Outline, Divergence Times, and Phylogenetic Analyses of Trechisporales (Agaricomycetes, Basidiomycota)

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Phylogenetic analyses inferred from the nuc rDNA ITS1-5.8S-ITS2 (ITS) data set and the combined 2-locus data set [5.8S + nuc 28S rDNA (nLSU)] of taxa of Trechisporales around the world show that Sistotremastrum family forms a monophyletic lineage within Trechisporales. Bayesian evolutionary and divergence time analyses on two data sets of 5.8S and nLSU sequences indicate an ancient divergence of Sistotremastrum family from Hydnodontaceae during the Triassic period (224.25 Mya). Sistotremastrum family is characterized by resupinate and thin basidiomata, smooth, verruculose, or odontoid-semiporoid hymenophore, a monomitic hyphal structure, and generative hyphae bearing clamp connections, the presence of cystidia and hyphidia in some species, thin-walled, smooth, inamyloid, and acyanophilous basidiospores. In addition, four new species, namely, Trechispora dentata, Trechispora dimitiella, Trechispora fragilis, and Trechispora laevispora, are described and illustrated. In addition, three new combinations, namely, Brevicellicium daweishanense, Brevicellicium xanthum, and Sertulicium limonadense, are also proposed.

Keywords: Hydnodontaceae, phylogenetic analysis, Trechispora, taxonomy, wood-rotting fungi

INTRODUCTION

Trechisporales K.H. Larss. was established by Hibbett et al. (2007). Most species in this order are corticioid fungi with smooth, grandinioid, odontioid, or hydnoid hymenophores, and others are polypores. All species have a monomitic or dimitic hyphal system with generative hyphae bearing clamp connections, and many species have rhizomorphs (mycelial cords) (Larsson, 2007).

At present, there is only an acknowledged and a named family belonging to Trechisporales, i.e., Hydnodontaceae Jülich. Hydnodontaceae contains 11 genera now, namely, Brevicellicium K.H. Larss. and Hjortstam, Dextrinocystis Gilb. and M. Blackw., Fibrodontia Parmasto, Pteridomyces Jülich, Luellia K.H. Larss. and Hjortstam, Porponyces Jülich, Scytinopogon Singer, Subulicyctidium Parmasto, Suillusporium Pouzar, Trechispora P. Karst., and Tubulicium Oberw (Larsson, 2007; Spirin et al., 2021).

Trechispora is the genus type of Trechisporales and Hydnodontaceae. It is the largest genus in this order, with more than 50 accepted species (Meiras-Ottoni et al., 2021; Zhao and Zhao, 2021). Identification keys for Trechispora species recorded in China and Brazil have been provided by
some fungal taxonomists (Chikowski et al., 2020; Meiras-Ottoni et al., 2021; Zong et al., 2021). *Trechispora* was typified with *Trechispora onusta* P. Karst. [= *Trechispora hymenocystis* (Berk. and Broome) K.H. Larss.] (Karsten, 1890). It is characterized by the resupinate basidiomata (a few species have stipitate, flabellate, and effused–reflexed basidiomata) with smooth granulinioid, odontioid, hydnoid, or poroid hymenophores, a monomitic or dimitic hyphal structure with clamped generative hyphae and smooth to verrucose or aculate basidiopores (Larsson, 1992; Larsson et al., 2004). Most species in *Trechispora* are soil-dwelling (Larsson et al., 2004). One remarkable character is the presence of ampullate septa on the subicular and especially on some hyphae of the mycelial cords. Above all, ampullate septa are only known from *Scytinopogon*, *Trechispora*, and *Porpomyces mucidus* (Pers.) Jülich within Trechisporales (Furtado et al., 2021; Meiras-Ottoni et al., 2021).

Larson (2007) used the term "Sistotremastrum family" for the first time to accommodate *Sistotremastrum suecicum* Litsch. ex J. Erikss. and *Sistotremastrum niveocremeum* [= *Sertulicium niveocremeum* (Höhn. and Litsch.) Spirin and K.H. Larss.]. Since then, "Sistotremastrum" family has been adopted by some taxonomists (Tellieria et al., 2013; Liu et al., 2019). In this work, the phylogeny of Trechisporales is carried out based on combined 5.8S + nLSU sequences. In addition, Bayesian evolutionary and divergence time analyses are also carried out to indicate the divergence time of Trechisporales, Hydnodontaceae, and *Sistotremastrum* family. We outline the *Sistotremastrum* family and discuss the difference between *Hydnodontaceae* and *Trechisporales*.

During investigations on the diversity of wood-rotting fungi, seven resupinate specimens were collected from China and Malaysia. Their morphology corresponds to the concept of *Trechispora*. To confirm their affinity, phylogenetic analyses based on the ITS sequences are carried out. Both morphological characteristics and molecular evidence demonstrate that these seven resupinate specimens represent the four new species of *Trechispora*.

In addition, we downloaded the type sequences of *Trechispora daweishanensis* C.L. Zhao, *Trechispora xantha* C.L. Zhao, and *Sistotremastrum limonadense* G. Gruhn and P. Alvarado from GenBank. We also studied the type specimens of *T. daweishanensis* and *T. xantha*. In conclusion, *T. daweishanensis* and *T. xantha* were transferred to *Brevicellicium*, while *S. limonadense* was transferred to *Sertulicium*.

**MATERIALS AND METHODS**

**Morphological Studies**

Macro-morphological descriptions are based on field notes and dry herbarium specimens. Microscopic structures are photographed using a Nikon Digital Sight DS-L3 (Japan) or Leica ICC50 HD (Japan) camera. Microscopic measurements are made from slide preparations of dry tissues stained with 1% Phloxine B (C$_{20}$H$_{24}$Br$_{2}$Cl$_{2}$K$_{2}$O$_{3}$) (Fan et al., 2021). We also use other reagents, such as Cotton Blue and Melzer’s reagent following Dai’s (2010) study. Spore measurements include both with ornamentation and without ornamentation. The following abbreviations are used: KOH = 5% potassium hydroxide; CB = Cotton Blue; CB(+) = weakly cyanophilous; CB− = acyanophilous in Cotton Blue; IKI = Melzer’s reagent; IKI− = neither amyloid nor dextrinoid in Melzer’s reagent; L = mean spore length (arithmetic average of all spores including ornamentation); W = mean spore width (arithmetic average of all spores including ornamentation); Q = a variation in the $L/W$ ratios between the specimens studied; $L′$ = mean spore length (arithmetic average of all spores excluding ornamentation); $W′$ = mean spore width (arithmetic average of all spores excluding ornamentation); $Q′$ = a variation in the $L′/W′$ ratios between the specimens studied; $n$ ($a/b$) = the number of spores ($a$) measured from a given number of specimens ($b$). When presenting spore size variation, 5% of measurements are excluded from each end of the range and these values are given in parentheses. Special color terms follow Petersen (1996). Herbarium abbreviations follow Thiers (2018). The studied specimens are deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC), and the herbarium of Southwest Forestry University (SWFC).

**DNA Extraction, Polymerase Chain Reaction Amplification, and Sequencing**

Total genomic DNA from the dried specimens is extracted by a CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Company Limited, Beijing, China) according to the manufacturer’s instructions with some modifications (Liu and Yuan, 2020; Du et al., 2021). The ITS regions are amplified with the primers ITS4 and ITS5 (White et al., 1990). The nLSU regions are amplified with the primers LR0R and LR7 (Vilgalys and Hester, 1990).

The polymerase chain reaction (PCR) procedure for ITS is as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s, and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, and a final extension of 72°C for 10 min (Zhao et al., 2015; Liu and Dai, 2021). The PCR products are purified and sequenced in the Beijing Genomics Institute, China, with the same primers used in the PCR reactions.

**Phylogenetic Analyses**

Two combined matrices, an ITS1-5.8S-ITS2 (ITS) data set and a two-gene data set (5.8S + nLSU), are used for phylogenetic analyses. Phylogenetic analyses are performed with maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) methods in the ITS data set. Phylogenetic analyses are performed with ML and BI methods in the combined two-gene data set (5.8S + nLSU). Species and strain sequences are adopted partly from 28S- and ITS-based tree topologies established by Meiras-Ottoni et al. (2021) and Spirin et al. (2021). New sequences generated in this study, along with reference sequences retrieved from GenBank (Table 1), are
### TABLE 1 Information of taxa used in phylogenetic analyses.

| Species                        | Collector ID (herbarium ID) | GenBank accession no. |
|--------------------------------|-----------------------------|-----------------------|
|                                |                             |ITS                    | nLSU                |
| Auricularia sp.                | PBM 2295                    | DQ200918              | AF634277            |
| Breviceillium atlanticum       | LISU 178566 (holotype)      | HE963777              | HE963774            |
| Breviceillium atlanticum       | LISU 178590                 | HE963775              | HE963776            |
| Breviceillium daweishanense    | CLZhao 18255 (SWFC)         | MW302338              | MW293867            |
| Breviceillium daweishanense    | CLZhao 17860 (SWFC, holotype) | MW302337              | MW293866            |
| Breviceillium exile            | MA-Fungi 26554 (holotype)   | HE963777              | HE963778            |
| Breviceillium olivascens       | KHL 8571 (GB)               | HE963792              | HE963793            |
| Breviceillium olivascens       | MA-Fungi 23498              | HE963877              | HE963878            |
| Breviceillium xanthum          | CLZhao 17781 (SWFC)         | MW302340              | MW293869            |
| Breviceillium xanthum          | CLZhao 2632 (SWFC, holotype) | MW302339              | MW293868            |
| Dextrinocystis calamicola      | He 5700 (BJFC)              | MK204534              | MK204547            |
| Dextrinocystis calamicola      | He 5693 (BJFC)              | MK204533              | MK204546            |
| Exidia recisa                  | EL 15-98 (GB)               | AF347112              | AF347112            |
| Exidiopsis calcea              | MW 331                      | AF291280              | AF291326            |
| Hyphodontia floccosa           | TFMF 24944 (holotype)       | KG282874              | KG282875            |
| Hyphodontia subalutacea        | DAI 12692 (BJFC)            | KG282874              | KG282875            |
| Porpomyces mucidus             | Cui 5183 (BJFC)             | KG282874              | KG282875            |
| Porpomyces submucidus          | Cui 5183 (BJFC)             | KG282874              | KG282875            |
| Pteridomyces gabini            | GB0150230                  | KG282874              | KG282875            |
| Pteridomyces gabini            | Berrinicha 8122 (GB)        | KG282874              | KG282875            |
| Scytinopogon angulispinosus    | TFB13611                    | KG282874              | KG282875            |
| Scytinopogon chartaceum        | JQ684661                    | KG282874              | KG282875            |
| Scytinopogon pallescens        | He 5192 (BJFC)              | KG282874              | KG282875            |
| Sertulicium chilense           | MA-Fungi 86368 (holotype)   | KG282874              | KG282875            |
| Sertulicium granuliferum       | He 3338                     | KG282874              | KG282875            |
| Sertulicium jacksonii          | Spiro 10425 (H)             | KG282874              | KG282875            |
| Sertulicium lateclavigerum     | LY 13467                    | KG282874              | KG282875            |
| Sertulicium limonadense        | LIP 0001683 (holotype)      | MT180961              | MT180978            |
| Sertulicium limonadense        | He 6276 (BJFC)              | OK296849*             | OK296847*           |
| Sertulicium rivocremnereum     | KHL13727 (GB)               | MN937563              | MN937563            |
| Sertulicium vernale            | Soderholm 3886 (H, holotype) | MT005311              | MT064174            |
| Sistotheastrum aculeatum       | Mettinen 10360.1 (H)        | MN937563              | MN937563            |
| Sistotheastrum aculeatum       | Cui 8401 (BJFC)             | MN937563              | MN937563            |
| Sistotheastrum aculeocrepitans | KHL 16004 (URM)             | MN937563              | MN937563            |
| Sistotheastrum confusum        | Motato-Vasquez 894 (SP, holotype) | MN937563              | MN937563            |
| Sistotheastrum denticulatum    | LIP 0001413 (holotype)      | MN937563              | MN937563            |
| Sistotheastrum fibrillosum     | LY 13467                    | KG937563              | KG937563            |
| Sistotheastrum fibrillosum s. l.| GUY113-119 (GB)             | KG937563              | KG937563            |
| Sistotheastrum fibrillosum s. l.| KHL 16988 (MG)             | KG937563              | KG937563            |
| Sistotheastrum geminum         | Mettinen 14333 (MAB, holotype) | KG937563              | KG937563            |
| Sistotheastrum induratum       | Spiro 8598 (H, holotype)    | MT002324              | MT664173            |
| Sistotheastrum mendax          | KHL 12022 (O, holotype)     | MN937570              | MN937570            |
| Sistotheastrum rigidum         | Motato-Vasquez 833 (SP, holotype) | MN937570              | MN937570            |
| Sistotheastrum suecicum        | Kunntu 5969 (H)             | MT002335              | MT002335            |
| Sistotheastrum suecicum        | Mettinen 14550.1 (H)        | MT002335              | MT002335            |
| Sistotheastrum suecicum        | KHL 11849 (GB)              | MN937571              | MN937571            |
| Sistotheastrum suigerans       | Fonneland 2011-78 (O, holotype) | MN937572              | MN937572            |
| Sistotheastrum suigerans       | Spiro 8778 (H)              | MN937572              | MN937572            |
| Subuckystinum tropicalum       | He 3968 (BJFC)              | MK204531              | MK204544            |
| Sulphoponum cystidiatum        | Spiro 3830 (H)              | MN937573              | MN937573            |
| Trechispora alnicola           | AFTOL-ID 665                | DG411529              | AF347084            |
| Trechispora araneosa           | KHL8570 (GB)               | AF347084              | AF347084            |
| Trechispora bambuicolora        | CLZhao 3302 (SWFC)          | MG546021              | MG520171            |
| Trechispora bispore            | CBS 142.63 (holotype)       | MG585241              | MG58642             |
| Trechispora cohaerens          | TU 110332                   | UD808249              | –                   |
| Trechispora cohaerens          | TU 115568                   | UD8018241             | –                   |
| Trechispora confinis           | KHL11084 (GB)              | AF347081              | AF347081            |

(Continued)
TABLE 1 | (Continued)

| Species | Collector ID (herbarium ID) | GenBank accession no. |
|---------|---------------------------|----------------------|
|         |                           | ITS                  | nLSU                 |
| Trechispora copiosa | AMO456                    | MN701019             | MN687976             |
| Trechispora copiosa | AMO422 (holotype)         | MN701013             | MN687971             |
| Trechispora cyathæse | FR-0219442                | UB024014             | UB024014             |
| Trechispora cyathæse | FR-0219443 (holotype)     | UB024015             | UB024015             |
| **Trechispora dentata** | Dai 22565 (BJFC)         | **OK298649**         | **OM049408**         |
| **Trechispora dimitiella** | Dai 21931 (BJFC)         | **OK298649**         | **OK298649**         |
| Trechispora echinospora | MA-Fungi 02485 (holotype)| OK298492             | OK298948             |
| Trechispora echinospora | KHL 8793 (GB)            | AF347089             | AF347089             |
| Trechispora echinospora | KHL 8451 (GB)            | AF347082             | AF347082             |
| Trechispora fimbriata | CLZhao 9796 (SWFC)       | MW544024             | MW540174             |
| Trechispora fimbriata | CLZhao 4154 (SWFC, holotype) | MW544023           | MW540173             |
| Trechispora fissurata | CLZhao 4571 (SWFC, holotype) | MW544027           | MW540177             |
| Trechispora fissurata | CLZhao 9795 (GB)         | MW544026             | MW540176             |
| **Trechispora fragilis** | Dai 20535 (BJFC)         | **OK298649**         | **OK298650**         |
| Trechispora gelatinosa | AMO1139 (holotype)       | MN701021             | MN687978             |
| Trechispora gelatinosa | AMO824                    | MN701020             | MN687977             |
| Trechispora havencampi | SFSU DED8300 (holotype)  | NR_154418            | NG_059993            |
| Trechispora hymenocystis | TL 11112 (holotype)     | UB000078             | UB000078             |
| Trechispora hymenocystis | KHL 8795 (GB)           | AF347090             | AF347090             |
| Trechispora incisa | GEBO090648               | KU747095             | KU747087             |
| Trechispora incisa | GEBO090521               | –                    | –                    |
| Trechispora kavinioides | KGN 181002 (GB)         | AF347086             | AF347086             |
| **Trechispora laevispora** | Dai 21655 (BJFC)        | **OK2986495**        | **OM108710**         |
| Trechispora miniapora | MEXU 28300 (holotype)    | MK288868             | MK328894             |
| Trechispora miniapora | MEXU 28301               | MK288869             | MK328895             |
| Trechispora mollis | URM 85884 (holotype)     | MK184545             | MK188003             |
| Trechispora mollusca | DILL2011-186 (CFMP)     | –                    | –                    |
| Trechispora mollusca | DILL2010-077 (CFMP)     | –                    | –                    |
| Trechispora nivea | GEBO102684               | KU747096             | AY586720             |
| Trechispora nivea | MA-Fungi 74044           | JX082832             | JX082833             |
| Trechispora papillosa | AM0713                    | MN701022             | MN687979             |
| Trechispora papillosa | AM0785 (holotype)        | MN701023             | MN687981             |
| Trechispora regularis | KHL10881 (GB)           | AF347087             | AF347087             |
| Trechispora rigida | URM 85754               | MT-006381            | MH279999             |
| Trechispora sp. | AMQ799                   | MN701008             | MN687969             |
| Trechispora sp. | AMQ440                   | MN701006             | MN687967             |
| Trechispora sp. | KHL16968 (C)            | MH290763             | MH290763             |
| **Trechispora sp.** | Dai 22173 (BJFC)        | **OK2986496**        | **OK298651**         |
| **Trechispora sp.** | Dai 22174 (BJFC)        | **OK2986497**        | **OK298652**         |
| Trechispora stevensioni | TU 115499               | UB016467             | UB016467             |
| Trechispora stevensioni | MA-Fungi 70669          | JX082841             | JX082842             |
| Trechispora subsphaerospora | KHL 8511 (GB)         | AF347080             | AF347080             |
| Trechispora termophilæ | AM0396 (holotype)      | MN701025             | MN687983             |
| Trechispora termophilæ | AM0309                   | MN701024             | MN687962             |
| Trechispora torendæ | URM 85886 (holotype)    | MK180004             | MH180004             |
| Tubulicium raphidisporum | He 3191 (BJFC)         | MK204537             | MK204545             |

*Newly generated sequences for this study. New species and new combinations or putatively new species are in bold.

aligned by MAFFT 7 (Katoh et al., 2019\(^1\)) using the “G-INS-i” strategy and manually adjusted in BioEdit (Hall, 1999). Unreliably aligned sections are removed before analyses and attempts are made to manually inspect and improve alignment. The data matrix is edited in Mesquite v3.70 software (Maddison and Maddison, 2021). The sequence alignment is deposited at TreeBase (submission ID 29141 and 29142). Sequences of *Auricularia* sp., *Exidia recisa* (Ditmar) Fr., and *Exidiopsis calcea* (Pers.) K. Wells are included in phylogenetic analyses. They belong to another order, Auriculariales Bromhead. The order is close to Trechisporales (Sulistyo et al., 2021). We add these three sequences in the combined two-gene data set (5.8S + nLSU) to demonstrate that Trechisporales forms a strongly supported sister clade to Auriculariales. Sequences of *Hyphodontia floccosa*
Brevicellicium atlanticum analysis. Two sequences of ∗ and the tree construction procedure is performed in PAUP.

Approaches to phylogenetic analysis follow Liu and Dai (2021), used as outgroups to root trees in the ITS analysis. Tellería, M. Dueñas and M.P. Martín obtained from GenBank are used as outgroups to root trees in the 5.8S analysis.

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Bayesian posterior probabilities (BPP) of the clades.

Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) are calculated for each maximum parsimony tree (MPT) generated.

Maximum likelihood research is conducted with RAxML-HPC v. 8.2.3 (Stamatakis, 2014) and RAxML-HPC through the CIPRES Science Gateway (Miller et al., 2009). Statistical support values (BS) are obtained using nonparametric bootstrapping with 1,000 replicates (Felsenstein, 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) are calculated for each maximum parsimony tree (MPT) generated.

The optimal substitution models for the combined data set are determined using the Akaike information criterion (AIC) implemented in MrModeltest 2.3 (Posada and Crandall, 1998; Nylander, 2004) after scoring 24 models of evolution by PAUP* version 4.0 beta 10 software (Swofford, 2002). The selected model applied in the BI analyses and ML analyses is the model GTR + I + G.

Branches that received BT support for ML (BS), MP (BP), and BPP greater than 65% (BS), 70% (BP), and 0.9 (BPP) are considered as significantly supported, respectively. Additionally, the ML analysis results in the best tree, and only the ML tree is presented along with the support values from the MP and BI analyses. FigTree v1.4.4 (Rambaut, 2018) is used to visualize the resulting tree.

**Divergence Time Estimation**

Divergence time is estimated with the BEAST v2.6.5 software package (Bouckaert et al., 2019) with 5.8S and nLSU sequences representing all main lineages in Basidiomycota (Table 2). Sequences of the species are adopted partly from the topology established by Wang et al. (2021). *Neurospora crassa* Shear and B.O. Dodge from Ascomycota are designated as outgroup taxon

![Table 2](http://www.phylo.org)

| Species                      | Specimen no. | ITS      | nLSU     |
|------------------------------|--------------|----------|----------|
| Amylocorticium cebennense    | HHH-2808     | GU187505 | GU187561 |
| Anomoloma mycelosum          | MUL-4413     | GU187500 | GU187559 |
| Athelia arachnoidea          | CBS 418.72   | GU187504 | GU187557 |
| Auricularia heimuer          | XIAHEIMAO    | LT178074 | KY141890 |
| Auricularia sp.              | PB2295       | DQ200918 | AY634277 |
| Australovulnemia cocconeae   | MG79         | HM046875 | HM046931 |
| Boletopsis leucomelegana     | AFTOL-ID 1527| DQ484064 | DQ154112 |
| Bondarzewia montana          | AFTOL-ID 452 | DQ200923 | DQ234539 |
| Brevicellicium atlanticum    | LISU 178666  | NR_119820| HE063774 |
| Brevicellicium atlanticum    | LISU 178690  | HE063775 | HE063776 |
| Brevicellicium daweishanense | CLZhao 17860 | MW302337 | MW293866 |
| Brevicellicium daweishanense | CLZhao 18255 | MW302328 | MW293867 |
| Brevicellicium exile         | MA-Fungi 26554| HE063777 | HE063778 |
| Brevicellicium olvascens     | KHL8571      | HE063792 | HE063793 |
| Brevicellicium olvascens     | MA-Fungi 23496| HE063787 | HE063788 |
| Brevicellicium xanthum       | CLZhao 17781 | MW302340 | MW293869 |
| Brevicellicium xanthum       | CLZhao 2632  | MW302339 | MW293868 |
| Bridgeoporus senonis         | Cu10013      | KY131832 | KY131891 |
| Calicera cornea              | AFTOL-ID 438 | AY789083 | AY701526 |
| Coltricia perennis           | Cu10319      | KU306067 | KU306063 |
| Coltricia defensens          | Dai 10944    | KY963737 | KY693737 |
| Corticium roseum             | MG34         | GU590877 | AY463401 |
| Craterocolla cerasi          | TUB 020203   | KF061265 | KF061265 |
| Cryptococcus_humicola        | AFTOL-ID 1552| DG645516 | DG645514 |
| Dacryopinax spathulata       | AFTOL-ID 454 | AY854070 | AY701525 |
| Dextraunicots calamicalica    | He 5700      | MK204534 | MK204547 |
| Dextraunicots calamicalica    | He 5693      | MK204533 | MK204546 |
| Eridria excida               | EL 15-98     | AF347112 | AF347112 |
| Exidiplosis calceae          | MW 331       | AF291280 | AF291236 |
| Fasciodontia brasiliensis    | MSK-F 7245a  | MK572024 | MK598734 |
| Fasciodontia bugellensis     | MSK-F 5548   | MK572024 | MK598736 |
| Fibrodontia alba             | TNME 24944   | KC928274 | KC928275 |
| Fibrodontia gossypina        | AFTOL-ID 599 | DG249274 | AY646100 |
| Fomitiporia hartigii         | MUCL 5555    | JX093799 | JX093833 |
| Fomitiporia mediterraneana   | AFTOL 888    | AY854080 | AY684157 |
| Gloeophyllum sepiarium        | Wilcox-3BB   | HMB36091 | HMB36061 |
| Gloeophyllum striatum         | ARIZAN 027866| HMB36092 | HMB36063 |
| Grimmola frondosa            | AFTOL-ID 701 | AY854084 | AY629318 |
| Gymnopolis plicatus          | ZRL 135501   | LT716066 | KY141882 |
| Hymenochaete rubiginosa       | He1049       | JQ716407 | JQ729667 |
| Hyphodontia densispora        | LWZ 20170098-5| MT319426 | MT319160 |
| Hyphodontia zhixiangi         | LWZ 20170818-13| MT319420 | MT319151 |
| Jaspiella argilaceae          | CBS 252.74   | GU187524 | GU187581 |
| Gomphidius roseus            | MB 95-036    | DO534570 | DO534699 |
| Kneiffia barba-jovis         | KHL 11730    | DO783609 | DO876360 |
| Kneiffia subalutacea          | LWZ 20170816-9| MT319407 | MT319139 |
| Lepista cristata             | ZRL 20151133| LT716026 | KY141884 |
| Leptosporomyces raunkeiseri  | HHH-7628     | GU187528 | GU187588 |
| Leucocephellus hobsoni       | Cu 6468      | KT203288 | KT203309 |
| Lyomyces macrorosus          | LWZ20170817-2| MT319459 | MT319194 |
| Multitubula mucida           | AFTOL-ID 1130| DQ521417 | AY885613 |
| Neotrodelgia gyeepsi         | Cu 10372     | KT203290 | MT319196 |
| Neotrodelgia thujae          | Dai 5065     | KT203293 | MT319197 |
| Neurospora crassa            | OR74A        | HO271348 | AF296411 |

(Continued)
Phylogenetic Analyses

The concatenated 5.8S + nLSU data set contains 50 5.8S and 50 nLSU sequences from 52 fungal specimens representing 35 taxa in Trechisporales. The data set has an aligned length of 1,528 characters, of which 1,126 are constant, 89 are variable but parsimony-uninformative, and 313 are parsimony-informative. The average standard deviation (SD) of split frequencies is 0.005271 (BI). Three new combinations, namely, Breviscellium daweishanense, Breviscellium xanthum, and Sertulicium limonadense, are proposed based on the examination of type materials and phylogenetic analyses of type sequences (Figure 1).

The ITS data set contains sequences from 58 fungal specimens representing 36 Trechispora taxa (4 new species and another 32 Trechispora taxa). The data set has an aligned length of 753 characters, of which 284 are constant, 72 are variable but parsimony-uninformative, and 397 are parsimony-informative. MP analysis yields 13 equally parsimonious trees (TL = 2,318, CI = 0.39, RI = 0.638, RC = 0.254, and HI = 0.602). The average SD of split frequencies in BI analyses is 0.006959 (BI). The phylogenetic tree (Figure 2) reveals four new and independent lineages represented by our specimens, indicating that they are phylogenetically distinct from the species currently known in the genus. In addition, another taxon (Dai 22173 and Dai 22174) is treated as Trechispora sp.

The combined data set for the molecular clock analysis includes 100 collections, of which 47 belonged to Trechisporales. This data set results in a concatenated alignment of 1,588 characters with GTR as the best-fit evolutionary model. The MCC tree is used to study divergence time. The tree shows that Trechisporales occurs in a mean stem age of 270.85 Mya with a 95% highest posterior density (HPD) of 234.1–307.93 Mya (Figure 3). The tree also shows that the Sistotremastrum family and Hydnodontaceae occur in a mean stem age of 224.25 Mya with a 95% posterior probability (PP) = 0.8) with a 95% HPD of 182.47–266.75 Mya.

Taxonomy

**Sistotremastrum family**

*Type genus*: Sistotremastrum J. Erikss.

**Habitat**: It grows on rotten angiosperm and gymnosperm wood.

Basidioma are resupinate, thin, pruinose, or waxy. Hymenophores are smooth, verruculose, or odontiod-sepimorpid. The hyphal structure is monomitic; generative hyphae bear clamp connections, CB(+). Cystidia and hyphidia conducted for 10 billion generations, producing log files and trees files. The log file is analyzed in Tracer 1, and a maximum clade credibility (MCC) tree is interpreted in TreeAnnotator by trees file, removing the first 10% of the sampled trees as burn-in, and viewed in FigTree v1.4.2.
FIGURE 1 | Phylogeny of Trechisporales generated by maximum likelihood (ML) analyses based on combined 5.8S + nLSU sequences. Branches are labelled with ML bootstrap (BT) > 65%, and Bayesian posterior probabilities (BPP) > 0.90, respectively. New combinations, the sequence origin from holotype and the type status of the species in the genus are indicated in bold.
are present in some species. Basidia are clavate or cylindrical, often with a median constriction, mostly with 2–4 or 4–6 sterigmata, and rarely with 6–8 sterigmata. Basidiospores are narrowly ellipsoid, ovoid, or cylindrical, thin-walled (but the wall is distinct), smooth, inamyloid, and acyanophilous.

**Notes:** *Sistotremastrum* family accommodates the genera *Sistotremastrum* and *Sertulicium* in the order Trechisporales based on its distinct lineage in the phylogenetic analysis. The combined phylogeny of two-gene data (Figure 1) demonstrates that *Sistotremastrum* family forms a supported sister clade to *Hydnodontaceae*. Basidia of most species in the *Sistotremastrum* family have more than four sterigmata, and basidiospores are smooth, while basidia of species in *Hydnodontaceae* have four sterigmata and their basidiospores are smooth to verrucose or aculeate. In addition, ampullate septa are only present in *Scytinopogon*, *Trechispora*, and *P. mucida* in *Hydnodontaceae*.
**Trechispora dentata** Z.B. Liu and Yuan Yuan, sp. November

**MycoBank number:** MB 842865.

**Type:** China, Yunnan province, Sipsongpanna, Mengla County, XiShuangBanNa Tropical Botanical Garden, on soil, in southwestern China, ca. E 101° 25′, N 21° 41′, alt. 570 m. The vegetation is a natural tropical forest. 4 July 2021, Y.C. Dai 22565 (holotype BJFC 037139).

**Etymology:** Dentata (Lat.): It refers to the species having a dentate hymenophore.

**Basidioma:** They are annual, resupinate, soft when fresh, fragile when dry, easily separable from the substratum, up to 2.5-cm long, 2-cm wide, and less than 1-mm thick at the center; hymenial surface irpicoid, white when fresh, becoming cream (4A2/3) when dry; margin indistinct and fimbriate, mycelial cords absent; pores or aculei 3–4/mm; hymenophore lacerate to dentate; subiculum very thin to almost absent; tubes or aculei concolorous with a hymenial surface, less than 1 mm long.

**Hyphal structure:** Hyphal system is monomitic; generative hyphae bear clamp connections; ampullate septa occasionally present in subiculum and trama, up to 5-µm wide; all hyphae IKI−, CB− are unchanged in KOH; rhomboidal calcium oxalate crystals are scattered.

**Subiculum:** Generative hyphae hyaline, thin- to thick-walled, frequently branched, loosely interwoven, 2–4 µm in diameter.

**Tubes or aculei:** Generative hyphae in trama hyaline, thin- to thick-walled, frequently branched, loosely interwoven, 2–3 µm in diameter; cystidia and cystidioles are absent; basidia are clavate or barrel-shaped, hyaline, bearing four sterigmata and a basal clamp connection, 10–15 × 4–5 µm; basidioles are similar to basidia in shape but slightly shorter.

**Basidiospores:** They are ellipsoid, hyaline, thick-walled, aculeate, occasionally with one guttule, IKI−, CB−, (4.1–5 × (3–)3.2–4(–4.1) µm (including ornamentation), L = 4.46 µm, W = 3.66 µm, Q = 1.22 (n = 60/1); (2.2–)2.6–3.7(–3.8) × 2–2.5 µm (excluding ornamentation), L′ = 3.17 µm, W′ = 2.23 µm, and Q′ = 1.42 (n = 60/1).

**Notes:** *T. dentata* was discovered in the Yunnan Province of China. Phylogenetically, *T. dentata* is close to *Trechispora regularis* (Murrill) Liberta with strong support (96% BS, 96% BP, 1.00 BPP; Figure 2). However, *T. regularis* is strictly poroid (Liberta, 1973), and basidiospores of *T. dentata* are smaller than that of *T. regularis* [4.1–5 × 3.2–4 µm vs. 4–5.5 × 3.5–5 µm in *T. regularis* (including ornamentation); Liberta, 1973].

**Trechispora dimitiella** Z.B. Liu and Yuan, sp. November

**Figure 5**

**MycoBank number:** MB 842866.

**Type:** China, Hainan Province, Haikou, Jinniuling Park, on a rotten leaf, in southwestern China, ca. E 110° 19′, N 20° 1′, alt. 17 m. The vegetation is a plantation in tropical China. 7 November 2020, Y.C. Dai 21931 (holotype BJFC 035830).

**Etymology:** Dimitiella (Lat.): It refers to the species having a dimitic hyphal system.

**Basidioma:** They are annual, resupinate, soft when fresh, fragile when dry, easily separable from the substratum, up to 6-cm long, 4-cm wide, and approximately 3-mm thick at the center; the hymenial surface is poroid, pore surface white to cream (4A2/3) when fresh, becoming white to buff-yellow (4A4) when dry; margin indistinct, often with emerging mycelial cords; pores angular, 5–6/mm; dissepiments thin, lacerate; subiculum up to 1 mm thick; tubes concolorous with a poroid surface, up to 2 mm long.
FIGURE 4 | Trechispora dentata (holotype, Dai 22565). (A) A basidioma, (B) hyphae from subiculum, (C) hyphae from trama, (D) hyphae with ampullate septa (black arrow), (E) basidia and basidioles, and (F) basidiospores. Photo by Ya-Ping Lian and Zhan-Bo Liu.
FIGURE 5 | Trechispora dimitiella (holotype, Dai 21931). (A) A basidioma, (B) hyphae with ampullate septa from subiculum (black arrow), (C) hyphae from tubes, (D) basidia, (E) basidioles, and (F) basidiospores. Photo by Ya-Ping Lian and Zhan-Bo Liu.
**Hyphal structure**: Hyphal system is dimitic; generative hyphae bear clamp connections; ampullate septa occasionally present in subiculum and trama, up to 4.5 \( \mu \)m wide; all hyphae IKI–, CB– are unchanged in KOH; rhomboidal calcium oxalate crystals are scattered.

**Subiculum**: Generative hyphae hyaline, thin-walled, rarely branched, 2–3 \( \mu \)m in diameter; skeletal hyphae thick-walled with a wide lumen, unbranched, loosely interwoven, 2–4 \( \mu \)m diameter.

**Tubes**: Generative hyphae hyaline, thin-walled, rarely branched, 1.5–2.5 \( \mu \)m in diameter; skeletal hyphae thick-walled with a wide lumen, unbranched, loosely interwoven, 2–3 \( \mu \)m in diameter; cystidia and cystidioles are absent; basidia are barrel-shaped, hyaline, bearing four sterigmata and a basal clamp connection, 9.5–12 \( \times \) 4–5 \( \mu \)m; basidioles are similar to basidia in shape but slightly shorter.

**Basidiospores**: They are ellipsoid, hyaline, thick-walled, aculeate, IKI–, CB–, (3.5–)3.6–4\( \times \) (4–4.2) \( \times \) (2.5–)2.7–3.1 (3.2) \( \mu \)m (including ornamentation), \( L = 3.84 \mu m, W = 2.92 \mu m, Q = 1.31–1.33 (n = 60/2); (2.6–)2.7–3.4 (3.7) \( \times \) 2.6–(2.9) \( \mu \)m (excluding ornamentation), \( L' = 3.04 \mu m, W' = 2.18 \mu m, and Q' = 1.38–1.4 (n = 60/2).

Additional specimen examined (paratypes): China, Yunnan Province, Jinghong, Primeval Forest Park, on soil, 7 July 2021, Y.C. Dai 22601 (BJFC), Dai 22602 (BJFC). Malaysia, Selangor, Kota Damansara, Community Forest Reserve, on rotten angiosperm wood, 7 December 2019, Y.C. Dai 21181 (BJFC 032835).

**Notes**: *T. dimitiella* was discovered in China and Malaysia. Most species in *Trechispora* are corticioid fungi with a monomitic hyphal structure, but *T. dimitiella* is different. Morphologically, *T. dimitiella* and *Trechispora brasiliensis* (Corner) K.H. Larss. share the poroid hymenophore with a dimitic hyphal system and aculeate basidiospores. However, the basidiospores of *T. dimitiella* are smaller than that of *T. brasiliensis* [3.6–4 \( \times \) 2.7–3.1 \( \mu \)m vs. 4–4.5 \( \times \) 3–4 \( \mu \)m in *T. brasiliensis* (including ornamentation), Larsson, 1992]. Phylogenetically, *T. dimitiella* is close to *Trechispora incisa* K.H. Larss. (80% BS, 0.99 BPP; **Figure 2**), but *T. dimitiella* can be easily distinguished from *T. incisa* due to its poroid hymenophore with a dimitic hyphal system because *T. incisa* has arachnoid to farinose or minutely granulose hymenophore with a monomitic hyphal system (Larsson, 1996).

**Trechispora fragilis** Z.B. Liu and Yuan Yuan, sp. November **Figure 6**

_Mycobank number:_ MB 842867.

_Type_: China, Yunnan Province, Sipsongpanna, Mengla County, XiShuangBanNa Tropical Botanical Garden, on the ground of the forest, in southwestern China, ca. E 119\(^\circ\) 56’, N 21\(^\circ\) 41’, alt. 570 m. The vegetation is a natural tropical forest. 18 August 2019, Y.C. Dai 20535 (holotype BJFC 032203).

_Etymology_: *Fragilis* (Lat.): It refers to the species having fragile basidioles.

**Basidioma**: They are annual, resupinate, soft when fresh, fragile when dry, easily separable from the substratum, up to 3 cm long, 2 cm wide, and less than 1 mm thick at the center; the hymenial surface is odontoid, white when fresh, becoming cream (4A2/3) to buff-yellow (4A4) when dry; margin is indistinct and fimbriate, often with emerging mycelial cords; aculei sparse, 4–6/mm; subiculum very thin to almost absent; aculei concolorous with a hymenial surface, less than 1 mm long.

_Hyphal structure_: Hyphal system monomitic; generative hyphae bear clamp connections; ampullate septa occasionally present in subiculum and aculei, up to 7 \( \mu \)m wide; all hyphae IKI–, CB– are unchanged in KOH; rhomboidal calcium oxalate crystals are scattered.

**Subiculum**: Generative hyphae hyaline, thin- to thick-walled, frequently branched, loosely interwoven, 1.5–4 \( \mu \)m in diameter.

**Aculei**: Generative hyphae in trama hyaline, thin- to thick-walled, frequently branched, loosely interwoven, 1.5–3 \( \mu \)m in diameter; cystidia and cystidioles are absent; basidia are clavate shaped, hyaline, bearing four sterigmata, and a basal clamp connection, 12–14 \( \times \) 3.5–4 \( \mu \)m; basidioles are similar to basidia in shape but slightly shorter.

**Basidiospores**: Ellipsoid, hyaline, thick-walled, aculeate, IKI–, CB–, (3.2–)3.8–4\( \times \) (4–2.5) \( \times \) 2.4–2.5 \( \mu \)m (including ornamentation), \( L = 3.53 \mu m, W = 2.79 \mu m, Q = 1.27 (n = 60/1); (2.6–2.8–3.7 (4) \( \times \) 1.9–2.7 (3.1) \( \mu \)m (excluding ornamentation), \( L' = 3.16 \mu m, W' = 2.26 \mu m, and Q' = 1.40 (n = 60/1).

_Notes_: *T. fragilis* was discovered in the Yunnan Province of China. Phylogenetically, *T. fragilis* groups with *Trechispora termitophila* Meiras-Ottoni and Gibertoni and *Trechispora havencampii* (Desjardin and B.A. Perry) Meiras-Ottoni and Gibertoni (69% BS, 0.92 BPP; **Figure 2**). *T. termitophila* can be easily distinguished from *T. fragilis* due to its coralloid basidioma. In addition, the basidiospores of *T. fragilis* are smaller than that of *T. termitophila* [6.5–7.5 \( \mu \)m vs. 4.5–5 \( \mu \)m in *T. termitophila* (including ornamentation), Meiras-Ottoni et al., 2021]. *T. havencampii* can also be easily distinguished from *T. fragilis* due to its coralloid basidioma. In addition, basidiospores of *T. fragilis* are smaller than that of *T. havencampii* [3.8–4 \( \times \) 2.5–3 \( \mu \)m vs. 5.2–6.5 \( \times \) 3.5–4.2 \( \mu \)m in *T. havencampii* (including ornamentation), Desjardin and Perry, 2015].

**Trechispora laevispora** Z.B. Liu, Y.D. Wu and Yuan Yuan, sp. November **Figure 7**

_Mycobank number:_ MB 842868.

_Type_: China, Inner Mongolia Autonomous Region, Arxan, Bailang Feng Scenic Spot, on the charred trunk of *Larix*, in southwestern China, ca. E 119\(^\circ\) 56’, N 47\(^\circ\) 10’, alt. 1,511 m. The vegetation is a natural boreal forest. 25 August 2020, Y.C. Dai 21655 (holotype BJFC 035556).

_Etymology_: *Laevispora* (Lat.): It refers to the species having smooth basidiospores.

**Basidioma**: They are annual, resupinate, soft when fresh and dry, up to 8 cm long, 3 cm wide, and less than 1 mm thick at the center; the hymenial surface is smooth, white when fresh and dry; margin is indistinct and fimbriate, often with emerging mycelial cords; subiculum very thin to almost absent.

_Hyphal structure_: Hyphal system monomitic; generative hyphae bear clamp connections; ampullate septa frequently present in subiculum and hymenium, up to 7 \( \mu \)m wide; all hyphae...
Subiculum: Generative hyphae hyaline, thin-walled, frequently branched, loosely interwoven, 1.5–3 µm in diameter.

Hymenium: Generative hyphae in subhymenium hyaline, thin-walled, frequently branched, 1.5–3 µm in diameter; cystidia and cystidioles are absent; basidia are clavate shaped, hyaline, bearing four sterigmata and a basal clamp connection, 11.5–15 × 4–5 µm; basidioles are similar to basidia in shape but slightly shorter.

Basidiospores: Ellipsoid, hyaline, thin-walled, smooth, IKI−, CB−, (2.5−) 2.6–3.2(--3.3) × (1.8−)1.9–2.2(--2.5) µm, \( L = 2.92 \mu m, W = 2.04 \mu m \), and \( Q = 1.43 (n = 60/1) \).

Notes: *T. laevispora* was discovered in the Inner Mongolia Autonomous Region of China. Phylogenetically, *T. laevispora* groups with *Trechispora cohaerens* (Schwein.) Jülich and Stalpers...
with strong support (94% BS, 96% BP, 1.00 BPP; Figure 2). Both species share a smooth hymenophore, a monomitic hyphal system with smooth basidiospores. However, basidiospores of *T. cohaerens* are thick-walled and larger than that of *T. laevispora* (3.5–4 × 2.2–2.5 µm in *T. cohaerens*; Larsson, 1992).
**B. daweishanense** (C.L. Zhao) Z.B. Liu and Yuan Yuan, comb. November

*Mycobank number: MB 842869.

*Basionym: T. daweishanensis C.L. Zhao, Phytotaxa 479(2): 153 (2021).

*Type:* China. Yunnan Province, Honghe, Pingbian County, Daweishan National Nature Reserve, on the fallen branch of angiosperms, 1 August 2019, CLZhao 17860 (holotype SWFC).

*Description:* See Zong et al. (2021, as *T. daweishanensis*).

**B. xanthum** (C.L. Zhao) Z.B. Liu and Yuan Yuan, comb. November

*Mycobank number: MB 842870.

*Basionym: T. xantha C.L. Zhao, Phytotaxa 479(2): 155 (2021).

*Type:* China. Yunnan Province, Xuxi, Xinpeng County, Mopanshan National Forestry Park, on the trunk of *Albizia julibrissin*, 20 August 2017, CLZhao 2632 (holotype SWFC).

*Description:* See Zong et al. (2021, as *T. xantha*).

*Notes:* Zong et al. (2021) described *T. daweishanensis* and *T. xantha* as new species. However, in our phylogeny, they belong to the genus *Breviscillium* (98% BS, 1.00 BPP; Figure 1). The type specimens of abovementioned species are studied [CLZhao 17860 (SWFC); CLZhao 2632 (SWFC)]. We do not observe ampullate hyphae from type materials as mentioned by Zong et al. (2021). We suppose that Zong et al. (2021) confused basidioles with ampullate hyphae (ampullate septa on some generative hyphae), which are remarkable characters of *Trechispora*. In fact, *T. daweishanensis* and *T. xantha* have a smooth hymenophore, a monomitic hyphal structure with clamped generative hyphae, and the absence of ampullate septa. They fit Breviscillium well. Herein, we combine these two species in Breviscillium based on morphological and phylogenetic evidence (Figure 1).

*S. limonadense* (G. Gruhn and P. Alvarado) Z.B. Liu and Yuan Yuan, comb. November

*Mycobank number: MB 842871.

*Basionym: S. limonadense G. Gruhn and P. Alvarado, Phytotaxa 498(1): 36 (2021).

*Type:* French Guiana. On the bark of an unidentified dead trunk lying on the ground, October 22, 2013, LIP 0001683 (holotype).

*Description:* See Gruhn and Alvarado (2021, as *S. limonadense*).

*Notes:* Gruhn and Alvarado (2021) described *S. limonadense* as a new species. However, at the same time, Spirin et al. (2021) segregated the species around *S. niveocremeum*—(Höhn. and Litsch.) J. Erikss. into the new genus *Sertulicium*. In our phylogeny, *S. limonadense* groups with *Sertulicium granuliferum* (Hallenb.) Spirin and Volobuev *Sertulicium lateclavigerum* (Boidin and Gilles) Spirin and Viner (Figure 1). We did not study specimens, but *S. limonadense* is characterized by smooth to tuberculate hymenophore and basidia have 6–8 sterigmata (Gruhn and Alvarado, 2021) and fits *Sertulicium* better. Hence, we transfer *S. limonadense* to *Sertulicium*.

**DISCUSSION**

Larsson (2007) showed that *S. suecicum* and *S. niveocremeum* (≡ *S. niveocremeum*) formed a strongly supported sister clade (94% BS, 1.00 BPP) to Hydnodontaceae within Trechisporales. However, in his phylogenetic analysis of 5.8S + nLSU, there were a few species in Hydnodontaceae and Sistotremastrum to establish a new family for *S. suecicum* and *S. niveocremeum*. Hence, Larsson (2007) named this clade *Sistotremastrum* family. The same strongly supported topology was recovered by Telleria et al. (2013); Gruhn et al. (2018), and Meiras-Ottoni et al. (2021) by the nLSU phylogenetic analysis. Spirin et al. (2021) presented a comprehensive study of *Sistotremastrum* and *Sertulicium* with 17 species. They used the nLSU region to perform phylogenetic analyses of 16 species in the two genera (Figure 1 in Spirin et al., 2021), except for *Sertulicium chilense* (Telleria, M. Dueñas and M.P. Martin) Spirin and Volobuev because the nLSU sequences of *S. chilense* were absent. However, they were not able to generate high support values for the node connecting Sistotremastrum and Sertulicium (87% BS, 0.87 BPP, Figure 1 in Spirin et al., 2021). As a result, they gave up establishing a new family too.

ITS1-5.8S-ITS2 is an important marker used for the barcoding of fungal species (Liu et al., 2021; Wangsawat et al., 2021). However, the difficulty in aligning ITS sequences for fungi in Trechisporales is evident because it is a data set covering taxa in distinct taxonomic levels (Larsson, 2007). Therefore, it is not a good idea to run combined analyses of ITS + nLSU, so we use the most stable and conservative portion of ITS (5.8S) and nLSU to our phylogenetic analyses of *Sistotremastrum* and *Sertulicium* (5.8S + nLSU) (Figure 1). We add *S. chilense* and *S. limonadense* to phylogenetic analyses. Our results of the *Sistotremastrum* are the same as phylogenetic analyses by Spirin et al. (2021, Figure 1). However, our phylogenetic analyses of *Sertulicium* are a bit different from that by Spirin et al. (2021, Figure 1) because the data sets used in both studies are different. Above all, we generate high support values for the node connecting *Sistotremastrum* and *Sertulicium* from ML analysis (93% BS) based on 5.8S and nLSU sequences; however, BI fails to provide support for the node (0.76 BPP).

Divergence time is estimated with 5.8S and nLSU sequences representing all main lineages in Basidiomycota (Figure 3). The MCC tree shows that Basidiomycota occurs in a mean stem age of 509.57 Mya. Trechisporales occurs in a mean stem age of 270.85 Mya. The tree also shows that the *Sistotremastrum* family and Hydnodontaceae occur in a mean stem age of 224.25 Mya (PP = 0.8). Zhao et al. (2017) indicate that the divergence times of Basidiomycota are 530 Mya (the mean stem age). He et al. (2019) indicate that the divergence times of Trechisporales and Hydnodontaceae are 259 Mya (the mean stem age). Our experimental results agree with them. In this paper, we update the divergence times of Trechisporales and Hydnodontaceae and define the divergence time of the *Sistotremastrum* family.

Bayesian phylogenetic inference fails to provide support for the node of *Sistotremastrum* and *Sertulicium*, so we use the term “Sistotremastrum family” for the two genera without a formal description of the new family. In the future, we will sequence...
additional DNA regions or whole genomes, for a more robust phylogenetic analysis.

At present, there are only two species in the Sistotremastrum family ever been recorded from China, i.e., Sistotremastrum aculeatum Miettinen and Viner (Cui 8401) and S. granuliferum (He 3338; CLZhao 5531, 9771). Recently, we collected a specimen from the Yunnan Province of China (He 6276), and its morphological and DNA data demonstrated the specimen is S. limonadense. The species is a new record in China, and we have uploaded ITS and nLSU sequences of the specimen (He 6276) to GenBank. Above all, we study all the Chinese specimens of species in the Sistotremastrum family seriously, and their morphology fits the descriptions of Gruhn and Alvarado (2021) and Spriin et al. (2021). We also collected a specimen from the Hainan Province of China (Dai 17696). The ITS (OK298490) region is different from Sistotremastrum fibrillosum G. Gruhn and P. Alvarado by 6%, and morphologically it is similar to S. fibrillosum. However, we only have a single specimen, so for the time being we regard Dai 17696 as Sistotremastrum sp.

In this article, we use the whole ITS region in analyses of Trechispora to visualize the genetic distances among new taxa and those already described. T. dentata, T. dimitiella, T. fragilis, and T. laevispora are described as new to science based on morphological characteristics and molecular evidence (Figure 2).

Most of these new species are found in subtropical or tropical Asia and conform to the phenomenon that subtropical or tropical Asia harbors high taxonomic diversity for all wood-decaying fungi (Dai, 2012; Cui et al., 2019). We also collected two resupinate specimens (Dai 22173 and Dai 22174) from the Hainan Province of China. The morphology of the two specimens corresponds to the concept of Trechispora and forms a distinct lineage within the Trechispora clade (100% BS, 1.00 BPP; Figure 2). However, these specimens are sterile, so we regard Dai 22173 and Dai 22174 as Trechispora spp. temporarily here.

Molecular phylogenetic analyses in the present study show that Brevicicilium forms a monophyletic clade in which all Brevicicilium species are included (98% BS, 1.00 BPP; Figure 1). However, when we add sequences of T. xantha and T. daweishanensis, we find sequences of a two-species cluster with Brevicicilium with high support (100% BS, 1.00 BPP; Figure 1). We request and examine type specimens from Zhao and find T. xantha and T. daweishanensis corresponding to the concept of Brevicicilium and they should be transferred to the genus Brevicicilium (see the notes of B. daweishanense).

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

Z-BL: design of the research, performance of the research, and writing and revising this manuscript. Z-BL, HZ, Y-PL, Y-RW, C-GW, and W-LM: data analysis and interpretation. Z-BL, YY, and Y-DW: a collection of the materials. All authors contributed to the article and approved the submitted version.

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