

A new species and new records of *Hymenopellis* and *Xerula* (Agaricales, Physalacriaceae) from China (#87474)

1

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A new species and new records of *Hymenopellis* and *Xerula* (Agaricales, Physalacriaceae) from China


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Hymenopellis is the genus with the highest number of species in the *Xerula* / *Oudemansiella* complex, and the study of this complex is of great significance. Many species of *Hymenopellis* are edible, and some of them have been domesticated and cultivated. During a survey of macrofungal resources in Henan and Jilin provinces, China, we collected many specimens of *Hymenopellis*. Based on the results of morphological and molecular phylogenetic studies, we introduced a new species of *Hymenopellis* *biyangensis*, a new record species for China—*H. ssima* and two new record species for Henan Province, *H. raphanipes* and *Xerula strigosa*. Internal transcribed spacer (ITS) and large subunit ribosomal (LSU) were used to construct a phylogeny for the identification of these species. Detailed descriptions, field habitat maps and line drawings of these species are provided. Relationships of the new species to related taxa are discussed and a key to reported *Hymenopellis* species and *Xerula* species in China are provided.

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Abstract

Hymenopellis is the genus with the highest number of species in the *Xerula* / *Oudemansiella* complex, and the study of this complex is of great significance. Many species of *Hymenopellis* are edible, and some of them have been domesticated and cultivated. During a survey of macrofungal resources in Henan and Jilin provinces, China, we collected many specimens of *Hymenopellis*. Based on the results of morphological and molecular phylogenetic studies, we introduced a new species of *Hymenopellis* *biyangensis*, a new record species for China—*H. altissima* and two new record species for Henan Province, *H. raphanipes* and *Xerula strigosa*. Internal transcribed spacer (ITS) and large subunit ribosomal (nrLSU) were used to construct a phylogeny for the identification of these species. Detailed descriptions, field habitat maps and line drawings of these species are provided. Relationships of the new species to related taxa are discussed and a key to reported *Hymenopellis* species and *Xerula* species in China are provided.

Keywords Physalacriaceae, Molecular phylogeny, Morphology, New species

Introduction

Hymenopellis RH Petersen is a genus of the *Xerula* / *Oudemansiella* complex group which is characterized by a moist to glutinous pileus (Petersen & Hughes 2010). Many species of *Hymenopellis* have been recorded as edible (Wu et al. 2019). One mushroom called "Heipijizong" was identified as *Hymenopellis raphanipes* and has widely cultivated in China (Hao et al. 2016; Niego et al. 2021; Sun et al. 2016; Xiao et al. 2022).

The *Xerula* / *Oudemansiella* complex group can date back to 1880, Spegazzini established the genus *Oudemansia* Speg., and then renamed it *Oudemansiella* in 1881 (Spegazzini 1881). In 1933, Maire established the genus *Xerula* (Maire 1933). In the classical taxonomy stage, the epitaxial and subordinate classification system of the *Oudemansiella* has long been the focus of debate among researchers, especially the relationship between *Oudemansiella*, *Mucidula*,

Mycenella and *Xerula* (Boursier 1924; Cl  men  on 1979; D  rfelt 1979; Lange 1914; Moser 1955; Pegler & Young 1986; Singer 1964). Moser (1955) proposed the *Oudemansiella* s.l., and merged *Xerula* and *Mucidula* into *Oudemansiella*. Singer accepted this view and proposed the concept of subtribe *Oudemansiellinae*, which include subg. *Oudemansiella* and subg. *Xerula* (Singer 1964, 1986). Cl  men  on (1979) proposed a taxonomic system of five subtribes and nine groups, on the premise of accepting *Xerula* as a subgenus of *Oudemansiella* and *Mycenella* as a separate genus. However, Pegler and Young continued with the two subgenera treatments proposed by Singer and proposed a systematic treatment of two subgenera and five groups (Pegler & Young 1986). D  rfelt (1979) retained *Oudemansiella* and *Xerula* as two separate genera, and some researchers also accepted this view (Boekhout & Bas 1986; Contu 2000; D  rfelt 1979; Halling & Mueller 1999; Petersen 2008; Petersen & Nagasawa 2006).

In the 21st century, according to the results of morphological and molecular systematics, researchers showed that *Oudemansiella* and *Xerula* are two independent genera, and the large number of species previously placed in *Xerula* should be attributed to *Oudemansiella* (Redhead 1987; Wang et al. 2008). At the same time, Zhang showed that the *Oudemansiella* may be divided into three groups: sect. *Oudemansiella*, sect. *Dactylosporina* and sect. *Radicatae* based on (ITS) and (LSU) (Zhang 2006). Wang et al. (2008) redefined the genus *Xerula* s. str. in a narrow sense. Yang et al. (2009) excluded the narrowly defined genus *Xerula* from the genus *Oudemansiella* and divided the genus *Oudemansiella* into four groups: sect. *Oudemansiella*, sect. *Mucidula*, sect. *Dactylosporina* and sect. *Radicatae*. Petersen & Hughes (2010) revised the taxonomic relationships of the *Xerula* / *Oudemansiella* complex based on morphological evidence and molecular phylogenetic results from ITS and nrLSU, proposing 68 new taxa, including four new genera (*Hymenopellis*, *Paraxerula*, *Pointiculomyces*, *Protoxerula*), the concept of the genus *Xerula* is consistent with Young et al., and the results are still widely accepted today (He et al. 2019; Niego et al. 2021; Park et al. 2017).

Hymenopellis species are widely distributed in Europe and North America (Petersen & Hughes 2010), but they can also be found on other continents, such as China, Japan, Thailand, Australia, etc. (Niego et al. 2021; Yang 1993). So far, there are 41 species records in Index Fungorum (www.indexfungorum.org) of which 12 species have been recorded in China (Petersen & Hughes 2010; Yang 1993; Yao et al. 2020).

During a survey of macrofungal resources in the Henan and Jilin provinces of China, we collected some specimens of *Hymenopellis*. After a combined morphological and phylogenetic study, we propose one new species, one new record species from China and two new record species from Henan.

Materials and Methods

Sampling and morphological analysis


The specimens were collected from Biyang County, Henan Province and Shulan City, Jilin Province, China. Voucher specimens were stored in the Herbarium of Mycology of Jilin

Agricultural University (HMJAU). Macroscopic morphological features are derived from field records of fresh specimens, and microscopic morphology by light microscope, and the main reagents included 5% KOH solution, Meler's reagent solution and 1% Congo red solution. The description of basidiomata colors uses Kornerup and Wanscher's (Kornerup & Wanscher 1978) color coding. The basidiospores size is expressed as (a) b–c (d), where "a" is the minimum and "d" is the maximum, and 95% of the observed range falls between b–c. "Q" is the ratio of the length to width of the basidiospores, and "Q ± av" is the average Q ± standard deviation of all basidiospores (Dong & Bau 2022; Wang et al. 2022).

DNA extraction, PCR amplification, and sequencing

The total DNA was extracted from dried specimens using the NuClean Plant Genomic DNA kit (Kangwei Century Biotechnology Co., Ltd., Beijing, China). Primers for amplification ITS used ITS 4 and ITS 5 (He et al. 2023; Ward & Akrofi 1994), nrLSU were LROR and LR5 (Kausrud & Schumacher 2001; Wang & Yang 2023). Then, the PCR products were sent to Biotechnology Co., Ltd. (Shanghai, China) Sequencing was performed.

Data Analysis

Sequences obtained by sequencing were uploaded in NCBI (www.ncbi.nlm.nih.gov). ITS and nrLSU sequences of related taxa were retrieved from GenBank and related articles (Hao et al. 2016; Lin et al. 2021; Matheny et al. 2006; Niego et al. 2021; Petersen & Hughes 2010; Qin et al. 2014), and combined with the sequences generated in this study (Table 1). The sequences obtained were compared and manually modified by BIOEDIT (Hall et al. 2011; Ye & Bau 2022). The multi-locus dataset (ITS + nrLSU) in *Hymenopellis* was 898 bp for ITS and 872 bp for nrLSU. The multi-locus dataset (ITS + nrLSU) in *Xerula* was 806 bp for ITS and 870 bp for nrLSU. Using PartitionFinder 2 to determine the optimal model scheme for multi-locus datasets (Lanfear et al. 2017). For the multi-locus data set of *Hymenopellis*, the optimal model for ITS is HKY + F + I + G4, and the optimal model for nrLSU is K2P +  or the multi-locus data set of *Xerula*, the optimal model for ITS is HKY + G and the optimal model for nrLSU is GTR + I. Phylogenetic analysis was performed using Maximum likelihood (ML) and Bayesian inference (BI) methods. The Maximum likelihood method (ML) and Bayesian inference (BI) were performed by IQTree 1.6.8 (Nguyen et al. 2015) and MrBayes 3.2.6 (2,000,000 generations) (Deng et al. 2022; Ronquist et al. 2012) in PhyloSuite 1.2.2 (Zhang et al. 2020), respectively.

Results

Molecular Phylogeny

The two-locus gene dataset (ITS + LSU) of *Hymeopellis* contains 51 sequences and 1770 bp nucleotide sites. *Flammulina yunnanensis* ZW Ge & Zhu L. Yang and *Flammulina velutipes* (Curtis) Singer were chosen as outgroups. Because the topology of the ML tree is the same as the topology of the BI tree, we have chosen to show only the ML tree (Fig. 1). In the phylogenetic tree, *Hymenopellis biyangensis* and *H. altissima* are belonged to *Hymenopellis*, and each forms a

strong branch with strong support (BP = 99, PP = 1 and BP = 99, PP = 1).

Hymenopellis raphanipes collected from Biyang County, China is located on the same branch as *Hymenopellis raphanipes* distributed in two other localities (Fig. 1).

The two-locus gene dataset (ITS + LSU) of *Xerula* contains 16 sequences and 1676 bp nucleotide sites. *Paraxerula americana* (Dörfelt) RH Petersen and *Paraxerula hongoi* (Dörfelt) RH Petersen were selected as outgroups. Because the topology of the ML tree is the same as the topology of the BI tree, we have chosen to show only the ML tree (Fig. 2). In the phylogenetic tree, *Xerula strigosa* collected from Biyang County, China was located in the same clade as *X. strigosa* from Yunnan, forming a sister clade with *X. sinopudens*, which received high support (BP = 72, PP = 0.99).

Taxonomy

Hymenopellis biyangensis Y.J. Liu, B. Zhang & Xiao Li sp. nov.

Mycobank No: 848957

Figs 3a–3b, 4

Etymology. “biyangensis” refers to its type locality Biyang County.

Holotype. China, Henan Province, Zhumadian City, Biyang County, WanFenSi, 113°36'40"E, 32°51'45"N, 11 July, 2021 (HMJAU67048).

Description. Basidiomata small-to-large sized. Pileus 30–105 mm in diam, subumbonate, convex to plano-concave, with a nearly radial distribution of folds, brownish orange (6C3) to light brown (7D4), yellowish grey (3B2), with a darker center, brownish grey (10E2), sticky when wet. Context white (1A1). Lamellae adnate with decurrent tooth, white (1A1), subdistant, thick, not the same length. Stipe 75–100 × 4–7.5 mm, subcylindrical, tapering upward, white (1A1) to brown (7E4), pileus junction nearly white, squamulosus. Pseudorhiza at least 62 mm long, tapering downward, dirty white.

Trama lamellarum subregularis, composed of 3–30 µm diameter, colorless mycelium, without clamp connection. Basidia 40–70 × 9–19 µm, clavate, with 2-spored, rarely 4-spored, sterigmata 5–14 µm long, without clamp connection. Basidiospores (13) 14–20 (20.5) × (8.5) 11–15 (17) µm, [Q = (1.07) 1.13–1.52 (1.57), Q_m = 1.28 ± 0.12], subglobose, ellipsoid to broadly ellipsoid, colorless in 5% KOH, hyaline, smooth, non-amyloid, non-dextrinoid. Pleurocystidia 62–143 × 23–56 µm, paddle-shaped, obovoid. Cheilocystidia abundant with sterile bands, 48–137 × 10–36 µm, clavate, broadly utriform, colorless, hyaline. Pileipellis 100–160 µm thick, composed of sphaeropedunculate and clavate cells (23–90 × 12–30 µm), without clamp connection. Stipe trama monophyletic mycelial system, composed of parallel hyphae 5–30 µm wide, colorless, hyaline. Stipe epidermis 3–12 µm, composed of flavidus parallel hyphae. Caulocystidia 36–119 × 7–17 µm, narrowly clavate, clamp connectio is rare.

Habitat and distribution. Solitary or scattered on the soil of *Quercus acutissima* forest or *Quercus acutissima* and *Castanea mollissima* mixed forest.

Additional specimens examined. China, Henan Province, Zhumadian City, Biyang County, Minzhuan Forest Farm, 32°52'18"N, 113°36'25"E, 10 July 2021. ZhengXiang Qi (HMJAU67043, HMJAU67044), GuiPing Zhao (HMJAU67047); China, Henan Province, Zhumadian City, Biyang County, WanFenSi, 32°51'45"N, 113°36'40"E, 11 July 2021. ZhengXiang Qi (HMJAU67045, HUJAU67046).

Notes. *H. biyangensis* is characterized by a slightly raised in the center, and a nearly radial distribution of folds, cheilocystidia are abundant and form sterile bands. The pileipellis consists of sphaeropedunculate and clavate cells. *H. biyangensis* is morphologically similar to *H. amygdaliformis*, but *H. amygdaliformis* is characterized by amygdaliform spores and vase-like pleurocystidia (Petersen & Hughes 2010; Yang 1993). This species is also similar to *H. vinocontusa*, but the latter has no caulocystidia (Petersen & Hughes 2010). In addition, *H. amygdaliformis* and *H. vinocontusa* have 4-spored basidia, and *H. biyangensis* is 2-spored basidia.

Hymenopellis altissima (Masse) RH Petersen

Basionym: *Collybia altissima* Masse 1914. Kew Bull. Misc. 358.

≡ *Oudemansiella altissima* (Masse) Corner, Gdns' Bull., Singapore 46 (1): 56 (1994)

Figs 3c, 5

Description. Basidiomata small sized. Pileus diameter 35 mm, plano-convex, greyish brown (7D3) to reddish brown (8E6), darker in the middle, strongly radially wrinkled from middle onto limb. Context white (1A1). Lamellae white (1A1), adnate, disatant, thick, not the same length. Stipe 63 × 3.8 mm, subcylindrical, gradually thickening down to the pseudorhiza, covered with brown (7E4) squamules but nearly white at apex. Pseudorhiza at least 77 mm, 5 mm wide, slightly tapered downward, white (1A1) to light brown (7D4).

Lamellar trama regular, composed of hyphae 3–23 µm diameter, without clamp connection. Basidiospores (13) 14–17.5 (18) × (12.5) 13–16 (16.5) µm, [Q = (1) 1.03–1.21 (1.29), Q_m = 1.10 ± 0.06], globose, subglobose, a few broadly ellipsoid, colourless, smooth, thin-walled, non-amyloid. Basidia 47–63 × 14–17 µm, clavate, 2-spored, sterigmata 5–9 µm long, without clamp connection. Pleurocystidia 90–165 × 22–39 µm, fusiform, obviously capitulates, colorless or with yellow pigment, without clamp connection. Cheilocystidia abundant, 62–127 × 13–32 µm, clavate, narrowly utriform, colorless, hyaline, thin-walled. Pileipellis an ixohymeniderma of 100–150 µm thick, composed of sphaeropedunculate cells (43–104 × 19–30 µm), without pileocystidia. Stipitipellis composed of vertically arranged, colorless, hyaline, slightly thick-walled (≤ 1 µm), hyphae 3–16 µm broad. Caulocystidia 54–156 × 10–22 µm, clavate to narrowly utriform, thinning at the end, colorless, hyaline.

Habitat and distribution. Growing on the ground in a mixed forest of *Quercus mongolica* and *Tilia tuan*.

Specimens examined. China, Jilin Province, Shulan City, Jiulongshan Forest Park. 3 August 2022, Lei Yue (HMJAU67050).

Notes. *H. altissima* is characterized by strongly radially wrinkled from pileus middle onto

limb, basidiospores globose, subglobose, pleurocystidia fusiform, obviously capitatus, without clamp connection. Our specimens have longer pseudorhiza compared to the description of this species by Petersen and Hughes (Petersen & Hughes 2010) which corresponds to the description of this species by Corner (Corner 1994). Morphologically, *H. altissima* is relatively similar to *H. vinocontusa*, but *H. vinocontusa* basidiospores of ellipsoid, ovate to sublimoniform, cheilocystidia fusiform-capitulate (Petersen & Hughes 2010). In addition, *H. vinocontusa* have 4-spored basidia, and *H. altissima* is 2-spored basidia.

Hymenopellis raphanipes (Berk.) R.H. Petersen

Basionym: *Agaricus raphanipes* Berk., Hooker's J. Bot. Kew Gard. Misc. 2: 48, 1850

≡ *Collybia raphanipes* (Berk.) Sacc., Syll. fung. (Abellini) 5: 202, 1887

≡ *Xerula raphanipes* (Berk.) Dörfelt, Feddes Repert. 94(7–8): 557, 1983

≡ *Oudemansiella raphanipes* (Berk.) Pegler & T.W.K. Young, Trans. Br. mycol. Soc. 87(4): 596, 1987 (1986)

≡ *Xerula chiangmaiae* var. *raphanipes* (Berk.) R.H. Petersen, Rep. Tottori Mycol. Inst. 43: 20, 2006

≡ *Hymenopellis raphanipes* (Berk.) R.H. Petersen, Nova Hedwigia Beih. 137: 213, 2010

Synonym: *Xerula chiangmaiae* R.H. Petersen & Nagas., Rep. Tottori Mycol. Inst. 43: 17, figs. 15–20, 2006

≡ *Oudemansiella chiangmaiae* (R.H. Petersen & Nagas.) Zhu L. Yang, G.M. Muell., G. Kost & Rexer, Mycosystema 28: 7, 2009

Figs 3d, 3f and 6

Description. Basidiomata small-to-large sized. Pileus 23–95 mm in diam, initially hemispherical, later nearly expansus, slightly convex or concave, margin or upturned, margin dehiscence, greyish brown (6D2), brownish orange (7C3), light brown (6D4), brown (7E8) to dark brown (7F8), smooth, sometimes with reticulate “veins” near the margin. Context white to creamy white. Lamellae adnate to sinuate or slightly decurrent, subdistant, thick, white to creamy white, sometimes with yellow spots, unequal in length, not the same length. Stipe 64–200 × 4–12 mm, subcylindrical, tapering upward, white to grey (10C1, 13D1–16D1), surface densely covered with gray scales but nearly white at apex, densely covered with brownish felted squamules. Pseudorhiza tapering downward, white to dirty white.

Lamellar trama regular, composed of 3–20 µm diameter, colorless mycelium, without clamp connection. Basidia 32–75 × 9–19 µm, clavate, 2-spored, rarely 4-spored, sterigmata 5–12 µm long, thin-walled, colorless, hyaline. Basidiospores (14) 16–22 (23) × (7) 10–14 (15) µm, [Q = (1.25) 1.36–1.73 (1.75), Q_m = 1.52 ± 0.12], ovoid, ellipsoid to broadly ellipsoid, colorless, hyaline, non-amyloid, non-dextrinoid, smooth. Pleurocystidia 66–150 × 20–48 µm, pedicellate, fusiform with prominent capitate apex, slightly thick-walled, colorless, hyaline. Cheilocystidia 52–274 × 14–50 µm, pedicellate, fusiform, narrowly clavate to clavate, thin-walled, colorless, hyaline. Pileipellis composed of clavate, broadly clavate to sphaeropedunculate cells (22–70 × 8–24 µm), ixohymeniderm not observed. Pileocystidia 65–181 × 9–22 µm, narrowly utriform to

subcylindrical, thin-walled, hyaline. Stipitipellis consists of colorless hyphae arranged longitudinally with a diameter of 3–20 μm , without clamp connection. Caulocystidia 35–206 \times 7–19 μm , clavate, cylindrical, hyaline.

Habitat. Solitary or scattered on the soil of mixed forests of *Quercus robur* and *Torrey pine*.

Distribution. Australia, China, India, Japan, Thailand (Petersen & Hughes 2010).

Specimens examined. China, Henan Province, Zhumadian City, Biyang County, Tongshan Lake Forest Park, 32°45'31"N, 113°30'51"E, 9 July 2022, YaJie Liu (HMJAU67039), 13 July 2022, YaJie Liu (HMJAU67040), 23 July 2022, YaJie Liu (HMJAU67051), 27 July 2022, YaJie Liu (HMJAU67042).

Notes. *H. raphanipes* is characterized by basidiomata small-to-large sized. Pileus smooth. Basidiospores ovoid, ellipsoid to broadly ellipsoid, pleurocystidia fusiforms with prominent capitate apex. *H. raphanipes* is morphologically similar to *H. radicata*, but *H. radicata* differs from *H. raphanipes* by its utriform to tenpin-shaped pleurocystidia and the absent of pileocystidia (Hao et al. 2016; Petersen & Hughes 2010). *H. raphanipes* is also similar to *H. furfuracea*, but *H. furfuracea* differs from *H. raphanipes* in its utriform-pedicellate to tenpin-shaped pleurocystidia (Hao et al. 2016; Petersen & Hughes 2010).

Xerula strigosa Zhu L. Yang, L. Wang & G.M. Muell. 2008

Figs 3e, 7

Description. Basidiomata small sized. Pileus 15–30 mm in diam, convexus to plano-convexus, yellowish brown (5E5) to dark brown (6F7), dry, not viscid, with brown setae, the margin smooth, not stripes. Context white. Lamellae free to adnate, subdistant, white (1A1), with lamellulae. Stipe 100–125 \times 2.5–3 mm, cylindrical or slightly tapered upward, reddish brown (8E8) to dark brown (7F4), paler towards the apex, surface covered with setae of the same color as the stipe. Pseudorhiza tapering downward.

Lamellar trama subregular, composed of 3–33 μm diameter, colorless, hyaline. Basidiospores (11) 12–16 (17) \times (8) 9–12 (13) μm , [Q = (1.00) 1.09–1.52 (1.63), $Q_m = 1.30 \pm 0.15$], subglobose, broadly ellipsoid to ellipsoid, colorless, hyaline. Basidia 27–67 \times 13–18 μm , clavate, 4-spored, rarely 2-spored, sterigmata 5–9 μm long, without clamp connection at base. Pleurocystidia 78–135 \times 18–33 μm , narrowly utriform, capitate to subcapitate at the top, often with yellowish crystalline deposits, without clamp connection. Lamellar edge fertile, with scattered cheilocystidia. Cheilocystidia 75–106 \times 18–27 μm , narrowly utriform, hyaline, thick-walled (wall 2 μm thick), without clamp connection. Pileipellis over umbo composed of clavate, sphaeropedunculate cells (51–105 \times 13–20 μm) and pileocystidia. Pileocystidia 93–530 \times 8–16 μm , lanceolate, thick-walled (wall 1–3 μm thick), yellowish brown, without clamp connection. Pileipellis at pileus margin constructed of subfusiform, pyriform or sphaeropedunculate cells (43–63 \times 12–18 μm) and pileocystidia. Pileocystidia 98–540 \times 8–18 μm , lanceolate, thick-walled (wall 1–3 μm thick), yellowish brown, without clamp connection. Stipitipellis composed of vertically arranged, slightly yellow, hyaline, slightly thick-walled ($\leq 1 \mu\text{m}$), hyphae 3–11 μm

broad. Caulocystidia similar to pileocystidia, $40\text{--}236 \times 3\text{--}12 \mu\text{m}$, lanceolate, thick-walled ($\leq 2 \mu\text{m}$), yellowish brown.

Habitat. Growing on the ground in mixed broad-leaved forests dominated by *Quercus robur*.

Distribution. China, Pakistan (Krisai-Greilhuber et al. 2017).

Specimens examined. China, Henan Province, Zhumadian City, Biyang County, Minzhuan Forest Farm, $32^{\circ}52'18''\text{N}$, $113^{\circ}36'25''\text{E}$, 10 July 2021. ZhengXiang Qi (HMJAU67049).

Notes. *Xerula strigosa* is characterized by broadly ellipsoid to ellipsoid basidiospores, pleurocystidia narrowly utriform, capitate to subcapitate at the top, pileocystidia lanceolate. *X. strigosa* closely resembles *X. pudens* (Wang et al. 2008), compared with *X. pudens*, *X. strigosa* exhibits longer spores and thin-walled apex of pleurocystidia. *X. strigosa* also strongly resembles the *X. hispida*, *X. hispida* differs from *X. strigosa* by its 2-spored (rarely 4) basidia and pleurocystidia without a capitate apex.

A key to China species of *Hymenopellis*

1. Pileipellis with pileocystidia.....2
- Pileipellis without pileocystidia.....9
2. pileus flesh white.....3
- Flesh fuliginous to avellaneous beneath pileipellis and above lamellae, otherwise white.....
-*Hymenopellis colensoi*
3. Basidiospores amygdaliform.....*H. amygdaliformis*
- Basidiospores broadly ellipsoid, ovatus to sublimoniform.....4
4. Cheilocystidia narrowly clavate to clavate, fusiform.....5
- Cheilocystidia fusiform with flagelliform apex.....*H. hygrophoroides*
5. Pleurocystidia apically expanded into capitate, capitulate or subcapitate.....6
- Pleurocystidia apically not expanded into capitate, capitulate or subcapitate.....7
6. Pleurocystidia utriform.....*H. furfuracea*
- Pleurocystidia fusiform*H. raphanipes*
7. Pileus subumbonate, with stripes*H. megalospora*
- Pileus subumbonate, no stripes.....*H. bispora*
8. Stipe furnished with annulate veil.....*H. velata*
- Stipe without annulate veil.....9
9. Basidia 2-spored.....10
- Basidia 4-spored.....11
10. Pleurocystidia paddle-shaped, obovoid.....*H. biyangensis*
- Pleurocystidia fusiform, obviously capitulates.....*H. altissima*
11. Stipe caulocystidia absent.....*H. vinocontusa*
- Stipe have caulocystidia.....12
12. Cheilocystidia broadly cylindrical.....13
- Cheilocystidia narrowly utriform, fusiform, narrowly clavate to clavate.....14
13. Stipe longitudinally lined, usually twisted, sometimes reluctantly reddish brown in spots

- 316 or suffused below.....*H. radicata*
 317 – Stipe appearing minutely laccate as though viscid, with darker amor-phous patches.....
 318*H. orientalis*
 319 14. Basidiospores globose to subglobose.....*H.japonica*
 320 – Basidiospores subovate to sublimoniform.....*H. aureocystidiata*
 321
 322 A key to China species of *Xerula*
 323 1. Basidia 2-spored, basidiospores globose, subglobose to ovoid.....*Xerula hispida*
 324 – Basidia 4-spored, basidiospores subglobose to broadly ellipso.....2
 325 2. Pleurocystidia fusiform, often with crystalline deposited apex.....3
 326 – Pleurocystidia fusiform, without crystalline deposits.....*X. sinopudens*
 327 3. Clamp connections common.....*X. puden*
 328 – Clamp connections absent.....*X. strigosa*

329 DISCUSSION

330 In this study, a new species *Hymenopellis biyangensis*, a new record species of *H. altissima*
 331 for China and two new record species, *H. raphanipes* and *Xerula strigosa* for Henan province
 332 were discovered by morphology and molecular phylogeny.

333 In the phylogenetic analysis, the new species *H. biyangensis* formed an independent clade
 334 and a sister group with *H. altissima*, which received high support (BP = 99, PP = 1) (Fig. 1).
 335 Morphologically, the pileipellis of *H. altissima* has an ixohymeniderma while *H. biyangensis*
 336 does not. In addition, *H. biyangensis* and *H. altissima* can be distinguished by pleurocystidia and
 337 basidiospores. *H. biyangensis* has paddle-shaped, obovoid pleurocystidia and ellipsoid to broadly
 338 ellipsoid basidiospores, and *H. altissima* has fusiform, obviously capitulates pleurocystidia and
 339 globose to subglobose basidiospores.

340 Pleurocystidia of *Hymenopellis* species are mostly described as “utriform” , “jar-shaped”
 341 and “ten pin-shaped” (Petersen & Hughes 2010), while *H. biyangensis* has paddle-shaped to
 342 obovoid pleurocystidia. In addition, both *H. biyangensis* and *H. altissima* are relatively rare 2-
 343 spore species in *Hymenopellis*. Among the 2-spored species, the basidiospores of *H. altissima* are
 344 subglobose to globose different from the other species. *H. biyangensis*, *H. altissima* and *H.*
 345 *raphanipes* are similar in habitat, growing on the forest dominated by Fagaceae. In the
 346 description of Hao (Hao 2016), Petersen and Hughes (Petersen & Hughes 2010), thirteen species
 347 of *Hymenopellis* were described to grow in Fagaceae forests, especially *Fagus* and *Quercus*,
 348 which leads us to surmise that the growth of species of this genus may have some relationship
 349 with Fagaceae, which require studied later.

350 *Hymenopellis* is a polyphyletic taxon, whose infrageneric species were previously
 351 distributed in several parts of *Oudemansiella* (Petersen & Hughes 2010). Therefore, some
 352 researchers have also questioned the establishment of *Hymenopellis*. Hao (2016) proposed a new
 353 taxonomic system, *Oudemansiella* is a highly supported monophyletic lineage, and should be
 354 treated as a single genus. A new systematic arrangement with three sections, namely, sect.
 355 *Oudemansiella*, sect. *Dactylosporina* and sect. *Radicatae* has been proposed, *Hymenopellis*

should be subsumed into the genus *Oudemansiella* (Hao 2016). Therefore, more researches on this taxon is still needed in the follow-up.

Four species of *Xerula*—*Xerula hispida*, *X. pudens*, *X. sinopudens* and *X. strigosa*, have been reported previously in China (Mueller et al. 2001; Wang et al. 2008; Yao et al. 2020). *X. strigosa* has previously been reported only in China, and according to Irmgard Krisai-Greilhuber et al. (Krisai-Greilhuber et al. 2017), *X. strigosa* is also present in Pakistan. In our phylogenetic analysis, *X. strigosa* is sister with *X. sinopudens* (Fig. 2), which is consistent with the results of Qin et al. (Qin et al. 2014). Morphologically, they both have small basidiomata, both have setae on the pileus, lanceolate pileocystidia. However, *X. strigosa* can be distinguished from *X. sinopudens* by narrowly utriform pleurocystidia, capitate to subcapitate at the top, often with yellowish crystalline deposits (Lan et al. 2008; Liu et al. 2009).

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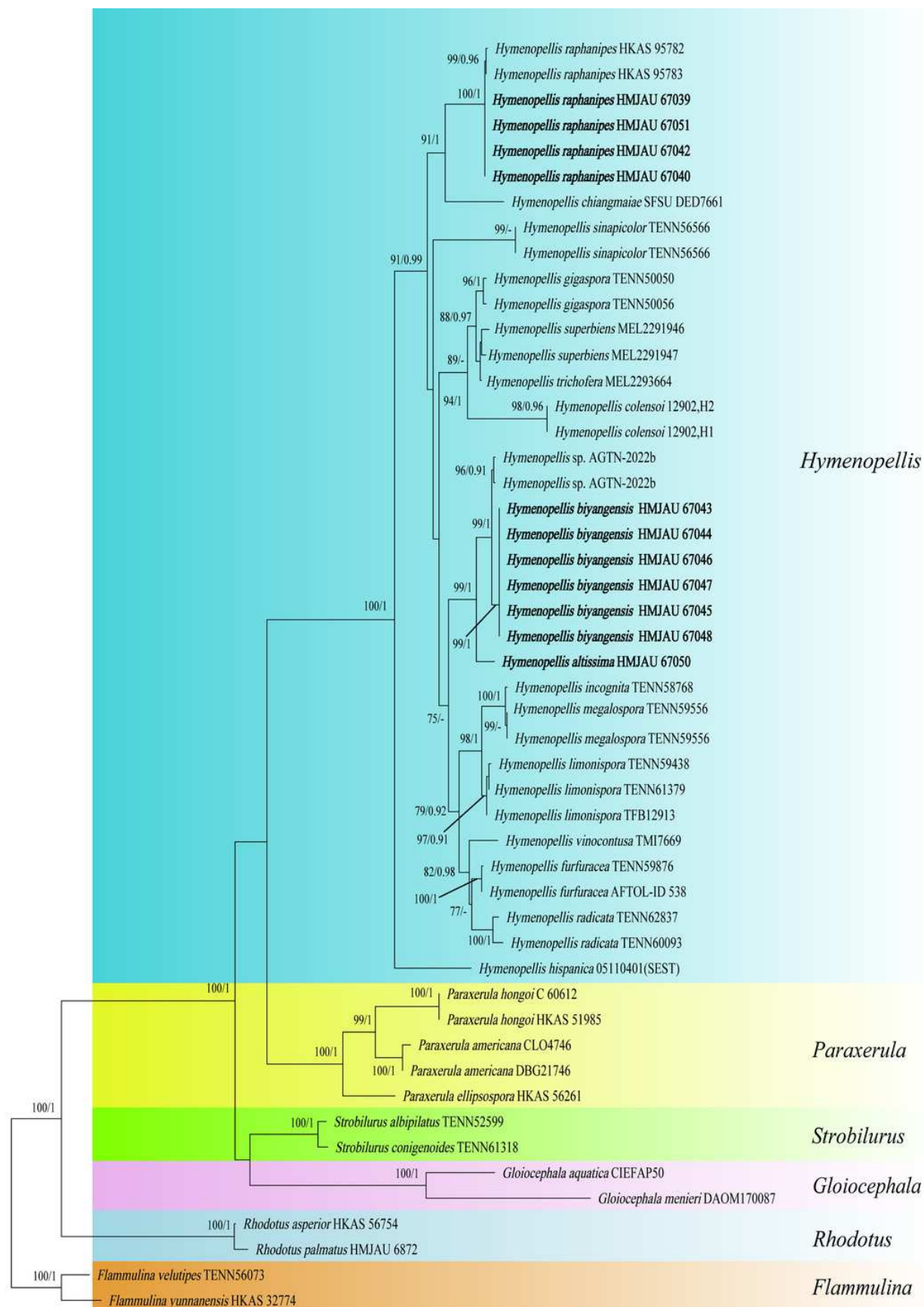
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Figure 1

Phylogenetic analysis of *Hymenopellis* inferred from ML analysis.

The two values of the internal node represent Maximum Likelihood bootstrap (MLBP > 70%) / Bayesian posterior probability (BIPP > 90%). Our species sequences are marked in bold.





0.04

Figure 2

Phylogenetic analysis of *Xerula* inferred from ML analysis.

The two values of the internal node represent Maximum Likelihood bootstrap (MLBP > 70 %) / Bayesian posterior probability (BIPP > 90 %). Our species sequences are marked in bold.

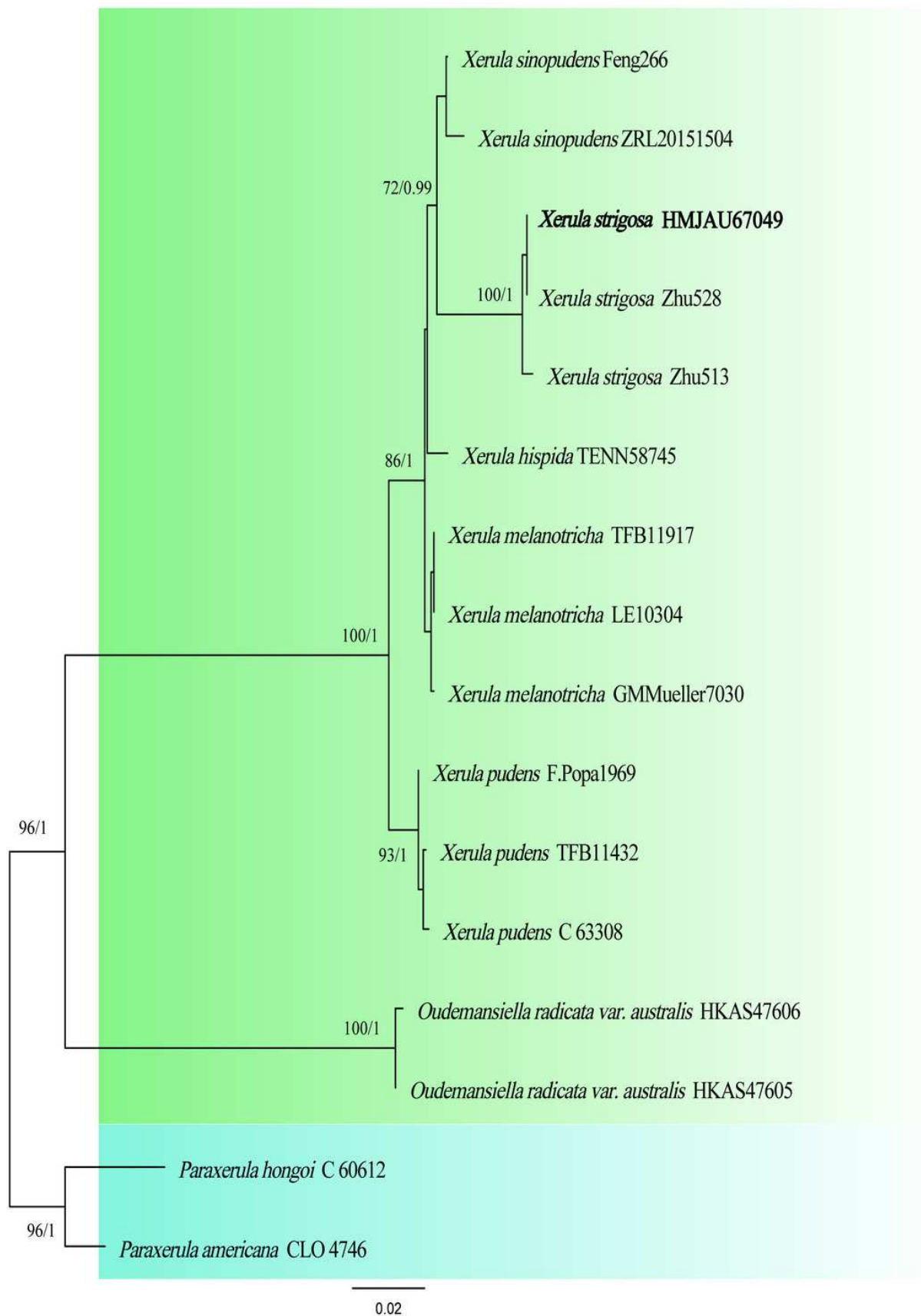


Figure 3

Basidiomata of *Hymenopellis* and *Xerula*

(a, b) *Hymenopellis biyangensis* (a HMJAU67045, b HMJAU67048) (c) *Hymenopellis altissima* (HMJAU67050) (d , f) *Hymenopellis raphanipes* (d HMJAU67041, f HMJAU67051) (e) *Xerula strigosa*

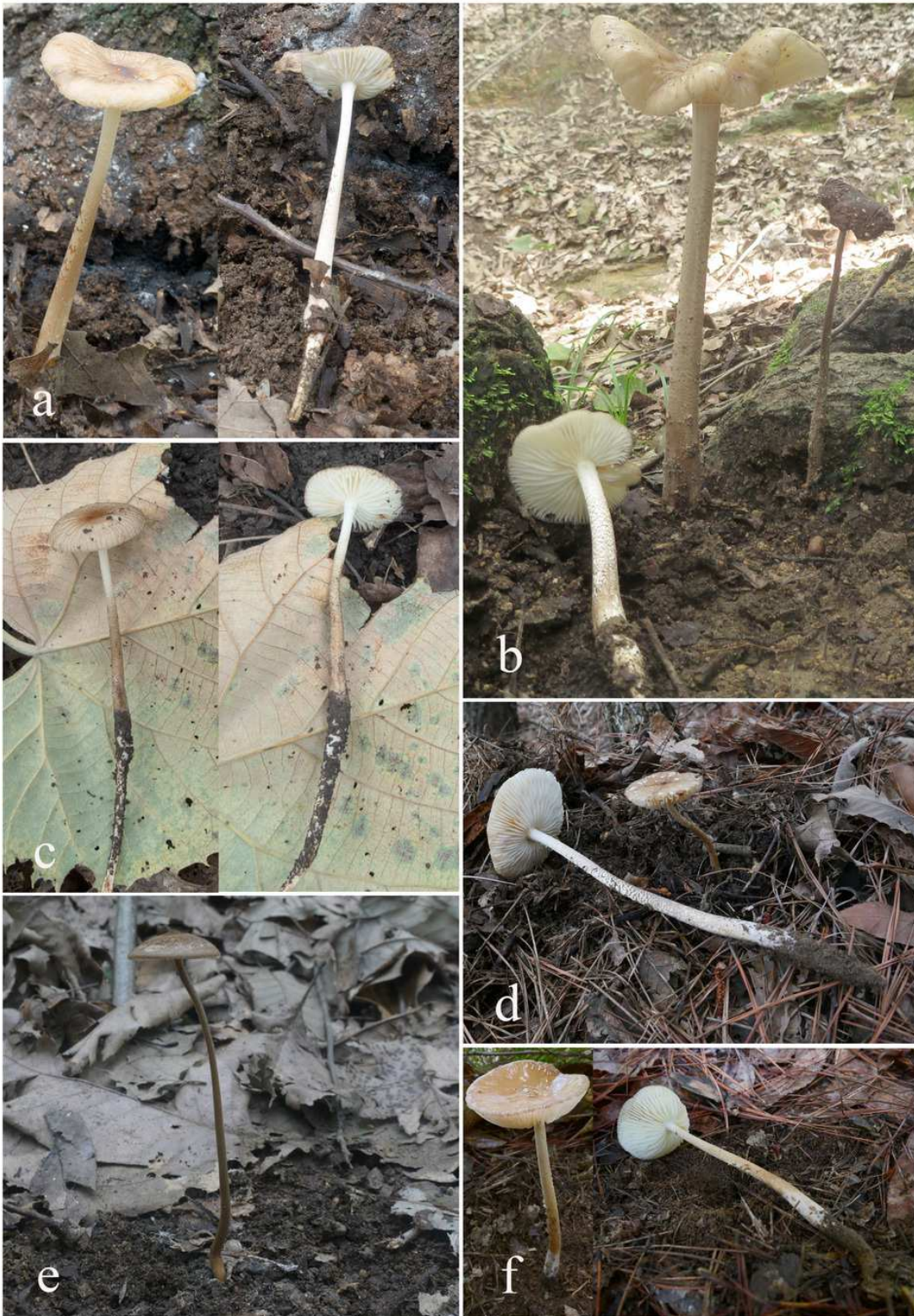


Figure 4

Microscopic features of *Hymenopellis biyangensis*.

(a) Pileipellis (b) Cheilocystidia (c) Basidiospores (d) Pleurocystidia (e) Hymenium (f) Caulocystidia. Scale bars: 20 μm .

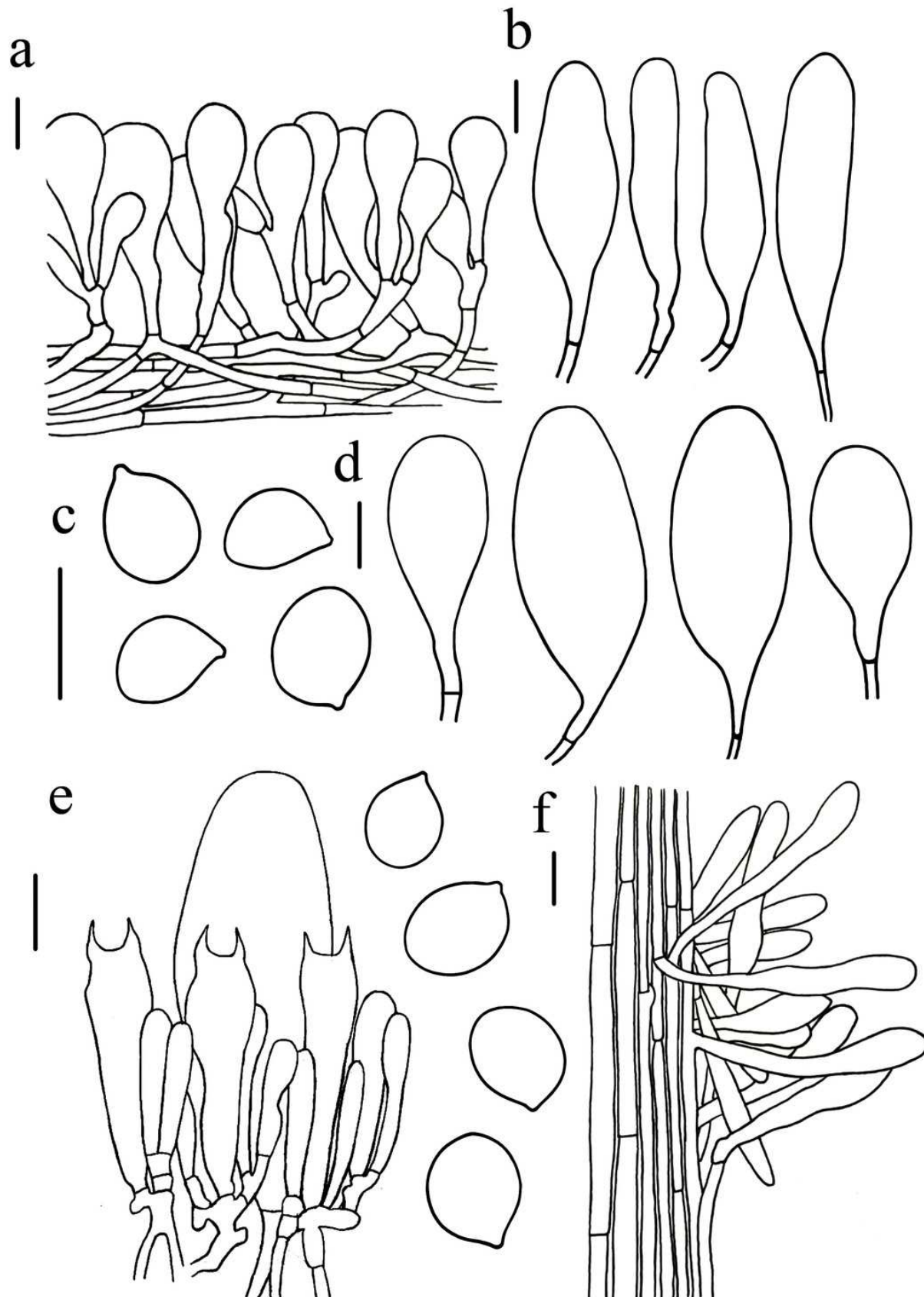


Figure 5

Microscopic features of *Hymenopellis altissima*.

(a) Pileipellis (b) Cheilocystidia (c) Pleurocystidia (d) Basidiospores (e) Hymenium (f) Caulocystidia. Scale bars: 20 μm .

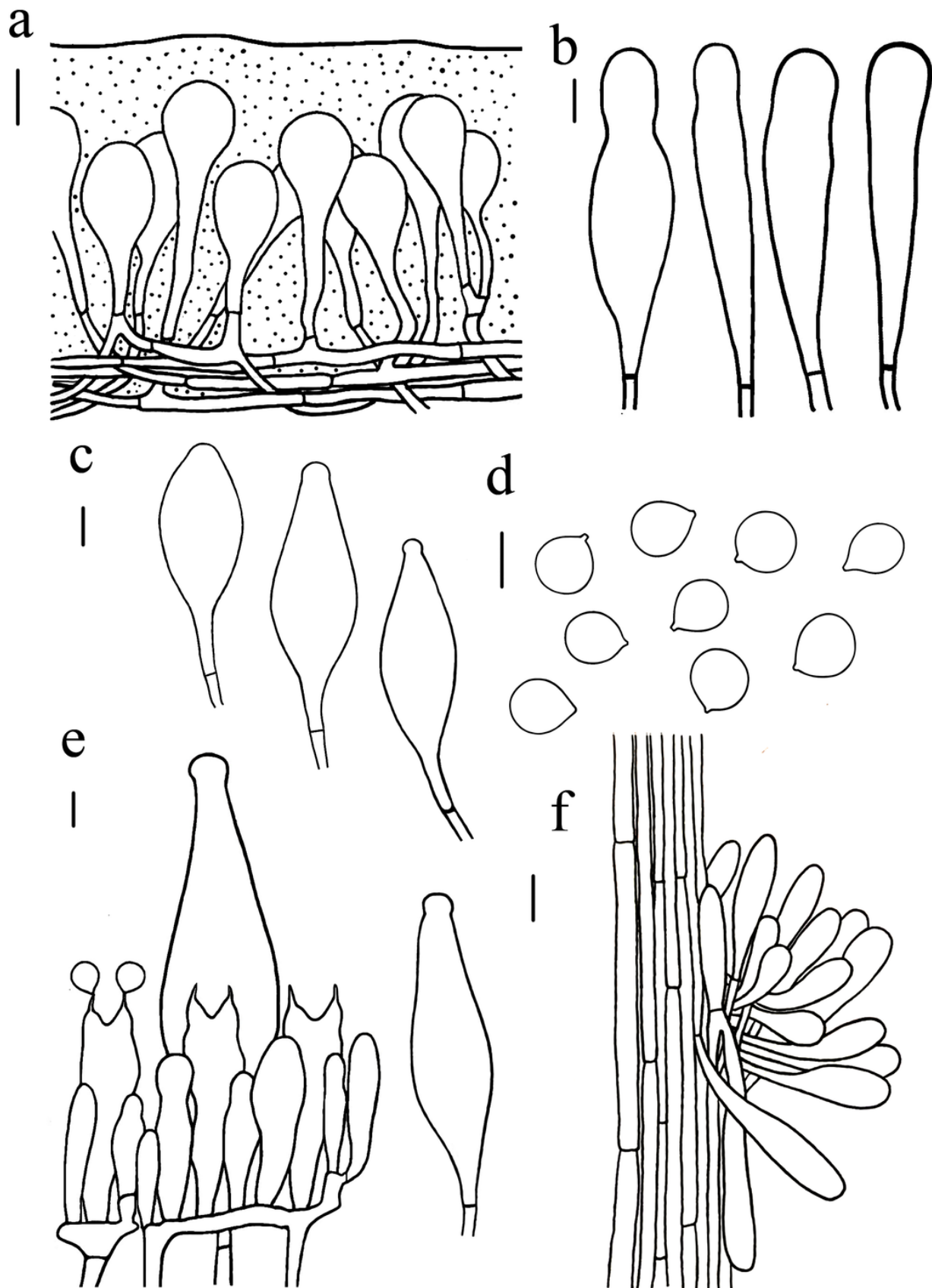


Figure 6

Microscopic features of *Hymenopellis raphanipes*.

(a) Pileipellis (b) Cheilocystidia (c) Basidiospores (d) Pleurocystidia (e) Hymenium (f) Caulocystidia. Scale bars: 20 μ m.

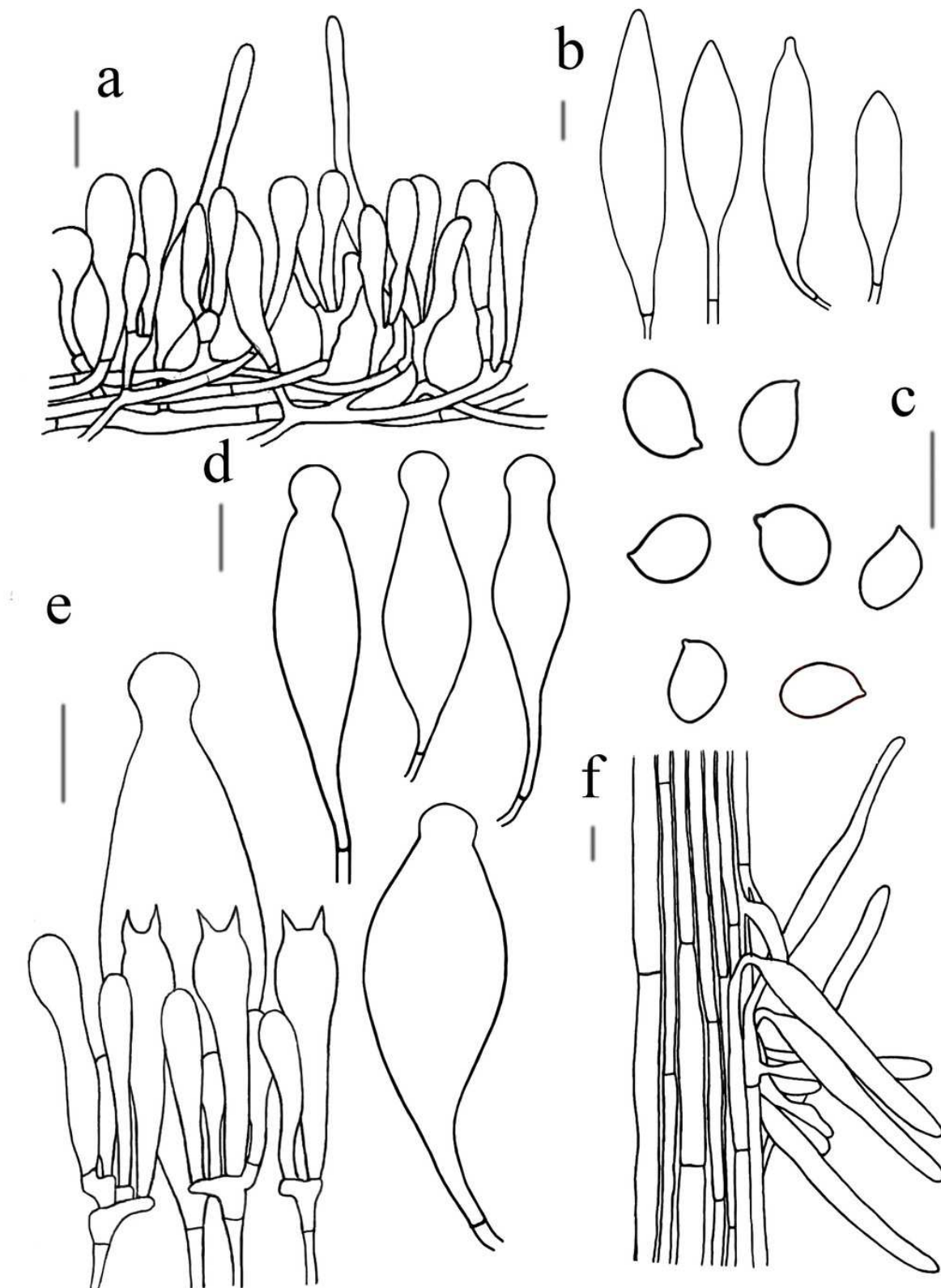


Figure 7

Microscopic features of *Xerula strigosa*.

(a) Pileipellis for pileus margin (b) Pileipellis for pileus disc (c) Pleurocystidia (d) Basidiospores (e) Cheilocystidia (f) Hymenium (g) Caulocystidia. Scale bars: 20 μm .

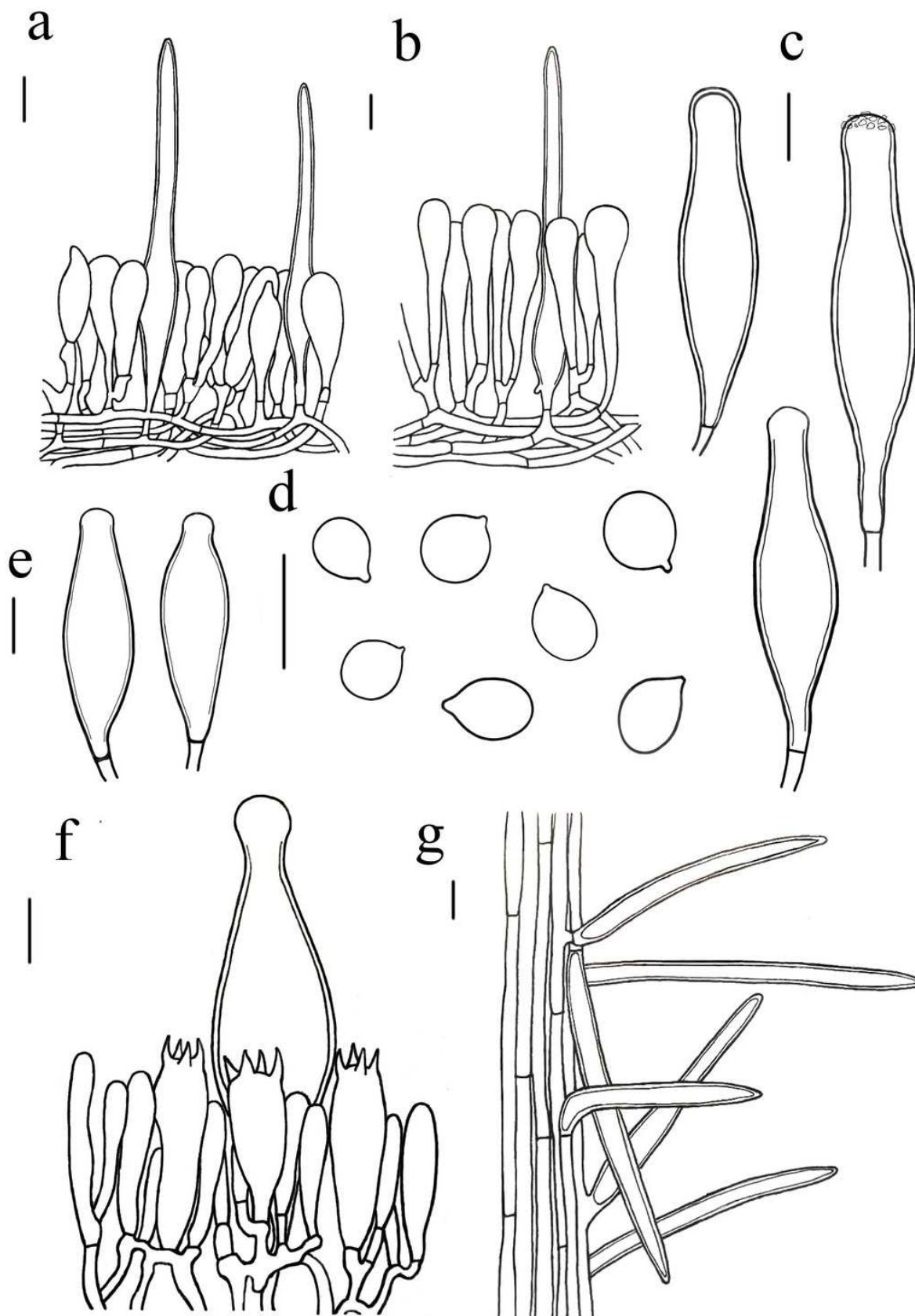


Table 1(on next page)

DNA sequence information for constructing phylogenetic trees.

“*” Sequence retrieved from GenBank. The newly generated sequences in this study are indicated in bold.

1 **Table 1.** DNA sequence information for constructing phylogenetic trees.

2

Taxon	Voucher	Locality	GenBank	
			ITS	nrLSU
<i>Flammulina velutipes</i>	TENN 56073	London, England	AF030877	HM005085
<i>F. yunnanensis</i>	HKAS 32774	China	DQ486704*	DQ457667*
<i>Gloiocephala aquatica</i>	CIEFAP 50	Argentina	DQ097356*	DQ097343*
<i>G. menieri</i>	DAOM 170087	Canada	DQ097358*	DQ097345*
<i>Hymenopellis altitude</i>	HMJAU 67050	Henan, China	OR0350776	OR036095
<i>H. biyangensis</i>	HMJAU 67043	Henan, China	OR0350770	OR036089
<i>H. biyangensis</i>	HMJAU 67044	Henan, China	OR0350771	OR036090
<i>H. biyangensis</i>	HMJAU 67045	Henan, China	OR0350772	OR036091
<i>H. biyangensis</i>	HMJAU 67046	Henan, China	OR0350773	OR036092
<i>H. biyangensis</i>	HMJAU 67047	Henan, China	OR0350774	OR036093
<i>H. biyangensis</i>	HMJAU 67048	Henan, China	OR0350775	OR036094
<i>H. chiangmaiae</i>	SFSU DED7661	Malasia	HM011506	HM005135
<i>H. colensoi</i>	12902,H2	New Zealand	HM005140	–
<i>H. colensoi</i>	12902,H1	New Zealand	HM005139	–
<i>H. furfuracea</i>	TENN 59876	TN, GSMNP, USA	GQ913367	HM005126
<i>H. furfuracea</i>	AFTOL-ID 538	Massachusetts, USA	DQ494703	AY691890
<i>H. gigaspora</i>	TENN 50050	NSW, Australia	GQ913359	–
<i>H. gigaspora</i>	TENN 50056	NSW, Australia	GQ913358	–
<i>H. hispanica</i>	05110401 (SEST)	Spain	–	HM005082
<i>H. incognita</i>	TENN 58768	TX, YSA	GQ913425	HM005105
<i>H. limonispora</i>	TENN 59438	TN, Knox County, USA	GQ913406	HM005133
<i>H. limonispora</i>	TENN 61379	TN, Knox County, USA	GQ913403	–
<i>H. limonispora</i>	TFB 12913	TN, Knox County, USA	–	HM005134
<i>H. megalospora</i>	TENN 59556	–	GQ913416	–
<i>H. megalospora</i>	TENN 59556	–	GQ913418	–
<i>H. radicata</i>	TENN 62837	Sweden	GQ913377	HM005125
<i>H. radicata</i>	TENN 60093	Russia	GQ913393	–
<i>H. raphanipes</i>	HMJAU 67039	Henan, China	OR035766	OR036085
<i>H. raphanipes</i>	HMJAU 67051	Henan, China	OR035767	OR036086
<i>H. raphanipes</i>	HMJAU 67041	Henan, China	OR035768	OR036087
<i>H. raphanipes</i>	HMJAU 67042	Henan, China	OR035769	OR036088
<i>H. raphanipes</i>	HKAS 95782	Hunan, China	KX688236	KX688263
<i>H. raphanipes</i>	HKAS 95783	Shandong, China	KX688238	KX688265
<i>H. sinapicolor</i>	TENN 56566	Thailand	GQ913352	HM005118

3 Continue **Table 1**

4

Taxon	Voucher	Locality	GenBank	
			ITS	nrLSU
<i>H. sinapicolor</i>	TENN 56566	Thailand	GQ913353	NG059449
<i>Hymenopellis</i> sp	AGTN-2022b	Thailand	OP265165	–
<i>Hymenopellis</i> sp	AGTN-2022b	Thailand	OP265164	–
<i>H. trichofera</i>	MEL2293664	Australia	GQ913354	HM005129
<i>H. vinocontusa</i>	TMI7669	Japan	GQ913370	–
<i>Oudemansiella radicata</i> var. <i>australis</i>	HKAS47605	Yunnan, China	AY961000*	AY960992*
<i>O. radicata</i> var. <i>australis</i>	HKAS47606	Yunnan, China	AY961001*	AY960993*
<i>Paraxerula americana</i>	CLO4746	New Mexico, USA	HM005142	HM005094
<i>P. americana</i>	DBG21746	Colorado, USA	HM005143	HM005093
<i>P. ellipsospora</i>	HKAS 56261	Yulong County, Yunnan, China	KF530557	KF530567
<i>P. hongoi</i>	HKAS 51985	Hokkaido, Japan	KF530561	KF530566
<i>P. hongoi</i>	C 60612	Japan	HM005144	HM005095
<i>Rhodotus asperior</i>	HKAS 56754	Yingjiang County, Yunnan, China	KC179737	KC179745
<i>R. palmatus</i>	HMJAU 6872	Antu County, Jilin, China	KC179742	KC179752
<i>Strobilurus albipilatus</i>	TENN52599	Canada	GQ892804	HM005089
<i>S. conigenoides</i>	TENN61318	North Carolina, USA	DQ097370	HM005091
<i>Xerula hispida</i>	TENN58745	Costa Rica, San Jose	HM005164*	HM005098*
<i>X. melanotricha</i>	TFB11917	Russia	HM005160*	HM005099*
<i>X. melanotricha</i>	LE10304	Russia	HM005159*	
<i>X. melanotricha</i>	GMMueller7030	–	AY665191*	AY804269*
<i>X. pudens</i>	Popa1969	Germany	MF063189*	MF063124*
<i>X. pudens</i>	TFB11432	Australia	HM005154	HM005097
<i>X. pudens</i>	C 63308	Spain	HM005155	–
<i>X. sinopudens</i>	Feng266	Xishuangbanna, Yunnan, China	KF530551	KF530571
<i>X. sinopudens</i>	ZRL20151504	–	LT716059*	KY418875*
<i>X. strigosa</i>	HMJAU67049	Henan, China	OR030918	OR030419
<i>X. strigosa</i>	Zhu528	Cheng county, Gansu China	KF530556*	KF530569*
<i>X. strigosa</i>	Zhu513	Cheng county, Gansu, China	KF530555	KF530568

5 “*” Sequence retrieved from GenBank. The newly generated sequences in this study are indicated in
6 bold.

