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

**CURRENT STATUS:** Under Review

Shi-Liang Liu, Shan Shen, Ji-Hang Jiang, Li-Wei Zhou

Shi-Liang Liu
Institute of Microbiology Chinese Academy of Sciences

Shan Shen
Institute of Microbiology Chinese Academy of Sciences

Ji-Hang Jiang
Institute of Microbiology Chinese Academy of Sciences

Li-Wei Zhou
Institute of Microbiology Chinese Academy of Sciences

✉️ liwei_zhou1982@im.ac.cn **Corresponding Author**

**ORCiD:** https://orcid.org/0000-0002-2851-2839

Prescreen

10.21203/rs.3.rs-29428/v1

**Subject Areas**

*Plant Physiology and Morphology* *Plant Molecular Biology and Genetics*
Keywords

*Hymenochaetaceae, phylogeny, species boundary, taxonomy, wood-inhabiting fungi*
Abstract

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

Introduction

Macrofungi are a group of fungi producing fruiting bodies visible by naked eyes. Many macrofungi are famous medicinal mushrooms and possess diverse medicinal functions (Wu et al. 2019a). Of them, “Sanghuang”, a kind of important wood-inhabiting medicinal mushrooms, has been utilized as folk medicines for the past two thousand years in China and adjacent countries (Zhou et al. 2020). After that modern scientific studies did reveal some medicinal functions from “Sanghuang”, including antitumor, antioxidant, anti-inflammation, immunomodulation and so on (Zhou et al. 2020), this kind of fungal resources attracts the attentions from European fungal chemists and pharmacologists (Chepkirui et al. 2018; Cheng et al. 2019). Secondary metabolites, such as, polysaccharides, polyphenols, pyrones and terpenes are in charge of these medicinal functions of “Sanghuang” (Zhou et al. 2020). Nowadays, “Sanghuang” are mainly consumed in a tea form of chips and pieces of cultivated basidiocarps and occasionally in an oral form of mycelial powders. Like other precious wood-inhabiting medicinal mushrooms, such as “Lingzhi” (Cao et al. 2012; Wang et al. 2012; Yao et al. 2013, 2020; Dai et al. 2017), “Niuchangchih” (Wu et al. 2012b, c) and “Fuhling” (Redhead and Ginns 2006), there was a hot debate about what the taxonomic identity of “Sanghuang” is. For now, most of fungal taxonomists have agreed that “Sanghuang” is represented by species in *Sanghuangporus* Sheng H. Wu, L.W. Zhou & Y.C. Dai (Zhou et al. 2020). A total of 14 species have been described and accepted as members of *Sanghuangporus*: 11 species are distributed in Asia, one in Africa, one in North America and one in Europe (Zhou et al. 2020). In addition, more new species of *Sanghuangporus* await to be described from Africa (Chepkirui et al. 2018; Cheng et al. 2019) and maybe also from other parts of the world. Besides morphological and ecological characters, ITS barcoding region provides the most powerful evidence for discriminating species of *Sanghuangporus* (Zhou et al. 2020).

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

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

### Materials And Methods

#### Molecular sequencing

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

| No. | Species name accepted here | Species name in GenBank |
|-----|---------------------------|-------------------------|
| 1.  | *Sanghuangporus alpinus*  | Cui 9646                |
| 2.  | *Sanghuangporus alpinus*  | Cui 9652                |
| 3.  | *Sanghuangporus alpinus*  | Cui 9658                |
| 4.  | *Sanghuangporus alpinus*  | Cui 9666                |
| 5.  | *Sanghuangporus alpinus*  | Cui 12444               |
| 6.  | *Sanghuangporus alpinus*  | Cui 12474               |

| GenBank No. | Host plant | Geographic origin |
|-------------|------------|-------------------|
| JQ860313*   | Angiosperm | Tibet, China      |
| JQ860309*   | Angiosperm | Tibet, China      |
| JQ860310*   | Angiosperm | Tibet, China      |
| JQ860311*   | Angiosperm | Tibet, China      |
| MF772782*   | Lonicera   | Sichuan, China    |
| MF772783*   | Lonicera   | Sichuan, China    |

| Type of material | Identifier |
|------------------|------------|
| Specimen         | Tian XM et al. |
| Specimen         | Tian XM et al. |
| Specimen         | Tian XM et al. |
| Specimen         | Tian XM et al. |
| Specimen         | Zhu L & Cui BK |
| Specimen         | Zhu L & Cui BK |
|   | Species | Collection Code | GenBank Accession | Location | Source | Authors |
|---|---------|-----------------|-------------------|----------|--------|---------|
| 7. | *Sanghuangporus alpinus* | Cui 12485 | MF772781* | Sichuan, China | Specimen | Zhu L & Cui BK |
| 8. | *Inonotus alpinus* | Yu 35 | JQ860312* | Tibet, China | Specimen | Tian XM et al. |
| 9. | *Inonotus alpinus* | Yuan 6396 | MT348577* | Qinghai, China | Specimen | This study |
| 10. | *Inonotus alpinus* | Yuan 6405 | MT348578* | Qinghai, China | Specimen | This study |
| 11. | *Inonotus alpinus* | Yuan 6438 | MT343579* | Qinghai, China | Specimen | This study |
| 12. | *S. baumii* | Tropicoporus linteus | ASI 26030 | South Korea | Strain | Han JG et al. |
| 13. | *Tropicoporus linteus* | ASI 26086 | KT862157 | Samchoek, South Korea | Strain | Han JG et al. |
| 14. | *Tropicoporus linteus* | ASI 26087 | KT862158 | Mokpo, South Korea | Strain | Han JG et al. |
| 15. | *Sanghuangporus baumii* | ASI 26108 | KT862162 | Inje, South Korea | Strain | Han JG et al. |
| 16. | *Inonotus baumii* | BZ-2029 | JN642565 | Purchased China | Strain | Wu SH et al. |
| 17. | *Inonotus baumii* | BZ-2030 | JN642566 | Purchased China | Strain | Wu SH et al. |
| 18. | *Inonotus baumii* | Cui 3573 | JQ860307* | Jilin, China | Specimen | Tian XM et al. |
| 19. | *Sanghuangporus baumii* | Cui 11769 | MF772784* | Heilongjiang, China | Specimen | Zhu L & Cui BK |
| 20. | *Sanghuangporus baumii* | Cui 11903 | KY328305* | Heilongjiang, China | Specimen | Zhu L & Cui BK |
| 21. | *Phellinus baumii* | Dai 2340 | AF534069 | | Strain | Lim YW et al. |
| 22. | *Inonotus baumii* | Dai 3683 | JN642567* | Heilongjiang, China | Strain | Wu SH et al. |
| 23. | *Inonotus baumii* | Dai 3684 | JN642568* | Heilongjiang, China | Strain | Wu SH et al. |
| No. | Genus                          | Accession   | Species | Location | Type       | Reference     |
|-----|-------------------------------|-------------|---------|----------|------------|---------------|
| 24. | *Inonotus baumii*             | Dai 3694    | N642569* | Syringa  | Heilongjiang, China | Strain Wu SH et al. |
| 25. | *Inonotus baumii*             | Dai 13360   | MT343580* | Prunus   | Shanxi, China  | Specimen This study |
| 26. | *Sanghuangporus baumii*       | Dai 16900   | MF772785* | Syringa  | Heilongjiang, China | Specimen Zhu L & Cui BK |
| 27. | *Inonotus baumii*             | FS 656165   | HM584807 |          |             | Strain Yu TW   |
| 28. | *Inonotus baumii*             | FS 656164   | GU903007 |          |             | Strain Yu TW   |
| 29. | *Inonotus baumii*             | HLJU        | KC312696 |          |             | Strain Liu Y et al. |
| 30. | *Sanghuangporus baumii*       | KUC 10644   | MH168100 |          |             | Strain Heo YM et al. |
| 31. | *Inonotus baumii*             | KUC 20130809-20 | KJ668511 |          | South Korea Specimen | Jiang Y & Kim JJ |
| 32. | *Inonotus baumii*             | LWZ 20190722-18 | MT348581* | Angiosper | Beijing, China Specimen | This study |
| 33. | *Inonotus baumii*             | MDJCBS 84   | DQ103887 |          |             | Strain Jiang J et al. |
| 34. | *Inonotus baumii*             | SFC 050511-32 | AY972811 |          |             | Strain Jung HS & Lee JS |
| 35. | *Inonotus baumii*             | SFC 050527-67 | AY972812 |          |             | Strain Jung HS & Lee JS |
| 36. | *Phellinus baumii*            | SFC 960405-4 | AF534068 |          |             | Strain Lim YW et al. |
| 37. | *Phellinus linteus*           | SFC 970527-1 | AF534073 |          |             | Strain Lim YW et al. |
| 38. | *Sanghuangporus baumii*       | SFCC 50029  | AY558608 |          |             | Strain Jeong WJ et al. |
| 39. | *Inonotus baumii*             | SH 3        | FJ190412 |          |             | Strain Zou L et al. |
| 40. | *Inonotus baumii*             | Wu 0910 – 54 | N642570* | Syringa  | Beijing, China | Strain Wu SH et al. |
|   |   | **Inonotus baumii** | Yuan 2444 | **X069836** | Angiosperm | Shanxi, China | Specimen | Tian XM et al. |
|---|---|---|---|---|---|---|---|---|
|41. |   | Sanghuangpors baumii | Yuan 4909 | **KY328310** | Angiosperm | Heilongjiang, China | Specimen | Zhu L & Cui BK |
|42. |   | Sanghuangpors baumii | Yuan 4929 | **KY328306** | Alnus | Heilongjiang, China | Specimen | Zhu L & Cui BK |
|43. | S. ligneus | Sanghuangpors ligneus | MG 12 | **KR073081** | Lonicera caucasica | Iran | Strain | Ghabbad-Nejhad M |
|44. |   | Sanghuangpors ligneus | MG 13 | **KR073082** | Lonicera caucasica | Iran | Strain | Ghabbad-Nejhad M |
|45. | S. lonicericolap | Inonotus baumii | BM-3753 | HQ845063 | China | Strain | Hu W & Deng X |
|46. |   | Inonotus baumii | BM-8335 | HQ845064 | China | Strain | Hu W & Deng X |
|47. |   | Sanghuangpors lonicericolap | Cui 10994 | MF772786 | China | Specimen | Zhu L & Cui BK |
|48. |   | Inonotus lonicericolap | Dai 8322 | **N642571** | Lonicera | Heilongjiang, China | Specimen | Wu SH et al. |
|49. |   | Inonotus lonicericolap | Dai 8335 | **N642573** | Lonicera | Heilongjiang, China | Specimen | Wu SH et al. |
|50. |   | Inonotus lonicericolap | Dai 8340 | **N642574** | Lonicera | Heilongjiang, China | Specimen | Wu SH et al. |
|51. |   | Inonotus lonicericolap | Dai 8376 | **Q860308** | Lonicera | Heilongjiang, China | Specimen | Tian XM et al. |
|52. |   | Sanghuangpors lonicericolap | Dai **17304** | **MT348582** | Lonicera | Liaoning, China | Strain | This study |
|53. |   | **Phellinus** sp. | HN100K9 | KF589300 | South Korea | Strain | Kang HW & Kim JK |
|54. |   | **Phellinus** ribis | SFCC 50032 | AY558643 | | Strain | Jeong WJ et al. |
|55. |   | Inonotus lonicericolap | TAA 105317 | **N642572** | Lonicera ruprechtiana | Russian Far East | Specimen | Wu SH et al. |
|56. | S. lonicerinus | Sanghuangpors lonicerinus | Dai 17093 | **MF772788** | Lonicera | Uzbekistan | Specimen | Zhu L & Cui BK |
|   |   | Sanghuangporus lonicerinus | Dai 17095 | MF772787* | Lonicera | Uzbekistan | Specimen | Zhu L & Cui BK |
|---|---|-----------------------------|-----------|-----------|----------|-----------|----------|-----------|
|58. |   | Sanghuangporus lonicerinus | MG 280 | KU213573* |          |           | Specimen | Langer EJ & Ghobad-Nejhad M |
|59. |   | Sanghuangporus lonicerinus | MG 281 | KU213574* |          |           | Specimen | Langer EJ & Ghobad-Nejhad M |
|60. |   | Inonotus sp. | TAA 55528 | KU6452575* | Lonicera | Turkmenistan | Strain | Wu SH et al. |
|61. |   | Inonotus lonicerinus | TAA 55696 | MT348583* | Lonicera | Turkmenistan | Specimen | This study |
|62. |   | Phellinus linteus | TAA-104264 | AF534074 |          |           | Strain | Lim YW et al. |
|63. |   | S. microcystideus | Sanghuangporus microcystideus | O 915609 | KP030787* | Olea africana | Tanzania | Specimen | Zhou LW et al. |
|64. |   | S. pilatii | Phellinus pilatii | BRNM 771989 | KT428764* | Populus alba | Czech Republic | Specimen | Tomšovský M |
|65. |   | S. quercicola | Phellinus rhabarbarinus | CBS 282.77 | AY558642 |          | Strain | Jeong WJ et al. |
|66. |   | Sanghuangporus quercicola | Dai 13947 | KY328309* |          | Chongqing, China | Specimen | Zhu L & Cui BK |
|67. |   | Sanghuangporus quercicola | Li 445 | KY328311* | Angiosperm | Henan, China | Specimen | Zhu L & Cui BK |
|68. |   | Sanghuangporus quercicola | Li 1149 | KY328312* | Quercus | Henan, China | Specimen | Zhu L & Cui BK |
|69. |   | Sanghuangporus quercicola | LWZ 20170821-13 | MT348584* | Angiosperm | Hubei, China | Specimen | This study |
|70. |   | Sanghuangporus quercicola | LWZ 20170821-14 | MT348585* | Angiosperm | Hubei, China | Specimen | This study |
|71. |   | Sanghuangporus quercicola | LWZ 20170821-15 | MT348586* | Angiosperm | Hubei, China | Specimen | This study |
|72. |   | Sanghuangporus | LWZ 20170821-16 | MT348587* | Angiosperm | Hubei, China | Specimen | This study |
| No. | Species                  | Accession | Country          | Status       | Author          |
|-----|--------------------------|-----------|------------------|--------------|----------------|
| 73. | *Sanghuangporus quercicola* | Wei 7575  | Henan, China     | Strain       | This study      |
| 74. | *Sanghuangporus sp.*      | Wu 1805-2 | Hubei, China     | Specimen     | Wu SH et al.   |
| 75. | *Sanghuangporus sp.*      | Wu 1805-3 | Hubei, China     | Specimen     | Wu SH et al.   |
| 76. | *Sanghuangporus sp.*      | Wu 1805-5 | Hubei, China     | Specimen     | Wu SH et al.   |
| 77. | *Sanghuangporus sp.*      | Wu 1807-2 | Hubei, China     | Specimen     | Wu SH et al.   |
| 78. | *Sanghuangporus sp.*      | Wu 1807-3 | Hubei, China     | Specimen     | Wu SH et al.   |
| 79. | *Sanghuangporus sp.*      | Wu 1807-4 | Hubei, China     | Specimen     | Wu SH et al.   |
| 80. | *Sanghuangporus sp.*      | Wu 1807-5 | Hubei, China     | Specimen     | Wu SH et al.   |
| 81. | *S. sanghuang*            | AH1       | Cultivated       | Anhui, China| This study      |
| 82. | *S. sanghuang*            | AH2       | Cultivated       | Anhui, China| This study      |
| 83. | *S. sanghuang*            | AH3       | Cultivated       | Anhui, China| This study      |
| 84. | *S. sanghuang*            | AH4       | Cultivated       | Anhui, China| This study      |
| 85. | *S. sanghuang*            | AH5       | Cultivated       | Anhui, China| This study      |
| 86. | *Phellinus igniarius*     | ASI 26010 | Jeongseon, South Korea | Strain | Han JG et al. |
| 87. | *Tropicoporus linteus*    | ASI 26011 | India         | Strain       | Han JG et al.  |
| 88. | *Tropicoporus linteus*    | ASI 26016 | South Korea    | Strain       | Han JG et al.  |
|   | Species          | Accession | Location       | Strain Type | Authors         |
|---|------------------|-----------|----------------|-------------|----------------|
|89.| Tropicoporus linteus | ASI 26021 | KT862138 | Hongcheon, South Korea | Strain Han JG et al. |
|90.| Tropicoporus linteus | ASI 26022 | KT862139 | Hongcheon, South Korea | Strain Han JG et al. |
|91.| Tropicoporus linteus | ASI 26025 | KT862140 | Wonju, South Korea | Strain Han JG et al. |
|92.| Tropicoporus linteus | ASI 26026 | KT862141 | Wonju, South Korea | Strain Han JG et al. |
|93.| Tropicoporus linteus | ASI 26039 | KT862143 | Pyeongchang, South Korea | Strain Han JG et al. |
|94.| Tropicoporus linteus | ASI 26046 | KT862144 | Hongcheon, South Korea | Strain Han JG et al. |
|95.| Tropicoporus linteus | ASI 26049 | KT862145 | Hongcheon, South Korea | Strain Han JG et al. |
|96.| Tropicoporus linteus | ASI 26054 | KT862147 | Hongcheon, South Korea | Strain Han JG et al. |
|97.| Tropicoporus linteus | ASI 26062 | KT862148 | Hwacheon, South Korea | Strain Han JG et al. |
|98.| Tropicoporus linteus | ASI 26063 | KT862149 | Jeongseon, South Korea | Strain Han JG et al. |
|99.| Tropicoporus linteus | ASI 26066 | KT862150 | Inje, South Korea | Strain Han JG et al. |
|100.| Tropicoporus linteus | ASI 26067 | KT862151 | Inje, South Korea | Strain Han JG et al. |
|101.| Tropicoporus linteus | ASI 26070 | KT862152 | | Strain Han JG et al. |
|102.| Tropicoporus linteus | ASI 26071 | KT862153 | | Strain Han JG et al. |
|103.| Tropicoporus linteus | ASI 26073 | KT862154 | South Korea | Strain Han JG et al. |
|104.| Tropicoporus linteus | ASI 26074 | KT862155 | Seongnam, South Korea | Strain Han JG et al. |
|105.| Tropicoporus linteus | ASI 26082 | KT862156 | Mokpo, South Korea | Strain Han JG et al. |
| No. | Species | Accession | Country | Strain Type | Strain Name | Author(s) |
|-----|---------|-----------|---------|-------------|-------------|-----------|
| 106. | Tropicoporus linteus | ASI 26088 | South Korea | Strain | Sancheong, South Korea | Han JG et al. |
| 107. | Tropicoporus linteus | ASI 26114 | South Korea | Strain | | Han JG et al. |
| 108. | Tropicoporus linteus | ASI 26115 | South Korea | Strain | | Han JG et al. |
| 109. | Phellinus linteus | ATCC 26710 | South Korea | Strain | | Kim GY et al. |
| 110. | Sanghuangporus sanghuang | Batch 1-12192170-1 | USA | Strain | | Raja HA et al. |
| 111. | Sanghuangporus sanghuang | Batch 2-10221252-2 | USA | Strain | | Raja HA et al. |
| 112. | Sanghuangporus sanghuang | Batch 2-12192170-1 | USA | Strain | | Raja HA et al. |
| 113. | S. sanghuang | BJ | Cultivated | China | | This study |
| 114. | Inonotus sp. | BZ-A | Morus | Hunan, China | | Wu SH et al. |
| 115. | Inonotus sp. | BZ-C | Morus | Hunan, China | | Wu SH et al. |
| 116. | Inonotus sp. | CA | Morus | Jiangxi, China | | Wu SH et al. |
| 117. | Inonotus sp. | CB | Morus | Jiangxi, China | | Wu SH et al. |
| 118. | Inonotus sp. | CC | Morus | Jiangxi, China | | Wu SH et al. |
| 119. | Sanghuangporus sanghuang | Cui 14419 | Morus | Shaanxi, China | | Zhu L & Cui BK |
| 120. | Sanghuangporus sanghuang | Cui 14420 | Morus | Shaanxi, China | | Zhu L & Cui BK |
| 121. | Inonotus sanghuang | Dai 12723 | Morus | Sichuan, China | | Tian XM et al. |

Northeast
| No. | Organism       | Strain Code | Accession Code | Cultivated | Country | Strain Code | Authors               |
|-----|----------------|-------------|----------------|------------|---------|-------------|-----------------------|
| 122 | *Phellinus* linteus | DB1         | MT421905*      | Cultivated | China   | Strain      | Chung JW et al.       |
| 123 | *Phellinus* linteus | DGUM25003   | AF082102       |            |         | Strain      | Chung JW et al.       |
| 124 | *Phellinus* linteus | DGUM25004   | AF080458       |            |         | Strain      | Chung JW et al.       |
| 125 | *Inonotus* linteus | FS 656160   | GU903004       |            |         | Strain      | Yu TW                |
| 126 | *Inonotus* linteus | FS 656161   | HM584806       |            |         | Strain      | Yu TW                |
| 127 | *Tropicoporus* linteus | FS 656179 | KU867779       |            |         | Strain      | Yu TW                |
| 128 | *Tropicoporus* linteus | FS 656180 | KU867780       |            |         | Strain      | Yu TW                |
| 129 | *S. sanghuang* | HB          | MT421907*      | Cultivated | Hubei, China | Strain      | This study            |
| 130 | *Phellinus* linteus | IFO 6980    | AF200226       |            |         | Strain      | Kim GY & Lee JD      |
| 131 | *Inonotus* linteus | IFO 6989    | AY640937       |            |         | Strain      | Lee JS & Jung HS      |
| 132 | *Phellinus* linteus | IMSNU 31014 | AF082101       |            |         | Strain      | Chung JW et al.       |
| 133 | *Sanghuangporus* sanghuang | JL-01 | MG062789       |            |         | Strain      | Xu X                 |
| 134 | *S. sanghuang* | JS1         | MT421908*      | Cultivated | Jiangsu, China | Strain      | This study            |
| 135 | *Inonotus* linteus | KAB-PL-01   | DQ462333       |            | Taiwan, China | Strain      | Chiou SJ & Yen JH    |
| 136 | *Phellinus* linteus | KCTC 6190   | AF077678       |            |         | Strain      | Chung JW et al.       |
| 137 | *Phellinus* igniarius | KCTC 16890 | AY189708       |            |         | Strain      | Nam BH et al.         |
| 138 | *Inonotus* linteus | KFDA 016    | AY436626       |            |         | Strain      | Yun JC et al.         |
| 139. | **Inonotus linteus** | KFDA P38 | AY513234 | Strain | Jin CY et al. |
| 140. | **Inonotus linteus** | KSSW01 | EF506943 | Strain | Park SY et al. |
| 141. | **Inonotus linteus** | LT-0802 | HQ845059 | South Korea | Hu W & Deng X |
| 142. | **Inonotus linteus** | LT-CBS83 | HQ845060 | South Korea | Hu W & Deng X |
| 143. | **Sanghuangporus sanghuang** LWZ 20180927-3 | MT348588* | Morus Yunnan, China | Specimen | This study |
| 144. | **Phellinus linteus** | MPNU 7016 | AF153009 | Strain | Kim GY et al. |
| 145. | **Inonotus linteus** | MUCL 47139 | GU461973 | Cuba | Strain | Amalfi M et al. |
| 146. | **Inonotus linteus** | NAAS00002 | JN043317 | Strain | Seok SJ et al. |
| 147. | **Phellinus linteus** | Namsan No1 | AF080457 | Strain | Chung JW et al. |
| 148. | **Inonotus linteus** | PL 0801 | FJ940906 | Strain | Xie LY et al. |
| 149. | **Inonotus linteus** | PL 5 | EF095712 | Strain | Park BW et al. |
| 150. | **Inonotus sp.** | PL 10 | JN642588* | China | Strain | Wu SH et al. |
| 151. | **Sanghuangporus sanghuang** S3 | MN153568 | Strain | Song JL et al. |
| 152. | **Phellinus sp.** | SA 01 | EF694971 | Strain | Zeng NK et al. |
| 153. | **Phellinus baumii** SFC 20001106-1 | AF534064 | Strain | Lim YW et al. |
| 154. | **Phellinus baumii** SFC 20010212-1 | AF534062 | Strain | Lim YW et al. |
| 155. | **Sanghuangporus sanghuang** SS | MG209821 | Strain | Cai C & Zhao G |
| 156. | Inonotus sp. | T004 | N642586* | Morus | Taiwan, China | Strain | Wu SH et al. |
| 157. | Inonotus sp. | TH | N642582* | Morus | Taiwan, China | Strain | Wu SH et al. |
| 158. | Inonotus sp. | TJ | N642585* | Morus | Taiwan, China | Strain | Wu SH et al. |
| 159. | Inonotus sp. | TM | N642583* | Morus | Taiwan, China | Strain | Wu SH et al. |
| 160. | Inonotus sp. | TN | N642584* | Morus | Taiwan, China | Strain | Wu SH et al. |
| 161. | Inonotus sp. | WD 1222 | N642576* | Morus | Japan | Strain | Wu SH et al. |
| 162. | Inonotus sp. | WD 2261 | N642577* | Morus | Japan | Strain | Wu SH et al. |
| 163. | Inonotus sp. | WD 2300 | N642578* | Morus | Japan | Strain | Wu SH et al. |
| 164. | Inonotus sp. | Wu 0903-1 | N794061* | Morus | Jilin, China | Strain | Wu SH et al. |
| 165. | Inonotus sp. | Zhangjiajie | MN242716 | Cultivated | | Strain | Wang Y |
| 166. | S. sanghuang | ZJ1 | MT421910* | Cultivated | Zhejiang, China | Strain | This study |
| 167. | S. sanghuang | ZJ2 | MT421911* | Cultivated | Zhejiang, China | Strain | This study |
| 168. | S. sanghuang | ZJ4 | MT421913* | Cultivated | Zhejiang, China | Strain | This study |
| 169. | S. sanghuang | ZJ5 | MT421914* | Cultivated | Zhejiang, China | Strain | This study |
| 170. | S. vaninii | Inonotus vaninii | HQ845058 | | China | Strain | Hu W & Deng X |
| 171. | Inonotus sp. | Beijing | MN242720 | Cultivated | China | Strain | Wang Y |
| 172. | Inonotus vaninii | BZ-2031 | N642593* | Populus | China | Strain | Wu SH et al. |
| 173. | Inonotus vaninii | CJC 01 | N642592* | Cultivated | Taiwan, China | Strain | Wu SH et al. |
| 174. | Sanghuangporus vaninii | Cui 9939 | MF772792* | | Jilin, China | Specimen | Zhu L & Cui BK |
| No.  | Species                     | Code  | Accession   | Host       | Location     | Type       | Source            |
|------|-----------------------------|-------|-------------|------------|-------------|------------|-------------------|
| 175. | Sanghuangporus vaninii     | Cui 14082 | MF772793*  | Populus    | Jilin, China | Specimen  | Zhu L & Cui BK     |
| 176. | Inonotus vaninii           | Dai 3624 | JN642590*  | Populus    | China       | Strain    | Wu SH et al.      |
| 177. | Inonotus vaninii           | Dai 7011 | JN642591*  | Populus davidiana | Jilin, China | Strain    | Wu SH et al.      |
| 178. | Sanghuangporus vaninii     | Dai 8236 | MF772791*  | Populus    | Jilin, China | Specimen  | Zhu L & Cui BK     |
| 179. | S. vaninii                 | DB2    | MT421906*  | Cultivated | Northeast China | Strain    | This study        |
| 180. | Inonotus baumii            | FS 656170 | GU903008   |            |             | Strain    | Yu TW            |
| 181. | Fiscoporia gilva           | FS 656175 | HM584811   |            |             | Strain    | Yu TW            |
| 182. | Sanghuangporus vaninii     | HZ-01  | MG062791   |            |             | Strain    | Xu X             |
| 183. | Inonotus sp.               | JinZhai | MN242717   | Cultivated | China       | Strain    | Wang Y           |
| 184. | S. vaninii                 | JS2    | MT421909*  | Cultivated | Jiangsu, China | Strain    | This study        |
| 185. | Inonotus sp.               | KangNeng | MN242721  | Cultivated | China       | Strain    | Wang Y           |
| 186. | Inonotus baumii            | KFDA 015 | AY436623   |            |             | Strain    | Yun JC et al.     |
| 187. | Inonotus baumii            | KFDA 022 | AY436624   |            |             | Strain    | Yun JC et al.     |
| 188. | Inonotus linteus           | KFDA 024 | AY436627   |            |             | Strain    | Yun JC et al.     |
| 189. | Inonotus baumii            | KFDA 029 | AY436625   |            |             | Strain    | Yun JC et al.     |
| 190. | Inonotus baumii            | KFDA P36 | AY509198   |            |             | Strain    | Jin CY et al.     |
| 191. | Inonotus baumii            | KFDA P40 | AY509199   |            |             | Strain    | Jin CY et al.     |
| 192. | Inonotus baumii            | KFDA P45 | AY509201   |            |             | Strain    | Jin CY et al.     |
| 193. | Inonotus sp. | Korea | MN242719 | Cultivated | China | Strain | Wang Y |
|---|---|---|---|---|---|---|---|
| 194. | Sanghuangporus baumii | LC 6686 | MK818502 | Strain | Li ZN |
| 195. | Inonotus linteus | LT-HG | HQ845061 | Strain | Hu W & Deng X |
| 196. | Fuscoporia gilva | MDJCBS87 | DQ103884 | Strain | Jiang J et al. |
| 197. | Phellinus baumii | MPNU 7004 | AF200229 | Strain | Kim GY & Lee JD |
| 198. | Phellinus baumii | MPNU 7005 | AF200230 | Strain | Kim GY & Lee JD |
| 199. | Phellinus baumii | MPNU 7006 | AF200231 | Strain | Kim GY & Lee JD |
| 200. | Phellinus sp. | MPNU 7007 | AF200235 | Strain | Kim GY & Lee JD |
| 201. | Phellinus sp. | MPNU 7010 | AF153007 | South Korea | Strain | Kim GY et al. |
| 202. | Phellinus sp. | MPNU 7012 | AF153008 | South Korea | Strain | Kim GY et al. |
| 203. | Phellinus sp. | MPNU 7013 | AF153011 | South Korea | Strain | Kim GY et al. |
| 204. | Inonotus baumii | PB 0802 | FJ940907 | Strain | Xie LY et al. |
| 205. | Inonotus baumii | PB 0803 | FJ940908 | Strain | Xie LY et al. |
| 206. | Inonotus baumii | PB 0806 | FJ940911 | Strain | Xie LY et al. |
| 207. | Inonotus baumii | PB 0808 | FJ940913 | Strain | Xie LY et al. |
| 208. | Inonotus baumii | PB 0809 | FJ940914 | Strain | Xie LY et al. |
| 209. | Inonotus sp. | QianDaoHu | MN242718 | Cultivated | China | Strain | Wang Y |
| 210. | Sanghuangporus vaninii | S1 | MN153566 | Strain | Song JL et al. |
| 211. | Sanghuangporus baumii | S2 | MN153567 | Strain | Song JL et al. |
|   | Genus          | Strain | Genus          | Strain | Genus          | Strain | Genus          | Strain | Genus          | Strain |
|---|---------------|--------|---------------|--------|---------------|--------|---------------|--------|---------------|--------|
| 212. | Fuscoporia  | S12    | MT275660      |        | Strain        | Li Y & Huo J |
| 213. | Phellinus sp. | SA 02  | EF694972      |        | Strain        | Zeng NK et al. |
| 214. | Phellinus sp. | SA 03  | EF694973      |        | Strain        | Zeng NK et al. |
| 215. | Phellinus sp. | SA 04  | EF694974      |        | Strain        | Zeng NK et al. |
| 216. | Inonotus baumii | SA 05 | EF694975      |        | Strain        | Zeng NK et al. |
| 217. | Phellinus sp. | SA 06  | EF694976      |        | Strain        | Zeng NK et al. |
| 218. | Phellinus sp. | SA 07  | EF694977      |        | Strain        | Zeng NK et al. |
| 219. | Phellinus linteus | SFC 970605 | AF534071   |        | Strain        | Lim YW et al. |
| 220. | Phellinus linteus | SFC 2001106-7 | AF534070 |        | Strain        | Lim YW et al. |
| 221. | Phellinus baumii | SFC 20010212-2 | AF534063 |        | Strain        | Lim YW et al. |
| 222. | Tropicoporus linteus | SFCC 10209 | AY558628 |        | Strain        | Jeong WJ et al. |
| 223. | Fuscoporia  | SH 1   | FJ190410      |        | Strain        | Zou L et al. |
| 224. | Inonotus baumii | SJ    | JN887691      |        | Strain        | Shin KS |
| 225. | Inonotus vaninii | Wei 3382 | JN169788* | Jilin, China | Specimen | Zhou LW & Qin WM |
| 226. | Inonotus vaninii | WN 0801 | HQ845054 | China | Strain | Hu W & Deng X |
| 227. | Inonotus vaninii | WN-1   | HQ845055 | China | Strain | Hu W & Deng X |
| 228. | Inonotus vaninii | WN-2   | HQ845056 | China | Strain | Hu W & Deng X |

17
| No. | Genus                  | Species     | Accession Number | Country | Location | Genus | Species | Accession Number | Country | Location | Genus | Species | Accession Number | Country | Location |
|-----|------------------------|-------------|------------------|---------|----------|--------|----------|------------------|---------|----------|--------|----------|------------------|---------|----------|
| 229 | *Inonotus vaninii*     |             | WN-4             | China   | Strain   | Deng X |         |                  |         |          |        |          |                  |         |          |
| 230 | *Inonotus vaninii*     |             | WN 8213          | China   | Strain   | Hu W & Deng X |        |                  |         |          |        |          |                  |         |          |
| 231 | *Inonotus vaninii*     |             | WN 8824          | China   | Strain   | Hu W & Deng X |        |                  |         |          |        |          |                  |         |          |
| 232 | *Inonotus vaninii*     |             | WN 3624          | China   | Strain   | Hu W & Deng X |        |                  |         |          |        |          |                  |         |          |
| 233 | *Sanghuangporus baumii*|             | XZ-01            | China   | Strain   | Xu X   |        |                  |         |          |        |          |                  |         |          |
| 234 | *Inonotus baumii*      |             | YC               | China   | Strain   | Shin KS |        |                  |         |          |        |          |                  |         |          |
| 235 | *Sanghuangporus vaninii*|             | Yuan 2764        | China   | Specimen | Zhu L & Cui BK | Quercus | Shaanxi, China |         |          |        |          |                  |         |          |
| 236 | *Sanghuangporus vaninii*|             | Yuan 5604        | China   | Specimen | Zhu L & Cui BK | Quercus | Jilin, China |         |          |        |          |                  |         |          |
| 237 | *S. vaninii*           |             | ZJ3              | China   | Strain   | This study | Cultivated | Zhejiang, China |         |          |        |          |                  |         |          |
| 238 | *S. weigelae*          | Sanghuangp | 420526MF0201    | China   | Specimen | Wang R et al. | Quercus | Hubei, China |         |          |        |          |                  |         |          |
| 239 | *Inonotus weigelae*    |             | Cui 6010         | China   | Specimen | Tian XM et al. | Lonicera | Jiangxi, China |         |          |        |          |                  |         |          |
| 240 | *Inonotus weigelae*    |             | Cui 6012         | China   | Specimen | Tian XM et al. | Lonicera | Jiangxi, China |         |          |        |          |                  |         |          |
| 241 | *Inonotus weigelae*    |             | Cui 7176         | China   | Specimen | Tian XM et al. | Syringa | Hebei, China |         |          |        |          |                  |         |          |
| 242 | *Inonotus weigelae*    |             | Dai 6352         | China   | Specimen | Tian XM et al. |         | Zhejiang, China |         |          |        |          |                  |         |          |
| 243 | *Inonotus weigelae*    |             | Dai 11694        | China   | Specimen | Tian XM et al. |         | Hunan, China |         |          |        |          |                  |         |          |
| 244 | *Sanghuangporus weigelae*|             | Dai 15770        | China   | Specimen | Zhu L & Cui BK | Weigela | Chongqing, China |         |          |        |          |                  |         |          |
| 245 | *Sanghuangporus weigelae*|             | Dai 16072        | China   | Specimen | This study | Weigela | Inner Mongolia, China |         |          |        |          |                  |         |          |
| 246. | Sanghuangporus weigelae | Dai 16077 | MF772794* | Weigela | Inner Mongolia, China | Specimen | Zhu L & Cui BK |
| 247. | Sanghuangporus weigelae | LWZ 20150802-3 | MT348590* | Weigela | Jiangxi, China | Specimen | This study |
| 248. | Sanghuangporus weigelae | LWZ 20150802-5 | MT348591* | Weigela | Jiangxi, China | Specimen | This study |
| 249. | Phellinus baumii | SFC 20000111-10 | AF534067 | | | | |
| 250. | Inonotus sp. | WD 1186 | N642597* | Weigela | Japan | Strain | Tian XM et al. |
| 251. | Inonotus sp. | WD 1187 | N642598* | Weigela | Japan | Strain | Tian XM et al. |
| 252. | Inonotus sp. | WD 1667 | N642594* | Weigela cordeenis | Japan | Strain | Wu SH et al. |
| 253. | Inonotus sp. | WD 1837 | N642595* | Weigela cordeenis | Japan | Strain | Wu SH et al. |
| 254. | Inonotus sp. | WD 1838 | N642596* | Weigela cordeenis | Japan | Strain | Wu SH et al. |
| 255. | Inonotus weigelae | Wei 2120 | JQ860314* | Coriaria | Hubei, China | Specimen | Tian XM et al. |
| 256. | Inonotus weigelae | Wei 2267 | JX069835* | Angiosperm | Hubei, China | Specimen | Tian XM et al. |
| 257. | Inonotus tenuicontextus | Yuan 5526 | N169786* | Angiosperm | Guizhou, China | Specimen | Zhou LW & Qin WM |
| 258. | S. weirianus | Sanghuangporus weirianus | CBS 618.89 | AY558654* | | | Jeong WJ et al. |
| 259. | Phellinus weirianus | IMSNU 32021 | AF110989* | | | | Chung JW et al. |
| 260. | S. zonatus | Inonotus zonatus | Cui 6631 | JQ860305* | Angiosperm | Hainan, China | Specimen | Tian XM et al. |
| 261. | Inonotus zonatus | Cui 8327 | JX069837* | Angiosperm | Yunnan, China | Specimen | Tian XM et al. |
| 262. | Inonotus | | | | | | Tian XM et al. |
| 262. |  | Inonotus | Dai 10841 | Q860306* | Angiosperm | China | Specimen | al. |
| 263. | S. sp. 1 | Inonotus sp. | AM-08 | F895464 | Ethiopia | Specimen | Assefa A et al. |
| 264. |  | Inonotus sp. | AM-19 | F895465 | Ethiopia | Specimen | Assefa A et al. |
| 265. |  | Inonotus linteus | F915611 | JX985739 | Ethiopia | Specimen | Assefa A et al. |
| 266. |  | Inonotus linteus | Teng 3279 | JX985738 | Xylosoma | China | Specimen | Assefa A et al. |
| 267. | S. sp. 2 | Phellinus sp. | DLL 2010–102 | JQ673184 | Populus tremuloides | USA | Strain | Brazee NJ et al. |
| 268. |  | Sanghuangporus vaninii | DLL 2010–102 | KU139197 | Populus tremuloides | USA | Strain | Brazee NJ |
| 269. | S. sp. 3 | Phellinus baumii | SFC 20001106-4 | AF534066 | South Korea | Strain | Lim YW et al. |
| 270. |  | not Sanghuangporus | DL 101 | KP974834 | China | Strain | Sun T et al. |
| 271. |  | not Sanghuangporus | WN-3 | HQ845057 | China | Strain | Hu W & Deng X |

New sequenced specimens and strains are in bold
* Sequences considered to be reliable for further analysis

**Downloading sequences from GenBank**

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

**Phylogenetic analyses**

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

Evaluation of genetic distances of ITS sequences

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

Identification of diagnostic ITS sequences

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

Results

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

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

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

Table 2

| Specie  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| S. alpinus | 0.0049 ± 0.0016 |     |     |     |     |     |     |     |     |     |     |     |     |
| S. baumii | 0.0445 ± 0.0073 | 0.0129 ± 0.0026 |     |     |     |     |     |     |     |     |     |     |     |
| S. ligneus | 0.0529 ± 0.0097 | 0.0439 ± 0.0084 |     |     |     |     |     |     |     |     |     |     |     |
| S. lonicericola | 0.0417 ± 0.0070 | 0.0315 ± 0.0059 | 0.0249 ± 0.0066 | 0.0045 ± 0.0016 |     |     |     |     |     |     |     |     |     |
| S. lonicerinus | 0.0156 ± 0.0042 | 0.0502 ± 0.0082 | 0.0600 ± 0.0102 | 0.0498 ± 0.0082 | 0.0046 ± 0.0017 |     |     |     |     |     |     |     |
| S. microcystideus | 0.1083 ± 0.0118 | 0.1166 ± 0.0119 | 0.1173 ± 0.0135 | 0.1104 ± 0.0121 | 0.1083 ± 0.0121 | n.a. |     |     |     |     |     |     |
| S. pilatii | 0.0476 ± 0.0079 | 0.0576 ± 0.0086 | 0.0532 ± 0.0097 | 0.0493 ± 0.0079 | 0.0508 ± 0.0085 | 0.1191 ± 0.0127 | n.a. |     |     |     |     |     |
|     | Species          | Distance 1 | Distance 2 | Distance 3 | Distance 4 | Distance 5 | Distance 6 | Distance 7 | Distance 8 | Distance 9 | Distance 10 | Distance 11 | Distance 12 | Distance 13 |
|-----|------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 8   | S. quercicola    | 0.0610     | ±0.0087    | 0.0654     | ±0.0087    | 0.0657     | ±0.0103    | 0.0662     | ±0.0090    | 0.0711     | ±0.0096    | 0.1313     | ±0.0126    | 0.0490     | ±0.0079    | 0.0044     | ±0.0014    |
| 9   | S. sanghuang     | 0.0390     | ±0.0069    | 0.0479     | ±0.0076    | 0.0581     | ±0.0100    | 0.0485     | ±0.0077    | 0.0391     | ±0.0074    | 0.1046     | ±0.0118    | 0.0370     | ±0.0071    | 0.0524     | ±0.0080    | 0.0010     | ±0.0003    |
| 10  | S. vaninii       | 0.0592     | ±0.0089    | 0.0686     | ±0.0096    | 0.0622     | ±0.0103    | 0.0628     | ±0.0092    | 0.0663     | ±0.0096    | 0.1210     | ±0.0126    | 0.0304     | ±0.0065    | 0.0590     | ±0.0084    | 0.0480     | ±0.0079    | 0.0049     | ±0.0012    |
| 11  | S. weigelae      | 0.0172     | ±0.0045    | 0.0474     | ±0.0078    | 0.0507     | ±0.0095    | 0.0438     | ±0.0075    | 0.0183     | ±0.0049    | 0.1064     | ±0.0119    | 0.0501     | ±0.0085    | 0.0696     | ±0.0094    | 0.0391     | ±0.0072    | 0.0667     | ±0.0095    | 0.0031     | ±0.0012    |
| 12  | S. weirianus     | 0.0605     | ±0.0086    | 0.0658     | ±0.0085    | 0.0631     | ±0.0102    | 0.0630     | ±0.0088    | 0.0622     | ±0.0090    | 0.1271     | ±0.0124    | 0.0540     | ±0.0081    | 0.0755     | ±0.0093    | 0.0416     | ±0.0069    | 0.0724     | ±0.0095    | 0.0585     | ±0.0086    | 0.0268     | ±0.0061    |
| 13  | S. zonatus       | 0.0695     | ±0.0091    | 0.0629     | ±0.0088    | 0.0672     | ±0.0105    | 0.0495     | ±0.0078    | 0.0769     | ±0.0101    | 0.1333     | ±0.0131    | 0.0803     | ±0.0101    | 0.0902     | ±0.0097    | 0.0763     | ±0.0103    | 0.0836     | ±0.0094    | 0.0712     | ±0.0108    | 0.0114     | ±0.0032    |

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

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

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

Discussion

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

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

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

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

Although intact mature specimens of “Sanghuang” are not difficult to be morphologically identified to a species level in a short time, most of commercial products are chips and pieces or even powders. Normally, it is impossible to rapidly determine which species such kind of commercial products really represents. Like other medicinal mushrooms (Raja et al. 2017), species names of Sanghuangporus are sometimes misapplied to certain products of “Sanghuang” (Shao et al. 2020). This confused situation to some extent restricts the industrial development of “Sanghuang” (Zhou 2020). Therefore, to standardize the industry of “Sanghuang”, ten candidate sequences were provided for HRCA based on the accurate boundaries among three commonly studied and cultivated species, viz. Sanghuangporus baumii, S. sanghuang and S. vaninii (Lin et al. 2017; Zhou et al. 2020). HRCA is an isothermal amplification approach and thus provides a rapid, simple and low-cost detection of specific nucleic acid sequences (Nilsson et al. 1994; Lizardi et al. 1998). This approach has been widely used for clinic detection of human-pathogenic microfungi (Zhou et al. 2008; Trilles et al. 2014; Rodrigues et al. 2015), and recently, was also reported for rapid detection of poisonous macrofungi (He et al. 2019a, 2019b). Regarding lethal Amanita species, a more than two-nucleotide-long difference was evidenced to be valid for identification of α-amanitin gene (He et al. 2019a). Here, to provide more candidates, two and more nucleotide differences are given, because it was reported that this approach could reveal single nucleotide differences (Nilsson et al. 1997). Hopefully, certain candidates will work well in future experiments.
Generally, to promote medicinal studies and industrial development, the ITS barcoding region of *Sanghuangporus* is comprehensively analyzed for accurate species identification. Firstly, the names of all available ITS sequences in GenBank related to “Sanghuang” are carefully corrected. Secondly, the intraspecific ITS difference for each species of *Sanghuangporus* but *S. weirianus* is evaluated to be below 1.50%, while the interspecific ITS difference is always above 1.50%. This provides a practical cut-off value for BLAST search-based species identification. Finally, ten potential diagnostic sequences are provided for HRCA assay to rapidly discriminate three commonly studied and cultivated species, viz. *Sanghuangporus baumii*, *S. sanghuang* and *S. vaninii*.

### Abbreviations

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

### Declarations

#### Acknowledgements

Drs. Yan Yang and He-Nan Zhang (Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, China) are thanked for kindly providing cultivated strains for sequencing. Profs. Yu-Cheng Dai (Beijing Forestry University) and Hai-Sheng Yuan (Institute of Applied Ecology, Chinese Academy of Sciences) are thanked for kindly forwarding specimens and strains as loans for sequencing.

#### Adherence to national and international regulations

Not applicable.

#### Authors’ contributions

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

#### Funding

The research was financed by the National Natural Science Foundation of China (No. 31970012), Youth Innovation Promotion Association of the Chinese Academy of Sciences (No. 2017240), and Biological Resources Programme, Chinese Academy of Sciences (KFJ-BRP-017-12).

#### Availability of data and materials

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

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests
Author details

1 State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, 2 Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China, 3 University of Chinese Academy of Sciences, Beijing, China

Supplementary Information

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

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

References

1. Cai C, Ma J, Han C, Jin Y, Zhao G, He X (2019) Extraction and antioxidant activity of total triterpenoids in the mycelium of a medicinal fungus, Sanghuangporus sanghuang. Sci Rep 9:7418. https://doi.org/10.1038/s41598-019-43886-0
2. Cao Y, Wu SH, Dai YC (2012) Species clarification of the prize medicinal Ganoderma mushroom “Lingzhi”. Fungal Divers 56:49–62. https://doi.org/10.1007/s13225-012-0178-5
3. 10.1016/j.phytol.2018.04.022 Chepkirui C, Cheng T, Matasyoh J, Decock C, Stadler M (2018) An unprecedented spiro [Furan-2,1’-indene]-3-one derivative and other nematicidal and antimicrobial metabolites from Sanghuangporus sp. (Hymenochaetaeaceae, Basidiomycota) collected in Kenya. Phytochem Lett 25:141–146. https://doi.org/10.1016/j.phytol.2018.04.022
4. Cheng T, Chepkirui C, Decock C, Matasyoh J, Stadler M (2019) Sesquiterpenes from an eastern African medicinal mushroom belonging to the genus Sanghuangporus. J Nat Prod 82:1283–1291. https://doi.org/10.1021/acs.jnatprod.8b01086
5. Dai YC, Zhou LW, Hattori T, Cao Y, Stalpers JA, Ryvarden L et al (2017) Ganoderma lingzhi (Polyporales, Basidiomycota): the scientific binomial for the widely cultivated medicinal fungus Lingzhi. Mycol Prog 16:1051–1055. https://doi.org/10.1007/s11557-017-1347-4
6. Edler D, Klein J, Antonelli A, Silvestro D (2019) raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. bioRxiv. https://doi.org/10.1101/800912
7. Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for Basidiomycetes: application to identification of mycorrhizal and rusts. Mol Ecol 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
8. Ghobad-Nejhad M (2015) Collections on Lonicera in Northwest Iran represent an undescribed species in the Inonotus linteus complex (Hymenochaetales). Mycol Prog 14:90. https://doi.org/10.1007/s11557-015-1100-9
9. Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696–704. https://doi.org/10.1080/10635150390235520
10. Han JG, Hyun MW, Kim CS, Jo JW, Cho JH, Lee KH et al (2016) Species identity of Phellinus linteus (sanghuang) extensively used as a medicinal mushroom in Korea. J Microbiol 54:290–295. https://doi.org/10.1007/s12275-016-5520-2
11. 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-amanitin gene sequences. Food Chem 298:125031. https://doi.org/10.1016/j.foodchem.2019.125031
12. 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. Front Microbiol 10:1523.
13. Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res 33:511-518. https://doi.org/10.1093/nar/gki198
14. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772-780. https://doi.org/10.1093/molbev/mst010
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547-1549. https://doi.org/10.1093/molbev/msy096
16. 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 Adv 7:7780-7788. https://doi.org/10.1039/c6ra27198g
17. 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. Nat Genet 19:225-232. https://doi.org/10.1038/898
18. Nilsson M, Krejci K, Koch J, Kwiatkowski M, Gustavsson P, Lane
deg U (1997) Padlock probes reveal single-nucleotide differences, parent of origin and in situ distribution of centromeric sequences in human chromosomes 13 and 21. Nat Genet 16:252-255. https://doi.org/10.1038/ng0797-252
19. Nilsson M, Malmgren H, Samiotaki M, Kwiatkowski M, Chowdhary BP, Lane
deg U (1994) Padlock probes: circularizing oligonucleotides for localized DNA detection. Science 265:2085-2088. https://doi.org/10.1126/science.7522346
20. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2010) How many bootstrap replicates are necessary? J Comput Biol 17:337-354. https://doi.org/10.1089/cmb.2009.0179
21. Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253-1256. https://doi.org/10.1093/molbev/msn083
22. Raja HA, Baker TR, Little JG, Oberlies NH (2017) DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification? Food Chem 214:383-392. https://doi.org/10.1016/j.foodchem.2016.07.052
23. 10.2307/25065702 Redhead SA, Ginns J (2006) (1738) Proposal to conserve the name Poria cocos against Daedalea extensa (Basidiomycota). Taxon 55:1027-1028. https://doi.org/10.2307/25065702
24. Rodrigues AM, Najafzadeh MJ, de Hoog GS, Camargo ZP (2015) Rapid identification of emerging human-pathogenic Sporothrix species with rolling circle amplification. Front Microbiol 6:1385. https://doi.org/10.3389/fmicb.2015.01385
25. Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029
26. Shao Y, Guo H, Zhang J, Liu H, Wang K, Zuo S et al (2020) The genome of the medicinal macrofungus Sanghuang provides insights into the synthesis of diverse secondary metabolites. Front Microbiol 10:3035. https://doi.org/10.3389/fmicb.2019.03035
27. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033
28. Stecher G, Tamura K, Kumar S (2020) Molecular Evolutionary Genetics Analysis (MEGA) for macOS. Mol Biol Evol 37:1237–1239. https://doi.org/10.1093/molbev/msz312
29. Tian XM, Yu HY, Zhou LW, Decock C, Vlasák J, Dai YC (2013) Phylogeny and taxonomy of the Inonotus linteus complex. Fungal Divers 58:159–169. https://doi.org/10.1007/s13225-012-0202-9
30. Tomšovský M (2015) Sanghuangporus pilatii, a new combination, revealed as European relative of Asian medicinal fungi. Phytotaxa 239:82–88. https://doi.org/10.11646/phytotaxa.239.1.8
31. Trilles L, Wang B, Firacative C, Lazéra MS, Wanke B, Meyer W (2014) Identification of the major molecular types of Cryptococcus neoformans and C. gattii by Hyperbranched rolling circle amplification. PLoS ONE 9:e94648. https://doi.org/10.1371/journal.pone.0094648
32. Wang XC, Xi RJ, Li Y, Wang DM, Yao YJ (2012) The species identify of the widely cultivated Ganoderma, ‘G. lucidum’ (Ling-zhi), in China. PLoS ONE 7:e40857. https://doi.org/10.1371/journal.pone.0040857
33. White TJ, Bruns TD, Lee SB, Taylor JW (1990) “Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) ” PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, pp 315–322
34. Wu F, Zhou LW, Yang ZL, Bau T, Li TH, Dai YC (2019a) Resource diversity of Chinese macrofungi: edible, medicinal and poisonous. Fungal Divers 98:1–76. https://doi.org/10.1007/s13225-019-00432-7
35. Wu SH, Chang CC, Wei CL, Jiang GZ, Cui BK (2019b) Sanghuangporus toxicodendri sp. nov. (Hymenochaetales, Basidiomycota) from China MycoKeys 57:101–111. https://doi.org/10.3897/mycokeys.57.36376
36. Wu SH, Dai YC, Hattori T, Yu TW, Wang DM, Parmasto E et al (2012a) Species clarification for the medicinally valuable ‘sanghuang’ mushroom. Bot Stud 53:135-149
37. Wu SH, Kirk PM, Redhead SA, Stalpers JA, Dai YC, Norvell LL et al (2012b) Resolution of the nomenclature for niu-chang-chih (Taiwanofungus camphoratus), an important medicinal polypore. Taxon 61:1305-1310. https://doi.org/10.1002/tax.616011
38. 10.1002/tax.616015
Wu SH, Yao YJ, Wang XC, Kirk PM, Redhead SA, Stalpers JA et al (2012c) (2101) Proposal to conserve the name Ganoderma camphoratum (Taiwanofungus camphoratus) (Polyporales) with a conserved type. Taxon 61:1321-1322. https://doi.org/10.1002/tax.616015
39. Yao YJ, Li Y, Du Z, Wang K, Wang XC, Kirk PM et al (2020) On the typification of Ganoderma sichuanense (Agaricomycetes)-the widely cultivated Lingzhi medicinal mushroom. Int J Med Mushrooms 22:45-54. https://doi.org/10.1615/IntJMedMushrooms.2019033189
40. Yao YJ, Wang XC, Wang B (2013) Epitypification of Ganoderma sichuanense J.D. Zhao & X.Q. Zhang (Ganodermataceae) Taxon 62:1025–1031. https://doi.org/10.12705/625.10
41. Zhou LW (2020) Systematics is crucial for the traditional Chinese medicinal studies and industry of macrofungi. Fungal Biol Rev 34:10-12. https://doi.org/10.1016/j.fbr.2019.10.002
42. Zhou LW, Ghabad-Nejhad M, Tian XM, Wang YF, Wu F (2020) Current status of ‘Sanghuang’ as a group of medicinal mushrooms and their perspective in industry development. Food Rev Int. https://doi.org/10.1080/87559129.2020.1740245
43. 10.1007/s11557-011-0792-8
Zhou LW, Qin WM (2012) Inonotus tenuicontextus sp. nov. (Hymenochaetaeaceae) from Guizhou, southwest China with a preliminary discussion on the phylogeny of its kin. Mycol Prog 11:791-798. https://doi.org/10.1007/s11557-011-0792-8
44. 10.1007/s13225-015-0335-8
Zhou LW, Vlasák J, Decock C, Assefa A, Stenlid J, Abate D et al (2016) Global diversity and taxonomy of the Inonotus linteus complex (Hymenochaetales, Basidiomycota): Sanghuangporus gen. nov., Tropicoporus excentrodendri and T. guanacastensis gen. et sp. nov., and 17 new combinations. Fungal Divers 77:335–347. https://doi.org/10.1007/s13225-015-0335-8
45. 10.1128/JCM.00420-08
Zhou X, Kong F, Sorrell TC, Wang H, Duan Y, Chen SC (2008) Practical method for detection and identification of Candida, Aspergillus, and Scedosporium spp. by use of rolling-circle amplification. J Clin Microbiol 46:2423–2427. https://doi.org/10.1128/JCM.00420-08
46. Zhu L, Song J, Zhou JL, Si J, Cui BK (2019) Species diversity, phylogeny, divergence time, and biogeography of the genus Sanghuangporus (Basidiomycota). Front Microbiol 10:812. https://doi.org/10.3389/fmicb.2019.00812
The phylogenetic tree inferred from 269 ITS sequences. The topology was generated from the maximum likelihood algorithm. The tips in blue color represent name-mislabeled specimens, while those in red color represent name-mislabeled strains.
Figure 2

The phylogenetic tree inferred from ITS sequences submitted by taxonomists. The topology was generated from the maximum likelihood algorithm, and bootstrap values and Bayesian posterior probabilities simultaneously above 50% and 0.8, respectively, are presented at the nodes.
Figure 3

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

Supplementary Files

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

- Additionalfile1TreeS1.tre
- Additionalfile2TreeS2.tre