence of these four collections and their affinity to S. baumii were also strongly supported (Fig. 2). Therefore, this undescribed lineage is described as a new species, S. subbaumii, below.

Molecular species delimitation was estimated on the tree generated from the new dataset with 122 selected ITS sequences. The mPTP method supported the independence of 11 species, while Sanghuangporus alpinus, S. lonicerinus and S. weigelae were recovered as a single species (Additional file 3: Fig. S1).

To further explore the species relationships among Sanghuangporus, the alignment with 122 selected ITS sequences underwent a genetic distance analysis. The ranges of the within and between species genetic distances are mostly non-overlapping (Additional file 4: Table S1). Sanghuangporus microcystideus and S. pilatii, each represented by a single collection, were excluded from the within species analysis. Regarding other species of Sanghuangporus, the genetic distances within S. vaninii, S. weirianus and S. zonatus were 0–1.72%, 2.68% and 0–1.71%, respectively, whereas those within other species were no more than 1.30% and as low as 0.00% within S. ligneus (Additional file 4: Table S1). Regarding the genetic distances between species, all were above 1.30% except that those between S. alpinus and S. lonicerinus, and S. baumii and S. subbaumii were 1.03–2.86% and 1.19–3.07%, respectively. Across all pairwise comparisons between species, most (84 of 91) had distances above the maximum within species distance of 2.68% (Additional file 4: Table S1). Furthermore, distances between S. microcystideus and all other species were more than 8.90% and those between S. pilatii and all other species were more than 2.69% (Additional file 4: Table S1).

Based on an integrative taxonomic approach, 14 species of Sanghuangporus are accepted here. Their taxonomic information and reliable ITS sequences (from holotypes where possible) are provided below. Regarding S. baumii, S. lonicericola, S. lonicerinus, S. microcystideus, S. pilatii, S. vaninii, and S. weirianus, their holotypes were too old (50 years old or more) and so were unlikely to be successfully sequenced. Moreover, certain institutions did not make holotypes available for sequencing. Therefore, we use ITS sequences from other reference collections as reliable ITS sequences for those species.

Fifty-four ITS sequences of S. baumii, S. sanghuang and S. vaninii, the most common species in medicinal studies and products (Zhou et al. 2020), were further retrieved from the dataset with 122 selected sequences. These 54 sequences were realigned and the alignment is presented with shaded background (Additional file 5: Fig. S2). From this alignment, ten potential diagnostic sequences with two to six nucleotide differences were identified for HRCA to differentiate species: two for S. baumii, two for S. sanghuang and six for S. vaninii (Additional file 5: Fig. S2, Table 2).

**TAXONOMY**

*Sanghuangporus alpinus* (Y.C. Dai & X.M. Tian) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 340 (2016).

*Basionym*: *Inonotus alpinus* Y.C. Dai & X.M. Tian, *Fungal Diversity* 58: 162 (2013).

*Type*: *China*: Tibet: Linzhi County, Lulang, on living angiosperm tree, 24 Sept. 2010, B.K. Cui, Cui 9658 (BJFC – holotype).

*ITS barcoding sequence*: JQ860310 (from holotype).

*Sanghuangporus baumii* (Pilát) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 340 (2016).

*Basionym*: *Phellinus baumii* Pilát, *Bull. trimest. Soc. mycol. Fr.* 48: 25 (1932).

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

*Type*: *Russia*: Primorsky Krai: Vladivostok, on trunk of *Syringae*, 5 June 1928, M.K. Ziling 267 (PRM 189012 – holotype).

*Reference collection*: *China*: Heilongjiang: Yichun, Fenglin nature reserve, on living trunk of *Syringa*, 8 Sept. 2002, Y.C. Dai, Dai 3683 (IFP)
Fig. 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.
Table 2  Diagnostic sequences with potential for discriminating *Sanghuangporus baumii*, *S. sanghuang*, and *S. vaninii* using Hyperbranched Rolling Circle Amplification. Label and position in alignment are as in Additional file 5: Fig. S2

| Label | Differentiated species | Diagnostic sequence | Position in alignment | Number of diagnostic nucleotides |
|-------|------------------------|----------------------|-----------------------|----------------------------------|
| 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               | 219–224               | 6                                |
| F     | *S. vaninii*           | CCCCC                | 264–278               | 4                                |
| G     | *S. vaninii*           | AG                   | 556–557               | 2                                |
| H     | *S. baumii*            | AGG                  | 650–652               | 2                                |
| I     | *S. vaninii*           | ACG                  | 664–666               | 2                                |
| J     | *S. sanghuang*         | TT                   | 690–691               | 2                                |

*ITS barcoding sequence:* JN642567 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

*Sanghuangporus ligneus* Ghob.-Nejh., *Mycol. Progr.* 14(90): 2 (2015).

*Type:* **Iran:** East Azerbaijan: Khoda-Afarin, Kalaleh-Eslami, Darana, deciduous forest with *Quercus macranthera*, *Lonicera*, *Cornus mas*, and *Crataegus*, on stem of living *Lonicera caucasica*, 10 May 2008, M. Ghobad-Nejhad, 1152 (IFP).

*ITS barcoding sequence:* KR073081 (from holotype).

*Sanghuangporus lonicericola* (Parmasto) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 45: 340 (2016).

*Basionym:* *Phellinus lonicericola* Parmasto, *Folia cryptog. Estonica* 38: 59 (2001).

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

*Type:* **Russia:** Primorsky Krai: Lazovsky Nature Reserve, Petrov island, on trunk of *Lonicera ruprechtiana* in *Taxus* mixed forest, 2 Sept. 1961, E. Parmasto (TAA-M 013933 – holotype).

*Reference collection:* **China:** Heilongjiang: Ningan County, Jingpohu National Scenic Area, on living trunk of *Lonicera*, 8 Sept. 2007, Y.C. Dai, Dai 8376 (IFP)

*ITS barcoding sequence:* JQ860308 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

*Sanghuangporus lonicericum* (Bondartsev) Imazeki, *Bull. Tokyo Sci. Mus.* 6: 107 (1943).

*Porodaedalea lonicera* (Bondartsev) Imazeki, *Col. Ill. Mushrooms Japan*, 2: 191 (1989).

*Inonotus lonicericus* (Bondartsev) Sheng H. Wu et al., *Bot. Studies (Taipei)* 53: 140 (2012).

*Type:* **Uzbekistan:** Samarkand, on trunk of *Lonicera tatarica*, 1926, E. Czerniakowski (LE 22512 – lectotype designated by Bondartsev 1953).

*Reference collection:* **Turkmenistan:** Bakharden: Bakharden, Arvaz, Montes Kopet-dagh, on *Lonicera*, 17 Oct. 1971, E. Trotter, (TAA 55428)

*ITS barcoding sequence:* JN642575 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

*Sanghuangporus microcystideus* (Har. & Pat.) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 340 (2016).

*Basionym:* *Phellinus microcystideus* Har. & Pat., *Bull. Mus. natn. Hist. nat., Paris* 15: 90 (1909).

*Synonym:* *Fomes microcystideus* (Har. & Pat.) Sacc. & Trotter, *Syll. Fung.* 21: 286 (1912).

*Type:* **Congo:** Moyen Oubangui: Grande Forêt, M.A. Chevalier 11431 (FH – holotype).

*Reference collection:* **Tanzania:** Arusha: Arusha National Park, Mount Meru, on trunk of *Olea africana*, 18 Feb. 1976, R. Harjula (O 915609)

*ITS barcoding sequence:* KP030787 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

*Sanghuangporus pilatii* (Černý) Tomšovský, *Phytotaxa* 239: 84 (2015).

*Basionym:* *Phellinus pilatii* Černý, *Česká Mykol.* 22(1): 2 (1968).

*Synonym:* *Porodaedalea pilatii* (Černý) Fasson & Niemelä, *Karstenia* 24(1): 26 (1984).
Type: **Czech Republic**: Břeclav: Tvrdonice, 8 Oct. 1955, A. Černý (PRM 628393 – holotype).
Reference collection: **Czech Republic**: Břeclav: Nové Mlýny, Křivé jezero National Nature Reserve, on *Populus alba*, 22 Oct. 2011, M. Tomášovský 41/2011 (BRNM 771989)

**ITS barcoding sequence**: KT428764 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

**Sanghuangporus quercicola** Lin Zhu & B.K. Cui, *Phytotaxa* **311**: 271 (2017).

Synonym: *Sanghuangporus toxicodendri* Sheng H. Wu et al., *MycoKeys* 57: 106 (2019).

Type: **China**: Henan: Neixiang County, Baotianman Nature Reserve, on dead tree of *Quercus*, 25 Aug. 2006, J. Li, Li 1149 (BJFC – holotype).

**ITS barcoding sequence**: KY328312 (from holotype).

**Sanghuangporus sanghuang** (Sheng H. Wu et al.)

Basionym: *Inonotus sanghuang* Sheng H. Wu et al., *Fungal Diversity* IMA Fungus **2013** (Sheng H. Wu et al.)

Synonym: *Sanghuangporus quercicola* and accepted here).

**ITS barcoding sequence**: JN794061 (from holotype).

**Sanghuangporus subbaumii** Shan Shen, Y.C. Dai & L.W. Zhou, **sp. nov.** (Figs. 3 and 4).

MycoBank MB838235.

Etymology: *subbaumii* (Lat.), refers to the similarity to *Sanghuangporus baumii*.

Diagnosis: Differing from *S. baumii* in having resupinate, effused-reflexed to pileate basidiomes, acute pileal margin and longer hymenial setae (>20 μm in length).

Type: **China**: Shanxi: Jiaocheng County, Pangquangou Nature Reserve, on fallen trunk of *Prunus* sp., 10 Aug. 2013, Y.C. Dai, Dai 13360 (BJFC – holotype; HMAS 281653 – isotype).

Description: Basidiomes perennial, resupinate, effused-reflexed to pileate, without odor or taste and hard corky when fresh, woody hard when dry; to 20 cm long and 5 cm wide when resupinate. Pilei dimidiate, ungulate in section, projecting to 3.5 cm wide, 6 cm long and 4 cm thick at base. Pileal surface dark brown and velutinate when juvenile, mouse-grey to black, glabrous and cracked with age, concentrically zonate and narrowly sulcate; margin yellow brown, acute. Pore surface yellowish brown, glancing; sterile margin distinct, yellowish; pores angular to circular, 5–7 per mm; dissepiments thin, entire. Context yellowish brown to dark brown, woody hard, to 3.5 cm thick. Tubes yellowish brown, darker than pore surface, woody hard, to 0.5 cm long.

Hyphal system monomitic in context, dimitic in trama; generative hyphae simple septate; tissue darkening but otherwise unchanged in KOH. Context generative hyphae occasionally slightly thick-walled with a wide lumen and yellowish, mostly thick-walled with a narrow lumen and yellowish brown, unbranched, frequently septate, more or less regularly arranged, 3.5–4.5 μm diam. Tubes generative hyphae thin to slightly thick-walled, hyaline, occasionally branched, frequently septate, 3–4.5 μm diam; skeletal hyphae dominant, thick-walled with a narrow lumen, yellowish brown, unbranched, rarely septate, subparallel along the tubes, 2.2–3.7 μm diam. Hymenial setae frequent in the mature hymenium, subulate to ventricose, dark brown, thick-walled, 20–35 × 7–12 μm. Cystidia subulate, with narrow and tapering apex, hyaline, 15–20 × 4–6 μm. Basidia barrel-shaped to broadly clavate, with four sterigmata and a simple septum at the base, hyaline, 20–25 × 7–9 μm; basidia in shape similar to basidia, but slightly smaller. Basidiospores broadly ellipsoid to subglobose, yellowish, slightly thick-walled, smooth, non-amyloid, non-dextrinoid, moderately cyanophilous, (3.8–)4–4.9(–5.2) × 3.1–3.8(–3.9) μm, L = 4.35 μm, W = 3.41 μm, Q = 1.24–1.31 (n = 60/2).

Notes: *Sanghuangporus subbaumii* mostly resembles *S. baumii*, but the latter species differs in having pileate basidiomes always, obtuse pileal margin and shorter hymenial setae (<20 μm in length; Dai 2010). The
resupinate to pileate basidiomes make *S. subbaumii* similar to *S. vaninii*, but *S. vaninii* lacks cystidioles and has a thin black zone separating heterogeneous context (Dai 2010). 

**ITS barcoding sequence:** MT348580 (from holotype).

**Additional specimen examined:** China: Beijing: Shangfangshan Forest Park, on fallen angiosperm trunk, 22 July 2019, L.W. Zhou, LWZ 20190722–18 (HAMAS 281654).

*Sanghuangporus vaninii* (Ljub.) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 340 (2016).

**Basionym:** *Phellinus vaninii* Ljub., *Bot. Mater.* 15: 115 (1962).

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

**Type:** Russia: Primorsky Krai: Shkotovsky District, watershed of the Maykhe river, Maykhinsky forestry, Verkhne-Maykhinskaya forest area, Peyshula, quarter 119, in valley of pine-broadleaved forest, on dried aspen tree, 14 Aug. 1951, L.V. Lyubarskii (LE 22523 – holotype).

**Reference collection:** China: Jilin: Antu County, Changbaishan, on fallen trunk of *Populus davidiana*, 26 Aug. 2005, Y.C. Dai, Dai 7011 (IFP)

**ITS barcoding sequence:** JN642591 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

*Sanghuangporus weigelae* (T. Hatt. & Sheng H. Wu) Sheng H. Wu et al., *Fungal Diversity* 77: 340 (2016).

**Basionym:** *Inonotus weigelae* T. Hatt. & Sheng H. Wu, *Bot. Studies (Taipei)* 53: 143 (2012).

**Synonym:** *Inonotus tenuicontextus* L.W. Zhou & W.M. Qin, *Mycol. Progr.* 11: 793 (2012).

**Type:** Japan: Nagano: Chino, Minoto, on *Weigela coraeensis*, 19 Sept. 1993, T. Hattori, F16899 (TFM – holotype).

**ITS barcoding sequence:** JN642596 (from holotype).
**Sanghuangporus weirianus** (Bres.) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 340 (2016).

**Basionym:** *Fomes weirianus* Bres., *Stud. Trent.*, Classe II, Sci. Nat. Econ. 7(1): 5 (1926).

**Synonyms:** *Phellinus weirianus* (Bres.) Gilb., *J. Ariz. Acad. Sci.* 7: 137 (1972).

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

**Type:** USA: New Mexico: on trunk of *Juglans rupelstris*, 25 Oct. 1911, G.G. Hedgcock & W.H. Long (BPI 235278 – holotype).

**Reference collection:** USA: Arizona: on *Juglans major*, 27 Aug. 1967, R.L. Gilbertson 6975-S (IMSNU 32021)

**ITS barcoding sequence:** AF110989 (from the reference collection cited above, proposed by Zhou et al. (2020) and accepted here).

**Sanghuangporus zonatus** (Y.C. Dai & X.M. Tian) L.W. Zhou & Y.C. Dai, *Fungal Diversity* 77: 341 (2016).

**Basionym:** *Inonotus zonatus* Y.C. Dai & X.M. Tian, *Fungal Diversity* 58: 165 (2013).

**Type:** China: Hainan: Jianfengling Nature Reserve, on living angiosperm tree, 11 May 2009, B.K. Cui, Cui 6631 (BJFC – holotype).

**ITS barcoding sequence:** IQ860305 (from holotype).

**DISCUSSION**

In this study, we summarized all available ITS barcoding sequences bearing the name “Sanghuang” in GenBank. A total of 271 ITS sequences related to “Sanghuang”, including 31 newly generated sequences from this study, were analyzed. In association with previous information of morphology, hosts, and multilocus-based phylogeny, 14 species are accepted as members of *Sanghuangporus* including the new species *S. subbaumii* described herein.

We also synonymize *S. toxicodendri* under *S. quercicola*.

*Sanghuangporus subbaumii* has a phylogenetically close relationship to *S. baumii*; however, these two species form two distinct lineages with strong support (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). Moreover, *S. subbaumii* and *S. baumii* were also estimated as two independent species using the mPTP method (Additional file 3: Fig. S1), and for ITS the interspecific distance is 1.19–3.07%, generally above the cut-off value of interspecific distances (1.30%) within *Sanghuangporus* (Additional file 4: Table S1). Besides molecular evidence, morphological differences between these two species are also clear. Geographically, *S. subbaumii* is only known from North China, whereas Chinese collections of *S. baumii* are distributed in north-east China (Table 1).

*Sanghuangporus toxicodendri* was recently described from specimens collected from *Toxicodendron* sp. in Hubei, central China (Wu et al. 2019b) and resembles *S. quercicola*, another species originally described from central China (Zhu et al. 2017). However, in the publication introducing *S. toxicodendri* (Wu et al. 2019b) the separation from *S. quercicola* was not well-supported phylogenetically. Moreover, the morphological differences between these two species are slight (such as for basidiospore length) or involve variable characters that do not have taxonomic signal (such as the surface color of the pileal margin) (Zhu et al. 2017; Wu et al. 2019b).

In the current phylogenetic analyses, the six specimens of *S. toxicodendri*, three specimens of *S. quercicola* and four additional collections merged in a fully supported clade (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). The mPTP-based estimation of species delimitation also treated *S. toxicodendri* and *S. quercicola* as a single species (Additional file 3: Fig. S1) and the intraspecific distances among ITS sequences under both names were 0–1.11%, well below the threshold of 1.30% (Additional file 4: Table S1). Therefore, *S. toxicodendri* and *S. quercicola* are considered conspecific, and *S. quercicola* has priority by publication date over *S. toxicodendri*.

The clade of *S. lonicericus* was present but not well-supported in our phylogenetic analyses (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2). Similarly, the clades of *S. alpinus* and *S. sanghuang* were not strongly supported by the ML algorithm (Fig. 2). For *S. lonicericus* and *S. alpinus*, despite the lack of support in one or both analyses, each formed a distinct clade, and for both species distances to other species were above the threshold of 1.30% (*S. lonicericus* minimum 2.19% and *S. sanghuang* minimum 2.90%; Additional file 4: Table S1). In addition, *S. alpinus*, *S. lonicerinus*, and *S. weigelae*, even though forming three independent lineages, were considered conspecific by the mPTP method (Additional file 3: Fig. S1). However, the interspecific distances for ITS between *S. weigelae* and each of *S. alpinus* and *S. lonicerinus* are above the cut-off value of interspecific distances (1.30%) within *Sanghuangporus* (Additional file 4: Table S1). Regarding the pair of *S. alpinus* and *S. lonicerinus*, for ITS the between species distance (1.03–2.86%) was generally above the intraspecific distances within either species (0–1.08% and 0–1.18%, respectively; Additional file 4: Table S1). Moreover, the monophyly of *S. alpinus* was strongly supported by the BI algorithm and that of *S. lonicerinus* was strongly supported by both the ML and the BI algorithms (Fig. 2). Besides, morphological delimitations among these five species are stable (Wu et al. 2012a; Tian et al. 2013; Zhou et al. 2016). Taking all this into account, we accept *S. alpinus*, *S. lonicericus*, *S. lonicerinus*, *S. sanghuang*, and *S. weigelae* as five independent species.

*Sanghuangporus vaninii*, *S. weirianus*, and *S. zonatus* are the only three species with intraspecific ITS
distances of more than 1.30% (0–1.72%, 2.68% and 0–1.71%, respectively; Additional file 4: Table S1). However, they all received strong support as independent species (Additional file 1: Tree S1, Additional file 2: Tree S2, Fig. 2, Additional file 3: Fig. S1). As one of the most commonly cultivated species, several cultivars of *S. vaninii* were included in the evaluation of genetic distances of ITS sequences (Zhou et al. 2020; Table 1). The procedure of cultivation with continuous passage culture can dramatically accelerate the accumulation of genetic variation, which may result in the higher intraspecific ITS difference in *S. vaninii*. Noteworthily, branch lengths of the only two available collections of *S. weirianus* were markedly different even though the two strains were from the same original isolate (Fig. 2). Regarding *S. zonatus*, two collections from Hainan, South China grouped together with full statistical support, and then formed a fully supported clade with a collection from Yunnan, Southwest China (Table 1, Figs. 1 and 2). Both *S. weirianus* and *S. zonatus* are poorly collected species, and a more comprehensive sampling of these two species in phylogenetic analyses will further clarify their intraspecific relationships. For now, we tentatively accept them as monophyletic species.

A study by Nilsson et al. (2006) revealed that about 10–21% of 51,000 fungal ITS sequences available at that time in the International Nucleotide Sequence Databases were annotated with incorrect taxonomic information. More recently, this proportion has increased to almost 30% (Hofstetter et al. 2019). Regarding “Sanghuang”, more than half (or say 146) of the ITS sequences labeled as such, were found to be mislabeled, implying that the proportion of incorrectly labeled ITS sequences for “Sanghuang” is much higher than the average proportion for all fungal groups. This phenomenon may be attributable to the medicinal properties of “Sanghuang”, which attracts much more attention from non-taxonomists who submit ITS sequences to GenBank. Consequently, the numerous errors result in chaos with BLAST searches, especially for non-taxonomists. Although the RefSeq Targeted Loci (RTL) database has been initiated for fungal ITS sequences from type collections (Schoch et al. 2014), only two species of *Sanghuangporus*, viz. *S. alpinus* and *S. zonatus* were reannotated and deposited under accession numbers of NR_158887 and NR_166366. Actually, ITS sequences from six holotypes of accepted *Sanghuangporus* species are available in GenBank. This number increases to eight, if two synonyms of other species of *Sanghuangporus*, viz. *Inonotus tenuicontextus* and *S. toxicodendri* are considered. In UNITE (Nilsson et al. 2019), tens of species hypotheses belonging to *Sanghuangporus* are available under various threshold values at species level; however, not all accepted species of *Sanghuangporus* (such as *S. ligneus*, *S. pilatii*, and *S. quercicola*) are referred to and the reference sequences for some species hypotheses are not always those from holotypes. Moreover, both RTL and UNITE are not familiar to mycologists working on medicinal studies and government officers in charge of the policy of medicinal fungi, who normally take the first hit of a BLAST search in GenBank as the species name. Therefore, the accuracy of ITS sequences of “Sanghuang” in GenBank is crucial for medicinal studies and commercial development of this fungal genus.

Compared with specimens, many more mislabeled ITS sequences of *Sanghuangporus* came from cultivated strains, and most of those sequences were submitted by non-taxonomists. A typical case is the recent paper on genome sequencing of “Sanghuang” that also submitted six ITS sequences to GenBank (Shao et al. 2020). In GenBank, all these six sequences were labeled as *Inonotus* sp. rather than species of *Sanghuangporus* (MN242716–MN242721), while the six strains generating these sequences were named as *S. sanghuang* (Shao et al. 2020). However, five of the six strains, including the one (labeled as KangNeng) subjected to genome sequencing, are actually *S. vaninii* (Fig. 1, Zhou et al. 2020); i.e. five out of six strains were wrongly identified to species level. Therefore, this species misidentification means that the whole genome sequence of “Sanghuang” may be misapplied in future studies. Shao et al. (2020) also stated that these six strains are commercially cultivated, which further results in the name chaos for commercial products of “Sanghuang”. Another publication on genome sequencing identified the genome sequenced strain S12 as *Phellinus gilvus* according to ITS barcoding region (Huo et al. 2020). However, the corresponding ITS sequence (MT275660) annotated as *Fuscoporia gilva* in GenBank represents *S. vaninii* (Fig. 1, Zhou et al. 2020). Another case is a paper devoted to 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 *S. 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.

Ten mislabeled ITS sequences found in the current study came from basidiomes. These errors were caused mainly by taxonomic revisions of certain species. Six sequences of specimens Wu 1805–2, Wu 1805–3, Wu 1805–5, Wu 1807–2, Wu 1807–3 and Wu 1807–4 that were originally labeled as *Sanghuangporus* sp. but later cited under *S. toxicodendri* by Wu et al. (2019b) are accepted to represent *S. quercicola*. Yuan 2444, previously considered as *S. baunii*, was nested within the lineage segregated from *S. baunii* as a new species *S. subbaunii* (Figs. 1 and 2, Additional file 3: Fig. S1). Consequently,
the ITS sequence of Yuan 2444 (JX069836) is corrected to S. subbaumii (Table 1). Another mislabeled sequence was generated from a species originally described as Inonotus tenuicontextus (Zhou and Qin 2012). Although this species was published online earlier than Inonotus weigelae (basionym of S. weigelae; Wu et al. 2012a; Tian et al. 2013), its online date is before 1 January 2012 and thus the name was not effectively published online according to Art. 29.1 of the ICNafp (Turland et al. 2018). Inonotus tenuicontextus was then treated as a later synonym of I. weigelae (Tian et al. 2013). Therefore, this mislabeled sequence is accepted to represent S. weigelae (Table 1).

Although intact mature basidiomes of “Sanghuang” are not difficult to identify to species level morphologically and in a short time by taxonomists working on this group, most of the commercial products are small pieces or even powders. Normally, it is impossible to rapidly determine which species those commercial products represent. As for other traditional 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 commercial development of “Sanghuang” (Zhou 2020). Therefore, to standardize the “Sanghuang” industry, ten reference sequences are provided for HRCA based on the accurate boundaries among three commonly studied and cultivated species, viz. S. 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) even for single nucleotide differences (Nilsson et al. 1997). This approach has been widely used for the clinical detection of human pathogenic microfungi (Zhou et al. 2008; Trilles et al. 2014; Rodrigues et al. 2015) and, recently, was also reported for the rapid detection of poisonous macrofungi (He et al. 2019a, 2019b). Regarding lethal Amanita species, nucleotide differences greater than two allowed species identification using the a-amanitin gene (He et al. 2019a). Here, for Sanghuangporus a set of candidates for future testing is provided that have diagnostic sequences containing between two and six nucleotide differences.

CONCLUSION
In order to promote medicinal studies and industrial development, the ITS barcoding region of Sanghuangporus species is here comprehensively analyzed to enable accurate species identification. Firstly, the ITS region is confirmed as an effective barcode in Sanghuangporus. Secondly, the names of all available ITS sequences in GenBank related to “Sanghuang” are carefully revised and where necessary corrected. Thirdly, the intraspecific ITS difference for each species of Sanghuangporus is evaluated to be up to 1.30% (except S. vaninii, S. weirianus, and S. zonatus), while the interspecific ITS difference is above 1.30% (except between S. alpinus and S. lonicerinus, and S. baumii and S. subbaumii). 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 differentiate the three commonly studied and cultivated species, viz. S. baumii, S. sanghuang, and S. vaninii. As a follow up, we will suggest reannotation of ITS sequences related to “Sanghuang” to the GenBank administrators, especially to ensure that sequences from holotypes and reference collections for each species of Sanghuangporus are designated as such. Further, we will liaise with UNITE to ensure that appropriate reference sequences are designated for UNITE species hypotheses within Sanghuangporus.

Abbreviations
BI: Bayesian inference; BPP: Bayesian posterior probability; CB: Cotton Blue; CTAB: Cetyl-trimethyl-ammonium bromide; KI: Melzer’s reagent; ITS: Nuclear ribosomal internal transcribed spacer; KOH: 5% potassium hydroxide; ML: Maximum likelihood; mPfP: Multi-rate Poisson Tree Processes; PCR: Polymerase chain reaction; RTL: RefSeq Targeted Loci

Supplementary Information
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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.

Additional file 3: Figure S1. Molecular species delimitation estimated from the Newick tree file of Fig. 2 using multi-rate Poisson Tree Processes method. The continuous red branches represent a single species.

Additional file 4: Table S1. Genetic distances of ITS sequences between and within species of Sanghuangporus.

Additional file 5: Figure S2. 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.

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SS, S-LL 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, 2, 3, 4 and 5. 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).

Declarations

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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References
Bondartsev AS (1953) Polyopes of the European part of the USSR and Caucasus. Academy of Sciences of URSS, Moskva & Leningrad
Cai C, Ma J, Han C, Jin Y, Zhao G, He X (2019) Extraction and antioxidant activity of total terpenoids in the mycelium of a medicinal fungus, Sanghuanggongus sanghuang. Scientific Reports 9(1):7418. https://doi.org/10.1038/s41598-019-43886-0
Cao Y, Wu SH, Dai YC (2017) Species clarification of the prize medicinal Ganoderma mushroom ‘Lingzhi’. Fungal Diversity 56(1):49–62. https://doi.org/10.1007/s13225-012-0178-5
Cheng T, Chepkirui C, Decock C, Matsayj J, Stadler M (2019) Sesquiterpenes from an eastern African medicinal mushroom belonging to the genus Sanghuanggongus. Journal of Natural Products 82(5):1283–1291. https://doi.org/10.1021/acs.jnatprod.8b00186
Chepkirui C, Cheng T, Matsayj J, Decock C, Stadler M (2018) An unprecedented spiro [Furan-2,1’-indene]-3-one derivative and other nematocidal and antimicrobial metabolites from Sanghuanggongus sp. (Hymenocharaeaceae, Basidiomycota) collected in Kenya. Phytochemistry Letters 25:141–146. https://doi.org/10.1016/j.phytol.2018.04.022
Dai YC (2010) Hymenocharaeaceae in China. Fungal Diversity 45(1):131–343. https://doi.org/10.1007/s13225-010-0066-9
Dai YC, Zhou LW, Hattori T, Cao Y, Stalpers JA, Ryvarden L, Buchanan P, Oberwinkler F, Hallenberg N, Liu PG, Wu SH (2017) Species identity of Phellinus linteus (sanghuang) extensively used as a medicinal mushroom in Korea. Journal of Microbiology 54(4):290–295. https://doi.org/10.1007/s12275-016-5520-2
He Z, Luo T, Fan F, Zhang P, Chen Z (2019a) Universal identification of lethal amanitas by using Hyperbranched rolling circle amplification based on α-amino acid gene sequences. Food Chemistry 295:1299–1303. https://doi.org/10.1016/j.foodchem.2019.125031
He Z, Su Y, Li S, Long P, Zhang P, Chen Z (2019b) Development and evaluation of isothermal amplification methods for rapid detection of lethal Amanita species. Frontiers in Microbiology 10:1523. https://doi.org/10.3389/fmicb.2019.01523
Hofstetter V, Buyck B, Eyssartier G, Schnee S, Gindro K (2019) The unbearable lightness of sequence-based identification. Fungal Diversity 96:1243–284. https://doi.org/10.1007/s13225-019-00428-3
Huo J, Zhong S, Du X, Cao Y, Wang W, Sun Y et al (2020) Whole-genome sequence of Phellinus giganteus (mulberry Sanghuang) reveals its unique medicinal values. Journal of Advanced Research 24:325–335. https://doi.org/10.1016/j.jare.2020.04.011
Kappel P, Lutteropp S, Zhang J, Robert K, Pavlidis P, Stamatakis A, Flouti T (2017) Multi-rate Poisson tree processes for single locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics 33:1630–1638. https://doi.org/10.1093/bioinformatics/btx025
Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33(2):518–521. https://doi.org/10.1093/nar/gkj133
Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4):772–780. https://doi.org/10.1093/molbev/mst010
Kumar S, Stadler M, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096
Lin WC, Deng JS, Huang SS, Wu SH, Lin HY, Huang GJ (2017) Evaluation of antioxidant, anti-inflammatory and anti-proliferative activities of ethanol extracts from different varieties of Sanghuang species. RSC Advances 7(13):7780–7788. https://doi.org/10.1039/c6ra27198b
Lizardi PM, Huang X, Zhu Z, Bray-Ward P, Thomas DC, Ward DC (1998) Mutation detection and single-molecule counting using isothermal rolling-circle amplification. Nature Genetics 19(3):225–232. https://doi.org/10.1038/8989
Nilsson M, Keiji K, Koch J, Kwiatkowska M, Gustavsson P, Landegren U (1999) Padlock probes reveal single-nucleotide differences, parent of origin and in situ distribution of centromeric sequences in human chromosomes 13 and 21. Nature Genetics 16(3):252–255. https://doi.org/10.1038/ng0797-252
Nilsson M, Malmgren H, Sarniotaki M, Kwiatkowska M, Chowdhry BP, Landegren U (1994) Padlock probes: circularizing oligonucleotides for localized DNA detection. Science 265(5185):2085–2088. https://doi.org/10.1126.science.752346
Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U (2006) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 44(D1):D259–D264. https://doi.org/10.1093/nar/gkj1022
Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Kõljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS One 1(1):e59. https://doi.org/10.1371/journal.pone.0000059
Pattengale ND, Allipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2010) How many bootstrap replicates are necessary? Journal of Computational Biology 17(3):337–354. https://doi.org/10.1089/cmb.2009.0177
Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25(7):1253–1256. https://doi.org/10.1093/molbev/msn083
Raja HA, Baker TR, Little JS, Oberlies NH (2017) DNA barcoding for identification of consumer-relevant mushrooms: a partial solution for product certification? Food Chemistry 214:383–392. https://doi.org/10.1016/j.foodchem.2016.07.052
