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## Molecular phylogeny and morphology reveal a new *Hymenochaete* species (*Hymenochaetaceae*, *Basidiomycota*) from China

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### Abstract

Wood-inhabiting fungi play an essential role in forest ecosystems as an important group of decomposers, which are found in living trees, dead standing trees, decorticated trunks, fallen branches, as well as manufactured wood. A new wood-inhabiting fungus *Hymenochaete sinensis*, belonging to the family Hymenochaetaceae, is described and illustrated based on the morphological characteristics and molecular data. *Hymenochaete sinensis* sp. nov. is characterized by annual, resupinate basidiomata with aurantium to coconut brown to brownish black hymenial surface, a monomitic hyphal system having the simple-septate with distinctly thick-walled generative hyphae, and ellipsoid to broadly ellipsoid basidiospores measuring as  $4\text{--}5 \times 2.5\text{--}3.5 \mu\text{m}$ . The phylogenetic analyses based on ITS+nLSU rDNA sequences confirmed that the new species belongs to the genus *Hymenochaete*. The ITS phylogenetic tree revealed that the new taxon is closely related to species of *Hymenochaete longispora* and *H. quercicola*.

**Key words:** 1 new species, *Basidiomycetes*, Molecular phylogeny, Wood-inhabiting fungi

### Introduction

Fungi are well-known as a diverse group of microorganisms that play important roles in forest ecosystems (Phookamsak *et al.* 2019). With the frequent inclusion of data from DNA sequences and by using both fresh material and cultures, the fungal classification of the kingdom fungi has been updated continuously. Mycologists recollected historic taxa and types to accomplish taxonomy and phylogeny in 20 phyla of fungi as *Aphelidiomycota*, *Ascomycota*, *Basidiobolomycota*, *Basidiomycota*, *Blastocladiomycota*, *Calcarisporiellomycota*, *Caulochytriomycota*, *Chytridiomycota*, *Entomophthoromycota*, *Entorrhizomycota*, *Glomeromycota*, *Kickxellomycota*, *Monoblepharomycota*, *Mortierellomycota*, *Mucoromycota*, *Neocallimastigomycota*, *Olpidiomycota*, *Rozellomycota*, *Sanchytriomycota* and *Zoopagomycota* (Wijayawardene *et al.* 2022). *Basidiomycota* constitute a major phylum of the kingdom fungi and is second in number of described species to *Ascomycota* (Wijayawardene *et al.* 2017, Yang *et al.* 2023, Andjèrèyir *et al.* 2024, He *et al.* 2024, Wang *et al.* 2024, Zhou *et al.* 2024). Approximately 60 new genera have recently been recognized for agarics, 40 for boletes, and 50 for bracket fungi in *Basidiomycota* (Wijayawardene *et al.* 2020, He *et al.* 2024).

Wood-inhabiting fungi are found in living trees, dead standing trees, decorticated trunks, fallen branches as well as manufactured wood products, in which these fungi of the cell walls and the components within the living cells secrete various enzymes that effectively degrade cellulose, hemicellulose and lignin into simple inorganic substances, in which as an important group of decomposers, they play an important role in forest ecosystems (Dai 2011, Yang *et al.* 2024, Deng *et al.* 2024, Zhang *et al.* 2024).

*Hymenochaete* Lév. was erected in 1846 and typified by *H. rubiginosa* (Dicks.) Lév. Léger (1998) wrote a worldwide monograph on *Hymenochaete*, providing a key to this genus. According to Index Fungorum ([www.indexfungorum.org](http://www.indexfungorum.org); accessed on 19 June 2024), the genus *Hymenochaete* has 360 registered names with 234 species have been accepted worldwide (Léger 1998, Parmasto 2001, Parmasto & Gilbertson 2005, He & Dai 2012, Parmasto 2012, He *et*

al. 2017, Nie *et al.* 2017, Pacheco *et al.* 2018, Miettinen *et al.* 2019, Du *et al.* 2021). *Hymenochaete* is characterized by annual to perennial, resupinate, effused-reflexed to pileate basidioma with smooth, tuberculate, lamellate, poroid or hydroid hymenophores; a monomitic or dimitic hyphal system; presence of setae, and hyaline, thin-walled, narrowly cylindrical to globose basidiospores (Léger 1998, Parmasto 2001, He & Dai 2012). Several characteristics can be used to circumscribe the genus, such as brown basidiocarps darkening in potassium hydroxide, simple-septate generative hyphae, brown setae, and causing a white rot (Léger 1998, Parmasto 2001).

Based on the early embrace of molecular systematics by mycologists, both the discovery and classification of fungi, among the more basal branches of the tree, are now coming to light from genomic analyses and environmental DNA surveys that have been conducted (James *et al.* 2020). The first phylogenetic study of *Hymenochaete* s.l. was conducted by Wagner & Fisher (2002), who separated *Pseudochaete* T. Wagner & M. Fisch. (2002: 100) from the genus *Hymenochaete* based on the analyses of 28S sequences. Their results were later confirmed by further studies (Larsson *et al.* 2006, He & Dai 2012, Baltazar *et al.* 2014, Parmasto *et al.* 2014). In phylogenetic trees of the family Hymenochaetaceae (Hymenochaetales), the species of *Hymenochaete* formed a monophyletic clade and was sister to *Hymenochaetopsis* S.H. He & Jiao Yang (2016: 2), in which they were almost indistinguishable in morphology (Larsson *et al.* 2006, He & Dai 2012, Baltazar *et al.* 2014, Parmasto *et al.* 2014, Yang *et al.* 2016).

During investigations on wood-inhabiting fungi in Yunnan Province, China, two specimens of *Hymenochaete* were collected. To clarify the placement and relationships of both specimens, we carried out a phylogenetic and taxonomic study on the genus *Hymenochaete*, based on the ITS and nLSU sequences. Both specimens are identified as an undescribed species of *Hymenochaete*, and the detailed description and illustrations of the new species are provided here.

## Materials and methods

### *Sample Collection and Herbarium Specimen Preparation*

Fresh basidiomata growing on angiosperm branches were collected from Qujing, Yunnan Province, P.R. China. The samples were photographed *in situ*, and macroscopic details of fresh basidiomata were recorded. Photographs were taken using a Jianeng 80D camera (Tokyo, Japan). Specimens were dried in an electric food dehydrator at 45°C (Hu *et al.* 2022). Once dried, the specimens were sealed in envelopes and zip-lock plastic bags and labeled (Zhao *et al.*, 2023). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

### *Morphology*

The macromorphological descriptions were based on field notes and photos captured in the field and lab. The color terminology follows Petersen (1996). The micromorphological data were obtained from the dried specimens observed under a Nikon Eclipse E100 light microscope following Dai (2010). The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB- = acyanophilous, IKI- = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied, and n = a/b (number of spores (a) measured from given number (b) of specimens).

### *Molecular Phylogeny*

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain genomic DNA from the dried specimens according to the manufacturer's instructions. The nuclear ribosomal of the internal transcribed spacer (ITS) region was amplified with the primer pair ITS5/ITS4 (White *et al.* 1990), the nuclear large subunit (nLSU) region with the primer pair LR0R/LR7 (Vilgalys & Hester 1990). The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s, and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, P.R. China). All newly generated sequences were deposited in GenBank (Table 1).

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

Species name	Specimen number	ITS	nLSU	References
<i>Hydnoporia tabacina</i>	He 390	JQ279610	JQ279625	He <i>et al.</i> 2017
<i>Hymenochaete berteroi</i>	He 1488	KU975459	KU975498	He <i>et al.</i> 2017
<i>Hymenochaete biformisetosa</i>	He 1445	KF908247	KU975499	He & Li 2014
<i>Hymenochaete cana</i>	He 1305	KF438169	KF438172	He <i>et al.</i> 2017
<i>Hymenochaete cinnamomea</i>	He 755	JQ279548	JQ279658	He <i>et al.</i> 2017
<i>Hymenochaete colliculosa</i>	Dai 16427	MF370595	MF370602	He <i>et al.</i> 2017
<i>Hymenochaete colliculosa</i>	Dai 16428	MF370596	MF370603	He <i>et al.</i> 2017
<i>Hymenochaete colliculosa</i>	Dai 16429	MF370597	MF370604	He <i>et al.</i> 2017
<i>Hymenochaete contiformis</i>	He 1166	KU975461	KU975501	He <i>et al.</i> 2017
<i>Hymenochaete cruenta</i>	He 766	JQ279595	JQ279681	He <i>et al.</i> 2017
<i>Hymenochaete cyclolamellata</i>	Cui 7393	JQ279513	JQ279629	He <i>et al.</i> 2017
<i>Hymenochaete denticulata</i>	He 1271	KF438171	KF438174	He <i>et al.</i> 2017
<i>Hymenochaete duportii</i>	AFTOL ID666	DQ404386	AY635770	He <i>et al.</i> 2017
<i>Hymenochaete epichlora</i>	He 525	JQ279549	JQ279659	He & Dai 2012
<i>Hymenochaete floridea</i>	He 536	JQ279597	JQ279683	He & Dai 2012
<i>Hymenochaete fulva</i>	He 640	JQ279565	JQ279648	He & Dai 2012
<i>Hymenochaete huangshanensis</i>	He 432	JQ279533	JQ279671	He & Dai 2012
<i>Hymenochaete japonica</i>	He 245	JQ279590	JQ279680	He & Dai 2012
<i>Hymenochaete innexa</i>	He 555	JQ279584	JQ279674	He & Dai 2012
<i>Hymenochaete legeri</i>	He 960	KU975469	KU975511	He <i>et al.</i> 2017
<i>Hymenochaete longispora</i>	He 217	JQ279537	KU975514	He & Dai 2012
<i>Hymenochaete luteobadia</i>	He 8	JQ279569	KU975515	He & Dai 2012
<i>Hymenochaete megaspora</i>	He 302	JQ279553	JQ279660	He & Dai 2012
<i>Hymenochaete minor</i>	He 933	JQ279555	JQ279654	He & Dai 2012
<i>Hymenochaete minuscula</i>	He 253	JQ279546	KU975516	He & Dai 2012
<i>Hymenochaete murina</i>	He 569	JQ716406	JQ716412	He <i>et al.</i> 2017
<i>Hymenochaete nanospora</i>	He 475	JQ279531	JQ279672	He & Dai 2012
<i>Hymenochaete ochromarginata</i>	He 47	JQ279579	JQ279666	He & Dai 2012
<i>Hymenochaete tabacina</i>	Dai 11635	JQ279563	JQ279647	He & Dai 2012
<i>Hymenochaete orientalis</i>	He 4601	KY425677	KY425685	He <i>et al.</i> 2017
<i>Hymenochaete parmastoi</i>	He 867	JQ780063	KU975518	He <i>et al.</i> 2017
<i>Hymenochaete paucisetigera</i>	Cui 7845	JQ279560	JQ279644	He & Dai 2012
<i>Hymenochaete quercicola</i>	He 373	KU975474	KU975521	He <i>et al.</i> 2017
<i>Hymenochaete rhabarbarina</i>	He 280	JQ279574	KY425688	He & Dai 2012
<i>Hymenochaete rhododendricola</i>	He 389	JQ279577	JQ279653	He & Dai 2012
<i>Hymenochaete rubiginosa</i> *	He 1049	JQ716407	JQ279667	Yang <i>et al.</i> 2016
<i>Hymenochaete rufomarginata</i>	He 1489	KU975477	KU975524	He <i>et al.</i> 2017
<i>Hymenochaete sinensis</i>	CLZhao 26040	OR659001	PP425893	Present study
<i>Hymenochaete sinensis</i>	CLZhao 26652	PQ060540	—	Present study
<i>Hymenochaete separabilis</i>	He 460	JQ279572	JQ279655	He & Dai 2012
<i>Hymenochaete spathulata</i>	He 685	JQ279591	KU975529	He <i>et al.</i> 2017
<i>Hymenochaete sphaericola</i>	He 303	JQ279599	JQ279684	He & Dai 2012
<i>Hymenochaete sphaerospora</i>	He 715	JQ279594	KU975531	He <i>et al.</i> 2017
<i>Hymenochaete tasmanica</i>	He 449	JQ279582	JQ279663	He <i>et al.</i> 2017
<i>Hymenochaete tongbiguanensis</i>	He 1552	KF908248	KU975532	He <i>et al.</i> 2017
<i>Hymenochaete tropica</i>	He 574	JQ279587	JQ279675	He <i>et al.</i> 2017

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**TABLE 1.** (Continued)

Species name	Specimen number	ITS	nLSU	References
<i>Hymenochaete ulmicola</i>	He 864	JQ780065	KU975534	He <i>et al.</i> 2017
<i>Hymenochaete unicolor</i>	He 468a	JQ279551	JQ279662	He <i>et al.</i> 2017
<i>Hymenochaete verruculosa</i>	Dai 17052	MF370594	MF370601	He <i>et al.</i> 2017
<i>Hymenochaete verruculosa</i>	Dai 17047	—	MF370600	Du <i>et al.</i> 2021
<i>Hymenochaete villosa</i>	He 537	JQ279528	JQ279634	He <i>et al.</i> 2017
<i>Hymenochaete yunnanensis</i>	He 1447	KU975486	KU975538	He <i>et al.</i> 2017
<i>Hymenochaete acerosa</i>	He 338	JQ279543	JQ279657	He <i>et al.</i> 2017
<i>Hymenochaete adusta</i>	He 207	JQ279523	KU975497	He <i>et al.</i> 2017
<i>Hymenochaete angustispora</i>	Dai 17045	MF370592	MF370598	He <i>et al.</i> 2017
<i>Hymenochaete angustispora</i>	Dai 17049	MF370593	MF370599	He <i>et al.</i> 2017
<i>Hymenochaete anomala</i>	He 592	JQ279566	JQ279650	He <i>et al.</i> 2017
<i>Hymenochaete asetosa</i>	Dai 10756	JQ279559	JQ279642	He <i>et al.</i> 2017
<i>Hymenochaete attenuata</i>	He 28	JQ279526	JQ279633	He <i>et al.</i> 2017
<i>Hymenochaete bambusicola</i>	He 4116	KY425674	KY425681	He <i>et al.</i> 2017
<i>Hymenochaete boddingii</i>	MEH 66068	MN030343	MN030345	Du <i>et al.</i> 2021
<i>Hymenochaete boddingii</i>	MEH 69996	MN030341	MN030347	Du <i>et al.</i> 2021
<i>Hymenochaete boddingii</i>	MEH 66150	MN030344	—	Du <i>et al.</i> 2021
<i>Hymenochaete borbonica</i>	CBS 731.86	MH862026	MH873716	Du <i>et al.</i> 2021
<i>Hymenochaete conchata</i>	MEH 70144	MF373838	—	Du <i>et al.</i> 2021
<i>Hymenochaete damicornis</i>	URM 84261	KC348466	—	Du <i>et al.</i> 2021
<i>Hymenochaete damicornis</i>	URM 84263	KC348467	—	Du <i>et al.</i> 2021
<i>Hymenochaete dracaenicola</i>	Dai 22090	MW559797	MW559802	Du <i>et al.</i> 2021
<i>Hymenochaete dracaenicola</i>	Dai 22096	MW559798	MW559803	Du <i>et al.</i> 2021
<i>Hymenochaete fuliginosa</i>	He 1188	KU975465	KU975506	Du <i>et al.</i> 2021
<i>Hymenochaete globispora</i>	He 911	—	KU975508	Du <i>et al.</i> 2021
<i>Hymenochaete macrochloae</i>	ARAN-Fungi 7079	MF990738	MF990743	Du <i>et al.</i> 2021
<i>Hymenochaete muroiana</i>	He 405	JQ279542	KU975517	Du <i>et al.</i> 2021
<i>Hymenochaete rheicolor</i>	Cui 8317	JQ279529	—	Du <i>et al.</i> 2021
<i>Hymenochaete setipora</i>	Cui 6301	JQ279515	JQ279639	Du <i>et al.</i> 2021
<i>Hymenochaete sharmae</i>	CAL 1535	KY929017	KY929018	Du <i>et al.</i> 2021
<i>Hymenochaete sharmae</i>	66088	MK588753	MK588836	Du <i>et al.</i> 2021
<i>Hymenochaete subferruginea</i>	Cui 8122	JQ279521	—	Du <i>et al.</i> 2021
<i>Hymenochaete subferruginea</i>	He 1598	KU975481	—	Du <i>et al.</i> 2021
<i>Hymenochaete tenuis</i>	He 779	JQ279538	JQ279641	Du <i>et al.</i> 2021
<i>Hymenochaete xerantica</i>	Cui 9209	JQ279519	JQ279635	Du <i>et al.</i> 2021

\* Indicates type specimen

The sequences were aligned in MAFFT version 7 (Katoh *et al.* 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). The dataset was aligned first, and then the sequences of ITS and nLSU were combined with Mesquite version 3.51. The combined ITS and nLSU sequences datasets were used to infer the position of the new species in the *Hymenochaete* and related species.

Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were applied to the combined datasets following a previous study (Zhao & Wu 2017), 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 bootstrap (BT) analysis with 1000 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. The multiple sequence alignment was also analyzed using maximum likelihood (ML) in RAxML-HPC2 (Miller *et al.* 2012). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each dataset for Bayesian inference (BI), which was performed using MrBayes 3.2.7a with a GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 1.9 million generations for the combined ITS and nLSU sequences with trees and parameters sampled every 1000 generations. The first one-fourth of all the generations were discarded as burn-ins. The majority-rule consensus tree of all the remaining trees was calculated. Branches were considered significantly supported if they received a maximum likelihood bootstrap value (BS) of  $\geq 70\%$ , a maximum parsimony bootstrap value (BP) of  $\geq 70\%$ , or Bayesian posterior probabilities (BPP) of  $\geq 0.95$ .

## Results

### *Phylogenetic analyses*

The dataset based on ITS+nLSU (Figure 1) comprises sequences from 80 fungal specimens representing 70 species. The dataset had an aligned length of 2,236 characters, of which 1,503 characters are constant, 227 are variable and parsimony-uninformative, and 506 are parsimony-informative. Maximum parsimony analysis yielded one equally parsimonious tree (TL = 3,431, CI = 0.3331, HI = 0.6669, RI = 0.5749, RC = 0.1915). Bayesian analysis and ML analysis resulted in a topology similar to MP analysis with the effective sample size (ESS) across the two runs double the average ESS (avg ESS) = 753.

The phylogenetic tree (Figure 1) inferred from ITS and nLSU sequences revealed that *Hymenochaete sinensis* was nested into the *Hymenochaete* (Hymenochaeteaceae). The dataset based on ITS (Figure 2) comprises sequences from 78 fungal specimens representing 69 species. The dataset had an aligned length of 853 characters, of which 340 characters are constant, 153 are variable and parsimony-uninformative, and 360 are parsimony-informative. Maximum parsimony analysis yielded one equally parsimonious tree (TL = 2776, CI = 0.3170, HI = 0.6830, RI = 0.4905, RC = 0.1555). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis, with the effective sample size (ESS) across the two runs is double the average ESS (avg ESS) = 996.

The phylogenetic tree (Figure 1) inferred from ITS sequences revealed that *Hymenochaete sinensis* was sister to *H. longispora* Parmasto (1986: 375) and *H. quercicola* S.H. He & Hai J. Li (2011: 485).

## Taxonomy

*Hymenochaete sinensis* Y.C. Li & C.L. Zhao, *sp. nov.* Figures 1 and 2.

Mycobank no.: MB 854216

**Etymology:**—*sinensis* (Lat.): refers to the country (China) where the type specimen was collected.

**Holotype:**—China. Yunnan Province, Qujing, Qilin District, Cuishan Forest Park, GPS coordinates: 25°55' N, 103°69' E, altitude: 2245 m, on a fallen branch of angiosperm, leg. C.L. Zhao, 5 November 2022, CLZhao 26040 (SWFC).

**Basidiomata:**—Annual, resupinate, closely adnate, ceraceous, without odor or taste, at first as small irregular patches up, later confluent up to 11 cm long, 2.5 cm wide, and 0.3 mm thick. Hymenial surface aurantium to coconut brown when fresh, coconut brown to brownish black upon drying. Sterile margin thinning, aurantium, up to 1 mm.

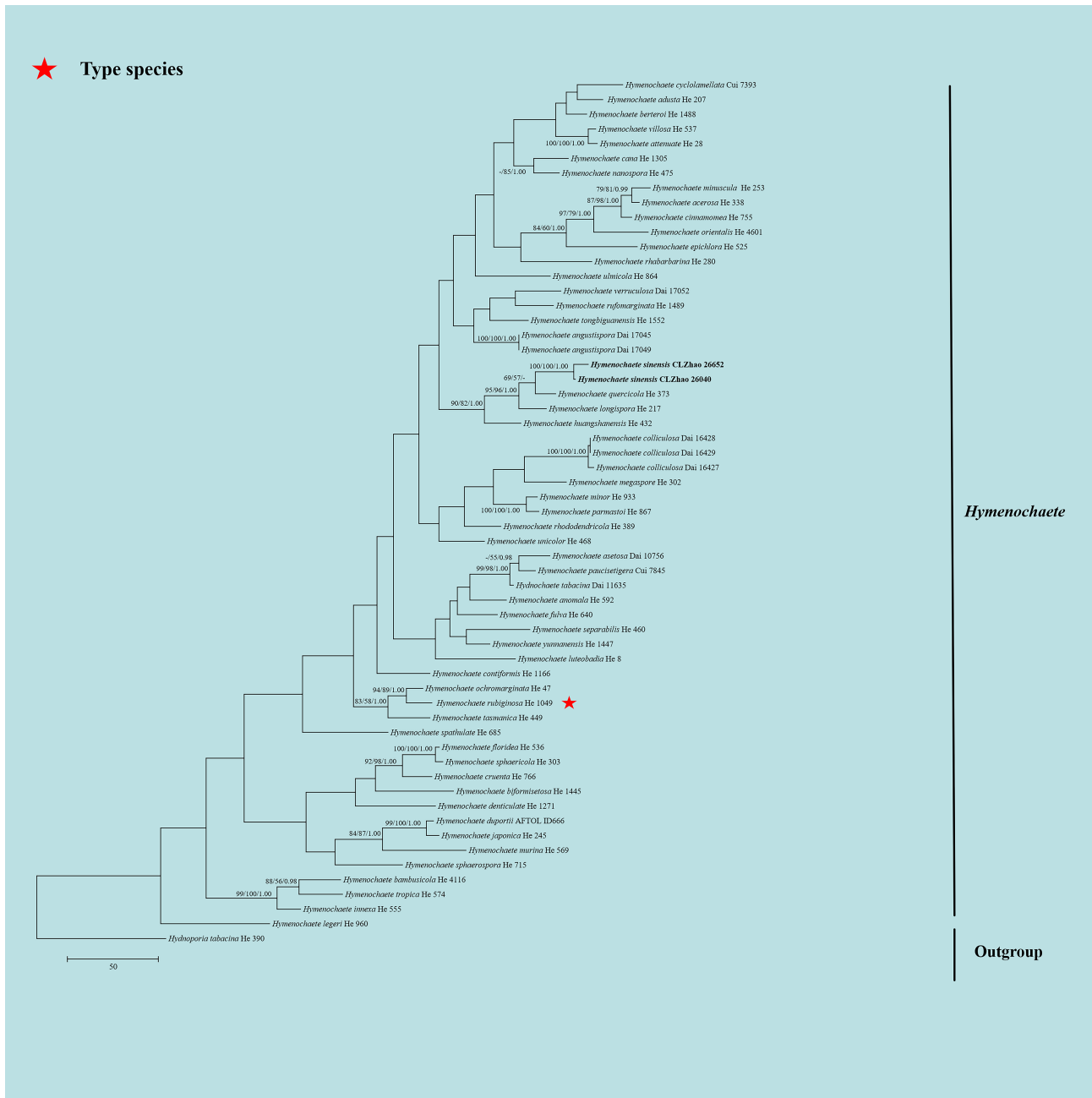
**Hyphal system:**—Monomitic; generative hyphae with simple-septate, colorless, distinctly thick-walled, frequently branched, interwoven, 2.5–3.5  $\mu\text{m}$  in diameter; IKI–, CB–, tissues unchanged in KOH.

**Hymenium:**—Cystidia absent, cystidioles abundant, fusiform, slightly sinuous, colorless, thin-walled, 17–21  $\times$  4.2–4.9  $\mu\text{m}$ . Basidia barrelled, colorless, thin-walled, with four sterigmata and a basal simple septum, 10–12  $\times$  3.1–3.8  $\mu\text{m}$ ; basidioles dominant, in shape similar to basidia, but slightly smaller. Setae abundant, distinctly thick-walled, subulate, with an acute tip, yellowish to reddish brown, 49–63  $\times$  6.7–7.5  $\mu\text{m}$ , projecting out of the hymenium up to 36  $\mu\text{m}$ .

★ Type species



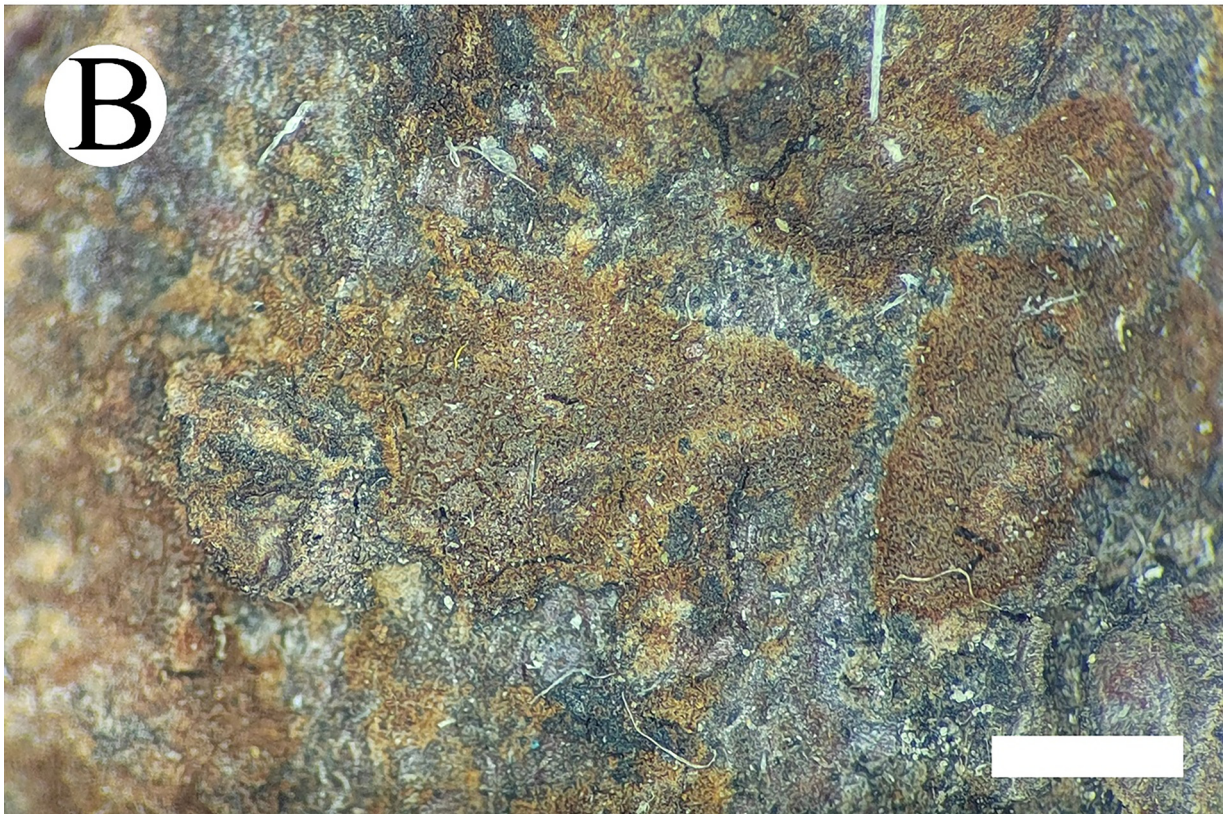
**FIGURE 1.** Maximum parsimony strict consensus tree illustrating the phylogeny of one new species and related species in *Hymenochaete* based on ITS+nLSU sequences. The branch is labeled with a maximum likelihood lead value equal to or greater than 70%, a reduced lead value equal to or greater than 50%, and a Bayesian posterior probability equal to or greater than 0.95. The red star represents the type species. The new species is in black bold.



**FIGURE 2.** Maximum parsimony strict consensus tree illustrating the phylogeny of one new species and related species in *Hymenochaete* based on ITS sequences. The branch is labeled with a maximum likelihood lead value equal to or greater than 70%, a reduced lead value equal to or greater than 50%, and a Bayesian posterior probability equal to or greater than 0.95. The red star is the type species. The new species is in black bold.

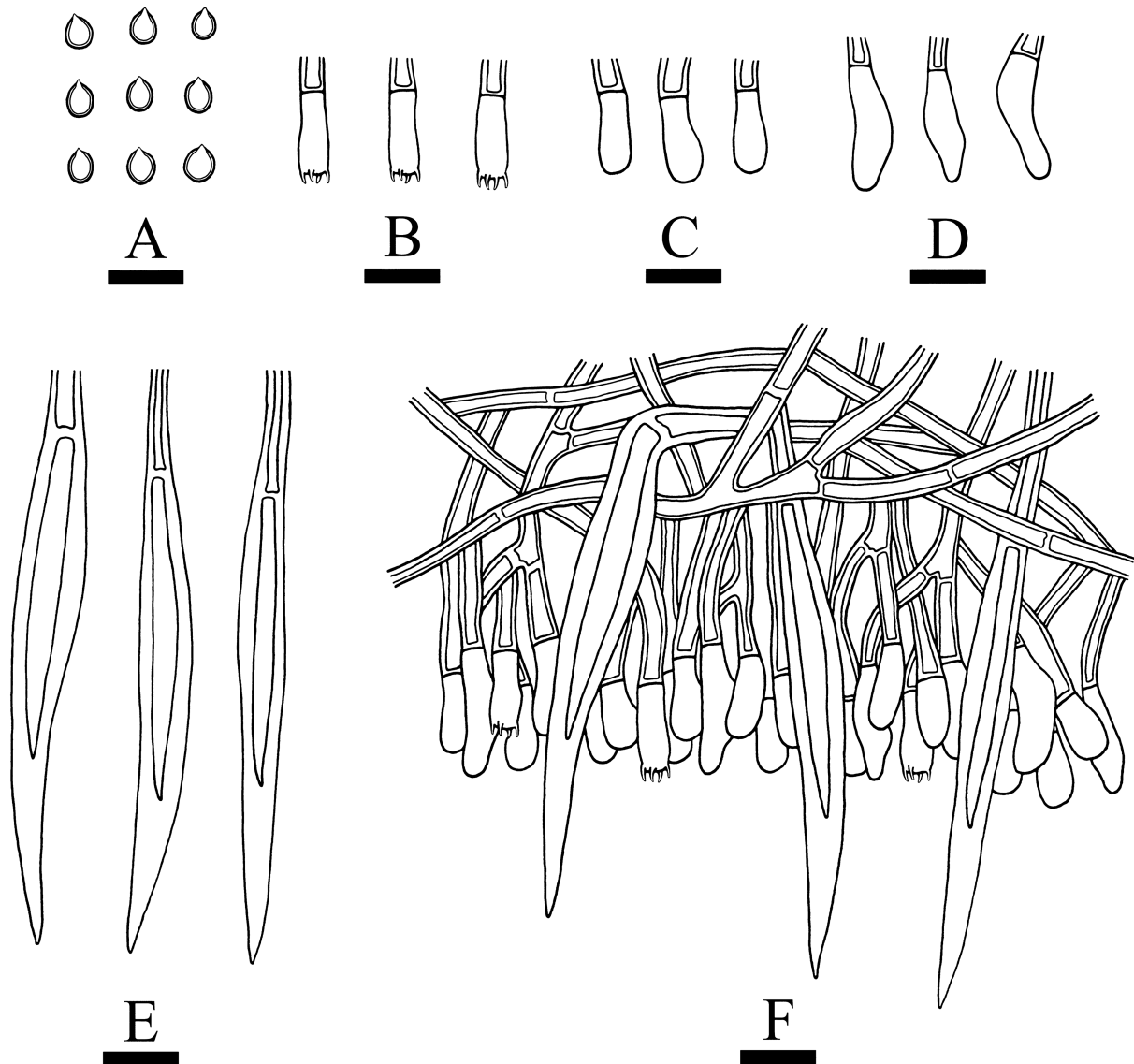
**Spores:**—Basidiospores ellipsoid to broadly ellipsoid, colorless, distinctly thick-walled, smooth, IKI–, CB–, 4–5 × 2.5–3.5 μm, L = 4.20 μm, W = 3.20 μm, Q = 1.31 (n = 30/1).

**Additional specimens examined (paratypes):**—China. Yunnan Province, Qujing, Qilin District, Cuishan Forest Park, GPS coordinates: 25°55' N, 103°69' E, altitude: 2245 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 5 November 2022, CLZhao 26652, 13 April 2024, CLZhao 35894 (SWFC).



**FIGURE 3.** *Hymenochaete sinensis* (holotype, CLZhao 26040): basidiomata on the substrate (A), macroscopic characteristics of hymenophore (B). Bars: (A) = 1 cm and (B) = 1 mm.





**FIGURE 4.** Microscopic structures of *Hymenochaete sinensis* (holotype, CLZhao 26040): basidiospores (A), basidia (B), basidioles (C), cystidia (D), setae (E), a section of the hymenium (F). Bars: (A–F) = 10  $\mu$ m.

## Discussion

The present study describes *Hymenochaete sinensis* as a new species based on phylogenetic analyses and morphological characteristics. There are currently 29 species of *Hymenochaete* in China, including the present new species.

In our study, the phylogenetic tree of ITS+nLSU (Figure 1) shows that the new species *Hymenochaete sinensis* is closely related to *H. longispora*, *H. quercicola* and *H. huangshanensis* S.H. He & Y.C. Dai (2021: 83). However, *Hymenochaete longispora* is distinct from *H. sinensis* by having brunneus to brown hymenial surface, larger setae measuring 80–130  $\times$  8–13  $\mu$ m, and longer basidiospores (8–10  $\times$  3–3.5  $\mu$ m, Léger 1998). The taxon *H. quercicola* differs from *H. sinensis* by its mouse-grey to vinaceous grey hymenial surface, longer setae (85–105  $\times$  7–10  $\mu$ m), and oblong-ellipsoid to cylindrical basidiospores (6.0–8.0  $\times$  3.0–3.6  $\mu$ m, He *et al.* 2011). The species *H. huangshanensis* is distinguished from *H. sinensis* by its larger setae measuring 70–150  $\times$  8–12  $\mu$ m, and cylindrical or allantoid, longer basidiospores (5.5–7  $\times$  2–2.8  $\mu$ m, He *et al.* 2012).

Morphologically, two species *Hymenochaete acerosa* S.H. He & Hai J. Li and *H. cystidiata* Parmasto are similar to *H. sinensis* in that they have distinctly thick-walled and acute setae. However, *Hymenochaete acerosa* is distinguished

from *H. sinensis* by its longer setae (85–170 × 5–8 µm) and larger basidiospores (7–8.5 × 4.8–6 µm, He *et al.* 2011). *Hymenochaete cystidiata* differs from *H. sinensis* by its smooth hymenial surface and longer setae (60–100 × 7–10 µm Parmasto 2012).

Recently, many significant research advances have been made in the studies of species diversity and divergence times of fungi (He *et al.* 2019, Dai *et al.* 2021). To date, about 155,000 species of fungi have been described, accounting for 6.20% of an estimated 2.5 million (Hawksworth & Lücking 2017, Hyde 2022, Niskanen *et al.* 2023). In addition, based on fossil evidence, determining the divergence times within *Basidiomycota* has provided a robust set of age estimates for higher taxa (He *et al.* 2019). Berbee and Taylor (2010) represented a minimum age of 125 Mya for *Hymenochaetaceae*. Meanwhile, the molecular dating studies of macrofungi widely pay attention to ectomycorrhizal fungi, saprotrophic fungi, and pathogenic fungi (Hibbett & Matheny 2009, He *et al.* 2019, Hyde 2022, Wijayawardene *et al.* 2022, Liu *et al.* 2023a). The discovery of new fungal species has rapidly increased with the development of molecular techniques, drawing attention to the enormous fungal diversity that exists on Earth (Cui *et al.* 2019, Dai *et al.* 2021, Wu *et al.* 2022, Liu *et al.* 2023b, Liu *et al.* 2023c, Zhao *et al.* 2023, Zhang *et al.* 2023, Duan *et al.* 2023, Yuan *et al.* 2023, Tian *et al.* 2024, Zhao *et al.* 2024, Zhou *et al.* 2024). The family *Hymenochaetaceae* is a core family of macrofungi that consists of approximately 670 poroid species (Wu *et al.* 2022) and is an interesting subject for species diversity studies (Dai 2010, Dai *et al.* 2021, Wu *et al.* 2022).

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