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# *Heteroradulum yunnanensis sp. nov.* (Auriculariales, Basidiomycota) evidenced by morphological characters and phylogenetic analyses in China

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## Abstract

A new wood-inhabiting fungal species, *Heteroradulum yunnanensis*, is proposed based on a combination of morphological features and molecular evidence. The species is characterized by an annual growth habit, resupinate basidiomata with odontoid hymenial surface (50–100 µm long), more or less pronounced yellow stains in older basidiomata, a monomitic hyphal system with thin-walled, clamped generative hyphae and two to three-celled basidia and cylindrical, hyaline, thin-walled, smooth, IKI–, CB– basidiospores measuring as 17–24 ×5–8 µm. Sequences of ITS and LSU nrRNA gene regions of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and bayesian inference methods. The phylogenetic analyses based on molecular data of ITS+nLSU sequences showed that *Heteroradulum yunnanensis* formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and then grouped with *H. adnatum*.

**Keywords:** *Exidiopsis*, phylogeny, taxonomy, wood-inhabiting fungi, Yunnan Province

## Introduction

Auriculariales Bromhead is a special wood-inhabiting fungal order with high morphological diversity, and it not only includes jelly species (Wu *et al*. 2015a, b), but many species that are previously called corticioid fungi belong to this order. *Heteroradulum* Lloyd ex Spirin & Malysheva (2017: 709) is one of the genera with corticoid hymenophore. The genus was typified by *H. kmetii* (Bres.) Spirin & Malysheva (2017: 711) (Malysheva & Spirin 2017), which is characterized by a combination of annual or perennial, resupinate or effused-reflexed basidiomata with a leathery consistencey, hymenophore smooth to odontioid, a monomitic hyphal structure (rare dimitic) with clamp connections on generative hyphae, and the presence of cystidia, basidia narrowly ovoid to obconical, longitudinally septate with a well-developed enucleate stalk and basidiospores hyaline, thin-walled, smooth, cylindrical, acyanophilous and negative in Melzer's reagent (Malysheva & Spirin 2017). So far six species have been accepted in the genus worldwide (Berkeley 1873, Berkeley & Broome 1875, Patouillard & Lagerheim 1893, Bresadola 1897, Bodman 1953, Malysheva & Spirin 2017).

Recently, molecular studies involving *Heteroradulum* have been carried out (Malysheva & Spirin 2017, Malysheva *et al.* 2018, Yuan *et al.* 2018). Phylogenetic study of the Auriculariales with stereoid basidiocarps revealed that *Heteroradulum* was validated and two new species, five combinations were introduced on the base of the combined data of the nuclear ribosomal LSU and the internal transcribed spacer (ITS) regions (Malysheva & Spirin 2017). Malysheva *et al.* (2018) revised of *Protohydnum* A. Möller (1895:173) and suggested that *Heteroradulum* grouped with *Exidiopsis* (Bref.) Möller (1895:85) and *Tremellochaete* Raitv (1964:29). Yuan *et al.* (2018) employed molecular study based on ITS+nLSU datasets to study of *Grammatus* H.S. Yuan & Decock (2018:32) in Auriculariales from tropical China and revealed that *Heteroradulum* grouped with *Exidiopsis* and *Grammatus*.

During investigations on wood-inhabiting fungi in southern China, an additional taxon was found which could

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not be assigned to any described species. In this study, the authors expand samplings from previous studies to examine taxonomy and phylogeny of this new species within the *Heteroradulum*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

**TABLE 1.** List of species, specimens, and GenBank accession numbers of sequences used in this study.

| Species name | Sample no. | GenBank accession no. | | References |
|---|---|---|---|---|
| | | ITS | nLSU | |
| *Exidiopsis effusa* | OM 19136 | KX262145 | KX262193 | Malysheva & Spirin 2017 |
| *Heteroradulum adnatum* | LR 23453 | KX262116 | KX262165 | Malysheva & Spirin 2017 |
| *H. deglubens* | LE 38182 | KX262112 | KX262162 | Malysheva & Spirin 2017 |
| *H. deglubens* | Solheim 1864 | KX262133 | KX262181 | Malysheva & Spirin 2017 |
| *H. kmetii* | Kmet | KX262124 | KX262173 | Malysheva & Spirin 2017 |
| *H. kmetii* | OF 295640 | KX262122 | KX262171 | Malysheva & Spirin 2017 |
| *H. kmetii* | LR 33201 | KX262117 | KX262166 | Malysheva & Spirin 2017 |
| *H. kmetii* | OF 295639 | KX262128 | KX262177 | Malysheva & Spirin 2017 |
| *H. yunnanensis* | CLZhao 4023 | MT215564 | MT215568 | This study |
| *H. yunnanensis* | CLZhao 8106 | MT215565 | MT215569 | This study |
| *H. yunnanensis* | CLZhao 9132 | MT215566 | MT215570 | This study |
| *H. yunnanensis* | CLZhao 9200 | MT215567 | MT215571 | This study |
| *Tremellochaete japonica* | LE 303446 | KX262110 | KX262160 | Malysheva & Spirin 2017 |

## Materials and methods

*Morphological studies*.—The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens, and observed under a light microscope following Dai (2012). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

*Molecular procedures and phylogenetic analyses*.—CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications that a small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 mL phenol-chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute the genome DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). 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 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 6 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 25001). Sequences of *Exidiopsis effusa* (Bref. ex Sacc.) Möller (1895:168) and *Tremellochaete japonica* (Lloyd) Raitv (1964:30). obtained from GenBank were used as outgroups to root trees following Malysheva & Spirin (2017) in the ITS+nLSU analysis (Fig. 1).

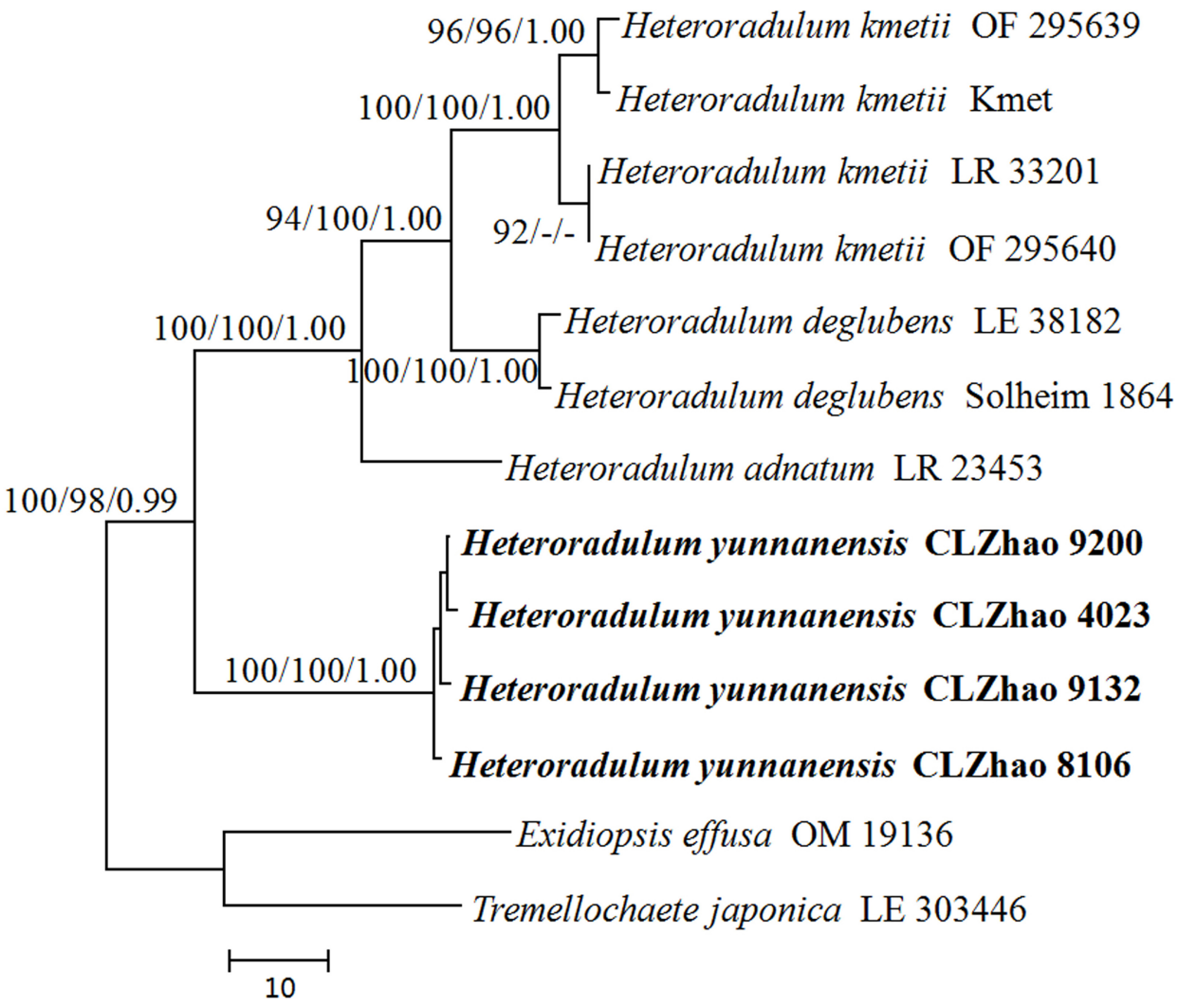


**FIGURE 1.** Maximum parsimony strict consensus tree illustrating the phylogeny of *Heteroradulum yunnanensis* and related species in *Heteroradulum* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap higher than 70%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95 respectively.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. Approaches to phylogenetic analysis followed Chen *et al.* (2016), and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). 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 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Sequences were also analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org, Miller *et al.* 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

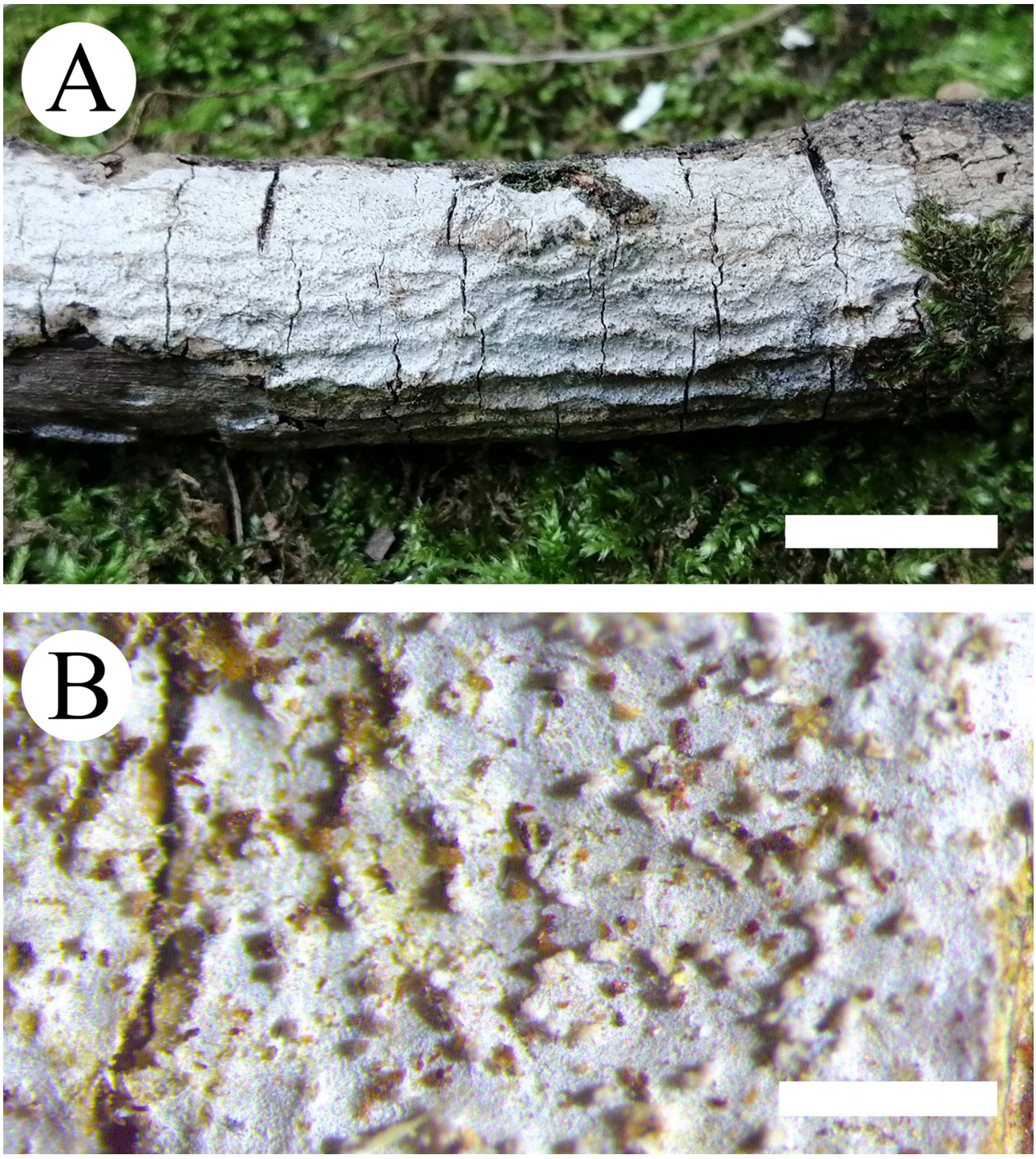


**FIGURE 2.** Basidiomata of *Heteroradulum yunnanensis* (holotype). Bars: A = 2 cm, B = 2 mm.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 with a general time reversible (GTR+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains run for 2 runs from random starting trees for 40, 000 generations (Fig. 1) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (ML) >70%, maximum parsimony bootstrap (MP) >50%, or Bayesian posterior probabilities (PP) >0.95.

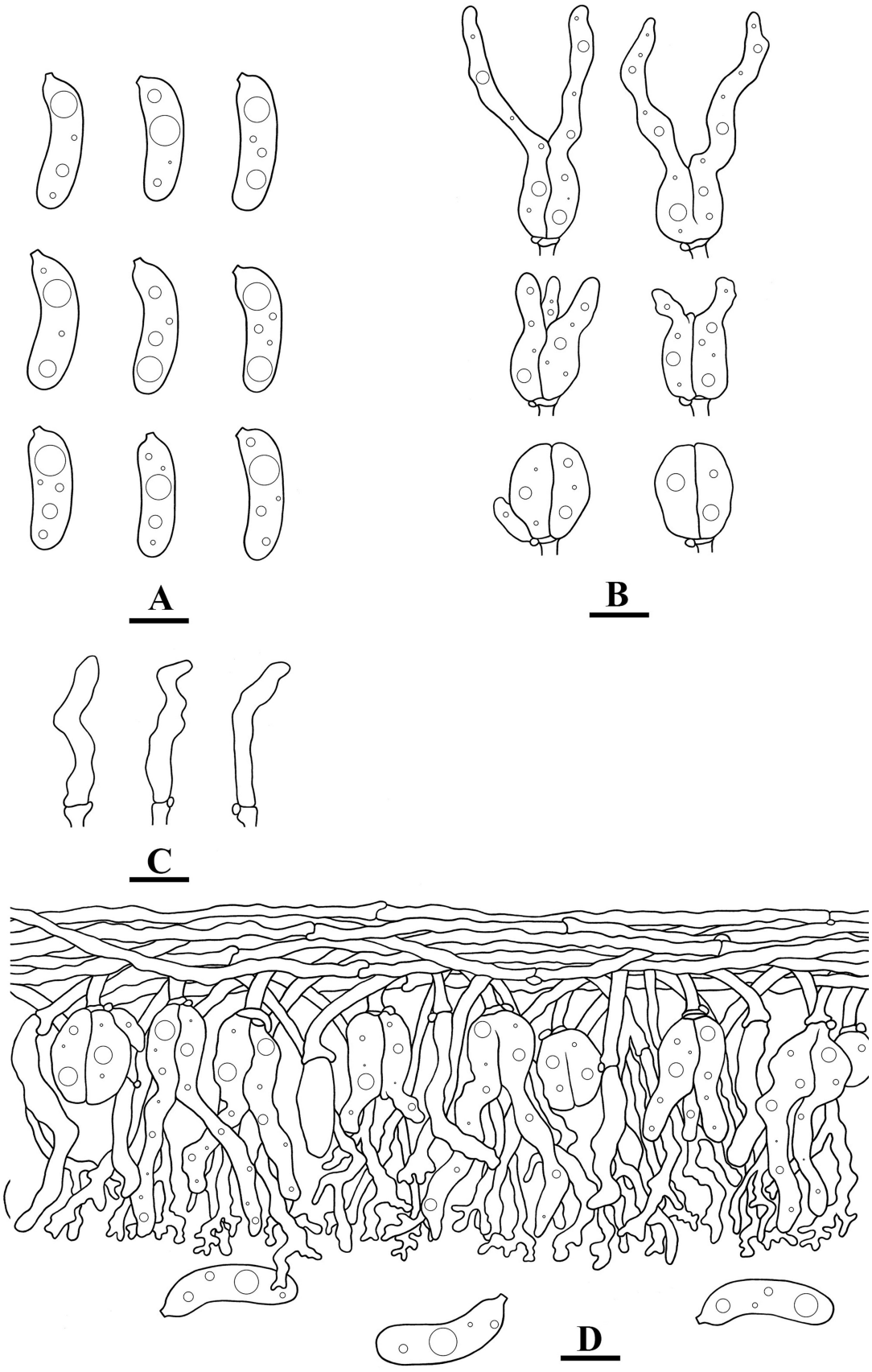


**FIGURE 3.** Microscopic structures of *Heteroradulum yunnanensis* (drawn from the holotype). a. Basidiospores. b. Basidia. c. Cystidia. d. A section of hymenium. Bars: a,b,c,d = 10 µm.

**TABLE 2.** A morphological comparison between *Heteroradulum yunnanensis* and four similar species in the genus.

| **Taxon** | **Habit** | Hymenial **surface** | Hyphal system | Basidia (µm) | Basidiospores **(µm)** | Cystidia (µm) |
|---|---|---|---|---|---|---|
| *Heteroradulum adnatum* | **Annual** | **White** | **Monomitic** | 17.3–21.8 × 9.2–11.3 | 11.4–14.2 × 5.2–7.2 | 26–95 × 4–10 |
| *H. deglubens* | **Annual or perennial** | Greyish pink | **Monomitic** | 26–44.8 × 9.3–14 | 13–20.4 × 6–8.2 | 31–44 × 5–6 |
| *H. kmetii* | **Perennial** | **Pinkish grey** | **Dimitic** | 27.4–45.4 × 9.7–14.7 | 14.1–24.9 × 5.8–9.7 | 27–40 × 4–7.5 |
| *H. spinulosum* | **Annual** | **Ochraceous to pinkish grey** | **Dimitic** | 17.4–29.8 × 9.2–12.4 | 11.7–19.8 × 5.9–7.4 | 23–45 × 6–7 |
| *H. yunnanensis* | **Annual** | **White** | **Monomitic** | 28–41 × 9–14 | 15–24.5 × 4.5–8.5 | 13–35 × 2–6 |

## Results

*Molecular phylogeny*

The ITS+nLSU dataset (Fig. 1) included sequences from 13 fungal specimens representing six species. The dataset had an aligned length of 1971 characters, of which 1793 characters are constant, 86 are variable and parsimony-uninformative, and 92 are parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (TL = 236, CI = 0.898, HI = 0.101, RI = 0.916, RC = 0.823). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009361 (BI).

The phylogenetic tree (Fig. 1) inferred from ITS+nLSU sequences, demonstrated four species of *Heteroradulum* and revealed that the new species formed a monophyletic lineage with high supports of 100% BS, 100% BP and 1.00 BPP and then grouped with *H. adnatum* Spirin & Malysheva.

## Taxonomy

***Heteroradulum yunnanensis*** C.L. Zhao, *sp. nov.* (Figs. 2, 3)
Mycobank no.: MB 834997

*Holotype*.—China. Yunnan Province: Yuxi, Xinping County, Jinshan Forestry Park, on a fallen angiosperm branch, 2 January 2019, *CLZhao 9132* (SWFC).

*Etymology*.—*Yunnanensis* (Lat.): referring to the locality (Yunnan) of the type specimens.

*Description*.—Basidiomata annual, resupinate, leathery, without odour or taste when fresh, becoming membranaceous upon drying, up to 15 cm long, 100–300 µm thick. Hymenial surface odontoid, 50–100 µm long, white to slightly grey when fresh, becoming white to smoke grey upon drying, older basidiomata with more or less pronounced yellow stains. Sterile margin smoke grey. Hyphal system monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH; subiculum almost absent or indistinct; hymenium thickening, hyphae colorless, more or less interwoven, thin-walled, unbranched, 2–4 µm in diameter.

*Hymenium*.—Cystidia clavate to fusiform, 13–35 × 2–6 µm; basidia narrowly ovoid to obconical, longitudinally septate, two to three-celled, embedded, often with a well-developed enucleate stalk, 28–41 × 9–14 µm. Basidiospores hyaline, thin-walled, smooth, cylindrical sometimes curved, with oily inclusions, IKI–, CB–, (15–)17–24(–24.5) × (4.5–)5–8(–8.5) µm, L = 20.6 µm, W = 6.71 µm, Q = 2.63–3.42 (n = 90/3).

*Ecology and distribution*.—Lignicolous, causing a white rot. Found in China.

*Additional specimens (paratypes) examined*.—China. Yunan Province: Yuxi, Xinping County, Jishan Forestry Park, on a fallen angiosperm branch, 2 January 2019, *CLZhao 9200* (SWFC), 21 August 2018, *CLZhao 8106* (SWFC); Puer, Jingdong County, Ailaoshan National Nature Reserve, on the fallen angiosperm branch, 4 October 2017, *CLZhao 4023* (SWFC).

## Discussion

In the present study, a new species, *Heteroradulum yunnanensis*, is described based on phylogenetic analyses and morphological characters.

Previously, seven corticioid genera in the Auriculariales were phylogenetically studied: *Amphistereum* Spirin & Malysheva (2017:696), *Eichleriella* Bres. (1903:115), *Exidiopsis*, *Heteroradulum*, *Hirneolina* (Pat.) Bres. (1905:208), *Sclerotrema* Spirin & Malysheva (2017:712) and *Tremellochaete* (Malysheva & Spirin 2017). Our result based on the combined ITS+nLSU sequence data (Fig. 1) demonstrated that *H. yunnanensis* was nested in the *Heteroradulum*.

Phylogenetically, *Heteroradulum yunnanensis* is closely related to *H. adnatum* in the rDNA based on the phylogeny (Fig. 1). But morphologically *H. adnatum* differs from *H. yunnanensis* by having the pink hymenial surface and smaller basidiospores (11.4–14.2 × 5.2–7.2 µm, Malysheva & Spirin 2017).

Morphologically, *Heteroradulum yunnanensis* resembles four similar species in this genus, *H. adnatum*, *H. deglubens* (Berk. & Broome) Spirin & Malysheva (2017:710), *H. kmetii* and *H. spinulosum* (Berk. & M.A. Curtis) Spirin & Malysheva (2017:712). A morphological comparison between *H. yunnanensis* and four species is presented in Table 2.

Wood-rotting fungi are an extensively studied group of Basidiomycota (Gilbertson & Ryvarden 1986, 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014, Wu *et al.* 2017, Shen *et al.* 2019), because some of them are economically important (Dai *et al.* 2007, Wu *et al*. 2019a),but the Chinese wood-rotting fungi diversity is still not well known, especially in subtropics and tropics, many recently described taxa of wood-rotting fungi were from these areas (Zhao & Wu 2017, Luo *et al*. 2018, Shen *et al*. 2018, Liu *et al.* 2019, Wu *et al.* 2019b). The new species in the present study, *Heteroradulum yunnanensis*, is from subtropics, too. It is possible that new taxa will be found after further investigations and molecular analyses, especially in some poorly known groups.

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