

# Morphological and molecular identification for four new species of wood-inhabiting macrofungi (Basidiomycota, Hydnodontaceae) from Yunnan-Guizhou Plateau, China

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## Research Article

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## Abstract

Four new wood-inhabiting fungi, *Trechispora albofarinosa*, *T. rhizomorpha*, *T. stipitata* and *T. wenshanensis* spp. nov., are proposed based on a combination of morphological features and molecular evidence. *Trechispora albofarinosa* is characterized by the farinosa basidiomata with flocculence hymenial surface, a monomitic hyphal system with clamped generative hyphae, and ellipsoid, warted basidiospores. *Trechispora rhizomorpha* is characterized by the membranous basidiomata with odontoid hymenial surface, rhizomorphic sterile margin, barrelled basidia and subglobose to broad ellipsoid, smooth basidiospores. *Trechispora stipitata* is characterized by the solitary or imbricate, laterally stipitate basidiomata, fan shaped pileus, radially striate covered the surface with appressed scales, odontoid hymenophore surface, and subglobose to broad ellipsoid, thin-walled, smooth basidiospores. *Trechispora wenshanensis* is characterized by a cottony basidiomata with a smooth hymenial surface, and ellipsoid, thin-walled, warted basidiospores. Sequences of ITS, nLSU, mt-SSU, GAPDH, RPB1, RPB2 markers of the studied samples were generated and phylogenetic analyses were performed with the maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogram based on the ITS+nLSU rDNA gene regions included fourteen orders within *Agaricomycetes* (*Basidiomycota*), in which the new species of *Trechispora* were grouped within the order *Trechisporales*. The phylogenetic tree inferred from the ITS sequences and the topology ITS+nLSU+mt-SSU+GAPDH+RPB1+RPB2 dataset highlighted that four new species were grouped into the genus *Trechispora*.

## Introduction

Fungi represent one of the most diverse groups of organisms on the earth, with an indispensable role in the processes and functioning of ecosystems (Hyde 2022). Wood-inhabiting fungi play an important role in the carbon cycle (Dai et al. 2015; Spirin et al. 2017; Wu et al. 2017; Ma and Zhao 2019; Huang and Zhao 2020; Lima et al. 2020; Luo and Zhao 2022a; Yu et al. 2023; Zhao et al. 2023). The wood-inhabiting fungal order *Trechisporales* K.H. Larss. is a species-poor order, compared with most other orders within *Agaricomycetes*, *Basidiomycota* (Wijayawardene et al. 2022).

*Trechispora* P. Karst. (*Hydnodontaceae*) typified by *T. onusta* P. Karst., which is characterized by resupinate to effused basidiomata; a smooth to hydroid to poroid hymenophore; ampullaceous septa; short cylindrical basidia; and smooth to verrucose or aculeate basidiospores (Karsten 1890; Bernicchia and Gorjón 2010). Currently, MycoBank and Index Fungorum have registered 160 recorded and 149 recorded intraspecific names in *Trechispora*, respectively. About 100 species are currently accepted in *Trechispora* worldwide (Karsten 1890; Bondartsev and Singer 1941; Rogers and Jackson 1943; Rogers 1944; Bondartsev 1953; Parker-Rhodes 1954; Liberta 1966, 1973; Parmasto 1968; Burdsall and Gilbertson 1982; Gilbertson and Budington 1970; Jülich 1975, 1976; Ryvarden 1975; Ryvarden and Liberta 1978; Hallenberg 1978, 1980; Jülich and Stalpers 1980; Rauschert 1987; Vries 1987; Larsson 1992, 1994, 1995, 1996; Hjortstam and Larsson 1995; Ryvarden 2002; Trichies and Schultheis 2002; Ryvarden et al. 2003; Miettinen and Larsson 2006; Dai 2011; Yuan and Dai 2012; Ordynets et al. 2015; Phookamsak et al. 2019; Xu et al. 2019; Chikowski et al. 2020; Haelewaters et al. 2020; Crous et al. 2021; de Meiras-Ottoni et al. 2021; Zhao and Zhao 2021; Liu et al. 2022; Luo and Zhao 2022a; Sommai et al. 2023), of which 31 species of the genus have been found in China (Dai 2011; Yuan and Dai 2012; Xu et al. 2019; Zhao and Zhao 2021; Luo and Zhao 2022a).

The high phylogenetic diversity on the corticioid *Agaricomycetes* based on two genes, 5.8S and 28S showed that nine taxa of *Trechispora* nested into trechisporoid clade (Larsson et al. 2004). The molecular systematics suggested that *Trechispora* belonged to *Hydnodontaceae* and was related to genera *Brevicellicium* K.H. Larss. & Hjortstam, *Porpomyces* Jülich, *Sistotremastrum* J. Erikss., and *Subulicystidium* Parmasto (Telleria et al. 2013). Based on the ITS and nLSU datasets, the phylogenetic study of *Trechispora* reported two new *Trechispora* species as *T. cyatheae* Ordynets, Langer & K.H. Larss. and *T. echinocrystallina* Ordynets, Langer & K.H. Larss., in La Réunion Island (Ordynets et al. 2015). The phylogeny of *Trechisporales* was inferred from a combined ITS-nLSU sequences, which revealed that two related genera *Porpomyces*, *Scytinopogon* Singer, grouped closely together with *Trechispora* and all of them nested within *Hydnodontaceae* (Liu et al. 2019). Based on ITS dataset, the three new species of *Trechispora* were described and used to evaluate the phylogenetic relationship with other species of this genus, in which *T. murina* was retrieved as a sister to *T. bambusicola* with moderate supports, and *T. odontioidea* formed a single lineage and then grouped with *T. fimbriata* and *T. nivea*, while *T. olivacea* formed a monophyletic lineage with *T. farinacea*, *T. hondurensis*, and *T. mollis* (Luo and Zhao 2022a). Recently, based on the morphological features and molecular evidence, three new species of *Trechispora* has been reported from Northern and Northeastern Thailand (Sommai et al. 2023).

During investigations on the wood-inhabiting fungi in the Yunnan–Guizhou Plateau of China, samples representing four additional species belonging to genus *Trechispora* were collected. To clarify the placement and relationships of the four species, we carried out a phylogenetic and taxonomic study on *Trechispora*, based on the ITS, nLSU, mt-SSU, GAPDH, RPB1, RPB2 sequences.

## Materials and methods

### Morphology

The specimens studied were deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. The macromorphological descriptions were based on field notes and photos captured in the field and laboratory. Color, texture, taste and odor of basidiomata were mostly based on authors' field trips. Color terminology followed Kornerup and Wanscher (1978). All materials were examined under a Nikon 80i microscope. Drawings were made with the aid of a drawing tube. The measurements and drawings of the microscopic structures were made (Wu et al. 2022). The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB = cotton blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = spore length (arithmetic average for all spores), W = spore width (arithmetic average for all spores), Q = L/W ratios of the specimens studied, and n = a/b (a = total number of spores measured, from b = number of specimens).

## Molecular phylogeny

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from the dried specimens following the manufacturer's instructions (Zhao and Wu 2017). The nuclear ribosomal ITS region was amplified with the primers ITS5 and ITS4 (White et al. 1990). The nuclear ribosomal LSU gene was amplified with the primers LR0R and LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994). The mt-SSU region with the primers MS1 and MS2 (White et al. 1990). The GAPDH region with the primers GAPDH-F and GAPDH-R (Kuuskeri et al. 2015). The RPB1 region with the primers RPB1-Af and RPB1-Cf (Matheny et al. 2002), and the RPB2 region with the primers bRPB2-6F and bRPB2-7.1R (Matheny 2005). The PCR procedure for ITS was 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. The PCR procedure for mt-SSU was as follows: initial denaturation at 94 °C for 2 min, followed by 36 cycles at 94 °C for 45 s, 52 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for GAPDH was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 50 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for RPB1 was as follows: (1) initial denaturation at 94 °C for 2 min, (2) denaturation at 94 °C for 40 s, (3) annealing at 60 °C for 40 s, (4) extension at 72 °C for 2 min, (5) repeat for 10 cycles starting at step 2, (6) denaturation at 94 °C for 45 s, (7) annealing at 55 °C for 1.5 min, (8) extension at 72 °C for 2 min, (9) repeat for 37 cycles starting at step 6, (10) leave at 72 °C for 10 min. The PCR procedure for RPB2 was as follows: (1) initial denaturation at 95 °C for 2.5 min, (2) denaturation at 95 °C for 30 s, (3) annealing at 52 °C for 1 min, (4) extension at 72 °C for 1 min (add 1 C per cycle), (5) repeat for 40 cycles starting at step 2, (6) extension at 72 °C for 1.5 min, (7) repeat for 40 cycles starting at step 6, (8) leave at 72 °C for 5 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Yunnan Province, China. All newly-generated sequences were deposited in NCBI GenBank (Table 1).

Table 1

List of species, specimens and GenBank accession numbers of sequences used in this study. [New species are shown in bold; \* type material].

| Species name                     | Specimen No.    | GenBank accession No. |          |        |       |      |      | References                |
|----------------------------------|-----------------|-----------------------|----------|--------|-------|------|------|---------------------------|
|                                  |                 | ITS                   | LSU      | mt-SSU | GAPDH | RPB1 | RPB2 |                           |
| <i>Aleurobotrys botryosus</i>    | CBS 336.66      | MH858812              | MH870451 | –      | –     | –    | –    | Vu et al. (2019)          |
| <i>Amaurodon viridis</i>         | TAA 149664      | AY463374              | AY586625 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Athelia epiphylla</i>         | EL 1298         | AY463382              | AY586633 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Athelopsis subinconspicua</i> | KHL 8490        | AY463383              | AY586634 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Chaetodermella luna</i>       | NH 8482         | EU118615              | –        | –      | –     | –    | –    | Larsson (2007)            |
| <i>Clavulina cristata</i>        | EL 9597         | AY463398              | AY586648 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Columnocystis abietina</i>    | KHL 12474       | EU118619              | –        | –      | –     | –    | –    | Larsson (2007)            |
| <i>Cystostereum murrayi</i>      | KHL 12496       | EU118623              | –        | –      | –     | –    | –    | Larsson (2007)            |
| <i>Dacrymyces stillatus</i>      | CBS 195.48      | MH856306              | MH867857 | –      | –     | –    | –    | Vu et al. (2019)          |
| <i>Dacryopinax spathularia</i>   | Miettinen 20559 | MW191976              | MW159092 | –      | –     | –    | –    | Unpublished               |
| <i>Erythricium laetum</i>        | NH 14530        | AY463407              | AY586655 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Fibrodontia alba</i>          | TNM F24944      | NR153983              | NG060401 | –      | –     | –    | –    | Yurchenko and Wu (2014)   |
| <i>Fibrodontia brevidens</i>     | TNMF 9008       | KC928276              | KC928277 | –      | –     | –    | –    | Yurchenko and Wu (2014)   |
| <i>Haplotrichum conspersum</i>   | KHL 11063       | AY463409              | AY586657 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Hymenochaete cinnamomea</i>   | EL 699          | AY463416              | AY586664 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Hyphodontia aspera</i>        | KHL 8530        | AY463427              | AY586675 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Inonotus radiatus</i>         | TW 704          | –                     | AF311018 | –      | –     | –    | –    | Wagner and Fischer (2001) |
| <i>Kavinia alboviridis</i>       | EL 1698         | –                     | AY463434 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Kavinia himantia</i>          | LL 98           | AY463435              | AY586682 | –      | –     | –    | –    | Larsson et al. (2004)     |
| <i>Lentaria dendroidea</i>       | SJ 98012        | EU118640              | EU118641 | –      | –     | –    | –    | Larsson (2007)            |
| <i>Lignosus hainanensis</i>      | Dai 10670       | NR154112              | GU580886 | –      | –     | –    | –    | Cui et al. (2011)         |
| <i>Merulicium fusisporum</i>     | Hjm s.n.        | EU118647              | –        | –      | –     | –    | –    | Larsson (2007)            |
| <i>Phellinus chrysoloma</i>      | TN 4008         | –                     | AF311026 | –      | –     | –    | –    | Wagner and Fischer (2001) |

| Species name                      | Specimen No.  | GenBank accession No. |          |          |       |      |          | References                    |
|-----------------------------------|---------------|-----------------------|----------|----------|-------|------|----------|-------------------------------|
|                                   |               | ITS                   | LSU      | mt-SSU   | GAPDH | RPB1 | RPB2     |                               |
| <i>Polyporus tubiformis</i>       | WD 1839       | AB587634              | AB368101 | —        | —     | —    | —        | Sotome et al. (2011)          |
| <i>Punctularia strigosozonata</i> | LR 40885      | AY463456              | AY586702 | —        | —     | —    | —        | Larsson et al. (2004)         |
| <i>Sistotrema alboluteum</i>      | TAA 167982    | AY463467              | AY586713 | —        | —     | —    | —        | Larsson et al. (2004)         |
| <i>Stereum hirsutum</i>           | NH 7960       | AF506479              | —        | —        | —     | —    | —        | Larsson and Larsson (2003)    |
| <i>Tomentellopsis echinospora</i> | KHL 8459      | AY463472              | AY586718 | —        | —     | —    | —        | Larsson et al. (2004)         |
| <i>Trametes suaveolens</i>        | CBS 279.28    | MH855012              | MH866480 | —        | —     | —    | —        | Vu et al. (2019)              |
| <i>Trechispora albofarinosa</i>   | CLZhao 4356   | OQ241383              | OQ282703 | —        | —     | —    | —        | This study                    |
| <i>Trechispora alnicola</i>       | AFTOL-ID 665  | DQ411529              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora araneosa</i>       | KHL 8570      | AF347084              | —        | —        | —     | —    | —        | Larsson et al. (2004)         |
| <i>Trechispora bambusicola</i>    | CLZhao 3302   | MW544021              | MW520171 | OQ252938 | —     | —    | OQ472008 | Zhao and Zhao (2021)          |
| <i>Trechispora bambusicola</i>    | CLZhao 3305   | MW544022              | MW520172 | —        | —     | —    | OQ686801 | Zhao and Zhao (2021)          |
| <i>Trechispora bispora</i>        | CBS:142.63    | MH858241              | MH869842 | —        | —     | —    | —        | Larsson et al. (2004)         |
| <i>Trechispora byssinella</i>     | UC 2023068    | KP814481              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora chartacea</i>      | FLOR 56185    | MK458775              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora clancularis</i>    | FRDBI 4426619 | MW487976              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora cohaerens</i>      | PDD 119021    | MW740327              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora cohaerens</i>      | HHB 19445     | MW740327              | —        | —        | —     | —    | —        | Unpublished                   |
| <i>Trechispora copiosa</i>        | AMO427        | MN701015              | MN687973 | —        | —     | —    | —        | de Meiras-Otoni et al. (2021) |
| <i>Trechispora copiosa</i>        | AMO450        | MN701017              | MN687974 | —        | —     | —    | —        | de Meiras-Otoni et al. (2021) |
| <i>Trechispora copiosa</i>        | AMO453        | MN701018              | MN687975 | —        | —     | —    | —        | de Meiras-Otoni et al. (2021) |
| <i>Trechispora copiosa</i>        | AMO456        | MN701019              | MN687976 | —        | —     | —    | —        | de Meiras-Otoni et al. (2021) |
| <i>Trechispora dentata</i>        | Dai 22565     | OK298491              | OM049408 | —        | —     | —    | —        | Liu et al. (2022b)            |
| <i>Trechispora dimitiella</i>     | Dai 21181     | OK298493              | OK298949 | —        | —     | —    | —        | Liu et al. (2022b)            |
| <i>Trechispora dimitiella</i>     | Dai 21931     | OK298492              | OK298948 | —        | —     | —    | —        | Liu et al. (2022b)            |

| Species name                   | Specimen No.    | GenBank accession No. |          |          |          |          |          | References                     |
|--------------------------------|-----------------|-----------------------|----------|----------|----------|----------|----------|--------------------------------|
|                                |                 | ITS                   | LSU      | mt-SSU   | GAPDH    | RPB1     | RPB2     |                                |
| <i>Trechispora echinospora</i> | MA-Fungi 82485a | JX392845              | JX392846 | —        | —        | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora echinospora</i> | MA-Fungi 82485b | JX392847              | JX392848 | —        | —        | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora echinospora</i> | MA-Fungi 82486a | JX392850              | JX392851 | —        | —        | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora echinospora</i> | MA-Fungi 82486b | JX392853              | JX392854 | —        | —        | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora farinacea</i>   | KHL 8451        | AF347082              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora farinacea</i>   | KHL 8454        | AF347083              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora farinacea</i>   | KHL 8793        | AF347089              | —        | —        | —        | —        | —        | Larsson et al. (2004)          |
| <i>Trechispora farinacea</i>   | MA-Fungi 79474  | JX392855              | JX392856 | —        | —        | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora fibrillosa</i>  | FRDBI 13394362  | MW487977              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora fimbriata</i>   | CLZhao 7969     | MW544024              | MW520174 | —        | —        | —        | OQ686802 | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 9006     | MW544025              | MW520175 | —        | —        | OQ787422 | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 4154     | MW544023              | MW520173 | —        | —        | OQ787420 | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 9102     | OQ241384              | —        | —        | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10297    | MT159997              | OQ282705 | —        | —        | OQ787424 | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10154    | MT159996              | OQ282704 | —        | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10373    | MT159998              | OQ282706 | —        | OQ766943 | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10389    | MT159999              | OQ282707 | —        | OQ766944 | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10398    | MT160000              | OQ282708 | —        | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 10442    | MT160001              | OQ282709 | OQ252939 | OQ766945 | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 15857    | OQ241385              | OQ282710 | OQ252940 | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fimbriata</i>   | CLZhao 16478    | MW581205              | —        | OQ252941 | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fissurata</i>   | CLZhao 995      | MW544026              | MW520176 | —        | —        | —        | —        | Zhao and Zhao (2021)           |
| <i>Trechispora fissurata</i>   | CLZhao 4571     | MW544027              | MW520177 | OQ252942 | OQ766958 | OQ787423 | OQ472007 | Zhao and Zhao (2021)           |
| <i>Trechispora fragilis</i>    | Dai 20535       | OK298494              | OK298950 | —        | —        | —        | —        | Liu et al. (2022b)             |
| <i>Trechispora gelatinosa</i>  | AMO824          | MN701020              | MN687977 | —        | —        | —        | —        | de Meiras-Ottoni et al. (2021) |

| Species name                    | Specimen No.         | GenBank accession No. |          |          |       |          |          | References                     |
|---------------------------------|----------------------|-----------------------|----------|----------|-------|----------|----------|--------------------------------|
|                                 |                      | ITS                   | LSU      | mt-SSU   | GAPDH | RPB1     | RPB2     |                                |
| <i>Trechispora gelatinosa</i>   | AMO1139              | MN701021              | MN687978 | —        | —     | —        | —        | de Meiras-Ottoni et al. (2021) |
| <i>Trechispora havencampii</i>  | SFSU DED8300         | NR154418              | NG059993 | —        | —     | —        | —        | Desjardin and Perry (2015)     |
| <i>Trechispora hondurensis</i>  | PUL HONDURAS19-F016  | NR178152              | NG081479 | —        | —     | —        | —        | Haelewaters et al. (2020)      |
| <i>Trechispora hondurensis</i>  | PUL HONDURAS19-F016a | MT571523              | MT636540 | —        | —     | —        | —        | Haelewaters et al. (2020)      |
| <i>Trechispora hymenocystis</i> | KHL 8795             | AF347090              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora hymenocystis</i> | KHL 16444            | MT816397              | —        | —        | —     | —        | —        | Larsson (2020)                 |
| <i>Trechispora incisa</i>       | GB0090521            | KU747093              | KU747086 | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora incisa</i>       | GB0105526            | KU747094              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora incisa</i>       | GB0090648            | KU747095              | KU747087 | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora invisitata</i>   | 5425_537             | ON963772              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora invisitata</i>   | UC2023088            | KP814425              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora invisitata</i>   | UC2022935            | KP814182              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora kavinioides</i>  | KGN 981002           | AF347086              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora laevispora</i>   | Dai 21655            | OK298495              | OM108710 | —        | —     | —        | —        | Liu et al. (2022b)             |
| <i>Trechispora mellina</i>      | URM85756             | —                     | MH280000 | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora microspora</i>   | FRDBI 18772216       | OL828778              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora mollis</i>       | URM85884             | MK514945              | MK514945 | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora mollusca</i>     | iNAT 30809943        | MZ269232              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora mollusca</i>     | CFMR:DLL2011-186     | KJ140681              | —        | —        | —     | —        | —        | Unpublished                    |
| <i>Trechispora murina</i>       | CLZhao 11736         | OL615003              | OQ282712 | OQ252943 | —     | OQ787425 | OQ686800 | Luo and Zhao (2022a)           |
| <i>Trechispora murina</i>       | CLZhao 11752         | OL615004              | OL615009 | —        | —     | —        | —        | Luo and Zhao (2022a)           |
| <i>Trechispora nivea</i>        | MA-Fungi 76238       | JX392824              | JX392825 | —        | —     | —        | —        | Telleria et al. (2013)         |
| <i>Trechispora nivea</i>        | MA-Fungi 76257       | JX392826              | JX392827 | —        | —     | —        | —        | Telleria et al. (2013)         |

| Species name                    | Specimen No.            | GenBank accession No. |          |          |       |          |      | References                    |
|---------------------------------|-------------------------|-----------------------|----------|----------|-------|----------|------|-------------------------------|
|                                 |                         | ITS                   | LSU      | mt-SSU   | GAPDH | RPB1     | RPB2 |                               |
| <i>Trechispora nivea</i>        | MA-Fungi 82480          | JX392829              | JX392830 | —        | —     | —        | —    | Telleria et al. (2013)        |
| <i>Trechispora nivea</i>        | MA-Fungi 74044          | JX392832              | JX392833 | —        | —     | —        | —    | Telleria et al. (2013)        |
| <i>Trechispora odontioidea</i>  | CLZhao 17890            | ON417458              | OQ282713 | —        | —     | OQ787427 | —    | Luo and Zhao (2022a)          |
| <i>Trechispora olivacea</i>     | CLZhao 17826            | ON417457              | OQ282714 | OQ252944 | —     | OQ787426 | —    | Luo and Zhao (2022a)          |
| <i>Trechispora pallescens</i>   | He442                   | —                     | MK204553 | —        | —     | —        | —    | Liu et al. (2019)             |
| <i>Trechispora pallescens</i>   | FLOR56184               | MK458767              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora pallescens</i>   | FLOR56188               | MK458774              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora papillosa</i>    | AMO713                  | MN701022              | MN687979 | —        | —     | —        | —    | de Meiras-Otoni et al. (2021) |
| <i>Trechispora papillosa</i>    | AMO795                  | MN701023              | MN687981 | —        | —     | —        | —    | de Meiras-Otoni et al. (2021) |
| <i>Trechispora patawaensis</i>  | VPapp-GF1901            | OL314550              | OL314546 | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora praefocata</i>   | FRDBI 18819116          | OL828784              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora regularis</i>    | KHL 10881               | AF347087              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora rhizomorpha</i>  | CLZhao 2522 *           | OQ241386              | —        | —        | —     | —        | —    | This study                    |
| <i>Trechispora rhizomorpha</i>  | CLZhao 7870             | OQ241387              | —        | —        | —     | —        | —    | This study                    |
| <i>Trechispora rigida</i>       | URM85754                | MT406381              | MH279999 | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora stellulata</i>   | 14153                   | MW023104              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora stellulata</i>   | MICH:352202             | ON364078              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora stellulata</i>   | UC2023099               | KP814451              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora stellulata</i>   | UC2023230               | KP814491              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora stevensonii</i>  | MA-Fungi 70669          | JX392841              | JX392842 | —        | —     | —        | —    | Telleria et al. (2013)        |
| <i>Trechispora stevensonii</i>  | MA-Fungi 70645          | JX392843              | JX392844 | —        | —     | —        | —    | Telleria et al. (2013)        |
| <i>Trechispora stipitata</i>    | CLZhao 4456             | OQ241388              | OQ282715 | —        | —     | OQ787421 | —    | This study                    |
| <i>Trechispora subhelvetica</i> | Tom Hellik Hofton 06435 | JN710601              | —        | —        | —     | —        | —    | Unpublished                   |
| <i>Trechispora subregularis</i> | VPapp-GF2103            | OL331097              | OL314548 | —        | —     | —        | —    | Unpublished                   |



| Species name                         | Specimen No. | GenBank accession No. |          |          |          |          |          | References                     |
|--------------------------------------|--------------|-----------------------|----------|----------|----------|----------|----------|--------------------------------|
|                                      |              | ITS                   | LSU      | mt-SSU   | GAPDH    | RPB1     | RPB2     |                                |
| <i>Trechispora subsphaerospora</i>   | KHL 8511     | AF347080              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora termitophila</i>      | AMO396       | MN701025              | MN687983 | —        | —        | —        | —        | de Meiras-Ottoni et al. (2021) |
| <i>Trechispora termitophila</i>      | AMO893       | MN701026              | MN687984 | —        | —        | —        | —        | de Meiras-Ottoni et al. (2021) |
| <i>Trechispora termitophila</i>      | AMO1165      | MN701027              | MN687985 | —        | —        | —        | —        | de Meiras-Ottoni et al. (2021) |
| <i>Trechispora termitophila</i>      | AMO1169      | MN701028              | MN687986 | —        | —        | —        | —        | de Meiras-Ottoni et al. (2021) |
| <i>Trechispora torrendii</i>         | URM85886     | MK515148              | MH280004 | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora torrendii</i>         | KHL 15384    | —                     | MH290760 | —        | —        | —        | —        | Unpublished                    |
| <i>Trechispora wenshanensis</i>      | CLZhao 11649 | OQ241389              | OQ282716 | —        | —        | —        | OQ686803 | This study                     |
| <i>Trechispora yunnanensis</i>       | CLZhao 210   | NR177488              | MN654918 | —        | —        | —        | —        | Xu et al. (2019)               |
| <i>Trechispora yunnanensis</i>       | CLZhao 214   | MN654922              | MN654919 | —        | —        | —        | —        | Xu et al. (2019)               |
| <i>Trechispora yunnanensis</i>       | CLZhao 215   | MN654923              | MN654920 | OQ252945 | —        | —        | —        | Xu et al. (2019)               |
| <i>Vuilleminia comedens</i>          | EL 199       | AY463482              | AY586725 | —        | —        | —        | —        | Larsson et al. (2004)          |
| <i>Xenasmatella ailaoshanensis</i>   | CLZhao 4839  | MN487106              | —        | —        | —        | —        | —        | Huang et al. (2019)            |
| <i>Xenasmatella ardosiacae</i>       | CLZhao 10450 | OQ241391              | —        | —        | OQ766941 | OQ787435 | OQ686805 | Unpublished                    |
| <i>Xenasmatella bambusicola</i>      | CLZhao 10985 | OQ371469              | OQ147009 | OQ252949 | OQ766942 | —        | —        | Yuan et al. (2023)             |
| <i>Xenasmatella borealis</i>         | UC2023132    | KP814274              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Xenasmatella christiansenii</i>   | TASM:YG-G36  | MT526342              | —        | —        | —        | —        | —        | Gafforov et al. (2020)         |
| <i>Xenasmatella gossypina</i>        | CLZhao 4316  | MW545959              | OQ282722 | OQ252953 | —        | —        | —        | Zong and Zhao (2021)           |
| <i>Xenasmatella nigroidea</i>        | CLZhao 18300 | OK045679              | OK045677 | —        | —        | —        | —        | Luo and Zhao (2022)            |
| <i>Xenasmatella rhizomorpha</i>      | CLZhao 9979  | OQ241403              | OQ282731 | —        | OQ766954 | OQ685934 | OQ686814 | Unpublished                    |
| <i>Xenasmatella roseobubalina</i>    | Dai 20506    | OM855607              | —        | —        | —        | —        | —        | Liu and Yuan (2022)            |
| <i>Xenasmatella tenuis</i>           | CLZhao 4528  | MT832960              | —        | —        | —        | —        | —        | Zong et al. (2021)             |
| <i>Xenasmatella vaga</i>             | DK 1566      | OL436894              | —        | —        | —        | —        | —        | Unpublished                    |
| <i>Xenasmatella wuliangshanensis</i> | CLZhao 4080  | MW545962              | —        | —        | —        | —        | —        | Zong and Zhao (2021)           |

| Species name                      | Specimen No. | GenBank accession No. |     |        |       |      |      | References         |
|-----------------------------------|--------------|-----------------------|-----|--------|-------|------|------|--------------------|
|                                   |              | ITS                   | LSU | mt-SSU | GAPDH | RPB1 | RPB2 |                    |
| <i>Xenasmattella xinpingensis</i> | CLZhao 2216  | MT832961              | —   | —      | —     | —    | —    | Zong et al. (2021) |
| <i>Xerocomus chrysenteron</i>     | KGP 62       | DQ822793              | —   | —      | —     | —    | —    | Peay et al. (2007) |

The sequences were aligned in MAFFT version 7 (Kato et al. 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). Each dataset was aligned separately at first and then the ITS, nLSU, mt-SSU, GAPDH, RPB1 and RPB2 regions were combined with Mesquite version 3.51. The combined dataset was deposited in TreeBASE (submission ID 30665). Sequences of *Dacrymyces stillatus* Nees and *Dacryopinax spathularia* (Schwein.) G.W. Martin retrieved from GenBank were used as an outgroup in the ITS + nLSU analysis (Fig. 1) (Luo and Zhao 2022b); Sequences of *Fibrodontia alba* Yurchenko & Sheng H. Wu and *F. brevidens* (Pat.) Hjortstam & Ryvarden retrieved from GenBank were used as an outgroup in the ITS analysis (Fig. 2) (Luo and Zhao 2022a); Sequences of *Fibrodontia alba* and *F. brevidens* retrieved from GenBank were used as an outgroup in the ITS + nLSU + mt-SSU + GAPDH + RPB1 + RPB2 analysis (Fig. 3) (Luo and Zhao 2022a).

Maximum parsimony analysis in PAUP\* version 4.0a169 (<http://phylosolutions.com/paup-test/>) was applied to ITS and the combined ITS + nLSU datasets following a previous study (Zhao and Wu 2017). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 pseudo replicates (Felsenstein 1985). Descriptive tree statistics - tree length (TL), composite consistency index (CI), composite retention index (RI), composite rescaled consistency index (RC) and composite homoplasy index (HI) - were calculated for each maximum parsimonious tree generated. The combined dataset was also analysed using Maximum Likelihood (ML) in RAxML-HPC2 through the CIPRES Science Gateway (Miller et al. 2012). Branch support (BS) for the ML analysis was determined by 1000 bootstrap pseudoreplicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each dataset for the purposes of Bayesian inference (BI), Bayesian inference was performed using MrBayes 3.2.7a with a GTR + I + G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). A total of four Markov chains were run for two runs from random starting trees for 4.5 million generations for ITS + nLSU (Fig. 1); 7 million generations for ITS (Fig. 2) and 4 million generations for ITS + nLSU + mt-SSU + GAPDH + RPB1 + RPB2 (Fig. 3) with trees and parameters sampled every 1,000 generations. The first quarter of all of the generations were discarded as burn-ins. A majority rule consensus tree was computed from the remaining trees. Branches were considered as significantly supported if they received a maximum likelihood bootstrap support value (BS) of > 70%, a maximum parsimony bootstrap support value (BT) of > 70% or a Bayesian posterior probability (BPP) of > 0.95.

## Results

### Molecular phylogeny

The ITS+nLSU dataset (Fig. 1) comprised sequences from 89 fungal specimens representing 89 taxa. The dataset had an aligned length of 2,137 characters, of which 1,016 characters were constant, 225 were variable and parsimony-uninformative and 896 (42%) were parsimony-informative. Maximum parsimony analysis yielded 5,000 equally parsimonious tree (TL = 6,686, CI = 0.3086, HI = 0.6914, RI = 0.5044 and RC = 0.1557). The best model of nucleotide evolution for the ITS + nLSU dataset estimated and applied in the Bayesian analysis was found to be GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis. The Bayesian analysis had an average standard deviation of split frequencies = 0.020839 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 270.5. The phylogram based on the ITS+nLSU rDNA gene regions (Fig. 1) include 14 orders within *Agaricomycetes* (*Basidiomycota*), which are *Agaricales*, *Atheliales*, *Boletales*, *Cantharellales*, *Corticiales*, *Gloeophyllales*, *Gomphales*, *Hymenochaetales*, *Polyporales*, *Russulales*, *Thelephorales*, *Trechisporales*, *Xenasmatales*, *Dacrymycetes*, in which *Trechispora* was grouped within the order *Trechisporales*.

The ITS dataset (Fig. 2) comprised sequences from 97 fungal specimens representing 50 taxa. The dataset had an aligned length of 641 characters, of which 191 characters were constant, 56 were variable and parsimony-uninformative and 394 (61%) were parsimony-informative. Maximum parsimony analysis yielded 5,000 equally parsimonious tree (TL = 3,545, CI = 0.2795, HI = 0.7205, RI = 0.6493 and RC = 0.1815). The best model of nucleotide evolution for the ITS dataset estimated and applied in the Bayesian analysis was found to be GTR + I + G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis. The Bayesian analysis had an average standard deviation of split frequencies = 0.007750 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 718. The phylogenetic tree (Fig. 2), inferred from the ITS sequences, highlighted that four new species were grouped into the genus *Trechispora*.

The ITS+nLSU+mt-SSU+GAPDH+RPB1+RPB2 dataset (Fig. 3) comprised sequences from 97 fungal specimens representing 50 taxa. The dataset had an aligned length of 5,571 characters, of which 2,563 characters were constant, 1,635 were variable and parsimony-uninformative and 1,373 (25%) were parsimony-informative. Maximum parsimony analysis yielded 5,000 equally parsimonious trees (TL = 8,491, CI = 0.5062, HI = 0.4938, RI = 0.5763 and RC = 0.2917). The best model of nucleotide evolution for the ITS dataset estimated and applied in the Bayesian analysis was found to be GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis. The Bayesian analysis had an average standard deviation of split frequencies = 0.031349 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 413.5. The topology (Fig. 3), based on ITS+nLSU+mt-SSU+GAPDH+RPB1+RPB2 sequences, revealed that four new species were grouped into the genus *Trechispora*.

## Taxonomy

***Trechispora albofarinosa*** K.Y. Luo & C.L. Zhao, sp. nov. (Figs. 4, 5 and 6)

MycoBank no.: MB 849463

*Holotype*: CHINA, Yunnan Province, Puer, Jingdong County, Huangcaoling, Wuliangshan National Nature Reserve, 24°23'N, 100°45'E, altitude 2350 m a.s.l., on the fallen branch of *Pinus*, leg. C.L. Zhao, 5 October 2017, CLZhao 4356 (SWFC).

*Etymology*: *albofarinosa* (Lat.)—referring to the farinosa basidiomata with white hymenial surface.

*Fruiting body*: Basidiomata annual, resupinate, farinosa, without odor or taste when fresh, up to 3.5 cm long, 1.5 cm wide, and 300–500 µm thick. Hymenial surface flocculence, white when fresh, white to cream on drying. Sterile margin indistinct, white, and up to 0.5 mm wide.

*Hyphal structure*: Hyphal system monomitic, generative hyphae with clamp connections, colorless, thick-walled, frequently branched, interwoven, 2–3.5 µm in diameter; IKI–, CB–, tissues unchanged in KOH.

*Hymenium*: Cystidia and cystidioles are absent; basidia clavate, with four sterigmata and a basal clamp connection, 6.5–10 × 3.5–5 µm.

*Spores*: Basidiospores ellipsoid, colorless, thin-walled, warted, IKI–, CB–, 2.5–3.5 (–4) × 2–2.5 (–3.5) µm, L = 3.18 µm, W = 2.44 µm, Q = 1.3 (n = 30/1).

***Trechispora rhizomorpha*** K.Y. Luo & C.L. Zhao, sp. nov. (Figs. 7 and 8)

MycoBank no.: MB 849464

*Holotype*: CHINA, Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, 23°56'N, 101°29'E, altitude 2200 m a.s.l., on the trunk of *Albizia julibrissin*, leg. C.L. Zhao, 20 August 2017, CLZhao 2522 (SWFC).

*Etymology*: *rhizomorpha* (Lat.)—referring to the rhizomorphic basidiomata.

*Fruiting body*: Basidiomata annual, resupinate, adnate, membranous, without odor or taste when fresh, up to 2.5 cm long, 1.5 cm wide, and 4 mm thick. Hymenial surface odontoid, cream. Sterile margin indistinct, white, rhizomorphic, and up to 0.5 mm wide.

*Hyphal structure*: Hyphal system monomitic, generative hyphae with clamp connections, colorless, slightly thick-walled, ampullate septa frequently present in subiculum and hymenium with crystals, up to 6 µm wide, branched, interwoven, 2.5–4 µm in diameter; IKI–, CB–, tissues unchanged in KOH.

*Hymenium*: Cystidia and cystidioles are absent; basidia barrelled, slightly constricted, with four sterigmata and a basal clamp connection, 6.5–14.5 × 3.5–5.5 µm.

*Spores*: Basidiospores subglobose to broad ellipsoid, colorless, slightly thick-walled, smooth, IKI–, CB–, (2–) 2.5–4 × 2–3.5 µm, L = 3.03 µm, W = 2.41 µm, Q = 1.23–1.28 (n = 60/2).

*Additional specimen (paratype) examined*: China, Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, 23°56'N, 101°29'E, altitude 2200 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 19 August 2018, CLZhao 7870 (SWFC).

***Trechispora stipitata*** K.Y. Luo & C.L. Zhao, sp. nov. (Figs. 9,10 and 11)

MycoBank no.: MB 849465

*Holotype*: CHINA, Yunnan Province, Puer, Jingdong County, Wuliangshan National Nature Reserve, 24°23'N, 100°45'E, altitude 2350 m a.s.l., on the trunk of angiosperm, leg. C.L. Zhao, 6 October 2017, CLZhao 4456 (SWFC).

*Etymology: stipitata* (Lat.)—referring to the stipitate basidiomata.

*Fruiting body:* Basidiomata annual, laterally stipitate, solitary or imbricate. Pileus fan shaped, cortical to cork, up to 1.5 cm long, 1 cm wide, and 2 mm thick, yellowish to yellowish brown, the surface radially striate covered with appressed scales, azonate; the hymenophore surface odontoid, yellowish brown, up to 1 mm long. Context cream, 1 mm thick. Sterile margin indistinct, slightly buff, and 0.5 mm wide.

*Hyphal structure:* Hyphal system monomitic, generative hyphae with clamp connections, colorless, thick-walled, frequently branched, interwoven, 2.5–4 µm in diameter, IKI–, CB–, tissues unchanged in KOH.

*Hymenium:* Cystidia and cystidioles are absent; basidia subcylindrical, constricted, with four sterigmata and a basal clamp connection, 5–7 × 2.5–4 µm.

*Spores:* Basidiospores subglobose to broad ellipsoid, colorless, thin-walled, smooth, IKI–, CB–, (2.5–) 2.8–5 (–5.5) × (2.5–) 3–4.7 µm, L = 4 µm, W = 3.56 µm, Q = 1.12 (n = 30/1).

***Trechispora wenshanensis*** K.Y. Luo & C.L. Zhao, sp. nov. (Figs. 12, 13 and 14)

MycoBank no.: MB 849466

*Holotype:* CHINA, Yunnan Province, Wenshan, Babao Town, Balao battle site, 23°22'N, 104°15'E, altitude 1300 m a.s.l., on the fallen branch of angiosperm, leg. C.L. Zhao, 19 January 2019, CLZhao 11649 (SWFC).

*Etymology: wenshanensis* (Lat.)—referring to the locality (Wenshan) of the type specimen.

*Fruiting body:* Basidiomata annual, resupinate, adnate, cottony, easily to separate from substrate, without odor or taste when fresh, up to 5.5 cm long, 4 cm wide, and 200–400 µm thick. Hymenial surface smooth, slightly cream when fresh, cream to buff on drying. Sterile margin indistinct, cream, and 1–2 mm wide.

*Hyphal structure:* Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin- to thick-walled, branched, interwoven, 1–2 µm in diameter, IKI–, CB–, tissues unchanged in KOH.

*Hymenium:* Cystidia and cystidioles are absent; basidia barrelled, with four sterigmata and a basal clamp connection, 7–10 × 3–5 µm.

*Spores:* Basidiospores ellipsoid, colorless, thin-walled, warty, IKI–, CB–, (2–) 2.5–3.7 (–4) × (1.5–) 2–3 µm, L = 3.02 µm, W = 2.37 µm, Q = 1.27 (n = 30/1).

## Discussion

Many recently described wood-inhabiting fungal taxa have been reported in the subtropics and tropics, including in the genus *Trechispora* (Ordynets et al. 2015; Phookamsak et al. 2019; Xu et al. 2019; Chikowski et al. 2020; Haelewaters et al. 2020; Crous et al. 2021; de Meiras-Ottoni et al. 2021; Zhao and Zhao 2021; Luo and Zhao 2022a; Sommai et al. 2023). The present study reports four new species in *Trechispora*, based on a combination of morphological features and molecular evidence.

Phylogenetically, the classification of corticioid fungi revealed that two taxa of *Trechispora farinacea* and *T. hymenocystis* nested into *Trechispora* located in *Hydnodontaceae* (*Trechisporales*) (Larsson 2007). In the present study, based on the ITS+nLSU data (Fig. 1), include 14 orders within *Agaricomycetes* (*Basidiomycota*), in which *Trechispora* was grouped within the order *Trechisporales*.

Based on ITS topology (Fig. 2) and ITS+nLSU+mt-SSU+GAPDH+RPB1+RPB2 phylogenetic tree (Fig. 3), four new species were grouped into the genus *Trechispora*, in which *T. albofarinosa* was sister to *T. araneosa* (Höhn. & Litsch.) K.H. Larss., and then grouped with the clade comprising *T. hymenocystis* (Berk. & Broome) K.H. Larss. and *T. mollusca* (Pers.) Liberta, However, morphologically, *T. araneosa* can be delimited from *T. albofarinosa* by its odontoid to poroid hymenial surface and larger basidiospores (5–6.5 × 4–5 µm; Larsson 1995). Further, *T. hymenocystis* differs from *T. albofarinosa* by its poroid hymenophore, shallow pores and larger basidiospores (4.5–5.5 × 3.5–4.5 µm; Larsson 1994); *T. mollusca* differs in its poroid hymenial surface, and ovoid to subglobose, broadly basidiospores (3.5–4 × 3–3.5 µm; Liberta 1973). The second new species *T. rhizomorpha* grouped closely with *T. laevispora* Z.B. Liu, Y.D. Wu & Yuan Yuan, and then clustered with *T. invisitata* (H.S. Jacks.) Liberta. However, morphologically, *T. laevispora* can be delimited from *T. rhizomorpha* by having the smooth hymenial surface, and thin-walled basidiospores (Liu et al. 2022); *T. invisitata* is different from *T. rhizomorpha* by its dimitic hyphal system and warty, longer basidiospores (4.5–5.5 × 3–4 µm; Liberta 1966). The third species *T. stipitata* formed a monophyletic lineage. The species *T. wenshanensis* grouped closely with *T. rigida* (Berk.) K.H. Larss. and *T. kavinioides* B. de Vries. However, morphologically, *T. rigida* can be delimited from *T. wenshanensis* by having the tuberculate hymenial surface and broadly ellipsoid, slightly thick-walled, larger basidiospores (5 × 4 µm; Larsson 1996). Further, *T. kavinioides* differs in its odontoid hymenial surface and narrower ellipsoid to lacrymiform basidiospores (Bernicchia and Gorjón 2010).

Morphologically, *Trechispora albofarinosa* resembles *T. olivacea* K.Y. Luo & C.L. Zhao and *T. yunnanensis* C.L. Zhao by sharing the farinosa basidiomata. However, *T. olivacea* differs from *T. albofarinosa* by olivaceous hymenial surface and thick-walled basidiospores (Luo and Zhao 2022a); *T. yunnanensis* can be delimited from *T. albofarinosa* due to its thick-walled, larger basidiospores (7–8.5 × 5–5.5 µm; Xu et al. 2019). The new species *T. albofarinosa* is similar to *T. bambusicola* C.L. Zhao, *T. fimbriata* C.L. Zhao, *T. fissurata* C.L. Zhao and *T. murina* K.Y. Luo & C.L. Zhao in its presence of ellipsoid basidiospores. *T. bambusicola* can be delimited from *T. albofarinosa* by odontoid hymenial surface with aculei cylindrical to conical (0.3–0.5 mm long), and thick-walled basidiospores (Zhao and Zhao 2021); *T. fimbriata* can be delimited from *T. albofarinosa* due to its hydroid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); *T. fissurata* is different from *T. albofarinosa* by hydroid hymenial surface and thick-walled, broadly basidiospores (3.3–4 × 2.8–3.5 µm; Zhao and Zhao 2021); *T. murina* can be delimited from *T. albofarinosa* due to its grandinoid hymenial surface and thick-walled basidiospores (Luo and Zhao 2022a).

*Trechispora rhizomorpha* is similar to *T. fastidiosa* (Pers.) Libertá by sharing the membranous basidiomata. However, *T. fastidiosa* differs from *T. rhizomorpha* by smooth hymenial surface and larger basidiospores (6–7 × 4.5–5.5 µm; Bernicchia and Gorjón 2010). *T. rhizomorpha* resembles *T. bambusicola* C.L. Zhao, *T. canariensis* Ryvarden & Libertá and *T. christiansenii* (Parmasto) Libertá in its monomitic hyphal system and presence of the crystals. However, *T. bambusicola* differs from *T. rhizomorpha* by its odontoid hymenial surface and ornamented basidiospores (Zhao and Zhao 2021); *T. canariensis* differs from *T. rhizomorpha* due to its larger basidia (15–20 × 5–6 µm) and thin-walled, larger basidiospores (5–7 × 3–3.5 µm; Ryvarden and Libertá 1978); *T. christiansenii* can be delimited from *T. rhizomorpha* by its larger basidia (15–20 × 6–7 µm) and larger basidiospores (5.5–7 × 4–4.5 µm; Libertá 1966).

*Trechispora stipitata* is similar to *T. byssinella* (Bourdot) Libertá, *T. kavinioides* B. de Vries, *T. silvae-ryae* (J. Erikss. & Ryvarden) K.H. Larss. and *T. subsphaerospora* (Litsch.) Libertá by sharing smooth basidiospores. However, *T. byssinella* differs from *T. rhizomorpha* by having narrower ellipsoid basidiospores (Bernicchia and Gorjón 2010); *T. kavinioides* can be delimited from *T. rhizomorpha* by its odontoid hymenial surface, and narrower ellipsoid to lacrymiform basidiospores (Bernicchia & Gorjón 2010); *T. silvae-ryae* is different from *T. rhizomorpha* by dimitic hyphal system (Bernicchia and Gorjón 2010); *T. subsphaerospora* differs from *T. rhizomorpha* by having angular basidiospores (Bernicchia and Gorjón 2010).

*Trechispora wenshanensis* resembles *T. fastidiosa* and *T. laevispora* Z.B. Liu, Y.D. Wu & Yuan Yuan by sharing smooth hymenial surface. However, *T. fastidiosa* differs from *T. wenshanensis* by larger basidiospores (6–7 × 4.5–5.5 µm; Bernicchia and Gorjón 2010); *T. laevispora* differs from *T. wenshanensis* by fimbriate margin of the basidiomata and smooth basidiospores (Liu et al. 2022). The new species *T. wenshanensis* is similar to *T. bambusicola* C.L. Zhao, *T. fimbriata* C.L. Zhao, *T. fissurata* C.L. Zhao, *T. murina* K.Y. Luo & C.L. Zhao and *T. yunnanensis* C.L. Zhao due to its ellipsoid basidiospores. However, *T. bambusicola* can be delimited from *T. wenshanensis* by odontoid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); *T. fimbriata* differs from *T. wenshanensis* due to its hydroid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); *T. fissurata* is different from *T. wenshanensis* by hydroid hymenial surface, and thick-walled, broadly basidiospores (3.3–4 × 2.8–3.5 µm; Zhao and Zhao 2021); *T. murina* can be delimited from *T. wenshanensis* due to its grandinoid hymenial surface, and thick-walled basidiospores (Luo and Zhao 2022a); *T. yunnanensis* is different from *T. wenshanensis* by farinaceous hymenial surface and thick-walled, larger basidiospores (7–8.5 × 5–5.5 µm; Xu et al. 2019).

#### Key to 35 accepted species of *Trechispora* in China

1. Basidiomata with clavarioid..... *T. longiramosa*
1. Basidiomata without clavarioid.....2
2. Basidiomata pileate-stipitate..... ***T. stipitata***
2. Basidiomata resupinate to effused.....3
3. Hymenophore poroid.....4
3. Hymenophore smooth, colliculose, irpicoid, grandinoid, odontoid, hydroid.....8
4. Hyphal system dimitic..... *T. dimitiella*
4. Hyphal system monomitic.....5
5. Subicular hyphae thick-walled.....6
5. Subicular hyphae thin-walled.....7
6. Ampullate septa present on subicular hyphae..... *T. mollusca*

|  |                               |
|--|-------------------------------|
| 6. Ampullate septa absent on subicular hyphae.....                         | <i>T. suberosa</i>            |
| 7. Crystals in subiculum as numerous rodlets.....                          | <i>T. candidissima</i>        |
| 7. Crystals in subiculum as rhomboidal plates or various shapes.....       | 8                             |
| 8. Sphaerocysts present in cords and the adjacent part of subiculum.....   | <i>T. hymenocystis</i>        |
| 8. Sphaerocysts absent.....  | <i>T. subhymenocystis</i>     |
| 9. Basidiospores smooth.....   | 10                            |
| 9. Basidiospores ornamented.....   | 12                            |
| 10. Basidiomata with rhizomorph.....                                       | <b><i>T. rhizomorpha</i></b>  |
| 10. Basidiomata without rhizomorph.....                                    | 11                            |
| 11. Basidiospores subglobose, angular to turbinate.....                    | <i>T. confinis</i>            |
| 11. Basidiospores ellipsoid.....   | <i>T. laevispora</i>          |
| 12. Basidiomata < 50 µm thick.....   | <i>T. gracilis</i>            |
| 12. Basidiomata > 50 µm thick.....   | 13                            |
| 13. Hymenophore smooth.....  | 14                            |
| 13. Hymenophore colliculose, irpicoid, grandinioid, odontoid, hydroid..... | 20                            |
| 14. Basidiospores slightly cyanophilous.....                               | <i>T. incisa</i>              |
| 14. Basidiospores acyanophilous.....                                       | 15                            |
| 15. Basidiospores > 6.5 µm long.....                                       | <i>T. yunnanensis</i>         |
| 15. Basidiospores < 6.5 µm long.....                                       | 16                            |
| 16. Generative hyphae < 2 µm in diameter.....                              | <b><i>T. wenshanensis</i></b> |
| 16. Generative hyphae > 2 µm in diameter.....                              | 17                            |
| 17. Generative hyphae thin-walled.....                                     | <i>T. larssonii</i>           |
| 17. Generative hyphae thick-walled.....                                    | 18                            |
| 18. Generative hyphae > 3.5 µm in diameter.....                            | <i>T. latehypha</i>           |
| 18. Generative hyphae < 3.5 µm in diameter.....                            | 19                            |
| 19. Basidiospores ellipsoid, thin-walled.....                              | <b><i>T. albofarinosa</i></b> |
| 19. Basidiospores broadly ellipsoid to globose, thick-walled.....          | <i>T. olivacea</i>            |
| 20. Hymenial surface colliculose, irpicoid or grandinioid.....             | 21                            |
| 20. Hymenial surface odontoid or hydroid.....                              | 23                            |
| 21. Generative hyphae thick-walled.....                                    | <i>T. murina</i>              |
| 21. Generative hyphae thin-walled.....                                     | 22                            |
| 22. Growth on bamboo.....  | <i>T. taiwanensis</i>         |
| 22. Growth on other plant.....   | <i>T. crystallina</i>         |

|   |                         |
|---|-------------------------|
| 23. Tramal hyphae thin-walled or slightly thick-walled.....                 | 24                      |
| 23. Tramal hyphae distinctly thick-walled.....                              | 28                      |
| 24. Crystals absent in trama.....   | <i>T. tropica</i>       |
| 24. Crystals present in trama.....  | 25                      |
| 25. Basidiospores subglobose to globose.....                                | <i>T. odontioidea</i>   |
| 25. Basidiospores ellipsoid or broadly ellipsoid.....                       | 26                      |
| 26. Tramal hyphae 3–6 µm wide, spines of basidiospores constricted.....     | <i>T. constricta</i>    |
| 26. Tramal hyphae 2–4 µm wide, spines of basidiospores not constricted..... | 27                      |
| 27. Cystidia present.....   | <i>T. chaibuxiensis</i> |
| 27. Cystidia absent.....  | <i>T. nivea</i>         |
| 28. Hymenophore aculei > 0.4 mm long.....                                   | 29                      |
| 28. Hymenophore aculei < 0.4 mm long.....                                   | 32                      |
| 29. Margin smooth.....  | <i>T. fissurata</i>     |
| 29. Margin fimbriate.....   | 30                      |
| 30. Basidiomata irpicoid.....   | <i>T. dentata</i>       |
| 30. Basidiomata odontoid or hydroid.....                                    | 31                      |
| 31. Hymenophore aculei sparse, cream to buff-yellow when fresh.....         | <i>T. fimbriata</i>     |
| 31. Hymenophore aculei dense, white when fresh.....                         | <i>T. fragilis</i>      |
| 32. Generative hyphae ampullate septa absent.....                           | <i>T. bambusicola</i>   |
| 32. Generative hyphae ampullate septa present.....                          | 33                      |
| 33. Basidiospores with sharp spines.....                                    | <i>T. subfissurata</i>  |
| 33. Basidiospores without sharp spines.....                                 | 34                      |
| 34. Spines of basidiospores constricted.....                                | <i>T. subsinensis</i>   |
| 34. Spines of basidiospores not constricted.....                            | <i>T. sinensis</i>      |

## Declarations

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**Data availability** Publicly available datasets were analyzed in this study. This data can be found here: [<https://www.ncbi.nlm.nih.gov/>; <https://www.mycobank.org/page/Simple%20names%20search>].

**Conflict of interest** The authors declare that they have no conflict of interest.

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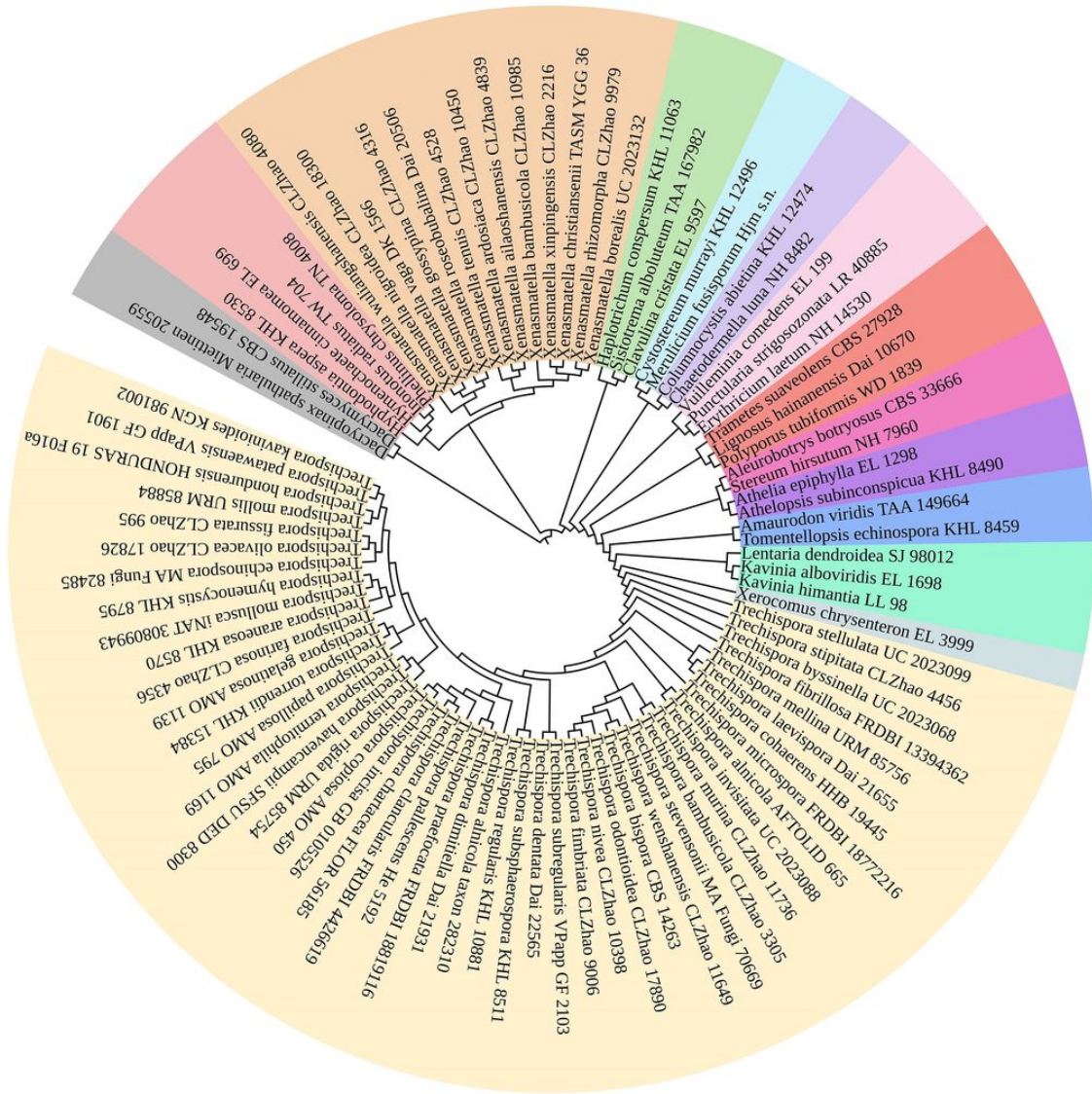
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## Figures

- Colored ranges**
- Hymenochaetales
  - Xenasmatales
  - Cantharellales
  - Agaricales
  - Gloeophyllales
  - Corticiales
  - Polyporales
  - Russulales
  - Atheliales
  - Thelephorales
  - Gomphales
  - Boletales
  - Trechisporales
  - Outgroup



**Figure 1**  
 Maximum parsimony strict consensus tree illustrating the phylogeny of *Trechisporales* and related order in the class *Agaricomycetes* based on ITS+nLSU sequences.

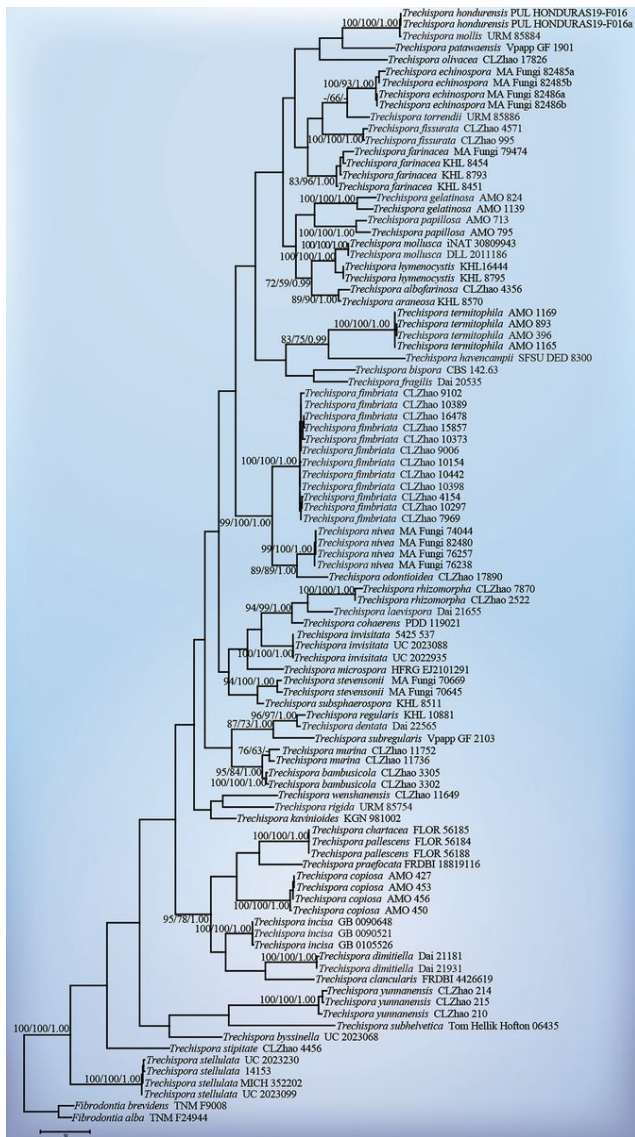
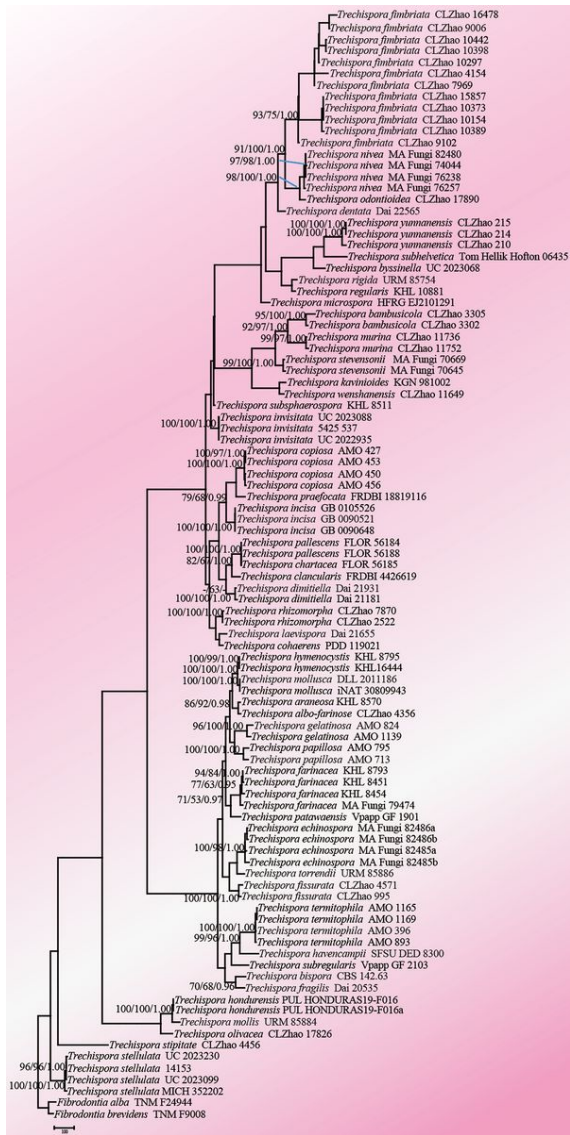


Figure 2

Maximum parsimony strict consensus tree illustrating the phylogeny of the four new species and related species in *Trechispora*, based on ITS sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95, respectively.



**Figure 3**  
 Maximum parsimony strict consensus tree illustrating the phylogeny of the four new species and related species in *Trechispora*, based on ITS+nLSU+mt-SSU+GAPDH+RPB1+RPB2 sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95, respectively.

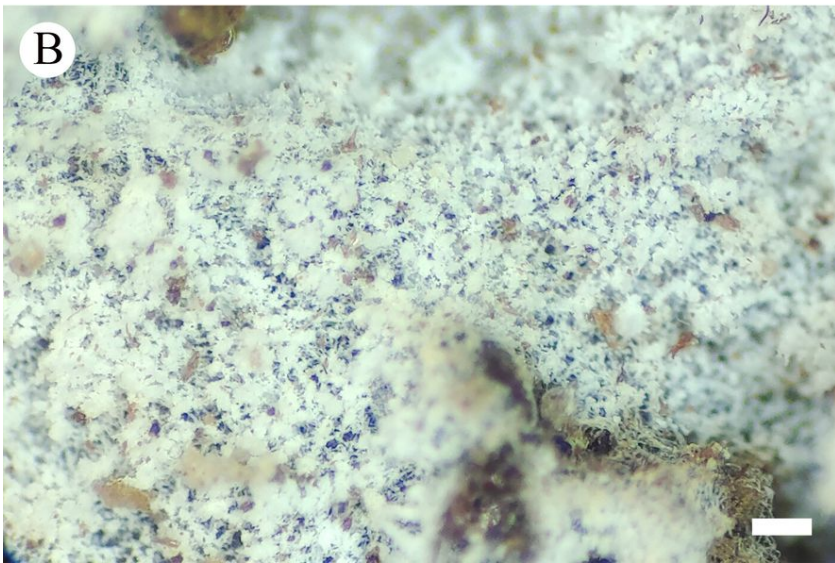
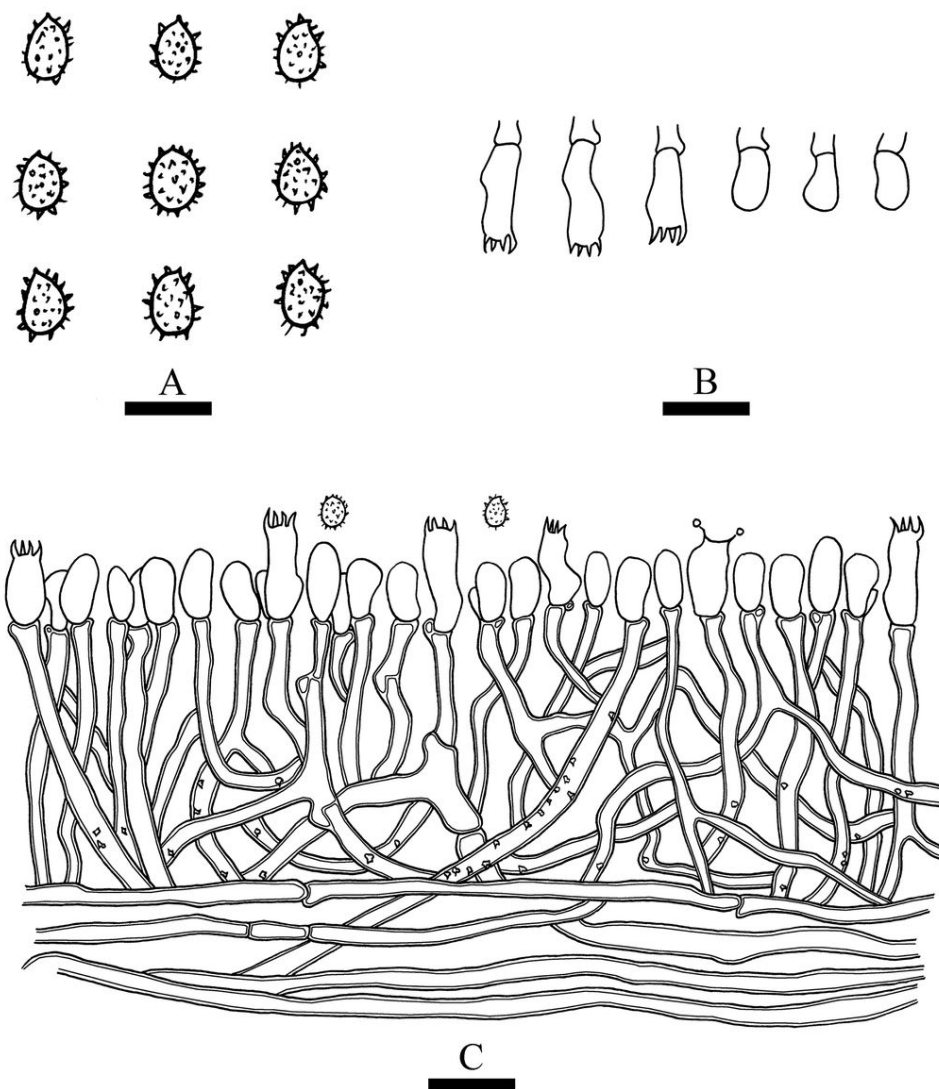


Figure 4

Basidiomata of *Trechispora albofarinosa* (holotype). Bars: A = 1 cm, B = 1 mm.



**Figure 5**

Microscopic structures of *Trechispora albofarinosa* (holotype). A Basidiospores. B Basidia and basidioles. C A section of hymenium. Bars: A = 5  $\mu\text{m}$ , B–C = 10  $\mu\text{m}$ .



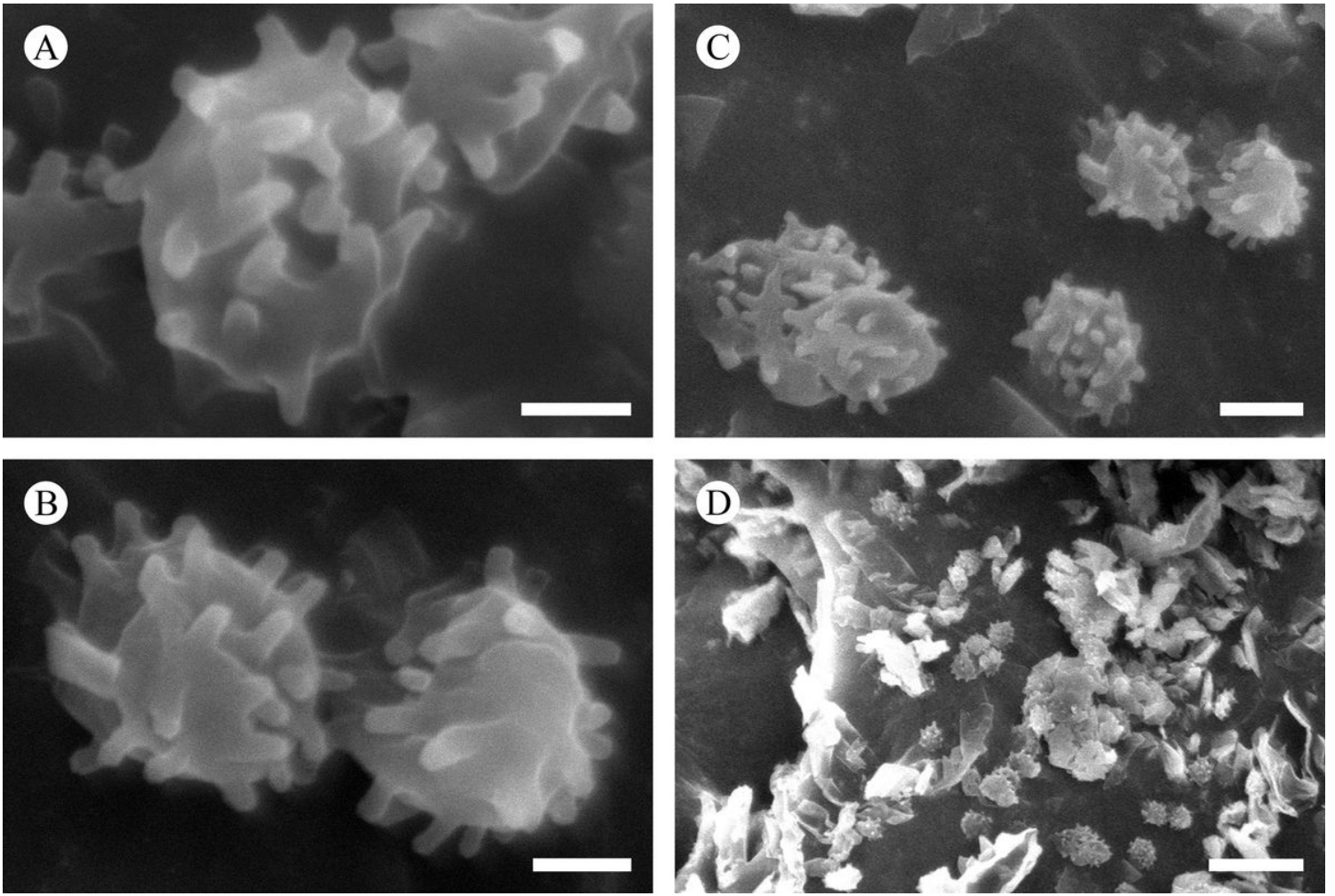


Figure 6

Scanning electron micrograph of basidiospores of *Trechispora albofarinosa* (holotype). Bars: A–B = 1  $\mu\text{m}$ , C = 2  $\mu\text{m}$ ; D = 10  $\mu\text{m}$ .

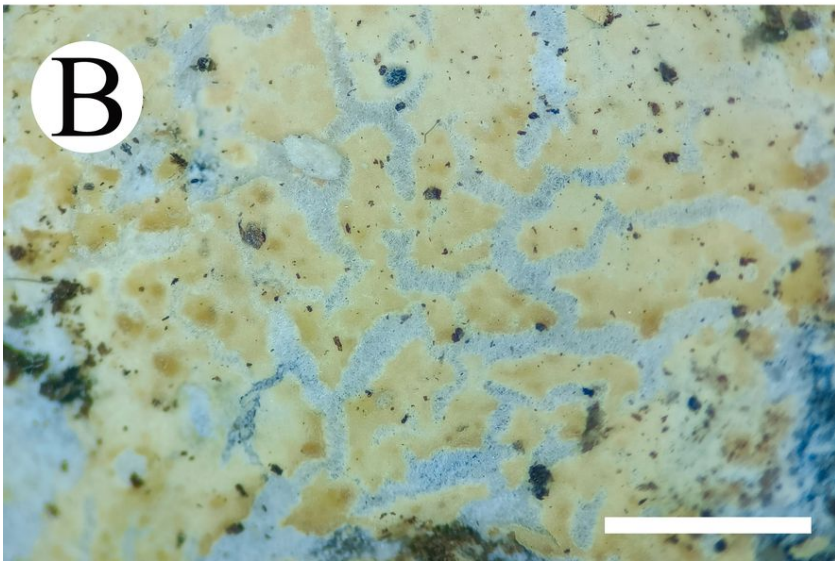
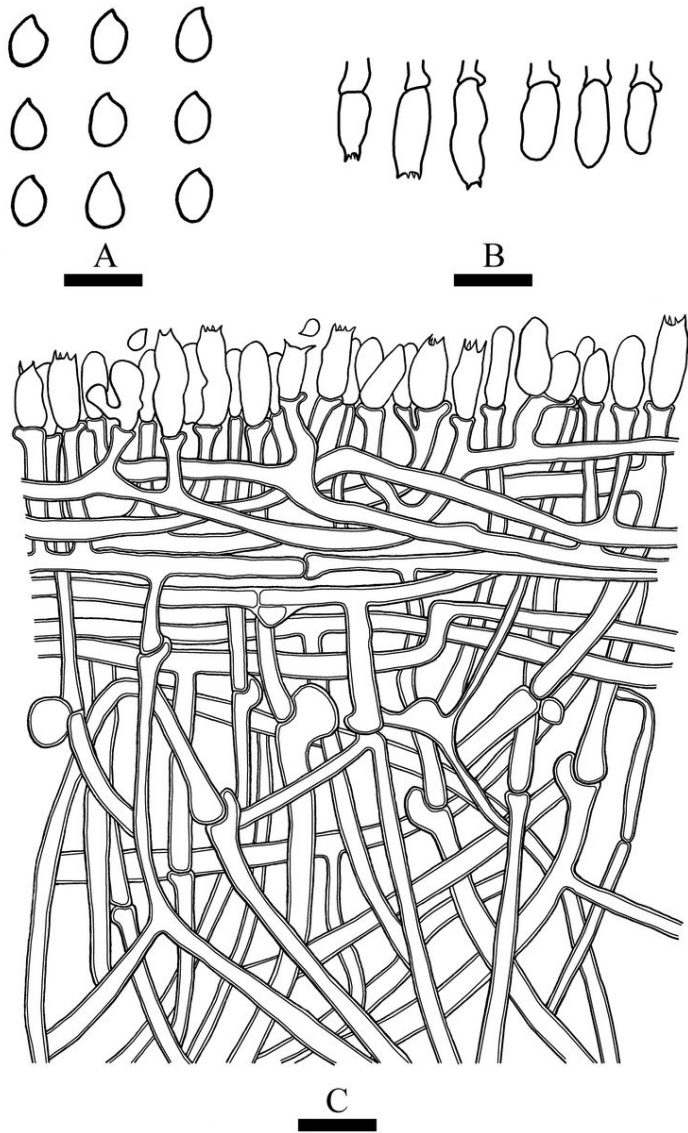


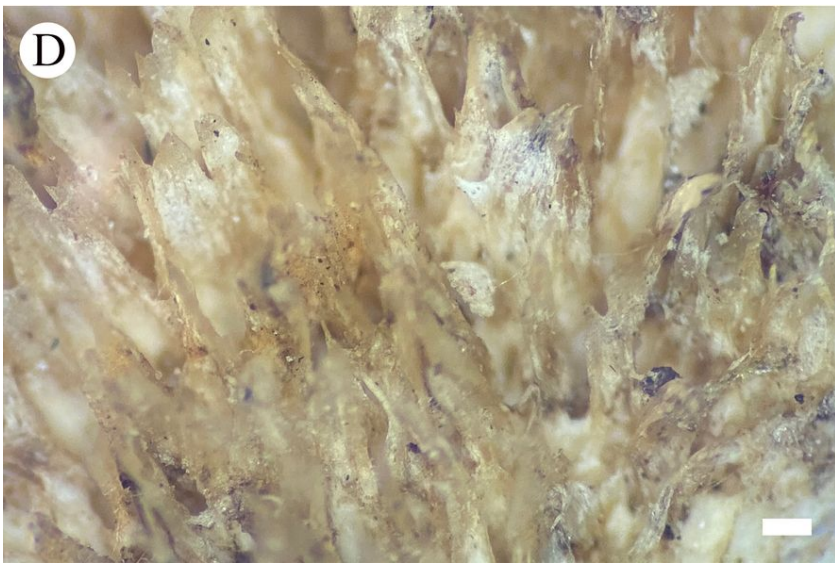
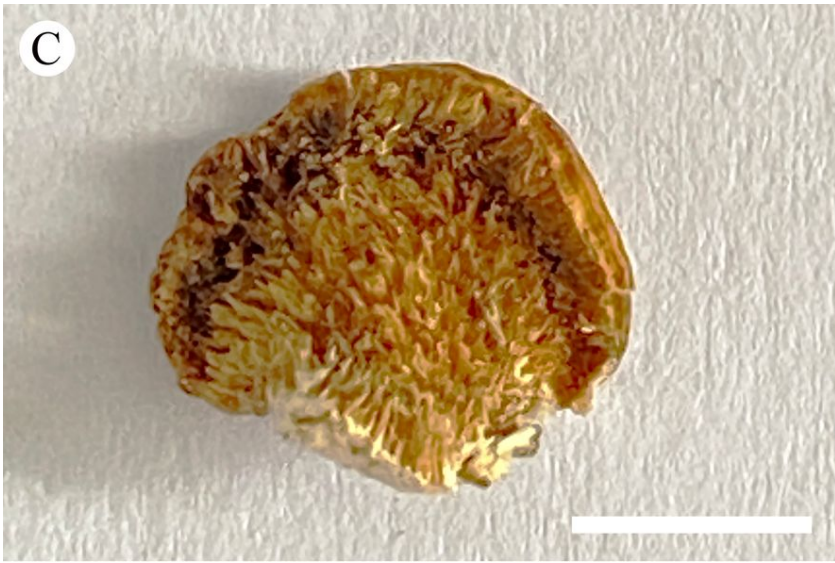
Figure 7

Basidiomata of *Trechispora rhizomorpha* (holotype). Bars: A = 1 cm, B = 1 mm.



**Figure 8**

Microscopic structures of *Trechispora rhizomorpha* (holotype). A Basidiospores. B Basidia and basidioles. C A section of hymenium. Bars: A = 5  $\mu\text{m}$ , B-C = 10  $\mu\text{m}$ .



**Figure 9**

Basidiomata of *Trechispora stipitata* (holotype). A–B The front of the basidiomata. Bars: A = 0.5 cm, B = 1 mm.

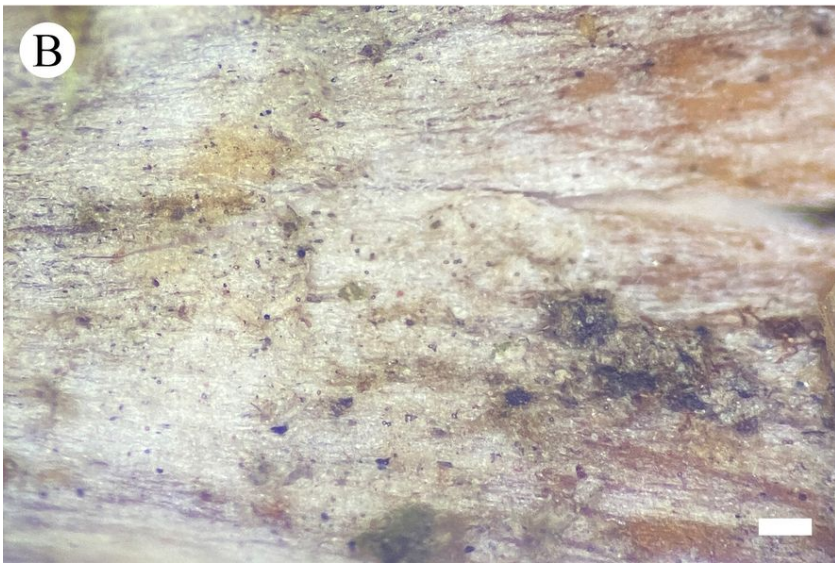
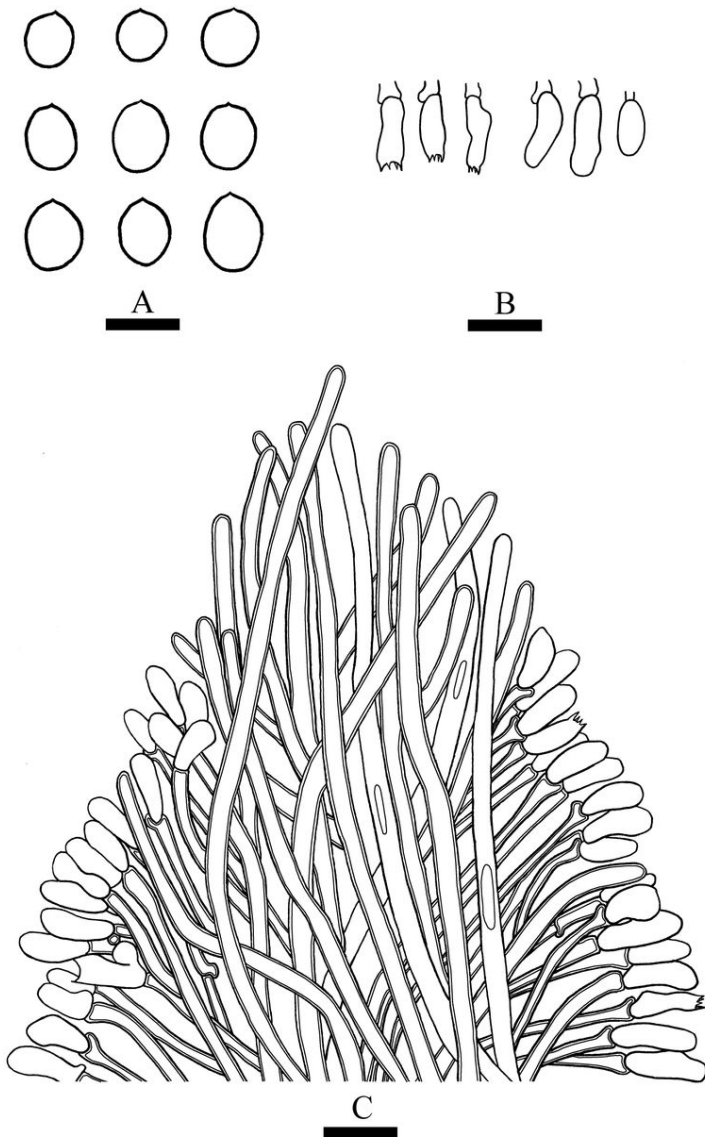


Figure 10

Basidiomata of *Trechispora stipitata* (holotype). C–D The back of the basidiomata. Bars: A = 0.5 cm, B = 1 mm.



**Figure 11**

Microscopic structures of *Trechispora stipitata* (holotype). A Basidiospores. B Basidia and basidioles. C A section of hymenium. Bars: A = 5  $\mu\text{m}$ , B–C = 10  $\mu\text{m}$ .

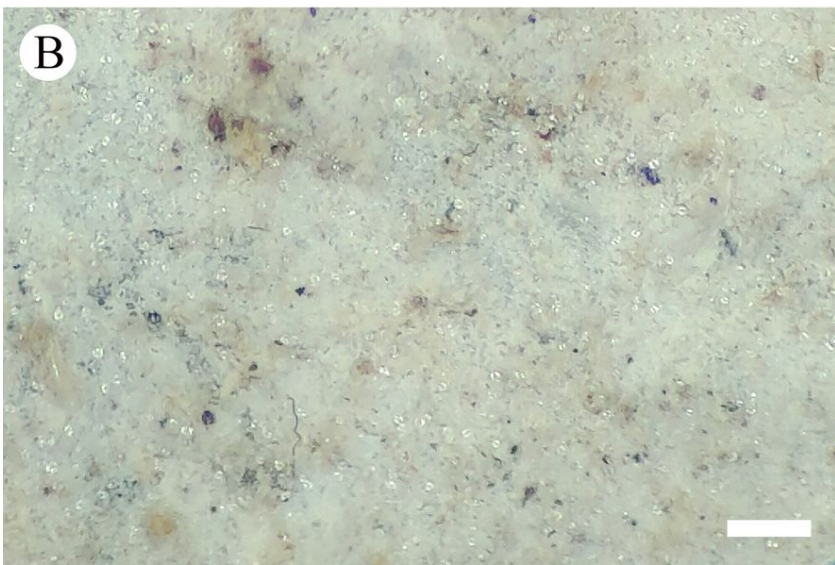


Figure 12

Basidiomata of *Trechispora wenshanensis* (holotype). Bars: A = 1 cm, B = 1 mm.

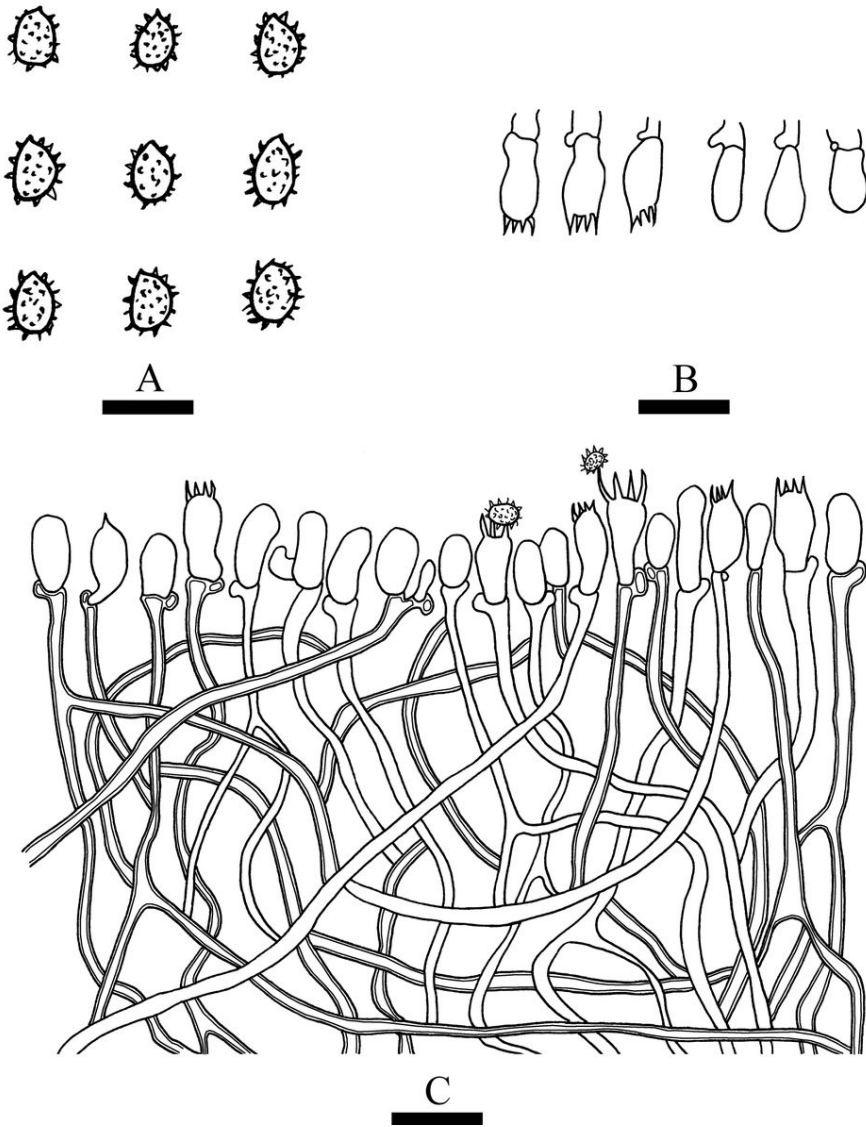


Figure 13

Microscopic structures of *Trechispora wenshanensis* (holotype). A Basidiospores. B Basidia and basidioles. C A section of hymenium. Bars: A = 5  $\mu\text{m}$ , B–C = 10  $\mu\text{m}$ .



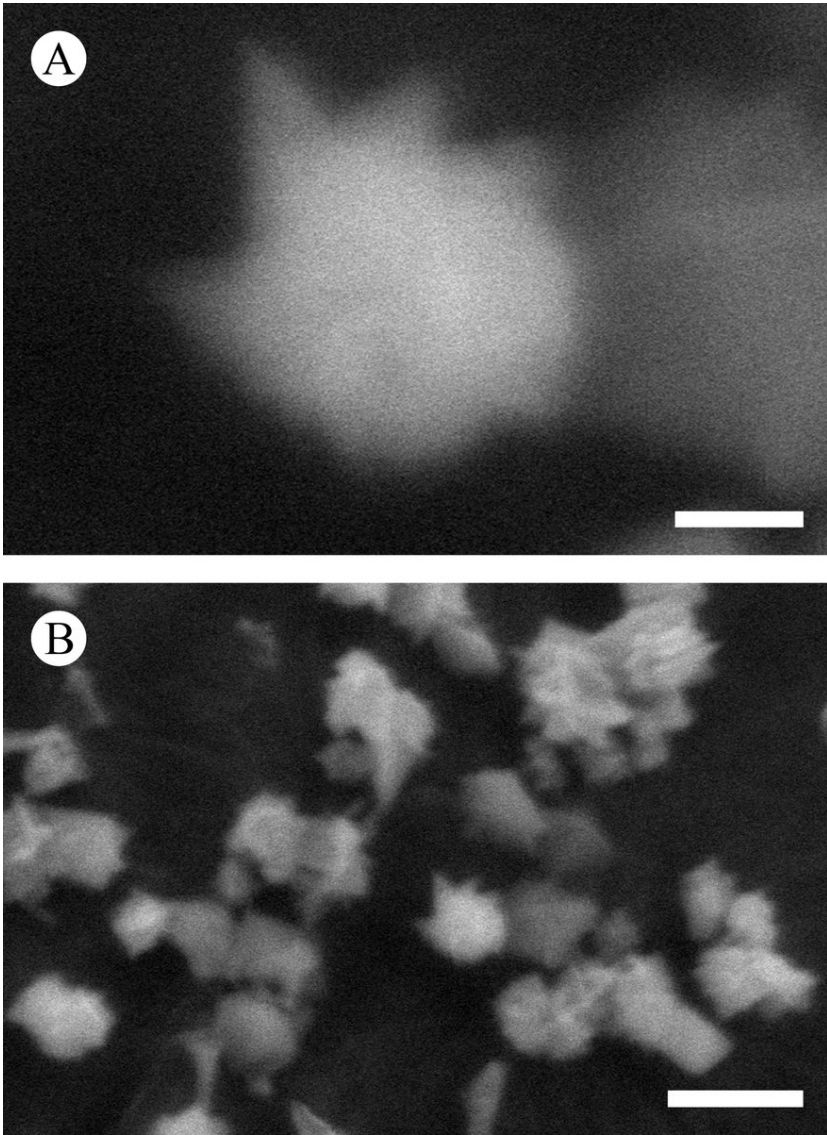


Figure 14

Scanning electron micrograph of basidiospores of *Trechispora wenshanensis* (holotype). Bars: A = 1  $\mu\text{m}$ ; B = 5  $\mu\text{m}$ .