

Molecular phylogeny and morphology reveal a new wood-rotting fungal species, *Cyathus wenshanensis* sp. nov. from the Yunnan-Guizhou Plateau

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Abstract

A new species of bird's nest fungus, *Cyathus wenshanensis* is proposed based on a combination of the morphological and molecular evidence. It is characterised by the obconical to cupulate basidiomata covered with hirsute hairs, striations on the outer and inner surface of the peridium, funicular peridioles, a trimitic hyphal system of peridium with generative hyphae having clamp connections, a dimitic hyphal system of peridiole middle, and subglobose, elliptical to ellipsoid-elongate, thick-walled basidiospores. Sequence of the internal transcribed spacers (ITS) gene region was generated, and the phylogenetic analysis was performed with maximum likelihood, maximum parsimony and Bayesian inference methods. The phylogenetic analyses inferred from ITS dataset indicated that *C. wenshanensis* nested within the genus *Cyathus*, in which it formed a monophyletic lineage and grouped with *C. albinus*, *C. amazonicus*, *C. badius*, *C. parvocinereus*, *C. pyristriatus* and *C. uniperidiolus*.

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INTRODUCTION

The genus *Cyathus* (Nidulariaceae, Nidulariales) was first introduced by Haller^[1] and later was adopted by Persoon^[2], typified by *C. striatus* (Huds.) Willd. *Cyathus* together with *Crucibulum* Tul. & C. Tul., *Mycocalia* J.T. Palmer, *Nidula* V.S. White, and *Nidularia* Fr., are commonly known as bird's nest fungi due to their cup-like basidiomata resembling bird nest and lenticular peridioles resembling eggs^[3–5]. It is characterized by having deeper or cuped, inverted bell-like basidiomata covered with shaggy or tomentose hairs on the outside; peridium composed of three layers of tissues, inside peridium filled with a number of dark-colored, small, hard lentil-shaped peridioles attached with funicular cords; colorless, thin-walled or thick-walled, smooth basidiospores^[3,6–11]. The species of *Cyathus* are saprobic, usually growing in decaying wood, on manure or directly on soil are a cosmopolitan group and have a rich diversity related to the high diversity of plants growing in boreal, temperate, subtropical, and tropical regions^[3,5,12–14]. Both MycoBank database (www.Mycobank.org; 23 June 2022) and Index Fungorum (www.indexfungorum.org; 23 June 2022) register 204 specific and infraspecific names in the genus *Cyathus*, but the actual number of species are about 60^[15], including 35 species from China^[5,16].

Molecular systematic studies of the genus *Cyathus* have been carried out previously^[14,17,18]. An overview of the phylogeny of the Agaricales presented based on a multilocus analysis of a six-gene region supermatrix revealed that the family Nidulariaceae was sister to Cystodermateae, in which *Cyathus striatus* and *Crucibulum laeve* grouped together within Nidulariaceae^[17]. Phylogenetic relationships within the genus *Cyathus* (bird's

nest fungi) were investigated with neighbor joining, maximum likelihood, weighted maximum parsimony and MrBayes analyses of the internal transcribed spacers (ITS) and large subunit (LSU) of ribosomal DNA sequences datasets, in which the morphological characters of the peridium plications and variations in peridium hair anatomy, peridiole structure and fruit-body color were not supported by the molecular data, while the ITS and LSU datasets supported the recognition of three infrageneric groups herein named the ollum, pallidum and striatum groups^[18]. Phylogenetic analyses based on ITS and LSU ribosomal DNA sequences revealed that three taxa *C. cheliensis*, *C. gansuensis*, and *C. megasporus* were respectively accepted as synonyms of *C. limbatus*, *C. pygmaeus*, and *C. poeppigii*^[5]. On the basis of the morphological and molecular data, Martin et al.^[19] discussed affinities among *Cyathus* species, which showed that this group formed a monophyletic group with high support. Phylogenetic reconstruction of *Cyathus* species based on alignment of 641 nucleotides of the ITS region indicated that three new species as *C. batistae* and *C. apiculatus*, *C. pedunculatus* were proposed, and discussed relationships with other species of *Cyathus*^[20]. Phylogenetic relationships of bird's nest fungi investigated with four commonly used loci (ITS, LSU, translation elongation factor (*TEF*), and RNA polymerase II second largest subunit (*RPB2*)) revealed that the family Nidulariaceae was resolved as a monophyletic group with Squamanitaceae as a potential sister taxon, and suggested that species concepts needed to be revisited and refined throughout Nidulariaceae and several bird's nest fungi species had global geographical distributions, whereas others may have more limited ranges, and the basic morphological characters of bird's nest fungi had likely been

lost or gained multiple times^[21]. The phylogenetic study using five loci (ITS, LSU, SSU, translation elongation factor 1- α (*TEF1*) and *RPB2*) revealed that a new genus *Retiperidiolia* to accommodate this phylogenetically and morphologically unique bird's nest fungus lineage, in which *Cyathus* formed a monophyletic lineage and then was sister to the genus *Retiperidiolia*^[14].

MATERIALS AND METHODS

Morphological studies

The fresh fruiting bodies of the bird's nest fungi were collected from Wenshan (Yunnan Province, P. R. China). The fresh specimens were dried in an electric food dehydrator at 40 °C, then sealed and stored in an envelope bag and deposited in the herbarium of the Southwest Forestry University (SWFC, Kunming, Yunnan Province, P.R. China).

The macromorphological descriptions were based on field notes and photos captured in the field and lab. The macromorphological descriptions are based on Brodie^[3]. The micromorphological data were obtained from the dried specimens and observed under Nikon Eclipse E100 light microscope following the methods of Zhao & Wu^[22]. Color terms follow Kornerup & Wanscher^[23]. Drawings were made with the aid of a fungus plotter. The measurements and drawings were made from slide preparations stained with Cotton Blue (0.1 mg aniline blue dissolved in 60 g pure lactic acid), Melzer's reagent (3 g potassium iodide, 1 g crystalline iodine, 44 g chloral hydrate, aq. dest. 40 ml) and 5% potassium hydroxide. In presenting spore size data, 5% of the measurements excluded from each end of the range are shown in parentheses. The following abbreviations are used: KOH = 5% potassium hydroxide; CB = cotton blue; CB- = acyanophilous; IKI = Melzer's reagent; IKI- = non-amyloid and non-dextrinoid; L = mean spore length (arithmetic average of all spores); W = mean spore width (arithmetic average of all spores); Q = L/W ratio; n = number of spores/measured from a given number of specimens.

Molecular procedures and phylogenetic analysis

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing, China) was used to obtain genomic DNA from dried fungal specimens, according to the manufacturer's instructions. The ITS region was amplified with the primer pair ITS5 and ITS4^[24]. The PCR cycling 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 products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). All newly generated sequences were deposited in GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to assemble and edit the generated sequence reads. Sequences were aligned in MAFFT 7 (<https://mafft.cbrc.jp/alignment/server/>) using the 'G-INS-I' strategy and manually adjusted in BioEdit^[36]. *Crucibulum laeve* (Huds.) Kambly and *Nidula niveotomentosa* (Henn.) Lloyd were selected as an outgroup for the phylogenetic analysis of the ITS phylogenetic tree^[25].

Maximum parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were applied to the ITS dataset sequences. Approaches to phylogenetic analyses followed^[22].

MP analysis was performed in PAUP* version 4.0b10^[37]. All of the 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. Maxtrees were set to 5000, branches of zero length were collapsed and all most-parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 replicates^[38]. Descriptive tree statistics tree length (TL), the consistency index (CI), the retention index (RI), the rescaled consistency index (RC) and the homoplasy index (HI) were calculated for each most-parsimonious tree generated. ML was inferred using RAXML-HPC2 through the Cipres Science Gateway (www.phylo.org)^[39]. Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates and evaluated under the gamma model.

MrModeltest 2.3^[40] was used to determine the best-fit evolution model for the dataset for Bayesian Inference (BI). Bayesian Inference was performed with MrBayes 3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites^[41]. Four Markov chains were used in each of two runs from random starting trees for 1.5 million generations (Fig. 1), with trees and parameters sampled every 100 generations. The first quarter of the generations were discarded as burn-in. A majority rule consensus tree of all remaining trees and posterior probabilities were calculated. Branches were considered significantly supported if they received maximum likelihood bootstrap value (BS) of > 70%, a maximum parsimony bootstrap value (BT) of > 70%, or Bayesian posterior probabilities (BPP) of > 0.95.

RESULTS

Molecular phylogeny

The ITS dataset (Fig. 1) included sequences from 49 fungal specimens representing 42 species. The dataset had an aligned length of 805 characters, of which 270 characters were constant, 232 were variable and parsimony-uninformative, and 303 parsimony-informative. The MP analysis yielded one equally parsimonious trees (TL = 1211, CI = 0.6474, HI = 0.3526, RI = 0.7855, RC = 0.5086). Best model for the ITS dataset estimated and applied in the Bayesian analysis: GTR+I+G, Iset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The bayesian and ML analyses resulted in a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.009975 (BI), and the effective sample size (ESS) across the two runs was double the average ESS (avg ESS) = 248. The phylogenetic tree (Fig. 1) inferred from ITS sequences revealed that *C. wenshanensis* nested within the genus *Cyathus*, in which it formed a monophyletic lineage and grouped with *C. albinus*, *C. amazonicus*, *C. badius*, *C. parvocinereus*, *C. pyristriatus* and *C. niperidiolus*.

Taxonomy

Cyathus wenshanensis Z.Y. Duan & C.L. Zhao, sp. nov. Figs 2–5.

Index Fungorum number: IF844702; Facesoffungi number: FoF12564

Etymology – *wenshanensis* (Lat.): referring to the provenance (Wenshan) of the type specimens.

Basidiomata obconical to cupulate, 5–15 mm high, 5–10 mm wide at the mouth, without expanding at the top or

A new wood-rotting fungal species

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

Species name	Sample no.	GenBank accession no.	References
<i>Crucibulum laeve</i>	SWFC 21261	DQ463357	Zhao et al. ^[18]
<i>Cyathus africanus</i>	DAOM 200370[T]	DQ463347	Zhao et al. ^[18]
<i>C. albinus</i>	UFRN-Fungos 2239	KY176371	Accioli et al. ^[25]
<i>C. amazonicus</i>	URM 80036[T]	KY495280	Accioli et al. ^[25]
<i>C. amazonicus</i>	UFRN-Fungos 2798	KY176375	Accioli et al. ^[25]
<i>C. annulatus</i>	MichaelKuo-8200901	MT444076	Kraisitudomsook et al. ^[21]
<i>C. apiculatus</i>	UFRN:Fungos 1448	KT365516	da Silva et al. ^[20]
<i>C. aurantogriseocarpus</i>	UFRN:Fungos:2798	KX966026	da Cruz et al. ^[26]
<i>C. badius</i>	KH:JPN15-1321	KX906250	da Cruz et al. ^[27]
<i>C. batistae</i>	UFRN:Fungos 1449	KT365515	daSilva et al. ^[20]
<i>C. berkeleyanus</i>	SWFC 20789	DQ463355	Zhao et al. ^[18]
<i>C. bulleri</i>	DAOMC 195062	MK020156	Vats & Mishra ^[28]
<i>C. canna</i>	CBS 370.80	MH861275	Vu et al. ^[29]
<i>C. colensoi</i>	DAOM 200423	DQ463344	Zhao et al. ^[18]
<i>C. crassimurus</i>	DAOM 200372[T]	DQ463350	Zhao et al. ^[18]
<i>C. discoideus</i>	AB 7831	KY652080	da Cruz ^[30]
<i>C. gansuensis</i>	SWFC 20880[T]	DQ463348	Zhao et al. ^[18]
<i>C. gansuensis</i>	Strain 69	KC869661	da Cruz et al. ^[27]
<i>C. gracilis</i>	AB7873	KY652081	da Cruz ^[30]
<i>C. hookeri</i>	SWFC 20799	DQ463346	Zhao et al. ^[18]
<i>C. hortensis</i>	UFRN:Fungos:1819	KX906252	da Cruz et al. ^[27]
<i>C. ibericus</i>	AH:48138	KX858598	Crous et al. ^[31]
<i>C. ibericus</i>	AH:48137[T]	KX858597	Crous et al. ^[31]
<i>C. intermedius</i>	UFRN:Fungos 1033	KT365519	da Silva et al. ^[20]
<i>C. jiyuguanensis</i>	SWFC 20846[T]	DQ463341	Zhao et al. ^[18]
<i>C. lignilantanae</i>	MA Fungi 87327	NR_154827	da Cruz et al. ^[27]
<i>C. limbatus</i>	UFRN-Fungos 2238	KY176373	Accioli et al. ^[25]
<i>C. magnomuralis</i>	UFRN:Fungos:1817	KX906251	da Cruz et al. ^[27]
<i>C. minimus</i>	AB7868	KY652082	da Cruz ^[30]
<i>C. novae-zeelandiae</i>	PDD-76442	MT444096	Kraisitudomsook et al. ^[21]
<i>C. olla</i>	PDD-86833	MT444086	Kraisitudomsook et al. ^[21]
<i>C. olla</i>	BPI 727227	DQ463345	Zhao et al. ^[18]
<i>C. pallidus</i>	KKUITN2	KU202745	Sutthisa & Sanoamuang ^[32]
<i>C. pallidus</i>	KKUITN3	KU202751	Sutthisa & Sanoamuang ^[32]
<i>C. parvocinereus</i>	UFRN:Fungos:1814	KX906253	da Cruz et al. ^[27]
<i>C. pedunculatus</i>	UFRN:Fungos 403	KT365518	da Silva et al. ^[20]
<i>C. poeppigii</i>	cp-457	KT962176	da Silva et al. ^[20]
<i>C. pyristriatus</i>	MFLUCC:14-0770	KU865513	Richter et al. ^[33]
<i>C. renweii</i>	SWFC 201406[T]	DQ463352	Zhao et al. ^[18]
<i>C. setosus</i>	DAOM 200815[T]	DQ463349	Zhao et al. ^[18]
<i>C. stercoreus</i>	NK-08	MT444037	Kraisitudomsook et al. ^[21]
<i>C. stercoreus</i>	DM4	KY706156	Hay et al. ^[34]
<i>C. striatus</i>	NK-61	MT444056	Kraisitudomsook et al. ^[21]
<i>C. subglobisporus</i>	BBH-14815	MT444063	Kraisitudomsook et al. ^[21]
<i>C. subglobisporu</i>	BBH18348	EF613553	Zhao et al. ^[5]
<i>C. triplex</i>	SWFC 21077	DQ463353	Zhao et al. ^[18]
<i>C. uniperidiolus</i>	AMH:10196	MN398297	Boonmee et al. ^[35]
<i>C. wenshanensis</i>	CLZhao 20202[T]	ON795104	This study
<i>Nidula niveotomentosa</i>	SWFC 3000	DQ463358	Zhao et al. ^[18]

tapering abruptly at the base; the base usually attached to the substrate by a slightly conspicuous emplacement, brown (5E6) to beige (4C3); *exoperidium* brown (5E6), hirsute, external wall striate near the mouth, 0.4–0.7 mm between folds, covered with brown (5E6) to dark brown (7F6), irregular and flexible tufts of hair; hair hyphae with clamp connections, colorless, thick-walled (wall up to 0.5–2 µm thick), 3.5–13.5 µm in diameter; *endoperidium* greyish brown (8F3) to black brown (7F4), conspicuously striate with 0.4–0.8 mm between the grooves; *mouth* finely fimbriate; *peridium walls* consist of three different layers: (1) outer wall layer, hyphal system trimitic, CB–,

IKI–, tissues unchanged in KOH; generative hyphae with clamp connections, colorless to pale brown, slight thick-walled, frequently branched, 1.5–4 µm in diameter; skeletal hyphae colorless to pale brown, thick-walled, unbranched, 2–4 µm in diameter; binding hyphae colorless to pale brown, thick-walled, unbranched, 1.5–2.5 µm in diameter; (2) inner wall layer, hyphal system trimitic, CB–, IKI–, tissues unchanged in KOH; generative hyphae with clamp connections, colorless to pale brown, slight thick-walled, rarely branched, 2–4 µm in diameter; skeletal hyphae colorless to pale brown, thick-walled, unbranched, 2.5–4.5 µm in diameter; binding hyphae colorless to pale

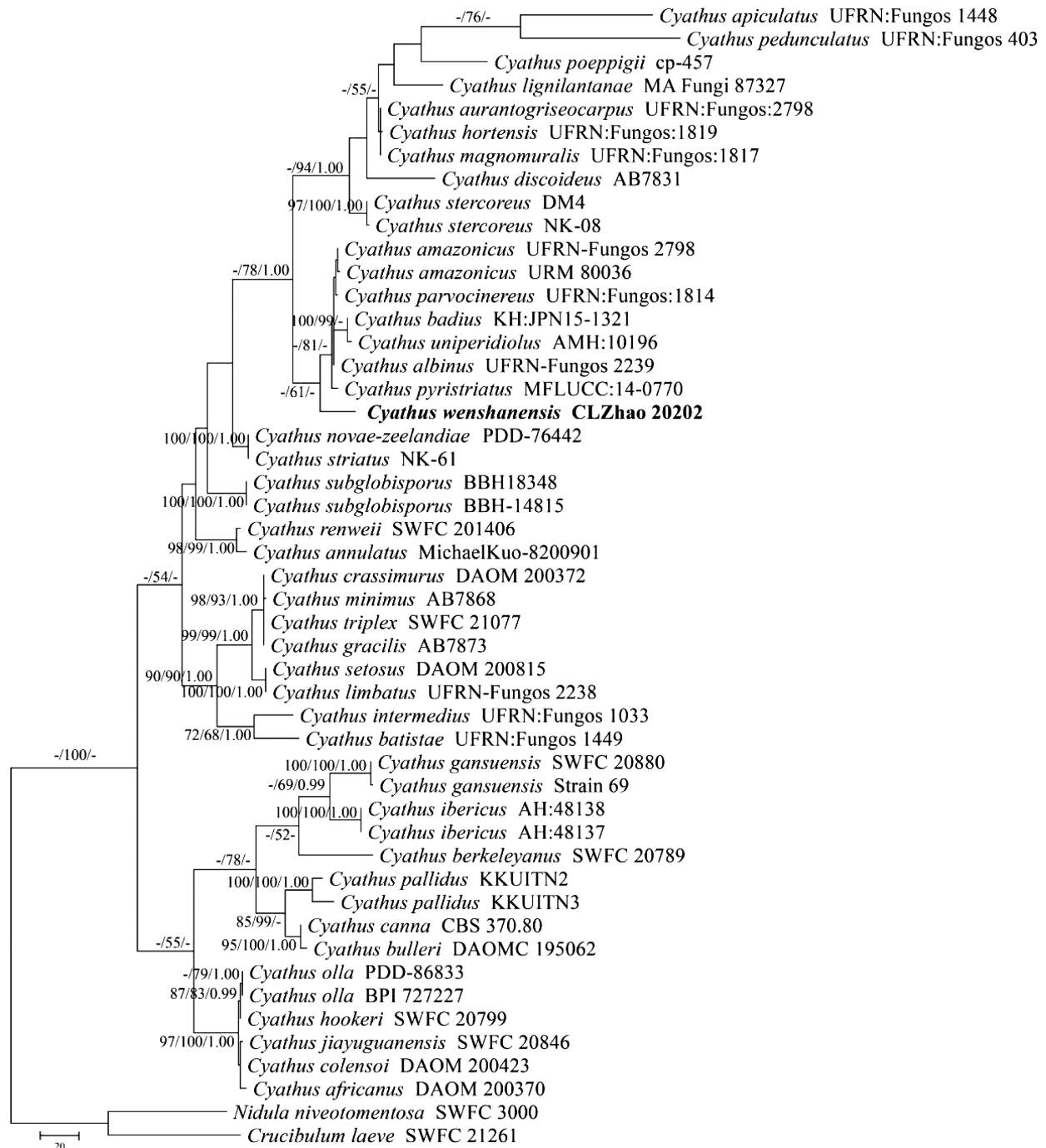


Fig. 1 Maximum parsimony strict consensus tree illustrating the phylogeny of the new species and related species in genus *Cyathus* based on ITS sequences. Branches are labelled with maximum likelihood bootstrap value > 70%, parsimony bootstrap value > 50% and Bayesian posterior probabilities > 0.95, respectively. The present species are in bold.

brown, thick-walled, rarely branched, 1.5–3 µm in diameter; (3) pseudopare-chymatous layer.

Peridioles depressed, shiny, angular to irregular, suborbicular, broadly ellipsoid to ovoid, dark grey (8F1) to black (6F3), surface smooth to wrinkled, tunica present, often inconspicuous, cortex single-layered, 2.5–3.5 × 2–3 mm; *funicular cord* present, funiculus hyphae with clamp connections, thick-walled, unbranched, pale yellowish, 1–3.5 µm in diameter; *hyphal system* of peridiole middle dimitic, generative hyphae with clamp connections, colorless, thin-walled, frequently

branched, with oil drops inside, 1–3 µm in diameter, CB–, IKI–, tissues unchanged in KOH; skeletal hyphae colorless, slight thick-walled, unbranched, with oil drops inside, 1.5–4 µm in diameter, CB–, IKI–, tissues unchanged in KOH.

Basidiospores subglobose, elliptical to ellipsoid-elongate, colorless, smooth, thick-walled (wall up to 1–5 µm thick), CB–, IKI–, with inclusions or oil-like globule, without apiculus, (10–)11–21(–22) × 9–14(–15) µm, L = 16.34 µm, W = 11.51 µm, Q = 1.4 (n = 60/1). Basidia not observed.

Known distribution – Thus far known only from China.



Fig. 2 Basidiomata of *Cyathus wenshanensis*. (a) Basidiomata, (b) outer corving of peridium. Scale bars: (a) = 1 cm, (b) = 1 mm.

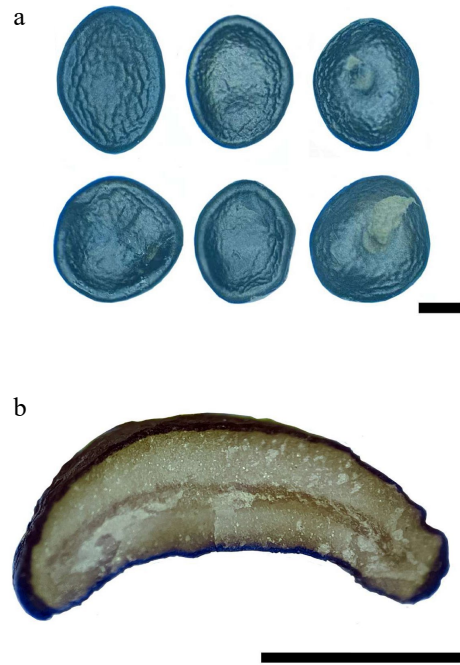


Fig. 3 Peridiole of *Cyathus wenshanensis*. (a) Peridioles with funicular cord, (b) transversal section of peridiole showing single-layered cortex. Scale bars: (a) = 1 mm, (b) = 1 mm.

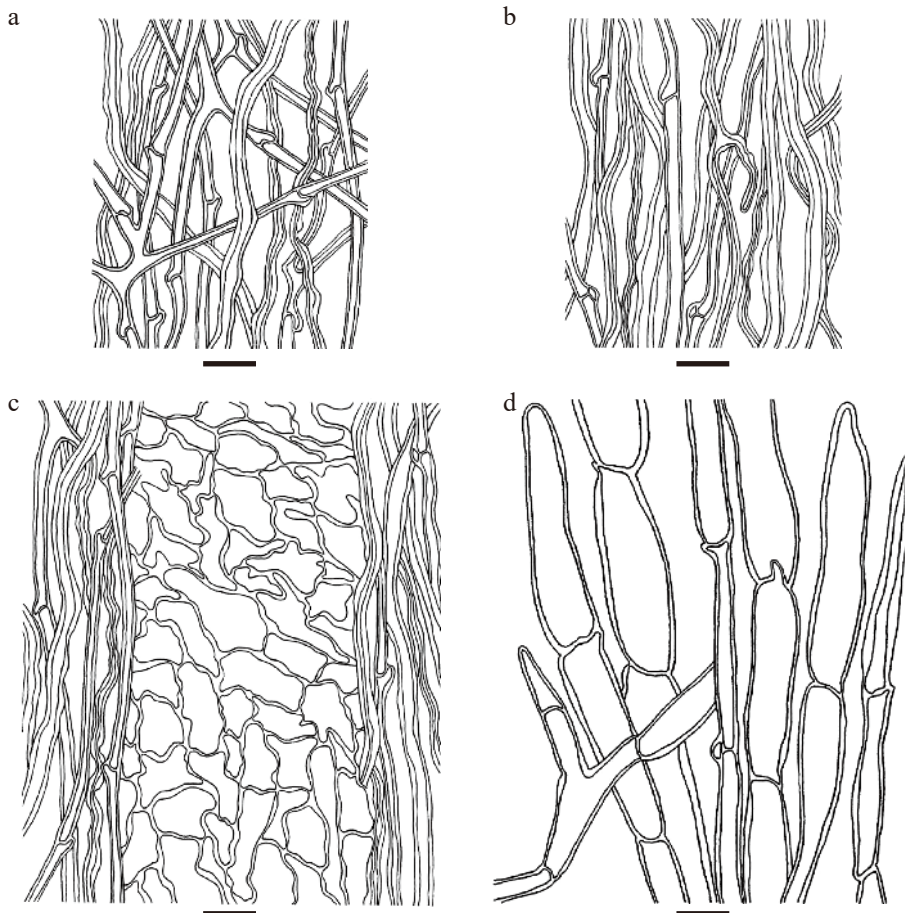


Fig. 4 Microscopic structures of *Cyathus wenshanensis*. (a) Outer wall of peridium, (b) inner wall of peridium, (c) three-layered peridium, (d) the structure of the hair. Scale bars: (a)–(d) = 10 μ m.

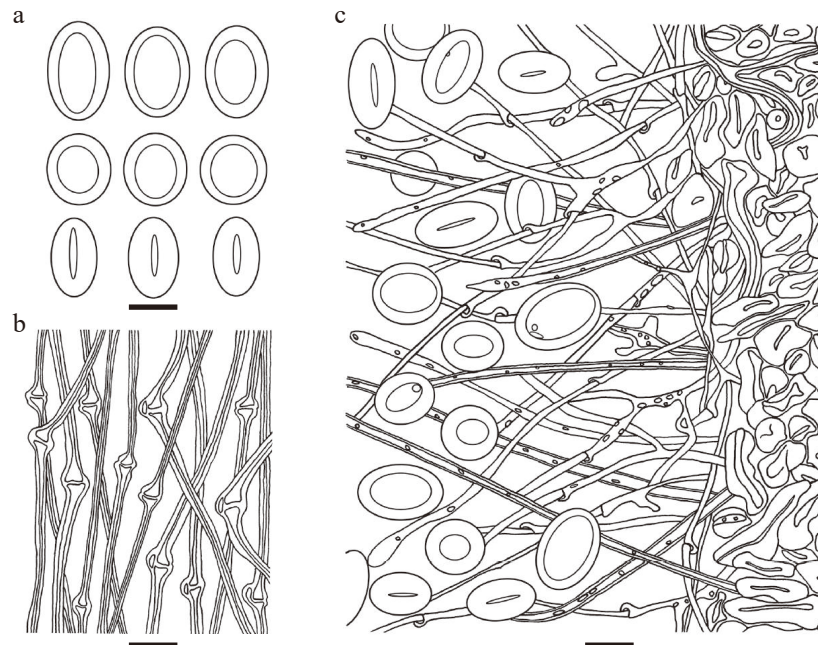


Fig. 5 Microscopic structures of *Cyathus wenshanensis*. (a) Basidiospores. (b) Funicular cord. (c) The internal structure of peridiole. Scale bars: (a)–(c) = 10 μ m.

Material examined – China. Yunnan Province, Wenshan, Pingba Town, Huguangqing Village, 23.26°N, 104.06°E, on the fallen branch of angiosperm, 12 August 2020, collected by C.L. Zhao. Specimen voucher number: CLZhao 20202 (SWFC 020202).

DISCUSSION

In the present study, *C. wenshanensis* sp. nov. is described based on the phylogenetic analyses and morphological characteristics.

Phylogenetically, the molecular systematics and taxonomic overview of the bird's nest fungi revealed that the family Nidulariaceae was resolved as a monophyletic group with Squamanitaceae as a potential sister taxon, in which *Cyathus* and *Crucibulum* each formed its own independent and well-supported clade, and *Nidula* and *Nidularia* formed a clade together, but each genus is polyphyletic^[21]. In the present study, *C. wenshanensis* nests within the genus *Cyathus* located in the family Nidulariaceae, in which it forms a monophyletic lineage and then groups with taxa *C. albinus*, *C. amazonicus*, *C. badius*, *C. parvocinereus*, *C. pyrstriatus* and *C. uniperidiolus*. However, morphologically *C. albinus* differs from *C. wenshanensis* by having the golden blond to dark blond exoperidium, brownish gray peridioles with double-layered cortex, and basidiospores with conspicuous apiculus^[25]. *C. amazonicus* differs in having very dark brown to grayish dark brown exoperidium and and gray, shiny endoperidium^[42]. *C. badius* differs in having the smooth exoperidium, light brown to orange endoperidium, ovoid basidiospores^[43]. *C. parvocinereus* differs in having the campanulate, smaller basidiomata (4–7 × 3.5–5 mm), pearl grey to brightness silvery endoperidium and greyish brown to grey peridioles with double-layered cortex^[44]. *C. pyrstriatus* differs in its clavate basidiomata with yellowish-brown or buff exoperidium, grey to dark grey endoperidium, greyish-brown peridioles, and ovoid basidiospores^[45]. *C. uniperidiolus* distinct from *C. wenshanensis* in having the globose to sub-globose

basidiomata with serrate margin at mouth, smooth peridium walls, and globose, smooth peridioles^[35] (see Table 2).

Morphologically, six taxa of *Cyathus* as *C. apiculatus*, *C. hortensis*, *C. limbatus*, *C. lignilantanae*, *C. pedunculatus*, and *C. poeppigii* are similar to *C. wenshanensis* on the basis of the character by having the obvious stripes on the inner and outer walls of peridium. However, *C. apiculatus* differs from *C. wenshanensis* by the basidiomata being expanded at the mouth and abruptly tapering to the base, silvery endoperidium, smaller peridioles (1–1.5 × 1.5–2 mm), and longer basidiospores (22–37 × 10–22 μ m)^[20]; *C. hortensis* is distinguished from *C. wenshanensis* by its basidiomata constricting abruptly at the base and forming a slender stipe, cinnamon exoperidium, smaller peridioles (1.2–2 × 1–1.5 mm) with double-layered cortex, and ovoid, wider basidiospores (17–34 × 13–20 μ m)^[44]; *C. limbatus* differs from *C. wenshanensis* by its double-layered peridioles, and basidiospores with apiculus^[46]; *C. lignilantanae* is different from *C. wenshanensis* by having a reddish brown exoperidium, brownish grey to greyish brown, smaller peridioles (2.1–2.3 × 1.8–2 mm) with double-layered cortex^[19]; *C. pedunculatus* is separated from *C. wenshanensis* by having the basidiomata abruptly tapering in the base forming a conspicuous pedicel, pale yellow to dark blond exoperidium, double-layered cortex, brownish grey, smaller peridioles (1.5–2 × 1–1.5 mm), and larger basidiospores (25–34 × 22–29 μ m)^[20]; *C. poeppigii* is distinguished from *C. wenshanensis* by having the narrowly obconical basidiomata with incurved mouths and a slender stipe at the base, and dark brown, smaller peridioles (1.5–2 mm) with double cortex, and larger basidiospores (30–45 × 18–30 μ m)^[47].

Several taxa, *Cyathus batistae*, *C. discoideus*, *C. gracilis*, *C. hookeri*, *C. magnomuralis*, *C. renweii* and *C. triplex* are similar to *C. wenshanensis* based on the character having the fimbriate of basidiomata mouth. However, *C. batistae* differs from *C. wenshanensis* by its expanded mouth of basidiomata, with the stipe, smooth exoperidium wall, double-layered cortex

Table 2. The comparison among *Cyathus wenshanensis* and phylogenetically related species.

	<i>C. wenshanensis</i>	<i>C. albinus</i>	<i>C. amazonicus</i>	<i>C. badius</i>	<i>C. parvocinereus</i>	<i>C. pyristriatus</i>	<i>C. uniperidiolus</i>
Basidiomata	5–15 × 5–10 mm	6–8.5 × 5–6.52 mm	9–11 × 5–7 mm	8–10 × 5–8 mm	4–7 × 3.5–5 mm	5.5–7 × 4–6 mm	2–12 × 2–3.5 mm
Size (high × wide)							
Shape	Obconical to cupulate	Infundibuliform	Obconical	Infundibuliform	Campanulate	Clavate to broadly obconic	Globose to sub-globose
Exoperidium	Brown	Golden blond to dark blond	Very dark brown to grayish dark brown	Brown	Reddish brown	Yellowish-brown or buff	Dark brown
Surface	Strigose Tufts; striate; 0.4–0.7 mm	Strigose tufts; striate; 0.3–0.5 mm	Strigose tufts; striate	Shaggy, wooly tufts; smooth to striate; 0.3 mm	Strigose tufts; striate; 0.4–0.5 mm	shaggy or fluffy hairs	Smooth to velvety
Mouth	Fimbriate	Fimbriate	Fimbriate	Fimbriate	Fimbriate		Serrate
Endoperidium	Greyish brown to black brown	Grayish brown	Gray to brownish gray	Light brown to orange	Platinum	Grey to dark grey	Dark brown
Surface	Striate, 0.4–0.8 mm	Striate, 0.3–0.6 mm	Striate	Smooth to minutely striate; 0.5 mm	Striate; 0.5 mm	Striate	Smooth
Peridioles	Lentil-shaped 2–3.5 mm	Lentil-shaped 1.8–2.6 mm	Lentil-shaped 1.7–3 mm	Lentil-shaped 2–2.5 mm	Lentil-shaped 1–2 mm	Lentil-shaped 3–3.5 mm	Globose 2–2.5 mm
Color	Dark grey to black	Brownish gray	Dark gray	Light grey to black	Greyish brown to grey	Greyish-brown to dark grey	Black
Cortex	Single layered	Double layered	Single layered	Single layered	Double layered	Double layered	Double layered
Basidiospores	Subglobose, elliptical to ellipsoid-elongate; apiculum absent	Ovoid to ellipsoid; apiculus present	Subglobose to broadly ellipsoid	Subglobose, ovoid to elliptical	elliptical, globose; apiculum absent	Ovoid, subglobose, ellipsoid to broadly ellipsoid	Oval, sub-globose, broadly ellipsoid to ellipsoid-elongate
Size	11–21 × 9–14 μm	14.8–20 × 10.4–14.3 μm	14–19 × 12–16 μm	13–19 × 9–11 μm	11.43–17.78 × 9–15.24 μm	14–17 × 8–10 μm	14.2–28.7 × 11.7–23.7 μm
Walls	1–5 μm thick	0.8–1.3 μm thick	thick-walled	1.9–3.2 μm thick	2–3.5 μm thick	1.5–3 μm thick	thick-walled
Distribution	China	Brazil	Amazon rainforest	Japan, Brazil	Brazil	Thailand	India
Reference	Present study	Accioly et al. ^[25]	Trierveiler-Pereira et al. ^[42]	da Cruz et al. ^[43]	da Cruz & Baseia ^[44]	Hyde et al. ^[45]	Boonmee et al. ^[35]

peridioles, and smaller basidiospores ($9\text{--}13 \times 5\text{--}8 \mu\text{m}$) with apiculus^[20]; *C. discoideus* differs from *C. wenshanensis* by having grey brown, smaller peridioles ($1.56\text{--}2.16 \times 1.41\text{--}1.74 \text{ mm}$)^[30]; *C. gracilis* is distinguished from *C. wenshanensis* by the basidiomata with slender base, umber to rusty outer surface of peridium, double-layered cortex peridioles, and basidiospores with apical notch^[48]; *C. hookeri* differs from *C. wenshanensis* by its smooth peridium walls, and smaller basidiospores ($9\text{--}13 \times 5\text{--}8 \mu\text{m}$)^[49]; *C. magnomuralis* is distinguished from *C. wenshanensis* by having the dark blond exoperidium, smaller peridioles ($1\text{--}1.5 \times 1\text{--}1.5 \text{ mm}$) with double-layered cortex, and ovoid, larger basidiospores ($27\text{--}49 \times 23\text{--}41 \mu\text{m}$) with small apiculus^[44]; *C. renweii* differs from *C. wenshanensis* by its greyish peridioles with the brown tunica, and longer basidiospores ($21\text{--}31 \times 10.5\text{--}13.5 \mu\text{m}$)^[50]; *C. triplex* is separated from *C. wenshanensis* by its smaller basidiomata ($5\text{--}8 \times 4.5\text{--}5 \text{ mm}$) with the slender base orbicular, flattened peridioles with double-layered cortex, and basidiospores with the apical notch^[25].

Eight species of the genus *Cyathus* as *C. africanus*, *C. colensoi*, *C. gansuensis*, *C. ibericus*, *C. jiyuguanensis*, *C. novae-zeelandiae*, *C. olla*, and *C. pallidus* are similar to *C. wenshanensis* in light of the characteristics of having single-layered cortex peridioles. However, *C. africanus* differs from *C. wenshanensis* by its peridium walls with woolly hairs, silvery peridioles, and broadly ovate, smaller basidiospores ($8.5\text{--}12 \times 6.5\text{--}8.5 \mu\text{m}$) with apiculus^[51]; *C. colensoi* differs from *C. wenshanensis* by its smooth peridium walls, and ovoid, smaller basidiospores ($8.5\text{--}11.5 \times 7\text{--}8.5 \mu\text{m}$)^[30,49]; *C. gansuensis* differs from *C. wenshanensis* by its narrow base basidiomata with grayish to dark smoke-gray interior, grayish, smaller peridioles ($1.5\text{--}2 \times 0.8\text{--}1.5 \text{ mm}$), and ovoid basidiospores^[52]; *C. ibericus* differs in its whitish to pale brownish grey external peridium with woolly hairs, smaller peridioles ($0.8\text{--}1.2 \text{ mm diam}$), and ovoid, smaller basidiospores ($7\text{--}9 \times 5\text{--}6 \mu\text{m}$)^[30]; *C. jiyuguanensis* differs from *C. wenshanensis* by its basidiomata with the short stipe, smoke-gray peridioles, and ovoid, smaller basidiospores ($8\text{--}11.5 \times 7\text{--}8.5 \mu\text{m}$)^[52]; *C. novae-zeelandiae* differs in *C. wenshanensis* by its basidiomata abruptly constricted into a stipe, peridioles with white tunica, and smaller basidiospores ($11\text{--}13 \times 5\text{--}6 \mu\text{m}$)^[4,53]; *C. olla* differs from *C. wenshanensis* by its peridium with tomentose outside, silver, smooth inside, pure silver peridioles, and the smaller basidiospores ($9.8\text{--}11.2 \times 6.4\text{--}8 \mu\text{m}$)^[54]; *C. pallidus* differs from *C. wenshanensis* by its smaller basidiospores ($6.8\text{--}14.5 \times 6\text{--}8.1 \mu\text{m}$)^[32].

Cyathus annulatus, *C. aurantogriseocarpus*, *C. minimus*, and *C. stercoreus* are similar to *C. wenshanensis* inferred from the characteristics of having thick-walled basidiospores without apiculus. However, *C. annulatus* is separated from *C. wenshanensis* by its expanded peridium at the top, ochraceous-tawny exoperidium, pale buff inner surface, subtriangular peridioles, and the striking dark-brown ring at the mouth^[55]; *C. aurantogriseocarpus* differs from *C. wenshanensis* by the orange-grey exoperidium with long tomentum, brownish grey, smaller peridioles ($1.5\text{--}1.75 \times 1.2\text{--}1.5 \text{ mm}$) with double-layered cortex, and larger basidiospores ($32.5\text{--}47 \times 22.5\text{--}28.5 \mu\text{m}$)^[26]; *C. minimus* differs from *C. wenshanensis* by the clay brown exoperidium, yellowish brown endoperidium, and reddish brown, coffee or brown tobacco, smaller peridioles ($1.3\text{--}1.37 \times 1.13\text{--}1.23 \text{ mm}$)^[30]; *C. stercoreus* differs in its smooth peridium walls, double-layered cortex peridioles and larger basidiospores ($30\text{--}41 \times 25\text{--}31 \mu\text{m}$)^[4].

The family Nidulariaceae is a characteristic group of Agaricomycetes (Basidiomycota), which has a number of macrofungi based on a result of the morphological, phylogenetic and cytological studies in China^[56,57], but the species diversity of macrofungi are still not well known, especially in subtropical and tropical areas of the country^[58–61]. The new species, *Cyathus wenshanensis* is from the subtropics. Therefore, the present paper enriches the fungal diversity in the Chinese ecosystem, and it is likely that more new taxa will be found after further fieldwork and molecular analyses.

In addition, the results of BLAST queries in NCBI based on ITS separately showed the sequences producing significant alignments descriptions: in ITS blast results, the top ten taxa are *C. pyristriatus* (Max score 1116; Total score 1116; Query cover 92%; E value 0.0; Ident 95.47%), *C. parvocinereus* (Maximum record descriptions: Max score 1081; Total score 1081; Query cover 89%; E value 0.0; Ident 95.71%), *C. amazonicus* (Maximum record descriptions: Max score 1077; Total score 1077; Query cover 87%; E value 0.0; Ident 96.11%), *C. uniperidiolus* (Maximum record descriptions: Max score 1075; Total score 1075; Query cover 91%; E value $1\text{e-}78$; Ident 94.84%), *C. albinus* (Max score 1053; Total score 1053; Query cover 85%; E value 0.0; Ident 96.01%), and *C. badius* (Maximum record descriptions: Max score 1000; Total score 1000; Query cover 83%; E value 0.0; Ident 95.15%).

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Conflict of interest

The authors declare that they have no conflict of interest.

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