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Research article

Punctochaete murina gen. et sp. nov. (Agaricomycetes, Basidiomycota) from southwestern China

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Abstract. Wood-inhabiting fungi play an integral role in wood degradation and the cycle of matter in the ecological system. They are considered a 'key player' in wood decomposition because of their ability to produce all kinds of enzymes that break down lignin, cellulose and hemicellulose. In this study, some wood-inhabiting mushrooms were collected from the Yunnan-Guizhou Plateau, Asia, and based on the morphological and molecular evidence, they are introduced as *Punctochaete murina* J.H.Dong & C.L.Zhao gen. et sp. nov. *Punctochaete* J.H.Dong & C.L.Zhao gen. nov. is characterized by resupinate basidiomata with a punctate hymenial surface, a monomitic hyphal system and generative hyphae bearing clamp connections, and cylindrical to allantoid, thin-walled, smooth, slightly curved basidiospores. Sequences of the internal transcribed spacer (ITS) and large subunit (nLSU) markers of the studied samples were generated, and phylogenetic analyses were performed with maximum likelihood, maximum parsimony and Bayesian inference methods. Phylogenetic analyses of ITS+nLSU nuclear RNA gene regions showed that *Punctochaete* forms a monophyletic lineage within the order Auriculariales in which it is sister to a clade comprising two closely related genera: *Exidiopsis* and *Tremellochaete*.

Keywords. Auriculariales, biodiversity, mushrooms, molecular systematics, taxonomy, wood-inhabiting fungi, Yunnan Province.

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Introduction

In forest ecosystems, fungi play an essential ecological role in driving carbon cycling in forest soils, mediating the mineral nutrition of plants and alleviating carbon limitations (Tedersoo *et al.* 2014). The fungal order Auriculariales Bromhead is a group mainly composed of wood-inhabiting Agaricomycetes Doweld (Basidiomycota R.T.Moore) (Hibbett *et al.* 2007). The type genus of this order is *Auricularia* Bull., which, together with several other gelatinous genera such as *Exidia* Fr., *Guepinia* Fr., and *Pseudohydnum* P.Karst., includes important edible and medicinal mushrooms (Wu *et al.* 2019; Liu *et al.* 2022). Therefore, interest in species diversity within the gelatinous genera has significantly increased in recent years (Chen *et al.* 2020; Shen & Fan 2020; Ye *et al.* 2020; Wang & Thorn 2021; Wu *et al.* 2021; Tohtirjap *et al.* 2023).

Contrary to the gelatinous genera, most species in the order Auriculariales are tough, including saprophytic species with resupinate, effused-reflexed, hydnoid, cerebriform, coralloid, or pileate basidiomata (Wells & Bandoni 2001; Miettinen *et al.* 2012; Zhou & Dai 2013; Hibbett *et al.* 2014; Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Liu *et al.* 2022; Tohtirjap *et al.* 2023). Species with stereoid basidiocarps are widely distributed in many orders of the Agaricomycetes, although they are certainly a minority in the Auriculariales (Malysheva & Spirin 2017).

Wood-inhabiting fungi are a cosmopolitan and highly diverse group recorded in boreal, temperate, subtropical and tropical regions (Bernicchia & Gorjón 2010; Dai *et al.* 2015, 2021; Cui *et al.* 2019; Liu *et al.* 2022; Zhao *et al.* 2023). The corticioid species of the order Auriculariales were traditionally placed in *Eichleriella* Bres., *Exidiopsis* (Bref.) Möller, and *Heterochaete* Pat. based on morphological characteristics (Burt 1915; Wells 1961; Raitviir & Wells 1966; Wells & Raitviir 1980). Subsequent morphological and phylogenetic studies led Malysheva & Spirin (2017) to revise the generic concepts of *Eichleriella*, *Hirneolina* (Pat.) Bres. and *Tremellochaete* Raitv., and to propose the new genus *Heteroradulum* Lloyd ex Spirin & Malysheva. While *Eichleriella* was accepted to be a monophyletic genus, *Exidiopsis* and *Heterochaete* were found to be synonymous, with *Heterochaete* having nomenclatural priority (Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Alvarenga & Gibertoni 2021). However, some species of *Exidiopsis*, including sequenced taxa such as *E. calcea* (Pers.) K.Wells and *E. grisea* (Bres.) Bourdot & Maire, still lack proper generic placement (Malysheva & Spirin 2017; Li *et al.* 2022; Liu *et al.* 2022). With the continuous study of the order Auriculariales, several new corticioid genera including *Adustochaete* Alvarenga & K.H.Larss., *Alloexidiopsis* L.W.Zhou & S.L.Liu, *Amphistereum* Spirin & Malysheva, *Crystallodon* Alvarenga, *Heteroradulum*, *Metulochaete* Alvarenga, *Proterochaete* Spirin & Malysheva and *Sclerotrema* Spirin & Malysheva have been established and described based on both morphological and phylogenetic studies (Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Alvarenga & Gibertoni 2021; Liu *et al.* 2022).

Recently, several genera and species of resupinate Auriculariales have been described or better defined using morphological and molecular analyses, showing the hidden diversity of this group (Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Spirin *et al.* 2019a, 2019b; Alvarenga & Gibertoni 2021; Liu *et al.* 2022).

During investigations of wood-inhabiting fungi on the Yunnan-Guizhou Plateau, China, rich material was collected. To clarify the placement and relationships of these specimens, we carried out a phylogenetic and taxonomic study of the order Auriculariales based on the ITS and nLSU sequences. The newly collected specimens could not be assigned to any known genus and form a monophyletic lineage within the order Auriculariales. Therefore, a new genus, *Punctochaete* gen. nov. with the type species *Punctochaete murina* gen. et sp nov., is proposed with descriptions and illustrations based on the morphological characteristics and phylogenetic analyses.

Materials and methods

Sample collection and herbarium specimen preparation

The fresh fruiting bodies were collected on fallen angiosperm branches from Zhaotong of the Yunnan Province, China. The samples were photographed in situ, and fresh macroscopic details were recorded (Rathnayaka *et al.* 2024). Photographs were taken with a Nikon D7100 camera. All the photos were focus-stacked using Helicon Focus software. Macroscopic details were recorded, and the specimens were transported to a field station where the fruiting bodies were dried in an electronic food dryer at 45°C (Hu *et al.* 2022). Once dried, the specimens were sealed in a paper envelope and zip-lock plastic bags and labeled (Dong *et al.* 2024a, 2024b, 2024c; Zhang *et al.* 2024). The dried specimens were deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China.

Morphology

Macro-morphological descriptions were based on field notes and photos taken in the field and lab, with color terminology following Petersen (1996). Micro-morphological data were obtained from dried specimens after observation under a light microscope at 1000 × magnification with oil immersion (Zhao *et al.* 2023). Sections were mounted in 5% KOH and 2% phloxine B ($C_{20}H_2Br_4C_{14}Na_2O_5$). Additional reagents, including Cotton Blue and Melzer's reagent, were used to observe micro-morphology following Wu *et al.* (2022b). To show the variation in spore sizes, 5% of measurements were excluded from each end of the range and shown in parentheses. At least thirty basidiospores were measured from each specimen. Basidia were measured excluding stalks, and basidiospore measurements excluded the hilar appendage.

The following abbreviations are used:
CB– = acyanophilous
IKI– = both inamyloid and indextrinoid
KOH = 5% potassium hydroxide water solution
L = mean spore length (arithmetic average for all spores)
n = a/b (number of spores (a) measured from given number (b) of specimens)
Q = variation in L/W ratios between the specimens studied
Q_m = mean Q of basidiospores ± standard deviation
W = mean spore width (arithmetic average for all spores)

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 internal transcribed spacer (ITS) region of nuclear ribosomal DNA was amplified with ITS5 and ITS4 primers (White *et al.* 1990). The nuclear large subunit (nLSU) region was amplified with the LR0R and LR7 primer pair (http://lutzonilab.org/nuclear-ribosomal-dna/, accessed on 18 May 2024).

The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles, each consisting of denaturation at 94°C for 40 s, annealing at 58°C for 45 s, and extension at 72°C for 1 min, and a final extension at 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, each consisting of denaturation at a 94°C for 30 s, annealing at 48°C for 1 min, and extension at 72°C for 1.5 min, and a final extension at 72°C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company (Yunnan Province, China). The newly generated sequences were deposited in NCBI GenBank (Table 1).

The sequences were aligned in MAFFT ver. 7 (Katoh *et al.* 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView ver. 1.27 (Larsson 2014). The dataset was aligned first, and then the sequences of ITS and nLSU were combined with Mesquite ver. 3.51. The alignment datasets were deposited in TreeBASE (submission ID 31403). The combined ITS + nLSU sequences and ITS datasets were used to infer the position of the new genus *Punctochaete* gen. nov. and related species in the order Auriculariales. Sequences of *Sistotrema brinkmannii* (Bres.) J.Erikss. was retrieved from GenBank and used as an outgroup in the ITS + nLSU analysis (Fig. 1) (Tohtirjap *et al.* 2023).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) analyses were applied to the combined three datasets following a previous study (Zhao & Wu 2017; Dong *et al.* 2024b), and the tree construction procedure was performed in PAUP* ver. 4.0b10 (Swofford 2002). All of the

Table 1 (continued on next page). List of species, specimens and GenBank accession numbers of sequences used in this study. New species is shown in **bold**; * = holotype.

Species Name	Sample No.	GenBank Accession No.		Country	References
		ITS	nLSU		
Adustochaete rava	*RC 841*	MK391516	–	Brazil	Alvarenga *et al.* 2019
Adustochaete rava	*KHL 15526*	MK391517	MK391526	Brazil	Alvarenga *et al.* 2019
Alloexidiopsis australiensis	*LWZ 20180513-22*	OM801933	OM801918	China	Liu *et al.* 2022
Alloexidiopsis calcea	*MW 331*	AF291280	AF291326	Germany	Weiss & Oberwinkler 2001
Amphistereum leveilleanum	*FP-106715*	KX262119	KX262168	USA	Malysheva & Spirin 2017
Amphistereum schrenkii	*HHB 8476*	KX262130	KX262178	USA	Malysheva & Spirin 2017
Aporpium caryae	*Miettinen 14774*	JX044145	–	Finland	Miettinen *et al.* 2012
Aporpium caryae	*WD 2207*	AB871751	AB871730	Japan	Sotome *et al.* 2014
Auricularia auricula-judae	*JT 04*	KT152099	KT152115	UK	Tohtirjap *et al.* 2023
Auricularia cornea	*Dai 13621*	MZ618936	MZ669905	China	Tohtirjap *et al.* 2023
Auricularia mesenterica	*Oberwinkler 25132*	AF291271	AF291292	Germany	Weiss & Oberwinkler 2001
Auricularia tibetica	*Dai 13336*	MZ618943	MZ669915	China	Tohtirjap *et al.* 2023
Auricularia polytricha	*TUFC 12920*	AB871752	AB871733	Japan	Sotome *et al.* 2014
Bourdotia galzinii	*Otto Miettinen X3067*	MG757511	MG757511	Spain	Malysheva *et al.* 2018
Crystallodon subgelatinosum	*RC 1609-URM93444*	MN475884	MN475888	Brazil	Alvarenga & Gibertoni 2021
Crystallodon subgelatinosum	*TBG BF-18001-URM93445*	MN475885	MN475889	Brazil	Alvarenga & Gibertoni 2021
Eichleriella bactriana	*TAAM 55071*	KX262121	KX262170	Russia	Malysheva & Spirin 2017
Eichleriella crocata	*TAAM 101077*	KX262100	KX262147	Russia	Malysheva & Spirin 2017
Eichleriella leucophaea	*Barsukova LE 303261*	KX262111	KX262161	Russia	Malysheva & Spirin 2017
Eichleriella tenuicula	*ValCB 1*	MK391515	MK391525	Brazil	Alvarenga *et al.* 2019
Elmerina cladophora	*Miettinen 14314*	MG757509	MG757509	Indonesia	Malysheva *et al.* 2018
Elmerina sclerodontia	*Miettinen 16431*	MG757512	MG757512	Malaysia	Malysheva *et al.* 2018
Exidia glandulosa	*YC Dai 21232*	MT663362	MT664781	China	Wu *et al.* 2020
Exidia glandulosa	*YC Dai 21233*	MT663363	MT664782	China	Wu *et al.* 2020
Exidia pithya	*MW 313*	AF291275	AF291321	Germany	Weiss & Oberwinkler 2001
Exidiopsis effusa	*OM 19136*	KX262145	KX262193	Finland	Malysheva & Spirin 2017
Grammatus labyrinthinus	*Yuan 1600*	KM379139	KM379140	China	Alvarenga *et al.* 2019
Grammatus semis	*OM10618*	KX262146	KX262194	China	Malysheva & Spirin 2017
Heteroradulum adnatum	*LR 23453*	KX262116	KX262165	Mexico	Tohtirjap *et al.* 2023
Heteroradulum deglubens	*LE 38182*	KX262112	–	Sweden	Malysheva & Spirin 2017
Heteroradulum deglubens	*Solheim 1864*	KX262133	KX262181	Norway	Malysheva & Spirin 2017
Heteroradulum kmetii	*VS 6466*	KX262104	KX262152	Russia	Malysheva & Spirin 2017
Heterochaete hirneoloides	*USJ 55480*	AF291283	AF291334	Germany	Weiss & Oberwinkler 2001
Hyalodon piceicola	*Spirin 2689*	MG735414	MG735422	Russia	Spirin *et al.* 2019b
Hyalodon piceicola	*Spirin 11063*	MG735415	MG735423	Russia	Spirin *et al.* 2019b
Metulochaete sanctae-catharinae	*AM 0678*	MK484065	MK480575	Russia	Spirin *et al.* 2019b
Mycostilla vermiformis	*Spirin 11330*	MG735417	MG735425	Russia	Spirin *et al.* 2019b
Mycostilla vermiformis	*OF 188059*	MG735418	–	Russia	Spirin *et al.* 2019b
Myxarium cinnamomescens	*OF160494*	KY801882	KY801909	Russia	Spirin *et al.* 2018
Myxarium grilletii	*VS9016*	MK098896	MK098944	Russia	Spirin *et al.* 2019a
Myxarium hyalinum	*TL2012 443455*	KY801880	KY801907	Russia	Spirin *et al.* 2018
Myxarium legonii	*VS 8986*	MK098899	MK098947	Russia	Spirin *et al.* 2019a
Proterochaete adusta	*CNOM 10519*	MK391519	–	Brazil	Alvarenga *et al.* 2019
Proterochaete adusta	*VS 9021*	MK391520	MK391528	Canada	Alvarenga *et al.* 2019
Protodaedalea foliacea	*Miettinen 13 054*	MG757507	MG757507	Finland	Malysheva *et al.* 2018

Table 1 (continued). List of species, specimens and GenBank accession numbers of sequences used in this study. New species is shown in **bold**; * = holotype.

Species Name	Sample No.	GenBank Accession No. ITS	GenBank Accession No. nLSU	Country	References
Protodaedalea hispida	*Spirin 5139*	MG757510	MG757510	Finland	Malysheva *et al.* 2018
Protodontia africana	*AS 171126 1104*	MK098978	MK098973	Russia	Spirin *et al.* 2019a
Protohydnum cartilagineum	*SP 467240*	MG735419	MG735426	Russia	Malysheva *et al.* 2018
Protomerulius dubius	*VS 3019*	MK484041	MK480553	Russia	Spirin *et al.* 2019b
Protomerulius minor	*KHL 15937*	MK484060	MK480569	Russia	Spirin *et al.* 2019b
Protomerulius substuppeus	*O 19171*	JX134482	JQ764649	China	Spirin *et al.* 2019b
Pseudohydnum gelatinosum	*F14063*	AF384861	AF384861	Canada	Weiss & Oberwinkler 2001
Pseudohydnum gelatinosum	*AFTOL ID1875*	DQ520094	DQ520094	Germany	Lutzoni *et al.* 2004
Punctochaete murina	***CLZhao 31670***	**PP819683**	**PP819698**	**China**	**Present study**
Punctochaete murina	***CLZhao 31873***	**PP819684**	**PP819699**	**China**	**Present study**
Punctochaete murina	***CLZhao 31880***	**PP819685**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 31985***	**PP819686**	**PP819700**	**China**	**Present study**
Punctochaete murina	***CLZhao 32023***	**PP819687**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 32113***	**PP819688**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 32118 ****	**PP819689**	**PP819701**	**China**	**Present study**
Punctochaete murina	***CLZhao 32189***	**PP819690**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 32347***	**PP819691**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 32814***	**PP819692**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 33101***	**PP819693**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 33389***	**PP819694**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 33403***	**PP819695**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 33703***	**PP819696**	–	**China**	**Present study**
Punctochaete murina	***CLZhao 33789***	**PP819697**	–	**China**	**Present study**
Sclerotrema griseobrunneum	*Niemela 2722*	KX262144	KX262192	Russia	Malysheva & Spirin 2017
Sclerotrema griseobrunneum	*Spirin 7674*	KX262140	KX262188	Russia	Malysheva & Spirin 2017
Stypellopsis farlowii	*Larsson 12337*	MG857095	MG857099	Russia	Spirin *et al.* 2018
Stypellopsis hyperborea	*J Norden 9751*	MG857097	MG857101	Russia	Spirin *et al.* 2018
Tremellochaete atlantica	*URM90199*	MG594381	MG594383	Brazil	Alvarenga *et al.* 2019
Tremellochaete japonica	*TAA 42689*	AF291274	AF291320	Russia	Weiss & Oberwinkler 2001
Tremiscus helvelloides	*AFTOL ID1680*	DQ520100	DQ520100	Germany	Lutzoni *et al.* 2004
Sistotrema brinkmannii	isolate 236	JX535169	JX535170	Netherlands	Alvarenga & Gibertoni 2021

characters were equally weighted, and gaps were treated as missing data. Using the heuristic search option with TBR branch swapping and 1000 random sequence additions, trees were inferred. 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.

jModelTest 2 (Darriba *et al.* 2012) was used to determine the best-fit evolutionary model for each data set for Bayesian inference (BI), which was performed using MrBayes ver.3.2.7a with a GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist *et al.* 2012). 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

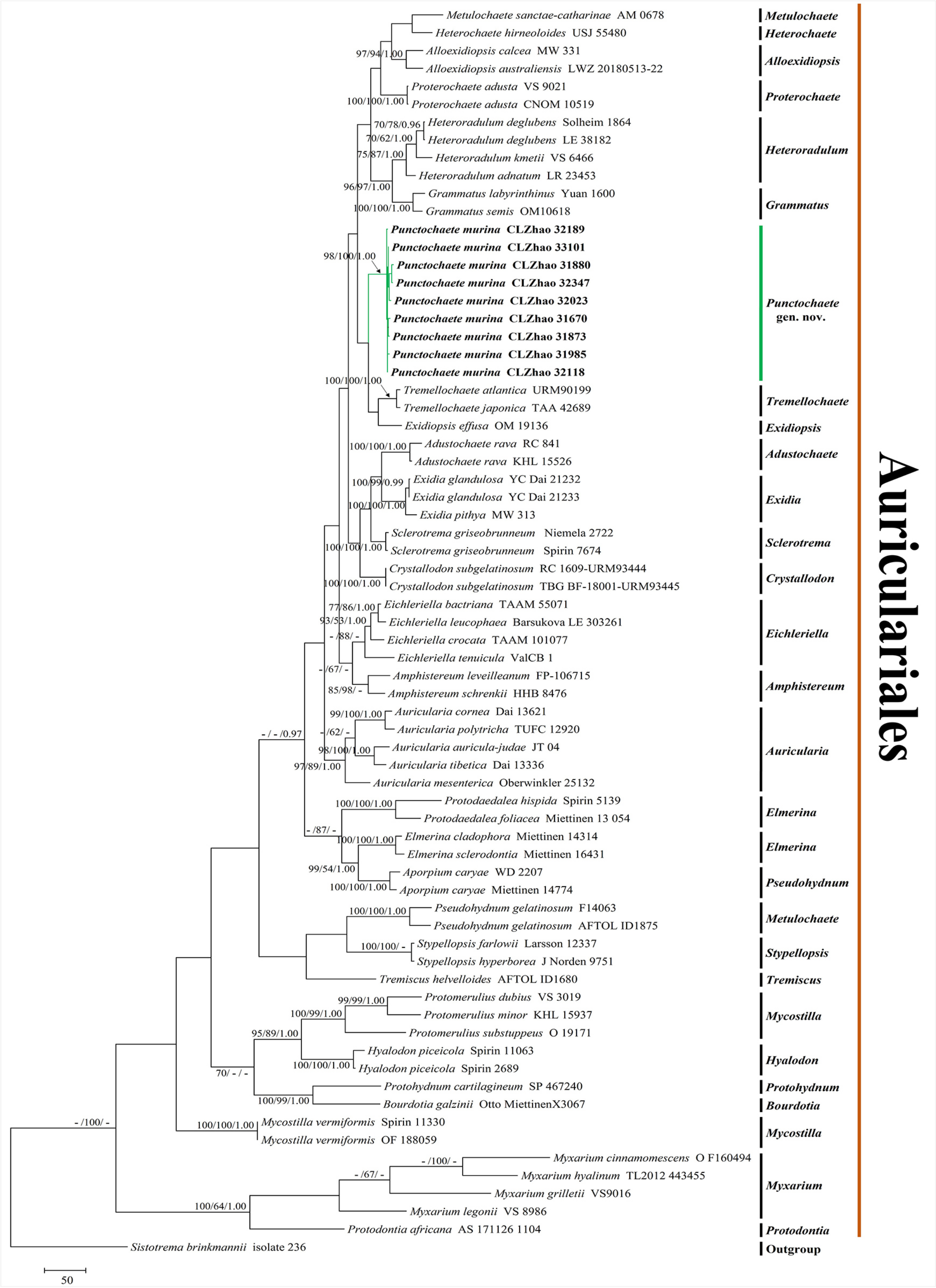

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Punctochaete* J.H.Dong & C.L.Zhao gen. nov. and related genera in the order Auriculariales Bromhead based on ITS + nLSU sequences. Branches are labeled with maximum likelihood bootstrap value ≥ 70%, Parsimony bootstrap value ≥ 50%, and Bayesian posterior probabilities ≥ 0.95.

a 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 aligned dataset comprised 70 specimens representing 52 species. Four Markov chains were run for 2 runs from random starting trees, each for 2.5 million generations for the combined ITS + nLSU data set with trees and parameters sampled every 1000 generations (Fig. 1). The dataset had an aligned length of 2273 characters, of which 1249 characters were constant, 387 were variable and parsimony uninformative, and 637 were parsimony informative. Maximum parsimony analysis yielded 24 equally parsimonious trees (TL = 4245, CI = 0.3972, HI = 0.6028, RI = 0.5209, and RC = 0.2069). The best model for the ITS + nLSU dataset, estimated and applied in the Bayesian analysis, was GTR+I+G (lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1)). Both Bayesian analysis and ML analysis resulted in a similar topology to MP analysis with an average standard deviation of split frequencies = 0.031877 (BI), and the effective sample size (ESS) for Bayesian analysis across the two runs was double the average ESS (avg ESS) = 309.5.

Phylogenetic analysis of combined ITS and nLSU sequences showed that the new genus *Punctochaete* gen. nov. forms a strongly supported monophyletic lineage (BS = 98%, BP = 100%, BPP = 1.00) within Auriculariales, closely related to *Exidiopsis* and *Tremellochaete* (Fig. 1).

Taxonomy

Phylum Basidiomycota R.T.Moore
Class Agaricomycetes Doweld
Order Auriculariales Bromhead
Family *incertae sedis*

Punctochaete J.H.Dong & C.L.Zhao gen. nov.
MycoBank: MB854043

Diagnosis

Punctochaete gen. nov. differs from other genera in the order Auriculariales by its membranaceous basidiomata, greyish white, punctate hymenial surface, monomitic hyphal system with clamp connections, subcylindrical to subclavate cystidia, frequently branched hyphidia, ellipsoid to ovoid, longitudinally septate basidia, and cylindrical to allantoid, slightly curved basidiospores.

Etymology

The name ‘*Punctochaete*’ refers to the punctate hymenial surface of the holotype.

Description

Basidiomata resupinate, membranaceous, very hard to separate from substrate. Hymenial surface punctate, greyish white when fresh, greyish white to cream to pale pink upon drying. Hyphal system monomitic; generative hyphae with clamp connections, colorless, thin-walled. Cystidia subcylindrical to subclavate, thin-walled. Hyphidia abundant, covering hymenium, frequently branched, thin-walled. Basidia ellipsoid to ovoid, longitudinally septate, two- to four-celled, colorless. Basidiospores cylindrical to allantoid, slightly curved, colorless, thin-walled, smooth, IKI–, CB–.

Punctochaete murina J.H.Dong & C.L.Zhao gen. et sp. nov.
MycoBank: MB854045
Figs 2–4

Diagnosis

Punctochaete murina gen. et sp. nov. is characterized by the membranaceous basidiomata with greyish white, smooth hymenial surface, monomitic hyphal system with clamp connections hyphae and cylindrical to allantoid, slightly curved basidiospores measuring 9–12 × 4.3–5.8 µm.

Etymology

The epithet '*murina*' refers to the murine hymenial surface of the holotype.

Type material

Holotype

CHINA – **Yunnan Province** • Zhaotong, Wumengshan National Nature Reserve; 28°03′ N, 104°20′ E; altitude 1500 m a.s.l.; 27 Aug. 2023; *CLZhao 32118*; on fallen angiosperm branches; GenBank nos: PP819689 (ITS), PP819701 (nLSU); SWFC [SWFCF00032118].

Paratypes

CHINA – **Yunnan Province** • same data as for holotype; 26 Aug. 2023; *CLZhao 31670*; on fallen angiosperm branches; GenBank nos: PP819683 (ITS), PP819698 (nLSU); SWFC [SWFCF00031670] • same data as for preceding; 27 Aug. 2023; *CLZhao 31873*; on fallen angiosperm branches; GenBank

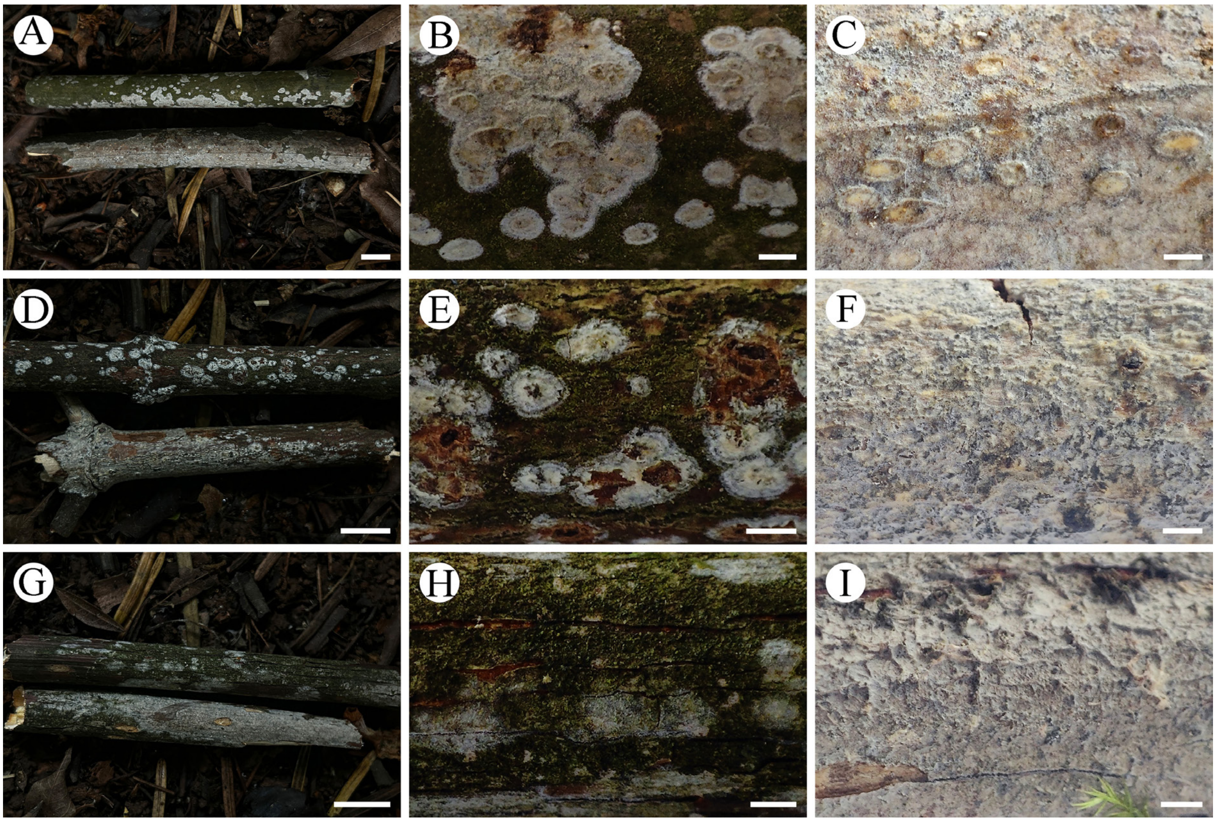

Fig. 2. Basidiomata of *Punctochaete murina* J.H.Dong & C.L.Zhao gen. et sp. nov. **A**–**C**. *CLZhao 32118* (holotype). **D**–**F**. *CLZhao 32189*. **G**–**I**. *CLZhao 31873*. Scale bars: A, D, G = 1 cm, B–C, E–F, H–I = 1 mm.

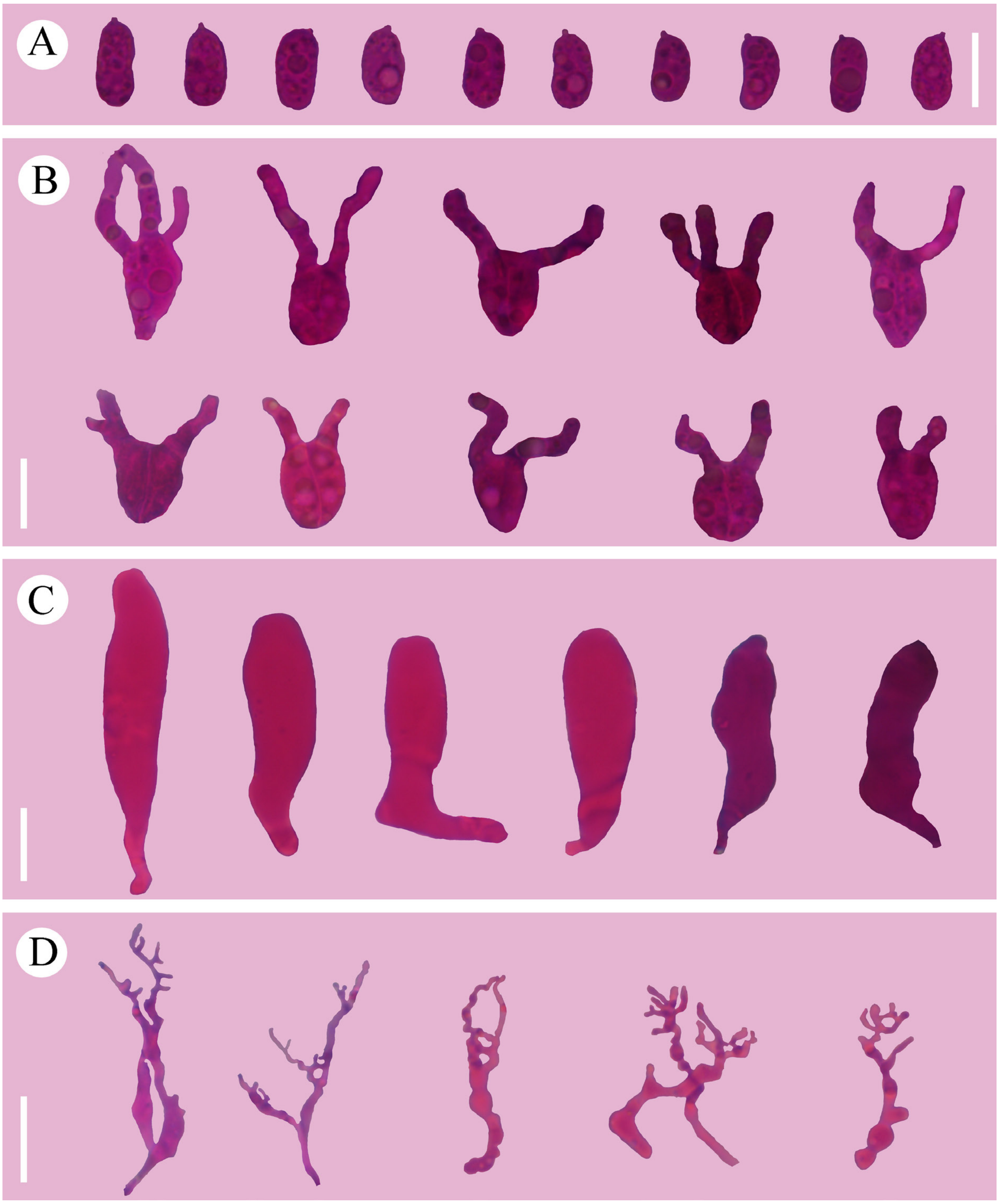

Fig. 3. Sections of hymenium of *Punctochaete murina* J.H.Dong & C.L.Zhao gen. et sp. nov. (holotype, *CLZhao 32118*). **A**. Basidiospores. **B**. Basidia. **C**. Cystidia. **D**. Hyphidia. Scale bars: A–D = 10 µm, 1000 × magnification with oil immersion.

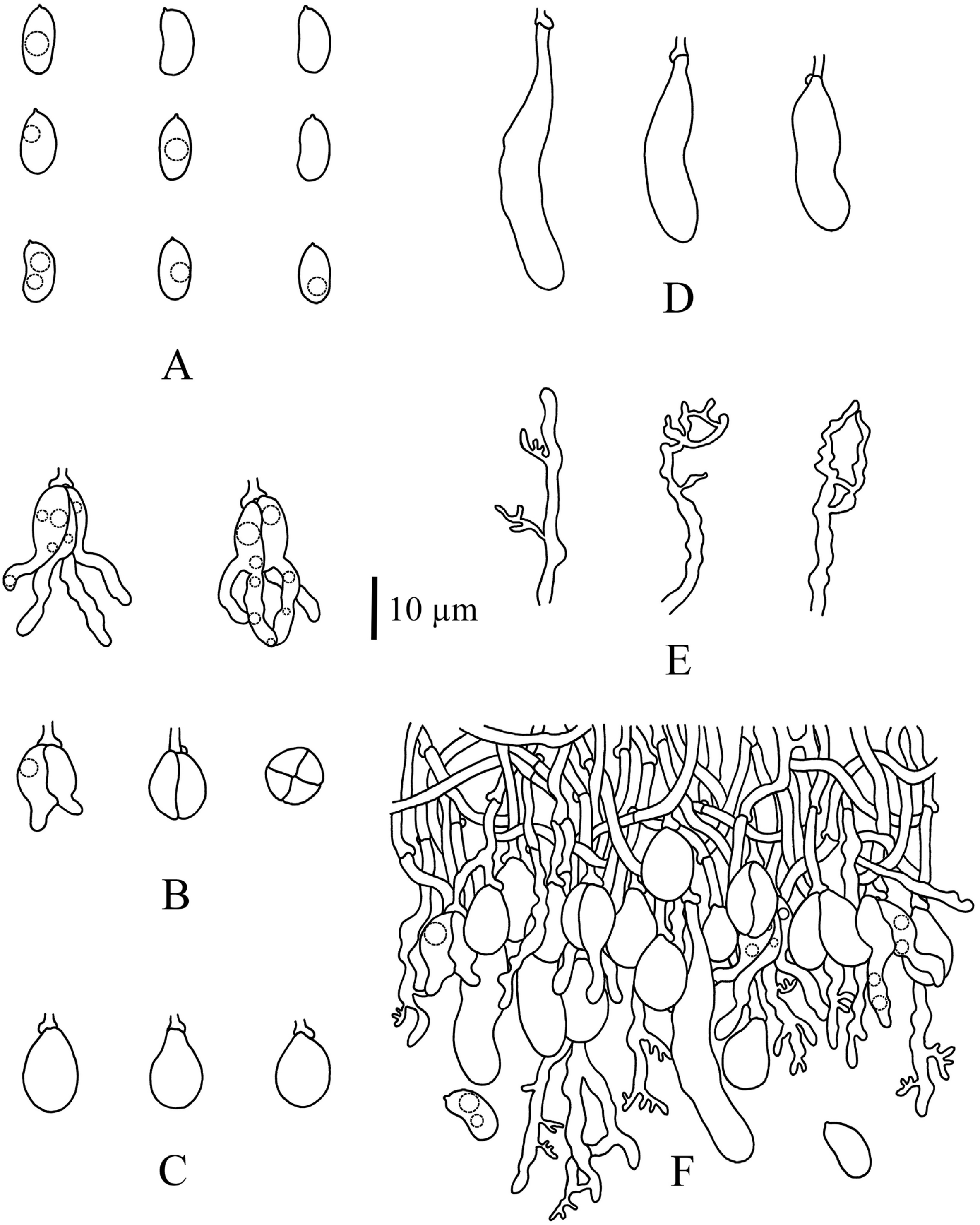

Fig. 4. Microscopic structures of *Punctochaete murina* J.H.Dong & C.L.Zhao gen. et sp. nov. (holotype, *CLZhao 32118*). **A**. Basidiospores. **B**. Basidia. **C**. Basidioles. **D**. Cystidia. **E**. Hyphidia. **F**. Part of the vertical section of hymenium.

nos: PP819684 (ITS), PP819699 (nLSU); SWFC [SWFCF00031873] • same data as for preceding; *CLZhao 31880*; on fallen angiosperm branches; GenBank no.: PP819685 (ITS); SWFC [SWFCF00031880] • same data as for preceding; *CLZhao 31985*; on fallen angiosperm branches; GenBank nos: PP819686 (ITS), PP819700 (nLSU); SWFC [SWFCF00031985] • same data as for preceding; *CLZhao 32023*; on fallen angiosperm branches; GenBank no.: PP819687 (ITS); SWFC [SWFCF00032023] • same data as for preceding; *CLZhao 32113*; on fallen angiosperm branches; GenBank no.: PP819688 (ITS); SWFC [SWFCF00032113] • same data as for preceding; 28 Aug. 2023; *CLZhao 32189*; on fallen angiosperm branches; GenBank no.: PP819690 (ITS); SWFC [SWFCF00032189] • same data as for preceding; *CLZhao 32347*; on fallen angiosperm branches; GenBank no.: PP819691 (ITS); SWFC [SWFCF00032347] • same data as for preceding; 29 Aug. 2023; *CLZhao 32814*; on fallen angiosperm branches; GenBank no.: PP819692 (ITS); SWFC [SWFCF00032814] • same data as for preceding; 19 Sep. 2023; *CLZhao 33101*; on fallen angiosperm branches; GenBank no.: PP819693 (ITS); SWFC [SWFCF00033101] • same data as for preceding; 20 Sep. 2023; *CLZhao 33389*; on fallen angiosperm branches; GenBank no.: PP819694 (ITS); SWFC [SWFCF00033389] • same data as for preceding; *CLZhao 33403*; on fallen angiosperm branches; GenBank no.: PP819695 (ITS); SWFC [SWFCF00033403] • same data as for preceding; 21 Sep. 2023; *CLZhao 33703*; on fallen angiosperm branches; GenBank no.: PP819696 (ITS); SWFC [SWFCF00033703] • same data as for preceding; *CLZhao 33789*; on fallen angiosperm branches; GenBank no.: PP819697 (ITS); SWFC [SWFCF00033789].

Description

Basidiomata annual, resupinate, closely adnate, membranaceous, very hard to separate from substrate, without odor or taste when fresh, becoming furfuraceous upon drying, up to 15 cm long, 3 cm wide, 50–100 µm thick. Hymenial surface smooth, punctate, greyish white when fresh, murine to cream to pale pink upon drying. Sterile margin distinct, greyish white, thinning out, up to 0.5 mm wide.

Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin-walled, branched, interwoven, 1.5–3 µm in diameter; IKI–, CB–, tissues unchanged in KOH. Cystidia numerous, thin-walled, subcylindrical to subclavate with an obtuse apex, occasionally sinuous in the basal position, 20.5–52.5 × 5–11 µm, with a clamp connection at base; cystidioles absent. Hyphidia arising from generative hyphae, nodulose, frequently branched, colorless, thin-walled, 1–4 µm in diameter. Basidia ellipsoid to ovoid, longitudinally septate, two to four-celled, 10.5–15.5 × 8–11 µm; basidioles dominant, similar to basidia in shape, but slightly smaller. Basidiospores cylindrical to allantoid, slightly curved, colorless, smooth, thin-walled, with 1–2 oil drops, IKI–, CB–, (8.5–)9–12(–12.5) × (4–)4.3–5.8(–6) µm, L = 10.49 µm, W = 4.96 µm, Q = 2.03–2.19, Q_m = 2.13 ± 0.18 (n = 150/5).

Discussion

In the present study, a new genus, *Punctochaete* gen. nov., is described based on phylogenetic analyses and morphological characteristics.

The corticioid species of the order Auriculariales were traditionally placed in *Eichleriella*, *Exidiopsis*, and *Heterochaete* based on morphological characteristics (Liu *et al.* 2022). Recent taxonomic revisions have led to the establishment of six new genera to place corticioid species (*Adustochaete*, *Alloexidiopsis*, *Amphistereum*, *Crystallodon*, *Proterochaete* and *Sclerotrema*) and reinstatement of three previously known genera (*Hirneolina*, *Heteroradulum* and *Tremellochaete*) (Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Alvarenga & Gibertoni 2021; Liu *et al.* 2022). A multilocus-based phylogeny with a wider sampling of various morphological groups in Auriculariales is urgently needed to achieve a more natural classification of this order, as in other orders within Agaricomycetes (Wang *et al.* 2021). Our result based on the combined ITS and nLSU sequence data (Fig. 1) reveals a new genus that forms a monophyletic lineage (98% BS, 100% BP, 1.00 BPP) within the order Auriculariales. Therefore, an additional genus is proposed here with description and illustrations based on the morphological characteristics and phylogenetic analyses.

Phylogenetically, the new genus *Punctochaete* gen. nov. forms a monophyletic lineage and is closely related to *Exidiopsis* and *Tremellochaete* in the ITS + nLSU analyses based on the phylogeny tree (Fig. 1). However, *Exidiopsis* differs morphologically from *Punctochaete* in having effused or effused-reflexed, waxy gelatinous, arid waxy or coriaceous basidiomata, with a hymenial surface that is smooth or with sterile spines, and cylindrical, clavate or fusiform cystidia (Möller 1895). The other genus, *Tremellochaete*, differs from *Punctochaete* in having gelatinous basidiomata and densely papillated hymenial surface (Raitviir 1964; Tohtirjap *et al.* 2023).

Morphologically, *Punctochaete* gen. nov. resembles eight similarly corticioid genera in the order Auriculariales, viz. *Adustochaete*, *Alloexidiopsis*, *Amphistereum*, *Crystallodon*, *Heteroradulum*, *Metulochaete*, *Proterochaete* and *Sclerotrema* (Table 2).

Fungi are one of the most diverse groups of organisms on earth and play a crucial role in ecosystem processes and functions (Hyde 2022). New DNA sequencing techniques have revolutionized the research of fungal taxonomy and diversity, in which about 165 000 species of fungi have been described (Hyde *et al.* 2024). In recent years, the wood-inhabiting fungi have become an extensively studied group of Basidiomycota, which includes a number of poroid, smooth, grandinoid, odontioid and hydnoid basidiomata in China (Dai *et al.* 2021; Ji *et al.* 2021; Wu *et al.* 2022a, 2022b; Dong *et al.* 2023a, 2023b; Duan *et al.* 2023; Liu *et al.* 2023; Mao *et al.* 2023; Yang *et al.* 2023, 2024; Yu *et al.* 2023; Yuan *et al.* 2023; Li *et al.* 2024; Zhang *et al.* 2024; Zhao *et al.* 2024; Zhou *et al.* 2024). Up to now, several corticioid genera have been reported and described in the order Auriculariales (Malysheva & Spirin 2017; Alvarenga *et al.* 2019; Alvarenga & Gibertoni 2021; Liu *et al.* 2022), but many new taxa remain undiscovered. So, the diversity of corticioid species in the order Auriculariales is still not well known in China, especially in the subtropical and tropical areas. This paper enriches our knowledge of the fungal diversity in this area. We anticipate that more undescribed corticioid taxa will be discovered throughout China after extensive collection combined with morphological and molecular analyses.

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Authors' contributions

Conceptualization, C.Z.; methodology, C.Z. and J.D.; software, C.Z. and J.D.; validation, C.Z. and J.D.; formal analysis, C.Z., J.D. and Q.L.; investigation, C.Z., J.D.; resources, C.Z., J.D. and Q.L.; writing – original draft preparation, C.Z., J.D., Q.L. and Q.S.; writing – review and editing, C.Z. and J.D.; visualization, C.Z. and J.D.; supervision, C.Z.; project administration, C.Z.; funding acquisition, C.Z. All authors have read and agreed to the published version of the manuscript.

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Table 2. Morphological comparison between *Punctochaete* J.H.Dong & C.L.Zhao gen. nov. and related corticioid genera in Auriculariales Bromhead.

Genus name	Basidiomata	Hymenial surface	Hyphal system	Basidiospores	Cystidia	Hyphidia	References
Adustochaete	Annual, small-sized, orbicular, waxy	Spiny or tuberculate, grayish to brownish	Monomitic	Cylindrical to broadly cylindrical, straight or curved	Clavate to fusiform, thin-walled	Variably branched	Alvarenga *et al.* 2019
Alloexidiopsis	Annual, effused, leathery	Smooth or with sterile spines, more or less grayish	Monomitic	Cylindrical to broadly cylindrical, slightly curved	Cylindrical to clavate, thin-walled	Nodulose or richly branched	Liu *et al.* 2022
Amphistereum	Annual or perennial, cupulate-orbicular, hard leathery	Smooth, pale	Dimitic	Cylindrical to broadly cylindrical, slightly curved	Rare, narrowly clavate, thin-walled	Richly branched	Malysheva & Spirin 2017
Crystallodon	Annual, effused, gelatinous to crustaceous	Covered by sharp-pointed sterile spines, brownish	Monomitic	Cylindrical to broadly cylindrical, slightly curved	Fusiform to cylindrical, often sinuous, thin-walled	Branched	Alvarenga & Gibertoni 2021
Heteroradulum	Annual or perennial, effused-reflexed, leathery	Smooth, with sterile spines, pinkish or reddish	Mono- or dimitic	Cylindrical to broadly cylindrical, sometimes curved	Clavate to fusiform, thin- to thick-walled	Richly branched	Malysheva & Spirin 2017
Metulochaete	Effused, gelatinous to waxy-arid	Smooth or covered by sterile spines, pale	Monomitic	Allantoid, straight to slightly curved	Metuloid, covering hymenial spines, thick-walled	Richly branched	Alvarenga *et al.* 2019
Proterochaete	Annual, orbicular, arid	Smooth or irregularly spiny, creamy to grayish or pale ochraceous	Monomitic	Cylindrical to broadly cylindrical, slightly curved	Occasional, sinuous, accidentally dichotomously branched, thin-walled	Richly or sparsely branched	Alvarenga *et al.* 2019
Punctochaete	Resupinate, closely adnate, membranaceous	Smooth, punctate, greyish white	Monomitic	Cylindrical to allantoid, slightly curved	Subcylindrical to subclavate, thin-walled	Nodulose or richly branched	**Present study**
Sclerotrema	Perennial, orbicular, leathery	Smooth or irregularly spiny, creamy to grayish or pale ochraceous	Monomitic	Allantoid, distinctly curved	Hyphoid to fusiform, thick-walled	Richly branched	Malysheva & Spirin 2017

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