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Phylogenetic analyses of *Hydnobolites* and new species from China

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Abstract: *Hydnobolites* is a symbiotic fungal genus with hypogeous ascomata in the family Pezizaceae (Pezizales). Molecular analyses of the genus *Hydnobolites* were conducted with single (ITS) and concatenated gene dataset (ITS-nLSU) available, including 92 newly gained sequences from Chinese specimens. Molecular evidence based on two dataset both revealed eight distinct phylogenetic clades, and ITS phylogenetic trees confirmed the presence of at least 42 phylogenetic species in genus *Hydnobolites*. Combined with the morphological examinations, five new species, (*Hydnobolites translucidus*, *H. subrufus*, *H. lini*, *H. sichuanensis H. tenuiperidius*) and one record species (*H. cerebriformis*) were described and illustrated from Southwest China ~~and the *H. cerebriformis* complex was proposed~~. Macro- and micromorphological analyses of ascomata revealed a few, but diagnostic differences between the complex, while similarities between complex ranged from 94.4% to 97.2% at the ITS region and phylogenetic analyses consistently present different well-supported taxa.

Keywords: **Ascomycota;** Pezizaceae; Hypogeous fungi; phylogeny; taxonomy

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1. Introduction

The genus *Hydnobolites* Tul. & C. Tul., with *H. cerebriformis* Tul. as the type species, was first described in 1843. It was characterized as having generally lobed or folded ascomata with a pseudoparenchymatous cortex that changed to a loose hyphal area toward the hymenium. The hymenium was poorly arranged around veins that led to the surface with openings generally between folds of the ascomata. Ellipsoid to pyriform, eight-spored asci were irregularly arranged between canals in the medullary area and globose spores with strongly projecting spines were scattered within the asci [1–6].

Hydnobolites has a complex, intertwined taxonomic history. The genus was previously placed in the Tuberaceae [7–12], in the Terfeziaceae [3,5,13–15] or in the Pyronemataceae [16]. Trappe [5] kept some hypogeous lines, as families, within his Pezizales, but other hypogeous taxa were placed alongside epigeous species in various mixed families, he regarded *Hydnobolite*s to be close to both Pachyphloeus and Terfezia. This idea was tested in a longlasting study based on both cytological and ultrastructural features of asci and ascospores by Kimbrough et al. [17] and summarized in 1994 [18], led to the placement of *Hydnobolites* in the Pezizaceae. With the molecular evidence in recent phylogenetic studies, the systematic delimitation of this genus belonging to Pezizaceae has been very clear [19–22].

There were three *Hydnobolites* species accepted in the Dictionary of Fungi [23], and 22 species name are listed in the Index Fungorum online database at present, five of these names, now are reclassified and transferred to other families and genera, such as

Discinaceae and Tuberaceae (http://www.indexfungorum.org /Names/Names.asp). To date, commonly accepted species including European *H. cerebriformis*, American *H. californicus* and a total of 5 species described from China: *Hydnobolites baodingensis* E. Wu & Z. Lan [24], *H. cerebriformis* [25], *H. canaliculatus* L. Fan, Meng Chen & Ting Li, *H. roseus* L. Fan, Meng Chen & Ting Li, *H. shanxiensis* L. Fan, Meng Chen & Ting Li, and *H. yunnanensis* L. Fan, Meng Chen & Ting Li [26]. Among them, *H. baodingensis* is considered to be a species of *Mattirolomyces* rather than *Hydnobolites* according to the morphological description and ecological habits [26–27]; *H. cerebriformis* could not be effectively identified based on voucher specimen and molecular evidence, but the EcM sequence from Hubei, China showed that this species was indeed distributed in China [26].

Geographically, *Hydnobolites* species are distributed across Europe, North America, and Asia and form mycorrhizal symbioses with both broad leaf and conifer trees that benefits the growth and survival of hosts. [21,26–28]. By including sequences derived from environmental samples in phylogenetic analyses, spore mats and EcM root tips data contributed to geographic distribution and habitat profiles for the genus *Hydnobolites*, and also revealed a greater diversity than was previously known [19,21,26,29–37].

Recently, several specimens of *Hydnobolites* were collected in southwest China, we compare these specimens with the previously recorded species of *Hydnobolites* and show them to represent different new species, which are described here as *Hydnobolites translucidus*, *H. subrufus*, *H. lini*, *H. sichuanensis H. tenuiperidius* and a new record species *H. cerebriformis*. By retrieving sequences from GenBank and comparing sequences of our own specimens from China, we established the phylogenetic framework of genus *Hydnobolites*, combined with detailed morphological features to clarify the phylogenetic position of five new taxa and one new record species, and further clarified and reassessed the species and geographical distribution diversity of this genus.

2. Materials and Methods

2.1. Sampling and Morphological Observations

Specimens were collected from Yunnan province, Sichuan province and Tibet Autonomous Region in Southwest China. The information of each species is described in Table 1 (Supplementary). Macroscopic descriptions were based on detailed field notes made on fresh ascomata. Microscopic characters were later examined from fresh and dried materials following the methods of Yang and Zhang [38]. Most species are fully described, with the exception of the immature new record species, for which only ascomata, peridium, gleba, paraphyses and asci characteristics were provided.

Hand-cut sections were mounted in 5% (w/v) KOH and examined under a light microscope (LM, Leica DM2500, Leica Microsystems, Wetzlar, Germany), while potassium hydroxide (2–5 %) Congo red was employed to enhance contrast in microscopical observations. For evaluation of the range of spore size, 60 ascospores were measured from the specimen. Measurements of ascospores are given as (a)b–c(d), where b–c includes a minimum of 90% of the measured values. Extreme values (a and d) are given in parentheses. The abbreviation 'Q' represents the range of ratio of spore length to spore width calculated for each spore and "$Q_m \pm av$" for the average Q of all spores ± sample standard deviation. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto double-sided tape, and this was mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed using a JSM-5600LV SEM (JEOL, Tokyo, Japan). The specimens are deposited at the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) or Yunnan Agricultural University (YNAU).

2.2. Molecular Identification

Total DNA was extracted from pieces of dried ascomata with a modified CTAB procedure [39]. Universal primer pairs ITS1F/ITS4 [39–40] and LROR/LR5 [41] were used for the amplifications of the internal transcribed spacers 1 and 2 with the 5.8S rDNA (ITS) and the large subunit of the nuclear ribosomal region (nrLSU), respectively. New sequences are listed in Table 1 highlighted in bold characters.

Polymerase chain reactions (PCR) were performed using the following procedure: 25 μL of PCR reaction solution contained 1 μL DNA, 1 μL (5 μm) of each primer pair, 2.5 μL 10 × buffer (Mg^{2+}plus), 1μL Dntp (1 mM), 0.5 ML BSA (0.1%), 0.5 μL $MgCl_2$, 1 U of Taq DNA polymerase (Takara Tag, Takara Biotechnology, Dalian, China). PCR reactions were run as follows: 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 1 min and 72 °C for 1 min. The final reaction was followed by an extension at 72 °C for 10 min. The PCR products were sent to Tsingke Biotechnology Co., Ltd. (Beijing, China) for purifying and sequencing.

2.3. Phylogenetic Analyses

An ITS and an ITS-nrLSU combined dataset were used to illustrate and identify the phylogenetic position of new species in this genus. Representatives of the ITS and nrLSU sequences of *Hydnobolites* species available in GenBank were retrieved and combined with our dataset. An ITS alignment of *Hydnobolites* was built including all lineages identified in the most recent phylogenetic works, as well as all sequences available originated from environmental samples (Tabel 1). Sequences derived from *Delastria* were selected and employed as outgroups. ITS and ITS-nrLSU alignments were made using the online version of the multiple sequence alignment program MAFFT v7 [42], applying the L–INS–I strategy, and were manually adjusted in BioEdit v.7.1.3.0 [43]. Alignments of all dataset used in this study were submitted to TreeBASE (No. 29854).

The phylogenetic relationships of taxa were inferred using maximum likelihood (ML) and Bayesian inference (BI). ML analyses on the ITS and ITS-LSU dataset were respectively performed in RAxML v.7.2.6 [44] and the GTR + GAMMA substitution model with parameters unlinked. ML bootstrap (BS) replicates (1000) were computed in RAxML with a rapid bootstrap analysis and search for the best-scoring ML tree. Bayesian analysis on the ITS and ITS-LSU dataset were performed in MrBayes v.3.1.2 [45] and the GTR + I + G model were selected as the best model under the Akaike Information Criterion (AIC) [46] implemented by MrModeltest v.2.3 [47]. Bayesian analysis was carried out using the selected model with four chains sampled every 100 generations and the average standard deviations of split frequencies were less than 0.01 at the end of the run and ESS (effective sampling size) values were > 200. A majority rule consensus tree was built after discarding trees from a 25% burning. Posterior probabilities (PPs) were calculated using the sumt command implemented in MrBayes. Nodes were annotated with support values from Maximum Likelihood and Bayesian analyses if at least one of these was considered significant. Support values were considered significant when ML bootstrap (BP) values were above 70 % or posterior probability (PP) values were above 0.90. Trees generated by the two analyses were viewed and exported in FigTree v1.3.1.

3. Results

3.1. Molecular Phylogenetic Analyses

ML and BI analyses based on two dataset yielded identical tree topologies respectively, and only the trees inferred from the ML analyses are shown (Figure 1, 2).

The ITS data matrix consisted of 147 taxa (including 46 sequences provided in this study) and 618 characters: 228 of ITS1 (complete), 148 of 5.8S (complete) and 242 of ITS2 (complete). ML of the general ITS dataset including all new sequences and those recovered from public databases (Figure 1) produced a phylogenetic tree consisting of eight distinct clades (Clade 1–Clade 8), six of them displayed significantly supported except for

lower support values for Clade 1 (BP 65, PP 1.0) and meaningless support values for Clade 3 (BP 47, PP 0.65). Specifically, the phylogenetic location and topologies of Clade 1 and 3 are similar with previous results [26] and Clade 1 has higher and meaningful bayesian support (PP 1.0) than before, which could be due to the increase of sampling. In terms of Clade 3, although more species sequences were included in the analysis, it was not supported by effective values compared with previous results [21,26], which could be caused by phylogenetic noise introduced by some sequences analyzed in the present work, or differences in the alignment of the highly variable ITS rDNA region.

Phylogenetic tree based on ITS dataset confirmed the presence of at least 42 phylogenetic species in genus *Hydnobolites* (Figure 1, red dots). Five new species clusters (*Hydnobolites translucidus*, *H. subrufus*, *H. lini*, *H. sichuanensis*, *H. tenuiperidius*) and one record (*H. cerebriformis*) were scattered in Clade 1 and Clade 3 together with species from Europe and North America. Moreover, in the Clade 1 (Figure 1), ITS rDNA sequences analyses showed that species between *H. cerebriformis* complex (red thick branch) share 94.4%–97.2% ITS sequence similarities while the similarities between sequences of *H. cerebriformis* were 97.2%–100% (subclade 1), and the topology also better presents the clustering characteristics of species coincided with the similarities and differences of ITS sequences. Another new species *H. sichuanensis* was separately formed an independent branch in Clade 1 with high support (BP 100, PP 1.0). Clade 2 includes five North American species represented by *H. californicus* with effective support (BP 92 PP 1.0). As for Clade 3, *H. tenuiperidius* was sister to *H. roseus*, and DNA sequence analyses showed that *H. tenuiperidius* and *H. roseus* (MK192825, holotype) share < 91.9% ITS sequence similarities. There were totally three phylogenetic species in the Clade 4, which were marked as subclade 2– subclade 4, ITS rDNA sequences analyses showed these species share <96.1% similarities, genetic information and topological structure indicated that they were not the same species, this is contrary to the results of previous studies [26]. Among the remaining branches, two are composed of American species (Clade 5, Clade 6) and two are composed of Chinese species (Clade 7, Clade 8), which is also consistent with the previous researched in the topology clustering [21,26].

ITS-nrLSU combined data matrix contained 79 taxa (including 46 sequences provided in this study) and 1419 characters after alignment and trimmed, with 618 of ITS and 801 of LSU. ML of the ITS-nrLSU combined dataset including all new sequences and the available sequences from public databases and the combined molecular data confirm the results generated from ITS analysis (Figure 2).

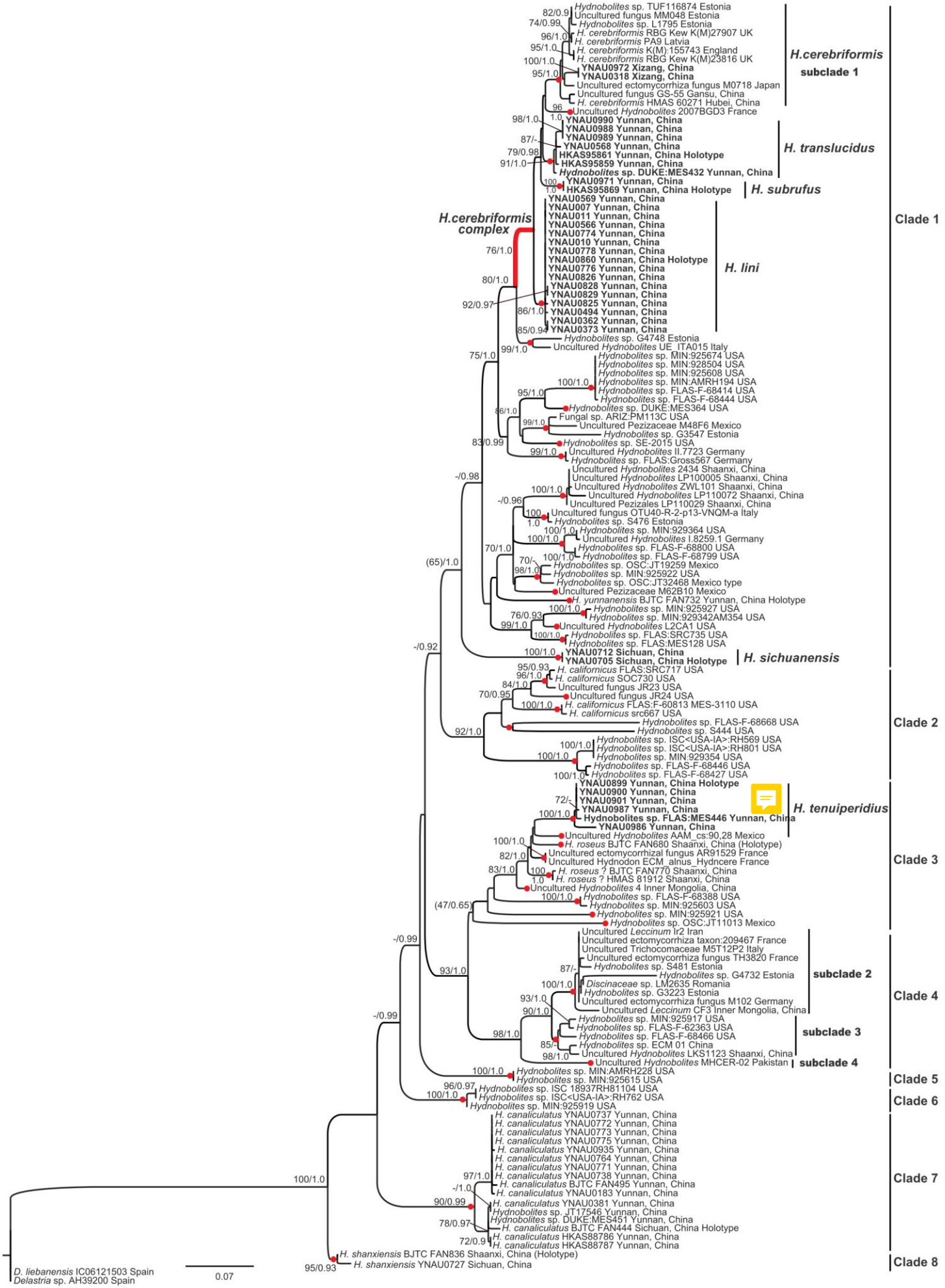
Hydnobolites sp. TUF116874 Estonia
Uncultured fungus MM048 Estonia
Hydnobolites sp. L1795 Estonia
H. cerebriformis RBG Kew K(M)27907 UK
H. cerebriformis PA9 Latvia
H. cerebriformis K(M):155743 England
H. cerebriformis RBG Kew K(M)23816 UK
YNAU0972 Xizang, China
YNAU0318 Xizang, China
Uncultured ectomycorrhiza fungus M0718 Japan
Uncultured fungus GS-55 Gansu, China
H. cerebriformis HMAS 60271 Hubei, China
Uncultured Hydnobolites 2007BGD3 France
H.cerebriformis subclade 1
YNAU0990 Yunnan, China
YNAU0988 Yunnan, China
YNAU0989 Yunnan, China
YNAU0568 Yunnan, China
HKAS95861 Yunnan, China Holotype
HKAS95859 Yunnan, China
Hydnobolites sp. DUKE:MES432 Yunnan, China
H. translucidus
YNAU0971 Yunnan, China
HKAS95869 Yunnan, China Holotype
H. subrufus
H.cerebriformis complex
YNAU0569 Yunnan, China
YNAU007 Yunnan, China
YNAU011 Yunnan, China
YNAU0566 Yunnan, China
YNAU0774 Yunnan, China
YNAU010 Yunnan, China
YNAU0778 Yunnan, China
YNAU0860 Yunnan, China Holotype
YNAU0776 Yunnan, China
YNAU0826 Yunnan, China
YNAU0828 Yunnan, China
YNAU0829 Yunnan, China
YNAU0825 Yunnan, China
YNAU0494 Yunnan, China
YNAU0362 Yunnan, China
YNAU0373 Yunnan, China
H. lini
Hydnobolites sp. G4748 Estonia
Uncultured Hydnobolites UE_ITA015 Italy
Hydnobolites sp. MIN:925674 USA
Hydnobolites sp. MIN:928504 USA
Hydnobolites sp. MIN:925608 USA
Hydnobolites sp. MIN:AMRH194 USA
Hydnobolites sp. FLAS-F-68414 USA
Hydnobolites sp. FLAS-F-68444 USA
Hydnobolites sp. DUKE:MES364 USA
Fungal sp. ARIZ:PM113C USA
Uncultured Pezizaceae M48F6 Mexico
Hydnobolites sp. G3547 Estonia
Hydnobolites sp. SE-2015 USA
Uncultured Hydnobolites II.7723 Germany
Hydnobolites sp. FLAS:Gross567 Germany
Uncultured Hydnobolites 2434 Shaanxi, China
Uncultured Hydnobolites LP100005 Shaanxi, China
Uncultured Hydnobolites ZWL101 Shaanxi, China
Uncultured Hydnobolites LP110072 Shaanxi, China
Uncultured Pezizales LP110029 Shaanxi, China
Uncultured fungus OTU40-R-2-p13-VNQM-a Italy
Hydnobolites sp. S476 Estonia
Hydnobolites sp. MIN:929364 USA
Uncultured Hydnobolites I.8259.1 Germany
Hydnobolites sp. FLAS-F-68800 USA
Hydnobolites sp. FLAS-F-68799 USA
Hydnobolites sp. OSC:JT19259 Mexico
Hydnobolites sp. MIN:925922 USA
Hydnobolites sp. OSC:JT32468 Mexico type
Uncultured Pezizaceae M62B10 Mexico
H. yunnanensis BJTC FAN732 Yunnan, China Holotype
Hydnobolites sp. MIN:925927 USA
Hydnobolites sp. MIN:929342AM354 USA
Uncultured Hydnobolites L2CA1 USA
Hydnobolites sp. FLAS:SRC735 USA
Hydnobolites sp. FLAS:MES128 USA
YNAU0712 Sichuan, China
YNAU0705 Sichuan, China Holotype
H. sichuanensis
H. californicus FLAS:SRC717 USA
H. californicus SOC730 USA
Uncultured fungus JR23 USA
Uncultured fungus JR24 USA
H. californicus FLAS:F-60813 MES-3110 USA
H. californicus src667 USA
Hydnobolites sp. FLAS-F-68668 USA
Hydnobolites sp. S444 USA
Hydnobolites sp. ISC<USA-IA>:RH569 USA
Hydnobolites sp. ISC<USA-IA>:RH801 USA
Hydnobolites sp. MIN:929354 USA
Hydnobolites sp. FLAS-F-68446 USA
Hydnobolites sp. FLAS-F-68427 USA
YNAU0899 Yunnan, China Holotype
YNAU0900 Yunnan, China
YNAU0901 Yunnan, China
YNAU0987 Yunnan, China
Hydnobolites sp. FLAS:MES446 Yunnan, China
YNAU0986 Yunnan, China
H. tenuiperidius
Uncultured Hydnobolites AAM_cs:90,28 Mexico
H. roseus BJTC FAN680 Shaanxi, China (Holotype)
Uncultured ectomycorrhizal fungus AR91529 France
Uncultured Hydnodon ECM_alnus_Hydncere France
H. roseus ? BJTC FAN770 Shaanxi, China
H. roseus ? HMAS 81912 Shaanxi, China
Uncultured Hydnobolites 4 Inner Mongolia, China
Hydnobolites sp. FLAS-F-68388 USA
Hydnobolites sp. MIN:925603 USA
Hydnobolites sp. MIN:925921 USA
Hydnobolites sp. OSC:JT11013 Mexico
Uncultured Leccinum lr2 Iran
Uncultured ectomycorrhiza taxon:209467 France
Uncultured Trichocomaceae M5T12P2 Italy
Uncultured ectomycorrhiza fungus TH3820 France
Hydnobolites sp. S481 Estonia
Hydnobolites sp. G4732 Estonia
Discinaceae sp. LM2635 Romania
Hydnobolites sp. G3223 Estonia
Uncultured ectomycorrhiza fungus M102 Germany
Uncultured Leccinum CF3 Inner Mongolia, China
subclade 2
Hydnobolites sp. MIN:925917 USA
Hydnobolites sp. FLAS-F-62363 USA
Hydnobolites sp. FLAS-F-68466 USA
Hydnobolites sp. ECM 01 China
Uncultured Hydnobolites LKS1123 Shaanxi, China
subclade 3
Uncultured Hydnobolites MHCER-02 Pakistan
subclade 4
Hydnobolites sp. MIN:AMRH228 USA
Hydnobolites sp. MIN:925615 USA
Hydnobolites sp. ISC 18937RH81104 USA
Hydnobolites sp. ISC<USA-IA>:RH762 USA
Hydnobolites sp. MIN:925919 USA
H. canaliculatus YNAU0737 Yunnan, China
H. canaliculatus YNAU0772 Yunnan, China
H. canaliculatus YNAU0773 Yunnan, China
H. canaliculatus YNAU0775 Yunnan, China
H. canaliculatus YNAU0935 Yunnan, China
H. canaliculatus YNAU0764 Yunnan, China
H. canaliculatus YNAU0771 Yunnan, China
H. canaliculatus YNAU0738 Yunnan, China
H. canaliculatus BJTC FAN495 Yunnan, China
H. canaliculatus YNAU0183 Yunnan, China
H. canaliculatus YNAU0381 Yunnan, China
Hydnobolites sp. JT17546 Yunnan, China
Hydnobolites sp. DUKE:MES451 Yunnan, China
H. canaliculatus BJTC FAN444 Sichuan, China Holotype
H. canaliculatus HKAS88786 Yunnan, China
H. canaliculatus HKAS88787 Yunnan, China
H. shanxiensis BJTC FAN836 Shaanxi, China (Holotype)
H. shanxiensis YNAU0727 Sichuan, China
D. liebanensis IC06121503 Spain
Delastria sp. AH39200 Spain
0.07
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Clade 8

Figure 1. Consensus phylogram of the Genus *Hydnobolites* obtained in RAxML of ITS rDNA. Nodes were annotated if supported by > 70 % ML BP or > 0.90 bayesian PP, but non-significant support values are exceptionally represented inside parentheses. New species are in bold font. Complex is reported as red thick branch.

Figure 2. Consensus phylogram of the Genus *Hydnobolites* obtained in RAxML of ITS-nrLSU. Nodes were annotated if supported by > 70 % ML BP or > 0.90 bayesian PP, but non-significant support values are exceptionally represented inside parentheses. New species are in bold font. Complex is reported as red thick branch.

3.2. Taxonomy

3.2.1. Hydnobolites translucidus S.P. Wan and F.Q. Yu, sp. nov.

MycoBank: 834662.

Etymology: In terms of the translucent gleba of the fruiting bodies (Figure 3).

Holotype: China, Yunnan Province, 25°53′19″ N and 103°29′24″ E, in humic soil under a pure *Quercus guyavifolia* Levl. forest, at about 2433 m, 14 August 2016, wsp761 (HKAS95861, GenBank accession: ITS = MT174247, LSU = OP642324).

Diagnosis: The new species *Hydnobolites translucidus* has deeply infolded and fused, white, dirty-white ascomata, translucent and watersoaked gleba, spores comparatively larger (up to 25.5 μm in length and 25 μm in width) than in its close relative *H. cerebriformis* (19–20–22 μm) [3], smaller than *H. subrufus* (up to 26.5 μm in length and 25.3 μm in width) and *H. lini* (up to 26.9 μm in length and 26.1 μm in width). *Hydnobolites translucidus* has whitish ascomata while *H. subrufus* has a red hue surface. The thinnest value of the peridium of *H. translucidus* is higher than *H. subrufus* and *H. lini*.

Description: Ascomata Ascomata irregularly globose, often grooved, cracked, deeply infolded and fused, softbut firm, surface smooth, glabrous, minutely downy to scurfy in the depression, 0.5–2.0 cm in diam., white, dirty-white, translucent when fresh, golden brown to brown when dried. Odour none. **Peridium** 43.0–182.0 μm thick, hyphae hyaline, pseudoparenchymatous, composed of subglobose, ellipsoid, and polygonal cells of 3.2–37.0× 3.0–20.0 μm. **Gleba** solid, transparent, watersoaked white and and stick when fresh, with whitish veins, convoluted and chambered, composed of intricately interwoven, hyaline and thin-walled hyphae, 4.0–13.0 μm diam, the cells irregularly subglobose, cylindrical interwoven to inflated, 3.0–16.0 × 3.0–12.5 μm. **Paraphyses** colourless, straight to bent, free from one another, septate, slightly swollen and blunt at tip, sparsely projecting to a distance of 25.0 μm above surface of peridium, 5.0–15.0 μm diam. **Asci** globose to subglobose, pyriform, ellipsoid or irregular, (74.0)75.0–106.0(–112.0) × (60.5–)63.0–92.8(–103.0) μm, hyaline, sessile or with a short stalk, 8 spored, crozier clearly seen in situ in immature asci. **Ascospores** spherical, white to light yellow, excluding their alveolate-reticulate ornamentation, (14.5–)16.0– 23.0(–25.5) × (14.0–)15.5–23.0(–25.0) μm, Q = 1.0–1.08 (Q_m ± 1.02), the ornaments of spores formed an alveolate-reticulum of 0.7–6.7 μm high, 3–7 meshes across the diameter when mature.

Habitat and distribution: Solitary to scattered in small groups, on humus-rich soil with dead leaves and wood under trees of *Q. guyavifolia* and *Pinus armandii* Franch.; where the fruiting bodies contacts with the soil, there are clumps of soil attached; only known from southwest China.

Additional material examined: China, Yunnan Province, 25°53′48″ N and 103°29′14″ E, in humic soil under a pure *P. armandii* forest, at about 2462 m, 14 August 2016, wsp758 (HKAS95859, GenBank accession: ITS = OM758120, LSU = OP642323). China, Yunnan Province, 25°58′19″ N and 102°43′32″ E, in soil under a *P. armandii* forest, at about 1920 m, 24 September 2021, wsp1435 (YNAU0568, GenBank accession: ITS = OM758130, LSU = OP642335). China, Yunnan Province, 25°61′15″ N and 100°25′28″ E, in soil under a *P. armandii* forest, 27 January 2022, wsp1919 (YNAU0988, GenBank accession: ITS = OP740750, LSU = OP799844). China, Yunnan Province, 25°61′37″ N and 100°25′54″ E, in soil under a *P. armandii* forest, 27 January 2022, wsp1920 (YNAU0989, GenBank accession: ITS = OP740751, LSU = OP799845). China, Yunnan Province, 25°61′55″ N and 100°25′73″ E, in soil under a *P. armandii* forest, 27 January 2022, wsp1923 (YNAU0990, GenBank accession: ITS = OP740752, LSU = OP799846).

Notes: *Hydnobolites translucidus* is characterized macroscopically by the characteristics of deeply infolded, fused, white to dirty-white ascomata, translucent and watersoaked gleba and microscopically by the morphology of its peridium and ascospores.

Morphologically, the new species *H. translucidus* is similar to its phylogeneticly close relatives *H. cerebriformis* and our newly described *H. subrufus* and *H. lini*. All species share the same grooved, cracked, infolded, fused, soft but firm ascomata, smooth and glabrous surface, and downy depression. However, *H. cerebriformis* has smaller ascospores (19–20–22 μm), as well as asci (85–100 × 60–75 μm) [3], can be clearly distinguished from the other three species. Despite *H. translucidus* was very similar with *H. subrufus* and *H. lini* in the stucture of pseudoparenchymatous peridium, the size and shape of asci and ascospores, but *H. translucidus* has whitish ascomata while *H. subrufus* has red to brownish red surface. Besides, the thinnest value of the peridium of *H. subrufus* and *H. lini* is smaller than that of *H. translucidus*, so the asci or spores of *H. subrufus* and *H. lini* can be seen directly from the dried ascomata surface. Phylogeneticlly, these four species all belong to the *H.*

cerebriformis complex according to this study based on both ITS and nrLSU, respectively, and the distinctions of species were also supported by the phylogenetic analyses (Figure 1, 2).

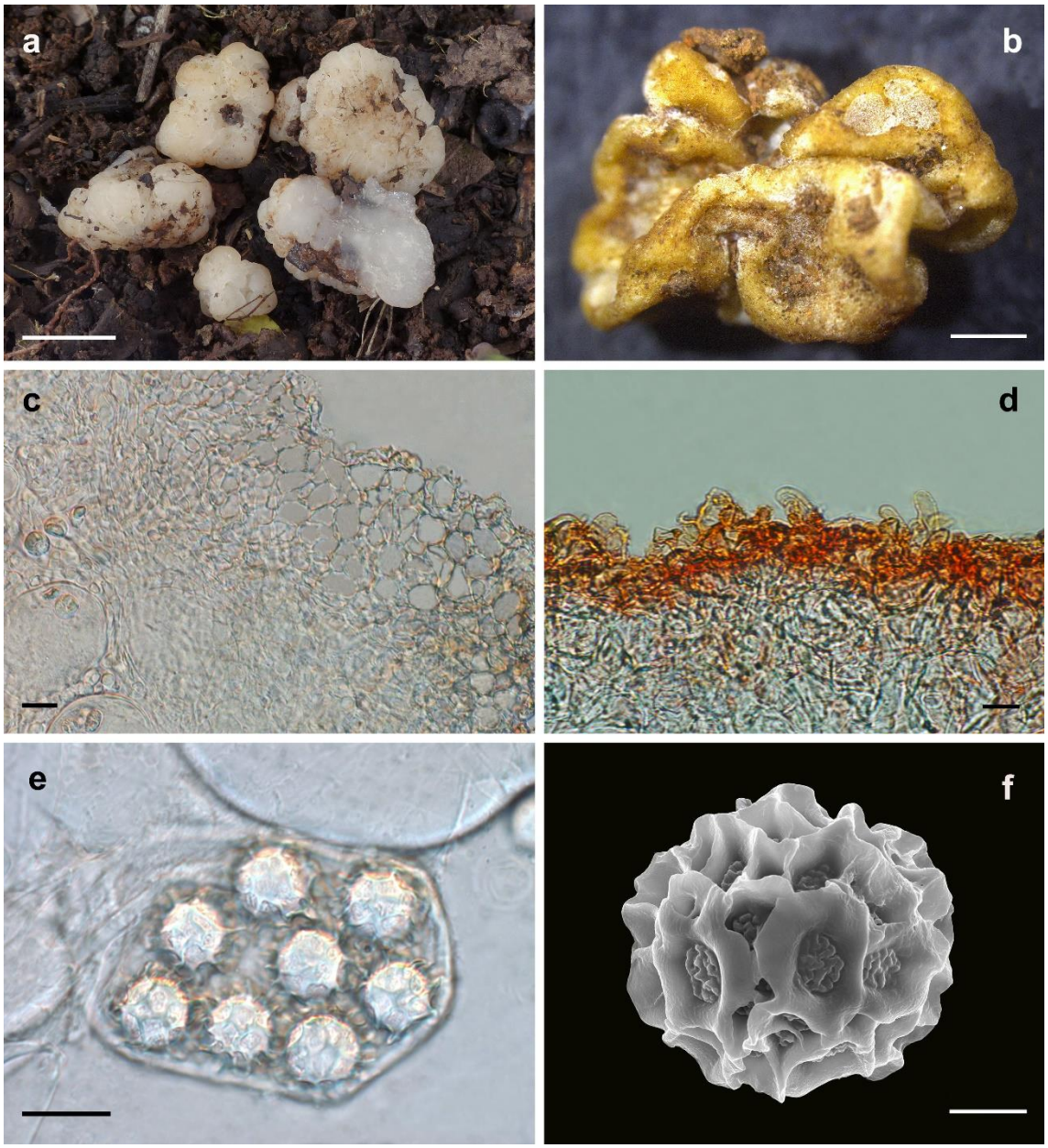

Figure 3. *Hydnobolites translucidus* (Holotype, HKAS95861). (a) Fresh ascomata and gleba; (b) Dried ascoma; (c) Peridium; (d) Paraphyses; (e) asci and ascospores under LM; (f) ascospore under SEM. Scale bars: a = 1 cm; b = 1 mm; c–e =20 μm; f =5 μm.

3.2.2. Hydnobolites subrufus S.P. Wan and F.Q. Yu, sp. nov.

MycoBank: 834663.

Etymology: In terms of the fruiting bodies suface with red tone (Figure 4).

Holotype: China, Yunnan Province, 25°53′50″ N and 103°28′35″ E, in humic soil under a pure *Q. guyavifolia* forest, at about 2354 m, 14 August 2016, wsp769 (HKAS95869, GenBank accession: ITS = MT174248, LSU = OP642325).

Diagnosis: The diagnostic characteristics of the new species *Hydnobolites subrufus* are white, red to brownish red ascomata with thin peridium (20.0–244.0 μm), the convex shape of the asci can be directly observed from the surface of the dried ascomata, which

can be well distinguished from *H. cerebriformis* and *H. translucidus. Hydnobolites lini* is well distinguished from *H. subrufus* by the clearly visible spores on the fruiting bodies surface.

Description: Ascomata irregularly ellipsoid to globose, grooved, cracked, infolded and fused, soft but firm, surface smooth, glabrous, minutely downy to scurfy in the depression, 0.5–1.0 cm in diam., white, red to brownish red when fresh, golden yellow to golden brown when dried and the surface showed obvious convex shape of the asci. Odour none. **Peridium** 20.0–244.0 μm thick, hyphae hyaline, pseudoparenchymatous, composed of subglobose, ellipsoid, and polygonal cells of 2.5–25.0 × 2.0–17.5 μm. **Gleba** solid, watery white when fresh, with whitish veins, grooved, cracked, composed of intricately interwoven, hyaline and thin-walled hyphae, 1.5–9.5 μm diam, the cells irregularly subglobose, square, cylindrical interwoven to inflated, 5.0–17.0 × 4.5–16.5 μm. **Paraphyses** colourless, straight to bent, free from one another, septate, slightly swollen and blunt at tip, sparsely projecting to a distance of 24.0 μm above surface of peridium, 5.0–10.0 μm diam. **Asci** globose to subglobose, pyriform, ellipsoid or irregular, (75.0–)80.0–105.0(–110.1) × (61.0–)67.0–93.5(–105.0) μm, hyaline, sessile or with a short stalk, 8 spored, crozier clearly seen in situ in immature asci. **Ascospores** spherical, white to light yellow, excluding their alveolate-reticulate ornamentation, (17.5–)18.5–25.5(–26.5) × (17.0–)18.0–25.3 μm, Q = 1.0–1.09 (Q_m ± 1.03), the ornaments of spores formed an alveolate-reticulum of 0.8–7.0 μm high, 3–7 meshes across the diameter when mature.

Habitat and distribution: Solitary to scattered in small groups, in humus-rich soil with dead leaves and wood under trees of *Q. guyavifolia*; where the fruiting bodies contacts with the soil, there are clumps of soil attached; only known from southwest China.

Additional material examined: China, Yunnan Province, 25°53′59″ N and 103°28′26″ E, in humic soil under a pure *Q. guyavifolia* forest, at about 2380 m, 14 August 2016, wsp769-1 (YNAU0971, GenBank accession: ITS = OM758121, LSU = OP642326).

Notes: Morphologically, the new species *Hydnobolites subrufus* is similar to *H. cerebriformis*, *H .lini* and *H. translucidus.* However, as described in the diagnosis and notes of *H. translucidus*, *H. subrufus* can be separated from *H. cerebriformis* [3] and *H. translucidus* in the color of ascomata, thickness of peridium and size of asci and spores. *Hydnobolites lini* differs from *H. subrufus* in its visible ascospores on the surface of ascomata. Another species (*H. roseus*) [26] described from northwest China also has red ascocarps, but differs from *H. subrufus* by its thicker peridium (55–325 μm), smaller asci (55–100 × 50–62.5 μm), larger spores extremum (up to 30 μm) and distant phylogenetic distance (Figure 1, 2).

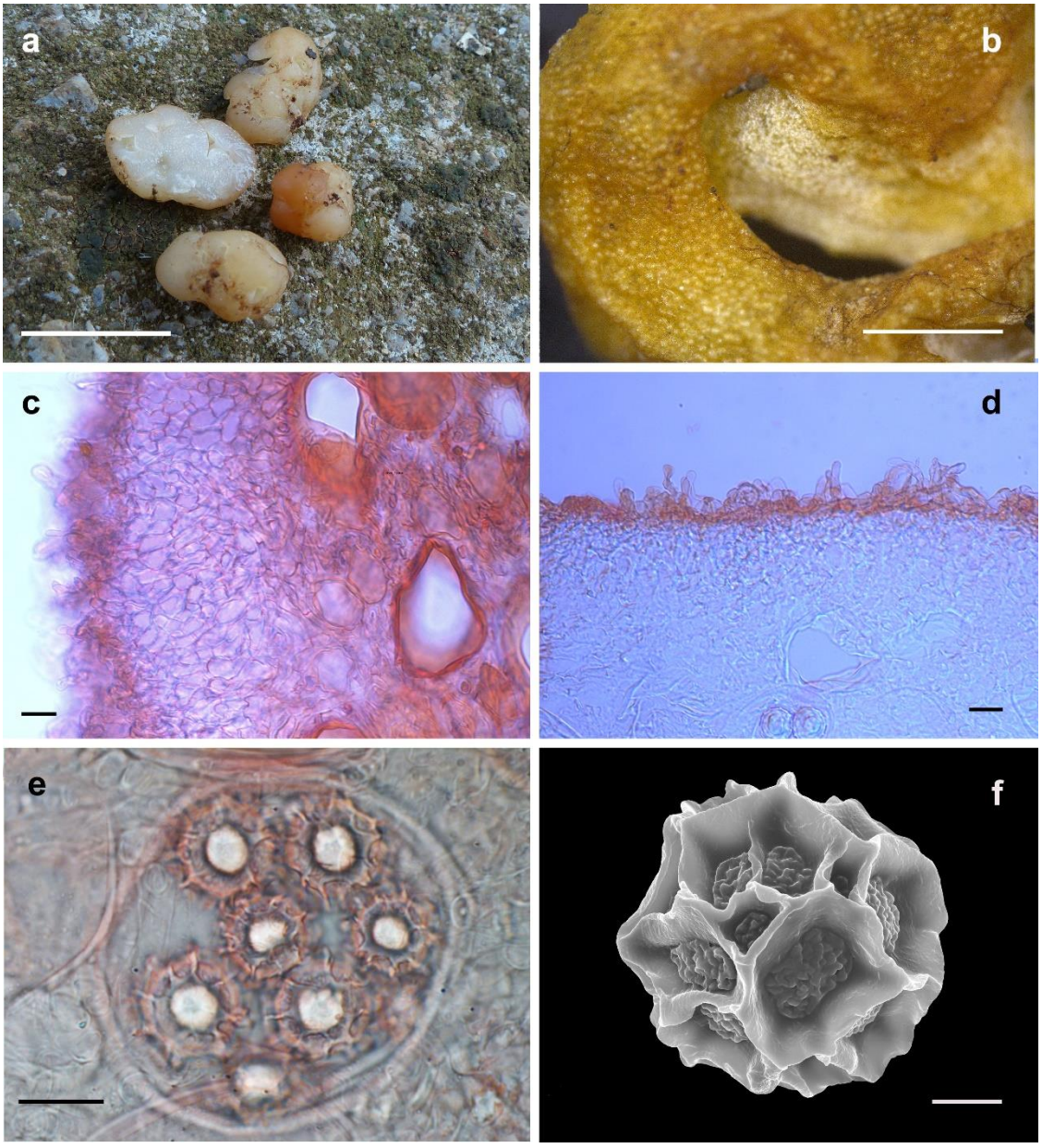

Figure 4. *Hydnobolites subrufus* (Holotype, HKAS95869). (a) Fresh ascomata and gleba; (b) Dried ascoma; (c,d) Peridium and paraphyses; (e) asci and ascospores under LM; (f) ascospore under SEM. Scale bars: a = 1 cm; b = 1 mm; c–e =20 μm; f =5 μm.

3.2.3. Hydnobolites lini S.P. Wan and F.Q. Yu, sp. nov.

MycoBank: 846353.

Etymology: In appreciation of Mr Lin Yongxiang, who has contributed many samples to the research of genus *Hydnobolites* (Figure 5).

Holotype: China, Yunnan Province, 25°58′20″ N and 102°45′21″ E, in humic soil under a pure *P. armandii* forest, at about 2270 m, 29 October 2021, wsp1723 (YNAU0860, GenBank accession: ITS = OM758149, LSU = OP642357).

Diagnosis: The new species *Hydnobolites lini* has obvious asci and ascospores on the surface of dried ascomata when compared with the known species.

Description: Ascomata irregularly globose to subglobose, grooved, cracked, infolded and fused, soft but firm, surface smooth, downy densely in grooves and depression, 0.8–2.5 cm in diam., white when fresh, reddish to light brown after a while, golden yellow to golden brown when dried. Odour none. **Peridium** 18.0–393.0 μm thick, hyphae

hyaline, pseudoparenchymatous, composed of subglobose, columnar, and polygonal cells of 4.5–40.0 × 3.5–25.0 μm. **Gleba** solid, white when fresh, light brown partly, with whitish veins, grooved, cracked, composed of intricately interwoven, hyaline and thin-walled hyphae, 3.4–28.0 μm diam, the cells subglobose, square to cylindrical, swollen and interwoven, 4.0–32.3 × 3.2–25.4 μm. **Paraphyses** colourless, straight to bent, free from one another, septate, blunt at tip and always swollen, sparsely projecting to a distance of 40.0 μm above surface of peridium, 3.5–9.0 μm diam. **Asci** globose to subglobose, pyriform, ellipsoid or irregular, (75.0–)80.0–105.0(–110.1) × (61.0–)67.0–93.5(–105.0) μm, hyaline, sessile or with a short stalk, 8 spored, crozier clearly seen in situ in immature asci. **Ascospores** spherical, white to light yellow, excluding their alveolate-reticulate ornamentation, (15.2–)16.3–26.6(–26.9) × (14.8–)15.5–26.0(–26.1) μm, Q = 1.0–1.17 (Q± = 1.05), the ornaments of spores formed an alveolate-reticulum of 0.7–5.5 μm high, 3–7 meshes across the diameterwhen mature.

Habitat and distribution: Solitary, in humus soil with *P. armandii*; where the fruiting bodies contacts with the soil, there are clumps of soil attached; only known from southwest China.

Additional material examined: China, Yunnan Province, 26°67′98″ N and 103°39′22″ E, in humic soil under pure *P. armandii*, at about 2245 m, 22 August 2021, wsp1246 (YNAU0362, GenBank accession: ITS = OM758125, LSU = OP642330). China, Yunnan Province, 26°67′55″ N and 103°39′18″ E, in humic soil under pure h *P. armandii*, at about 2159 m, 30 August 2021, wsp1257 (YNAU0373, GenBank accession: ITS = OM758126, LSU = OP642331). China, Yunnan Province, 26°46′32″ N and 103°26′91″ E, in humic soil under pure *P. armandii*, at about 2016 m, 22 September 2021,wsp1369 (YNAU0494, GenBank accession: ITS = OM758128, LSU = OP642333). China, Yunnan Province, 25°57′19″ N and 102°42′61″ E, in humic soil under trees dominated by *P. armandii* and associated with *Quercus* sp., at about 1899 m, 24 September 2021, wsp1433 (YNAU0566, GenBank accession: ITS = OM758129, LSU = OP642334). China, Yunnan Province, 25°57′08″ N and 102°42′34″ E, in humic soil under trees dominated by *P. armandii* and associated with *Quercus* sp., at about 1890 m, 24 September 2021, wsp1436 (YNAU0569, GenBank accession: ITS = OM758131, LSU = OP642336). China, Yunnan Province, 26°41′36″ N and 103°32′97″ E, in humic soil under *P. armandii*, at about 2073 m, 18 October 2021, wsp1635 (YNAU007, GenBank accession: ITS = OK598967, LSU = OP642343). China, Yunnan Province, 26°41′46″ N and 103°32′06″ E, in humic soil under *P. armandii*, at about 2011 m, 18 October 2021, 1641 (YNAU010, GenBank accession: ITS = OK598969, LSU = OP642344). China, Yunnan Province, 26°41′76″ N and 103°32′18″ E, in humic soil under *P. armandii*, at about 2022 m, 18 October 2021, wsp1642 (YNAU011, GenBank accession: ITS = OK598970, LSU = OP642347). China, Yunnan Province, 26°41′75″ N and 103°32′21″ E, in humic soil under *P. armandii*, at about 2019 m, 18 October 2021, wsp1642-2 (YNAU0774, GenBank accession: ITS = OM758141, LSU = OP642349). China, Yunnan Province, 26°41′06″ N and 103°32′79″ E, in humic soil under *P. armandii*, at about 1993 m, 18 October 2021, wsp1643-2 (YNAU0776, GenBank accession: ITS = OM758143, LSU = OP642351). China, Yunnan Province, 26°41′88″ N and 103°32′79″ E, in humic soil under *P. armandii*, at about 2004 m, 18 October 2021, wsp1643-4 (YNAU0778, GenBank accession: ITS = OM758144, LSU = OP642352). China, Yunnan Province, 26°16′91″ N and 102°49′60″ E, in humic soil under *P. armandii*, at about 1860 m, 29 October 2021, wsp1690 (YNAU0825, GenBank accession: ITS = OM758145, LSU = OP642353). China, Yunnan Province, 26°16′88″ N and 102°49′58″ E, in humic soil under *P. armandii*, at about 1862 m, 29 October 2021, wsp1691 (YNAU0826, GenBank accession: ITS = OM758146, LSU = OP642354). China, Yunnan Province, 26°16′86″ N and 102°49′55″ E, in humic soil under *P. armandii*, at about 1866 m, 29 October 2021, wsp1693 (YNAU0828, GenBank accession: ITS = OM758147, LSU = OP642355). China, Yunnan Province, 26°16′87″ N and 102°49′51″ E, in humic soil under *P. armandii*, at about 1868 m, 29 October 2021, wsp1694 (YNAU0829, GenBank accession: ITS = OM758148, LSU = OP642356).

Notes: The new species *Hydnobolites lini* differs from its close relative *H. cerebriformis*, *H. subrufus* and *H. translucidus* by the obvious asci and ascospores on the surface of dried ascomata. The other newly described species *H. tenuiperidius* in this paper also has the same characteristic but differs from *H. lini* by its smaller ascospores ((13.3–22.0(–23.0) × (12.5–)–22.0(–22.6) μm)) and distant phylogenetic distance (Figure 1, 2).

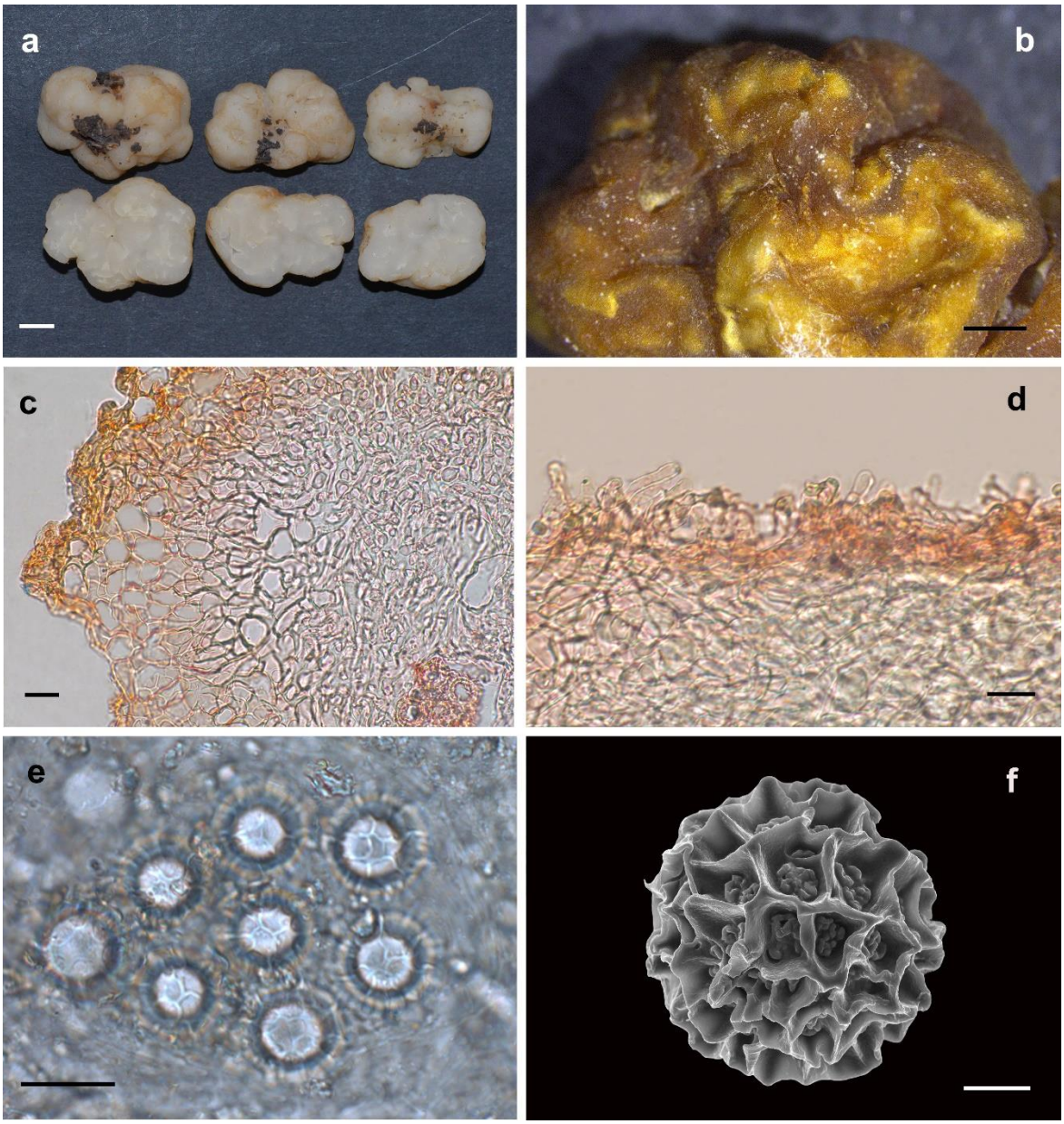

Figure 5. *Hydnobolites lini* (Holotype, YNAU0860). (a) Fresh ascomata and gleba; (b) Dried ascoma; (c) Peridium; (d) Paraphyses; (e) ascus and ascospores under LM; (f) ascospore under SEM. Scale bars: a = 1 cm; b = 1 mm; c–e =20 μm; f = 5 μm.

3.2.4. Hydnobolites sichuanensis S.P. Wan and F.Q. Yu, sp. nov.

MycoBank: 846354

Etymology: In reference to the location of the type collection (Figure 6).

Holotype: China, Sichuan Province, 30°05′22″ N and 100°78′53″ E, in humic soil under *Abies chensiensis* Tiegh. and *Picea wilsonii* Mast. forests, at about 3911 m, 10 October 2021, wsp1575 (YNAU 0705, GenBank accession: ITS = OM758132, LSU = OP642337).

Diagnosis: Differs from other species by possessing smaller spores ((15.0–)16.0–21.0 × (14.5–)15.5–20.5 μm) and thicker peridium (80.0–390.0 μm).

Description: Ascomata irregularly globose to subglobose, grooved, cracked, lobed and infolded, soft but firm, surface smooth but microvillus partly, downy densely to scurfy in the depression, 0.7–0.9 cm in diam., white to light orange when fresh, golden yellow to golden brown when dried. Odour none. **Peridium** 80.0–390.0 μm thick, hyphae hyaline, pseudoparenchymatous, composed of subglobose, ellipsoid, and polygonal cells of 5.5–41.0 × 4.0–34.0 μm. **Gleba** solid, watery white when fresh, with whitish veins, grooved, cracked, composed of intricately interwoven, hyaline and thin-walled hyphae, 1.4–9.8 μm diam, the cells irregularly subglobose, square to cylindrical, swollen and interwoven, 3.4–43.5 × 3.4–34.0 μm. **Paraphyses** colourless, subglobose to cylindrical, straight to bent or knobby, sometimes bulbous, free from one another, septate, blunt at tip and always swollen, sparsely projecting to a distance of 36.5 μm above surface of peridium, 3.8–10.5 μm diam. **Asci** globose to subglobose, pyriform, ellipsoid or irregular, (55.6–)62.0–83.5(–85.5) × (47.5–)52.5–74.5(–77.0) μm, hyaline, sessile or with a short stalk, 8 spored, crozier clearly seen in situ in immature asci. **Ascospores** spherical, white to light yellow, excluding their alveolate-reticulate ornamentation, (15.0–)16.0–21.0 × (14.5–)15.5–20.5 μm, Q = 1.0–1.08 (Q_m ± 1.02), the ornaments of spores formed an alveolate-reticulum of 0.7–6.7 μm high, 3–5 meshes across the diameter.

Habitat and distribution: Solitary, in humus-rich soil with dead leaves of *A. chensiensis* and *Pi. wilsonii*; where the fruiting bodies contacts with the soil, there are clumps of soil attached; only known from southwest China.

Additional material examined: China, Sichuan Province, 30°05′45″ N and 100°78′89″ E, in humic soil under trees dominated by *Q. guyavifolia* and associated with *Pi. wilsonii*, at about 3932 m, 10 October 2021, wsp1582 (YNAU0712, GenBank accession: ITS = OM758133, LSU = OP642338).

Notes: *Hydnobolites sichuanensis* is characterized microscopically by the morphology of its peridium and ascospores. The peridium are thicker (80.0–390.0 μm) than the konwn species. The ascospores of *H. sichuanensis* are regular spherical and relatively smaller ((15.0–)16.0–21.0 × (14.5–)15.5–20.5 μm) when compared with all known species except *H. californicus*, which has smaller ascospores (14–18 μm) and bigger alveoli (3–4 meshes) [2]. Furthermore, in phylogenetic analysis, *H. sichuanensis* forms a independently distinct branch with high significant support (BP 100, PP 1.0) (Figure 1, 2).

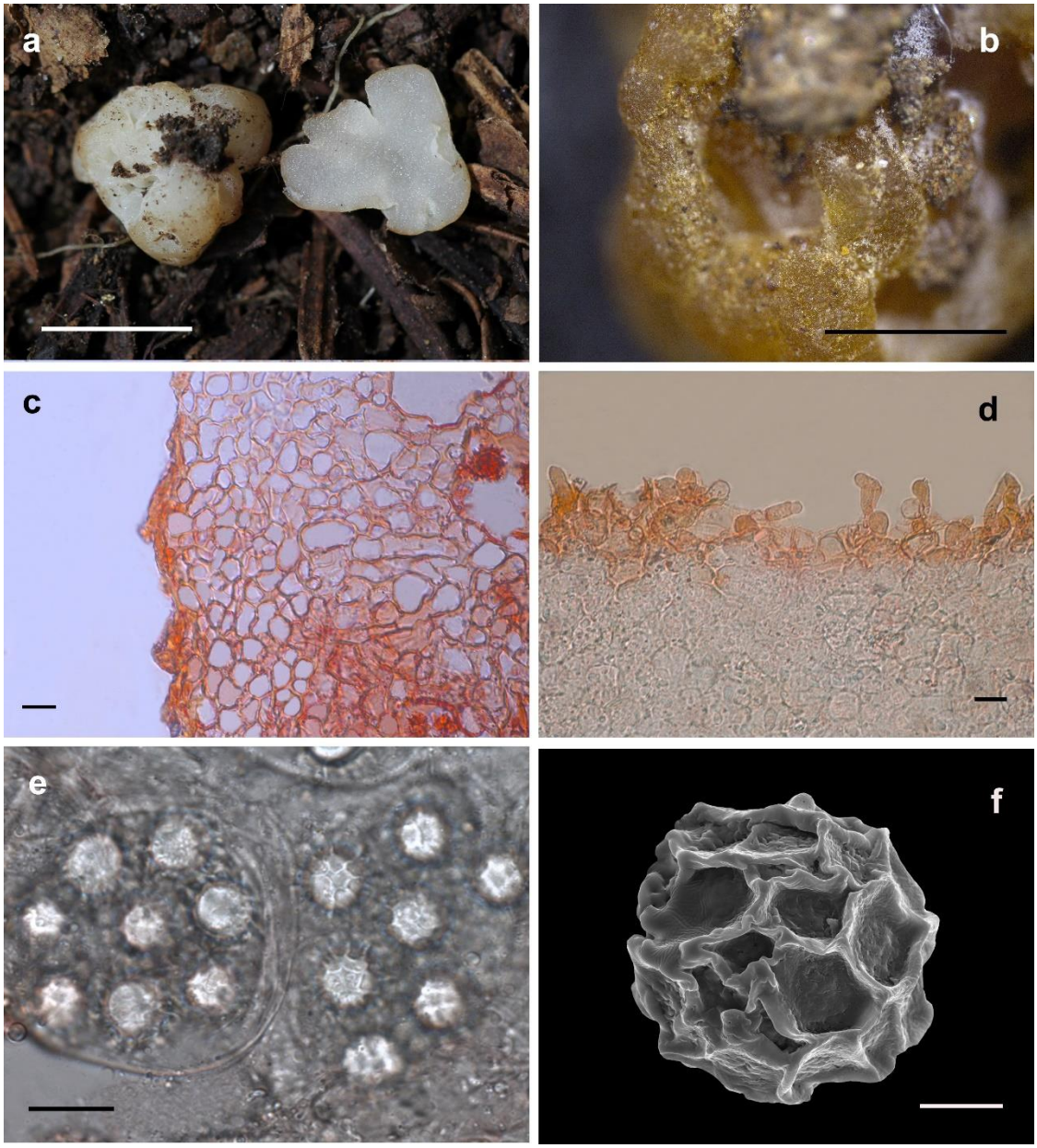

Figure 6. *Hydnobolites sichuanensis* (Holotype, YNAU0705). (a) Fresh ascoma and gleba; (b) Dried ascoma; (c) Peridium; (d) Paraphyses; (e) asci and ascospores under LM; (f) ascospore under SEM. Scale bars: a = 1 cm; b = 1 mm; c–e = 20 μm; f = 5 μm.

3.2.5. Hydnobolites tenuiperidius S.P. Wan and F.Q. Yu, sp. nov.

MycoBank: 846355

Etymology: In terms of the the thin-peridiumed *Hydnobolites* (Figure 7).

Holotype: China, Yunnan Province, 25°07′18″ N and 102°50′24″ E, in humic soil under a pure *P. armandii* forest, at about 2222 m, 14 November 2021, wsp1751 (YNAU0899, GenBank accession: ITS = OM758150, LSU = OP642358).

Diagnosis: Differs from other species by possessing extremely thin peridium (5.5–222.8 μm).

Description: Ascomata irregularly globose to subglobose, grooved, cracked, lobed and infolded, soft but firm, surface smooth, minutely downy in grooves and depression, 2.1–2.8 cm in diam., white when fresh, light red after a while, golden yellow to golden brown when dried. Odour none. **Peridium** 5.5–222.8 μm thick, hyphae hyaline, pseudoparenchymatous, composed of subglobose, columnar, and polygonal cells of 4.5–40.0 ×

3.5–25.0 μm. **Gleba** solid, white when fresh, with whitish veins, grooved, cracked, partly with white continuous interwoven mycelia which show rainbow luster after drying; gleba composed of intricately interwoven, hyaline and thin-walled hyphae, 4.5–24.4 μm diam, the cells subglobose, square to cylindrical, 4.7–44.6.0 × 3.7–30.0 μm. **Paraphyses** colourless, straight to bent, sometimes bulbous, free from one another, septate, blunt at tip and always swollen, sparsely projecting to a distance of 50.5 μm above surface of peridium, 5.5–18.6 μm diam.

Asci globose to subglobose, pyriform, ellipsoid or irregular, (50.6–)55.0–98.3(–99.0) × (37.8–)40.0–89.0(–90.0) μm, hyaline, sessile or with a short stalk, 8 spored, crozier clearly seen in situ in immature asci. **Ascospores** spherical, white to light yellow, excluding their alveolate-reticulate ornamentation, 13.3–22.0(–23.0) × (12.5–)–22.0(–22.6) μm, Q = 1.0–1.18 (Q± = 1.03), the ornaments of spores formed an alveolate-reticulum of 0.6–7.3 μm high, 3–7 meshes across the diameter.

Habitat and distribution: Solitary, in humus soil with *P. armandii*; where the fruiting bodies contacts with the soil, there are clumps of soil attached; only known from southwest China.

Additional material examined: China, Yunnan Province, 25°07′32″ N and 102°50′25″ E, in humic soil under pure *P. armandii*, at about 2238 m, 14 November 2021, wsp1752 (YNAU0900, GenBank accession: ITS = OM758151, LSU = OP642359). China, Yunnan Province, 25°07′15″ N and 102°50′19″ E, in humic soil under pure *P. armandii*, at about 2256 m, 14 November 2021, wsp1753 (YNAU0901, GenBank accession: ITS = OM758152, LSU = OP642360). China, Yunnan Province, 25°09′66″ N and 100°63′92″ E, in humic soil under pure *P. armandii*, at about 2256 m, 14 November 2021, wsp1829 (YNAU0986, GenBank accession: ITS = OP740748, LSU = OP799842). China, Yunnan Province, 25°09′61″ N and 100°63′ 90″ E, in humic soil under pure *P. armandii*, at about 2250 m, 14 November 2021, wsp1830 (YNAU0987, GenBank accession: ITS = OP740749, LSU = OP799843).

Notes: *Hydnobolites tenuiperidius* is characterized microscopically by the morphology of its peridium and ascospores. Some peridium areas are extremely thin (5.5 μm), so the spores can be clearly observed from the surface of the dried specimen. This characterictic was also shared by *H. lini*, but *H. lini* has larger size of asci ((75.0–)80.0–105.0(–110.1) × (61.0–)67.0–93.5(–105.0) μm) and spores ((15.2–)16.3–26.6(–26.9) × (14.8–)15.5–26.0(–26.1) μm).

In our phylogenetic analyses, six specimens of *H. tenuiperidius* forms a single branch with high molecular support (BS = 100, PP = 1.0) and clusters into the subclade 2 that includes *H. roseus* (Figure 1). The ITS similarities between *H. tenuiperidius* and *H. roseus* (ITS = MK192825, Holotype) are less than 91.9%. Morphologically, despite *H. tenuiperidius* shares the rose color ascomata and similar ascospores size characters with *H. roseus*, but *H. tenuiperidius* can be separated from *H. roseus* by the characteristics downy depression, thin peridium and 3–7 meshes, while *H. roseus* has nearly glabrous depression, thicker peridium, and 3–5 meshes across the spoers [26]. In addition, it should also be noted that there also two specimens (BJTC FAN770 and HMAS81912) were identified as *H. roseus* [26], but they were clusterd into a single branch and distinctly separated from the holotype of *H. roseus*, and they share less than 95.1% ITS similarities with the holotype sequence of *H. roseus*, so the molecular evidence suggests they may not be the same species.

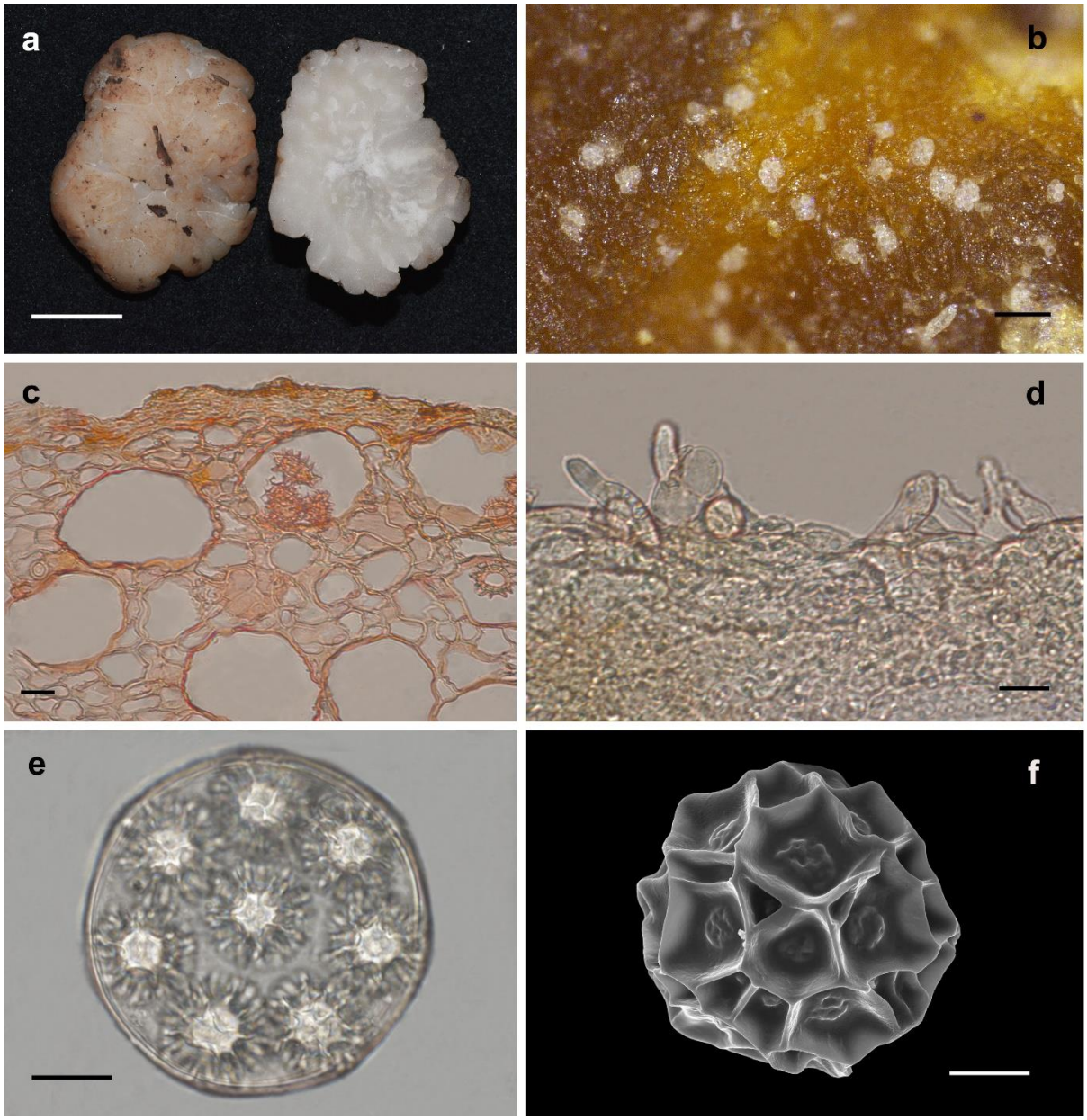

Figure 7. *Hydnobolites tenuiperidius* (Holotype, YNAU0899). (a) Fresh ascoma and gleba; (b) Surface of dried ascoma; (c) Peridium; (d) Paraphyses; (e) ascus and ascospores under LM; (f) ascospore under SEM. Scale bars: a = 1 cm; b = 0.1 mm; c–e =20 μm; f = 5 μm.

3.2.6. Hydnobolites cerebriformis Tul., Tul., Annales des Sciences Naturelles Botanique 19: 378 (1843) [1] (Figure 8).

MycoBank: 2389

Ascomata irregularly globose, grooved, cracked, much lobed and infolded, 0.7 to 1.0 cm diam., waxy, transparent partially, downy in the depression, at first white, then yellowish to sandy coloured when fresh, pale to light brown when dried. Odour none. **Peridium** 53.8–317.0 μm thick, hyphae hyaline, pseudoparenchymatous, composed of subglobose, ellipsoid, and polygonal cells of 1.7–34.6 × 1.7–19.7 μm. **Gleba** solid, white when fresh, with indistinct few whitish veins, connecting with folds, hyaline and thin-walled hyphae, 0.9–16.6 μm diam, the cells cylindrical interwoven to inflated, 2.0–24.8 × 1.9–20.7 μm. **Paraphyses** colourless, straight to bent, free from one another, septate, slightly swollen or blunt at tip, sparsely projecting to a distance of 65.0 μm above surface of peridium, 1.5–12.7 μm diam. **Asci** globose to subglobose, pyriform, ellipsoid or irregular, 83.0–96.5(–

101.8) × 62.1–72.0(–75.5) μm, hyaline, sessile or with a short stalk, crozier clearly seen in situ in immature asci and immature spores are occasionally visible.

Habitat and distribution: Solitary, in humus soil with *P. wallichiana* A. B. Jackson and associated with *Rosa* sp.; where the fruiting bodies contacts with the soil, there are clumps of soil attached. The specimen was collected in August, but it is still immature. Know from Tibet of China. Besides, according to the ITS sequences of Ecms and specimens in the public database, this species is widely distributed in Asia and Europe, including China (Hubei and Gansu province), Japan, Estonia, UK and Latvia.

Material examined: China, Tibet, 27°46′36″ N and 88°90′48″ E, in humic soil under trees of *P. wallichiana* and associated with *Rosa* sp., at about 3187 m, 4 August 2021, wsp1171 (YNAU0318, GenBank accession: ITS = OM758123, LSU = OP642328). China, Tibet, 27°46′37″ N and 88°90′51″ E, in humic soil under trees of *P. wallichiana* and associated with *Rosa* sp., at about 3185 m, 4 August 2021, wsp1171-1 (YNAU0972, GenBank accession: ITS = OM758124, LSU = OP642329).

Notes: *Hydnobolites cerebriformis* is originally described from Europe, and also reported from North America [3,12,17,18,21]. The occurrence of *H. cerebriformis* in China is confirmed from Tibet under *P. wallichiana* dominant forest based on molecular and morphological evidence. Despite the spores maturity were low and have not been described in detail, the other characteristics of *H. cerebriformis* observed in this study conform to those described by previous workers [2,8,12,17,20], on these basis, we also illustrate and described the characteristics of paraphyses.

A specimen (HMAS60271) ITS sequence (MK236552) from Hubei Province and an ITS sequence (LC623535) from the ECM of *P. tabulaeformis* Carr. in Gansu Province of China also match this species, in accordance with previous results [26]. Besides, ITS sequence (AB218135) from the ECM of *A. homolepis* Siebold & Zucc. also confirmed the distribution of this species in Japan of Asia. In general, sequences from different regions were clustered together as *H. cerebriformis* with high support (subclade 1, BP 100, PP 1.0), and the ITS similarities between these sequences were up to 97.2%–100%, well demonstrated that they are conspecific and widely distributed.

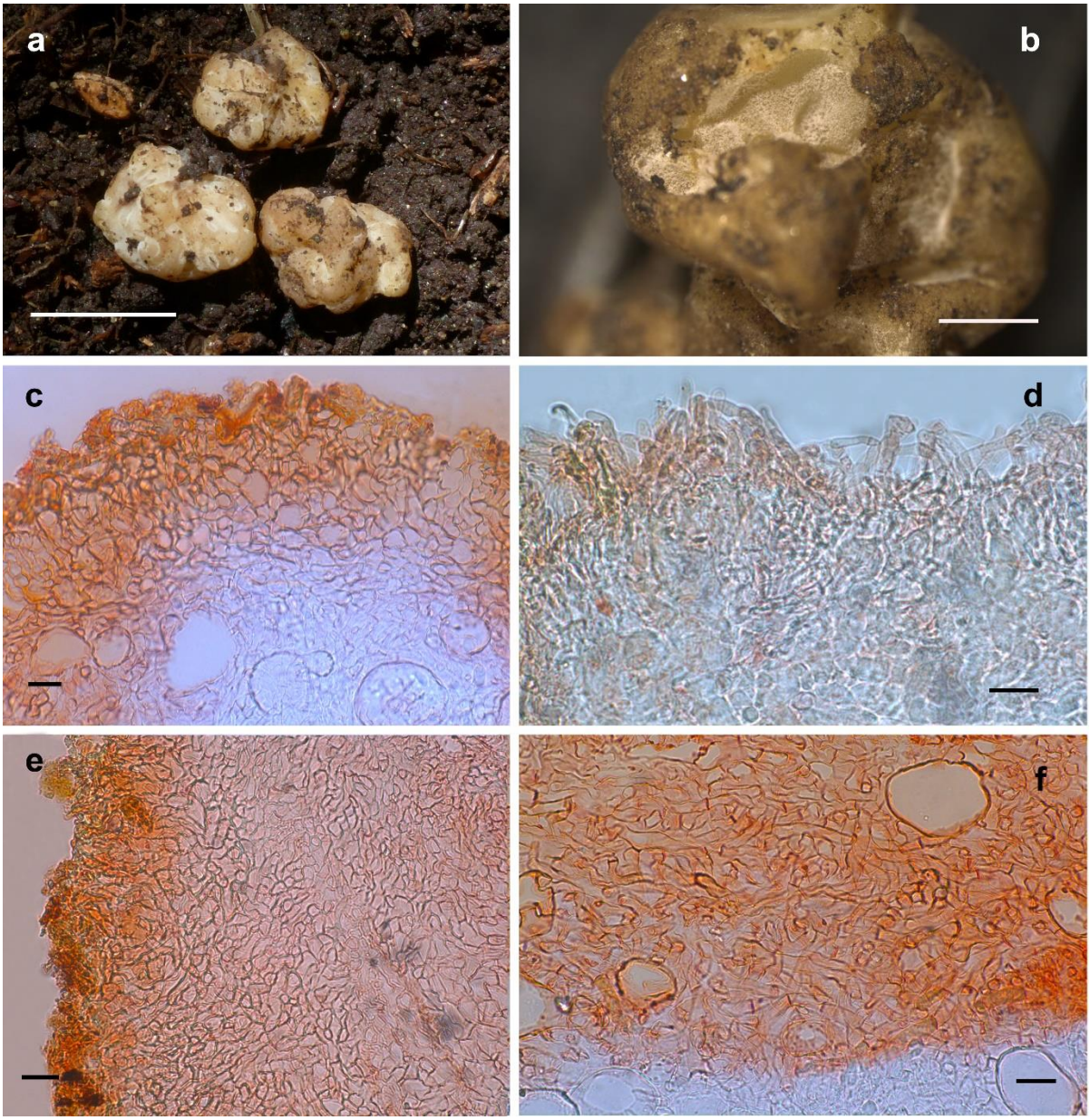

Figure 8. *Hydnobolites cerebriformis* (YNAU0318). (a) Fresh ascomata; (b) Dried ascoma; (c,e) Peridium; (d) Paraphyses; (f) hyphae of gleba. Scale bars: a = 1 cm; b = 1 mm; c–f =20 μm.

4. Discussion

Hydnobolites is a relatively small and infrequent genus [28]. Although the study of this genus can be traced back to 1834 [1], it is poorly understood and has not been thoroughly deeply studied in China. The high species diversity and broad geographic distribution of specimens and ECMs suggest that *Hydnobolites* has wider host, ecological adaptability and species formation potential [19,21,31–65].

Our phylogenetic analysis based on an ITS and ITS-nrLSU dataset both revealed that *Hydnobolites* species clustered into eight distinct clades (Figure 1, 2). Among them, Clade 1 received subsignificant values in the ML analyses but has significant supports in the bayesian results (Figure 1 BP 65, PP 1.0; Figure 2 BP 57, PP 0.99) while Clade 3 received subsignificant supports (Figure 1, BP 47, PP 0.65; Figure 2, BP 74, PP 0.84), which probably due to the insufficient phylogenetic signal in the species analyzed or gaps in the phylogenetic diversity because of an incomplete sampling, the other clades are well supported by effective supports both in the trees yielded by ITS and ITS-nrLSU analyses, respectively

(Figure 1, 2). Phylogenetic trees based on ITS dataset also confirmed the presence of at least 42 phylogenetic species distrubuted in Asia, Europe and North America. Of these, 10 are identified as known species, including one new record for China which was also shared across Japan, Europe and North America, and 5 species are identified as new in this paper. The others are not named due to they are from ECMs or lack of ascus material.

Although there were some subtle morphological differences, it may not be easy to define the close relationship between *H. cerebriformis* complex. However, according to the molecular evidence, significant divergences were observed in the ITS region, and to further determine their relationships within the complex, ITS-nrLSU combined dataset was used and different species of *H. cerebriformis* complex was clearly and consistently identified.

Based on previous research, *Hydnobolites* morphological characters with brain-like, pale ascomata, thin excipulum, pseudoparenchymatous peridium, lack of paraphyses or without well-differentiated paraphyses, apparent lack of basal mycelial tuft, globose spores with a complete reticulum ornamentation, 8 spores per mature ascus, and a tendency to turn orange with handling and as it dries [12,20,66]. In addition to the characteristics consistent with the above, we also observed well differentiated paraphyses, clearly visible spores and ascus on the surface and basal mycelial tuft adhering to the soil. The morphological characteristics of the genus have been further described and improved and it also further increases the understanding of this group.

Supplementary Materials: Table 1.

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Data Availability Statement: Data Availability Statement: Publicly available dataset were analyzed in this study. The resulting alignments were deposited in TreeBASE (http://www.treebase.org; accession number 29854). All newly generated sequences were deposited in GenBank (https://www.ncbi. nlm.nih.gov/genbank/, mentioned in the text, Table and in Figure 1, 2). All new taxa were deposited in MycoBank (https://www.mycobank.org/).

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