Two new species and a new record of *Astrocystis* from Southwestern China

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Abstract

In this study, three species of *Astrocystis* were collected from bamboo culms in Guizhou and Yunnan Provinces in China. Morphological characteristics and phylogenetic analyses (based on ITS, *rpb2*, β-tubulin and α-actin gene regions) supported the proposal of two new species *viz*., *Astrocystis pseudomirabilis* sp. nov. and *A. tessellati* sp. nov. and a new record of *A. sublimbata* from China. *Astrocystis pseudomirabilis* can be distinguished from *A. mirabilis* by its larger stromata (0.64–0.83 × 0.38–0.52 mm vs. 0.4–0.6 × 0.2–0.5 mm) and larger ascospores (11–14 × 6–8 µm vs. 10–12 × 4.5–6 µm). *Astrocystis tessellati* differs from *A. multiloculata* by its smaller ascospores (16.2–19.2 × 7.2–9 µm vs. 19–25 × 7–11 µm). *Astrocystis pseudomirabilis* and *A. tessellati* form two distinct clades within *Astrocystis*.

Keywords: bamboo, multi-gene, phylogeny, taxonomy, *Xylariaceae*

Li Q-R, Wu Y-P, Pi Y-H, Long S-H, Wijayawardene NN, Suwannarach N, Liu L-L (2022) Two new species and a new record of *Astrocystis* from Southwestern China. MycoAsia 2022/08.

Received: 25.08.2022 | Accepted: 28.08.2022 | Published: 28.09.2022

Handling Editor: Dr. Belle Damodara Shenoy

Reviewers: Dr. Gunjan Sharma, Dr. K. C. Rajeshkumar

Introduction

The family *Xylariaceae* Tul. & C. Tul. was introduced in 1863 (Tulasne and Tulasne 1863). It is one of the largest families of *Sordariomycetes*, which shows high diversity in tropical regions and comprises the highest number of bioactive secondary metabolite producers (Kuhnert et al. 2014, Maharachchikumbura et al. 2015, 2016, Senanayake et al. 2015, Daranagama et al. 2018, Wendt et al. 2018). In an updated classification of *Xylariaceae*, 30 genera were accepted by Wijayawardene et al.

(2021).

Astrocystis Berk. & Broome was introduced by Berkeley and Broome (1873) from dead culms of bamboo and *A. mirabilis* Berk. & Broome was designated as the type species. This genus is mostly confined to monocotyledonous plants and has uni- or rarely multi-peritheciate stromata, which may develop beneath the host cuticle and appear superficial. The asci have a relatively short stipe and the ascus apical apparatus is relatively small, amyloid and stopper-shaped (Smith et al. 2003). Morphologically, *Astrocystis* resembles *Collodiscula* Hino & Katumoto which has superficial carbonaceous stromata, and most of them were discovered from bamboo; meanwhile, the multi-gene analysis indicated that both genera have closer affinities in *Xylariaceae* (Li et al. 2015a, b, Hyde et al. 2017, Wu et al. 2021). But, species of *Collodiscula* have larger, one to multi-septate ascospores with a germ slit (except *C. leigongshanensis* Q.R. Li et al.) (Xie et al. 2020). Currently, more than 20 species of *Astrocystis* have been reported, of which *A. bambusicola* R.H. Perera & K.D. Hyde, *A. multiloculata* Y.P. Wu & Q.R. Li and *A. sinensis* Joanne E. Taylor et al. have been reported from China (Taylor and Hyde 2003, Hyde et al. 2019, Wu et al. 2021).

During an investigation of *Xylariaceae* taxa in southwestern China, two new species of *Astrocystis* and *A. sublimbata* (a new record from China) were collected in Guizhou and Yunnan Provinces in P. R. China. Morphological descriptions, illustrations and a phylogenetic tree to show the placements of the new collections are provided.

Materials and methods

Collection, isolation and specimen examination

Specimens were collected in Guizhou and Yunnan provinces of China. A Nikon SMZ 745 series stereomicroscope was used to observe the structure of stromata and photograph using a Canon 700D digital camera. Microphotography was carried out using a light microscope (Nikon) and digital camera (Canon 700D). More than 30 asci, ascospores and ascus apical apparatus were measured using the Tarosoft image framework (v. 0.9.0.7) for each specimen. All figures were processed with Adobe Photoshop CS6. Pure cultures were obtained using the single spore isolation method described in Daranagama et al. (2018). Materials were deposited at the Herbarium of Guizhou Medical University (GMB), Herbarium of Kunming Institute of Botany (KUN) or Guizhou Agricultural College (GACP). Living cultures grown on potato dextrose agar (PDA) or oat agar (OA) have been deposited in the Guizhou Medical University Culture Collection (GMBC).

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Genomic DNA was extracted from fungal mycelium using the BIOMIGA fungal genomic DNA extraction kit (GD2416, BIOMIGA, USA) following the manufacturer's protocol. DNA extraction was done from ascomata which were picked out with the help of a stereomicroscope using Lysis Buffer for Microorganism to Direct PCR kit (TAKARA RR047A, China). The internal transcribed spacer (ITS), partial RNA polymerase II second largest subunit (*rpb2*), partial β-tubulin (*tub2*) and partial α-actin gene (*act*) loci were subjected to PCR amplification and sequencing using specific primers and PCR conditions (Table 1). Amplified PCR fragments were sequenced by Sangon Biotech Co. (Shanghai). Sequences derived from this study were deposited at the NCBI GenBank nucleotide database (http://www.ncbi.nlm.nih.gov).

Table 1 Details of genes/loci with PCR primers and PCR conditions

Gene region	Primers	PCR conditions	Reference
ITS	ITS4/ITS5	a; 95 °C for 20 s, 52 °C for 30 s, 72 °C for 30 s (34 cycles); d	White et al. (1990)
rpb2	fRPB2-5F/ fRPB2-7cr	a; 95 °C for 15 s, 54 °C for 20 s, 72 °C for 45 s (35 cycles); d	Liu et al. (1999)
*tub*2	T1/T22	b; 95 °C for 20 s, 56.5 °C for 20 s, 72 °C for 30 s (35 cycles); d	O'Donnell and Cigelnik (1997)
act	ACT512F/ACT783R	c; 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s (35 cycles); d	Carbone and Kohn (1999)

Note: a, Initial denaturation step of 95 °C: 1 min. b, Initial denaturation step of 95 °C: 2 min. c, Initial denaturation step of 94 °C: 3 min. d, Final elongation step of 72 °C: 7 min and final hold at 12 °C.

Phylogenetic analyses

Sequence alignments for phylogenetic analyses were produced with BioEdit v. 7.2.6 and MAFFT (http://mafft.cbrc.jp/alignment/server/) (Hall 2006). The ITS, *rpb*2, *tub*2 and α-actin data matrices were combined for subsequent phylogenetic analyses. The sequences (Table 2) used for analyses were retrieved from NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) results and some relevant studies (Li et al. 2015a, b, Wendt et al. 2018, Xie et al. 2020, Wu et al. 2021). After exclusion of ambiguously aligned regions and long gaps, the final combined data matrix contained 3231 characters (1-444 nucleotides of ITS, 445-1466 nucleotides of *rpb*2, 1467-2348 nucleotides of *tub*2 and 2349-3231 nucleotides of α-actin).

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012) using the ML + rapid boot-strap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the different gene regions. Bayesian analyses were performed using MrBayes v3.2.2 (Huelsenbeck 2012). The model of evolution was performed by using MrModeltest v.2.3 (Posada and Crandall 1998). Posterior probabilities (PP) (Rannala and Yang 1996) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes. Six simultaneous markov chains were run for 2,000,000 generations and trees were sampled every 1000th generation. The first 25 % of trees were discarded as the burn-in phase of each analysis. The phylogenetic tree was visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/Figtree/) (Rambaut 2006) and edited using Adobe Photoshop CS6.

Results

Molecular analyses

The combined multi-locus alignment consisted of 56 taxa for representative species in *Xylariales*, including outgroup taxa with 3231 characters, of which 222 characters were variable, 1766 characters were constant, and 1243 parsimony-informative characters.

Table 2 Names, strain numbers, references, and corresponding GenBank accession numbers of the sequences used in phylogenetic analysis

Species	Strain number	GenBank accession numbers				References
		ITS	*rpb2*	*tub2*	*act*	
Amphirosellinia fushanensis	HAST 91111209[HT]	GU339496	GQ848339	GQ495950	GQ452360	Hsieh et al. (2010)
A. nigrospora	HAST 91092308[HT]	GU322457	GQ848340	GQ495951	GQ452361	Hsieh et al. (2010)
Astrocystis bambusae	HAST 89021904	GU322449	GQ844836	GQ495942	GQ449239	Hsieh et al. (2010)
A. bambusicola	MFLUCC:17-0127[HT]	MF467942	MF467946	N/A	N/A	Hyde et al. (2017)
A. cocoes		AY862571	N/A	N/A	N/A	Bahl et al. (2005)
A. concavispora	MFLUCC:14-0174[HT]	KP297404	KP340532	KP406615	N/A	Daranagama et al. (2015)
A. mirabilis	HAST 94070803	GU322448	GQ844835	GQ495941	GQ449238	Hsieh et al. (2010)
A. mirabilis	MFLUCC11-0636	KU940154	N/A	N/A	N/A	Dai et al. (2017)
A. multiloculata	GMB0032[HT]	MW732438	MW755331	MW755337	MW755346	Wu et al. (2021)
A. multiloculata	GMB0033	MW732439	MW755330	MW755336	MW755347	Wu et al. (2021)
A. pseudomirabilis	GMB0122[HT]	ON471845	ON462000	ON461996	ON462005	This study
A. pseudomirabilis	GMB0123	ON471846	ON462001	ON461997	ON462006	This study
A. pseudomirabilis	GACP QR21037	ON471847	ON462002	ON461998	ON462007	This study
A. sublimbata	CBS130006	MH865618	N/A	N/A	N/A	Vu et al. (2018)
A. sublimbata	GACP QR21028	ON471848	N/A	ON461999	ON462008	This study
A. sublimbata	HAST 89032207	GU322447	GQ844834	GQ495940	N/A	Hsieh et al. (2010)
A. tessellati	GMB0120[HT]	ON471849	ON462003	ON461994	ON462009	This study
A. tessellati	GMB0121	ON471850	ON462004	ON461995	ON462010	This study
A. thailandica	MFLUCC15-0009[HT]	KU246224	N/A	N/A	N/A	Li et al. (2016)
Biscogniauxia atropunctata	YMJ 128	JX507799	JX507778	AY951673	AY951785	Hsieh et al. (2005, 2010)

B. nummularia	MUCL 51395[ET]	KY610382	KY624236	KX271241	N/A	Wendt et al. (2018)
Camillea obularia	ATCC 28093	KY610384	JX507790	JX507795	N/A	Hsieh and Ju (direct submission)
Collodiscula bambusae	GZUH0102[HT]	KP054279	KP276675	KP276674	N/A	Li et al. (2015b)
C. chiangraiensis	MFLUCC16-1193[HT]	MF614126	N/A	N/A	N/A	Hyde et al. (2017)
C. fangjingshanensi	GZUH0109[HT]	KR002590	KR002592	KR002589	N/A	Li et al. (2015b)
C. japonica	CBS 124266	JF440974	JF440974	KY624273	KY624316	Jaklitsch and Voglmayr (2012)
C. lancangjiangensis	GMB0030[HT]	MW732442	N/A	MW755343	MW755348	Wu et al. (2021)
C. lancangjiangensis	GMB0031	MW732443	N/A	MW755342	MW755349	Wu et al. (2021)
C. leigongshanensis	GZUH0107[HT]	KP054281	KR002588	KR002587	N/A	Li et al. (2015b)
C. tubulosa	GACP QR0111[HT]	MN017302	MN018403	MN018405	MN018402	Xie et al. (2020)
C. tubulosa	GACP QR0097	MN017308	MN018404	MN018406	N/A	Xie et al. (2020)
Daldinia loculatoides	CBS 113279[ET]	AF176982	KY624247	KX271246	N/A	Han and Ma (2005)
D. macaronesica	CBS 113040[PT]	KY610398	KY624294	KX271266	N/A	Wendt et al. (2018)
Dematophora buxi	JDR 99	GU300070	GQ844780	GQ470228	GQ398228	Hsieh et al. (2010)
D. necatrix	CBS 349.36	AY909001	KY624275	KY624310	N/A	Pelaez et al. (2008)
Graphostroma platystomum	CBS 270.87[HT]	JX658535	KY624296	HG934108	HG934109	Wendt et al. (2018)
Hypoxylon rickii	MUCL 53309[ET]	KC968932	KY624281	KC977288	N/A	Kuhnert et al. (2014)
Kretzschmaria deusta	CBS 163.93	KC477237	KY624227	KX271251	N/A	Stadler et al. (2013)
K. guyanensis	HAST 89062903	GU300079	GQ844792	GQ478214	GQ408901	Hsieh et al. (2010)
Kretzschmariella culmorum	JDR 88	KX430043	KX430045	KX430046	KX430044	Johnston et al. (2016)
Nemania abortiva	BISH 467[HT]	GU292816	GQ844768	GQ470219	GQ374123	Hsieh et al. (2010)
N. sphaeriostoma	JDR 261	GU292821	GQ844774	GQ470224	GQ389696	Hsieh et al. (2010)
Podosordaria mexicana	176 WSP	GU324762	GQ853039	GQ844840	GQ455451	Hsieh et al. (2010)

P. muli	WSP 167[ET]	GU324761	GQ853038	GQ844839	GQ455450	Hsieh et al. (2010)
Poronia pileiformis	WSP 88113001[ET]	GU324760	GQ853037	GQ502720	GQ455449	Hsieh et al. (2010)
P. punctate	CBS 656.78[HT]	KT281904	KY624278	KX271281	N/A	Senanayake et al. (2015)
Stilbohypoxylon elaeicola	YMJ 173	EF026148	GQ844826	EF025616	EF025601	Hsieh et al. (2010)
S. elaeicola	HAST 94082615	GU322440	GQ844827	GQ495933	GQ438754	Hsieh et al. (2010)
S. quisquiliarum	YMJ 172	EF026119	GQ853020	EF025605	EF025590	Hsieh et al. (2010)
Xylaria adscendens	JDR 865	GU322432	GQ844818	GQ487709	GQ438746	Hsieh et al. (2010)
X. arbuscula	CBS 126415	KY610394	KY624287	KX271257	N/A	Fournier et al. (2011)
X. bambusicola	WSP 205[HT]	EF026123	GQ844802	AY951762	AY951873	Hsieh et al. (2010)
X. compuncta	CBS 359.61	KT281903	KY624230	KX271255	N/A	Senanayake et al. (2015)
X. curta	HAST 494	GU322444	GQ844831	GQ495937	GQ449233	Hsieh et al. (2010)
X. discolor	YMJ 1280[ET]	JQ087405	JQ087411	JQ087414	JQ087408	Hsieh et al. (2010)
X. hypoxylon	CBS 122620[ET]	KY610407	KY624231	KX271279	N/A	Sir et al. (2016)
X. polymorpha	MUCL 49884	KY610408	KY624288	KX271280	N/A	Wendt et al. (2018)

Notes: Type specimens are labeled with **HT** (holotype), **ET** (epitype), and **PT** (paratype). **N/A**: not available; **ATCC**: American Type Culture Collection; **CBS**: Westerdijk Fungal Biodiversity Institute (CBS-KNAW Fungal Biodiversity Centre), Utrecht, The Netherlands; **GACP**: Guizhou Agricultural College, China; **GMB**: Herbarium of Guizhou Medical University, China; **GZUH**: Herbarium of Guizhou University; **HAST**: herbarium, research Center for Biodiversity, Academia Sinica, Taipei; **JDR**: Herbarium of Jack D. Rogers; **MFLUCC**: Mae Fah Luang university Culture Collection, Thailand; **MUCL**: University Catholique de Louvain; **WSP**: Washington State University, U.S.A; **YMJ**: Herbarium of Yu-Min Ju; **Others**: information not available.

Daldinia loculatoides (CBS 113040) and *Hypoxylon rickii* (MUCL 53309) were selected as the outgroup taxa. RAxML bootstrap support value ≥75 % and Bayesian posterior probabilities (BYPP) value ≥0.90 are shown above the branches.

In the phylogenetic tree (Figure 1), *Astrocystis pseudomirabilis* formed a sister clade of *A. mirabilis* Berk. & Broome with high statistical support (100 % ML, 1 BYPP). *Astrocystis tessellati* formed an independent branch in the *Astrocystis* clade. Strain GACP QR21028 clustered with *A. sublimbata* CBS 130006 with high statistical support (Figure 1).

Taxonomy

Astrocystis pseudomirabilis Y.P. Wu & Q.R. Li, **sp. nov**. (Figs. 2 & 3)
MycoBank number: MB 843997

Type: CHINA. Guizhou Province: Dushan County, Mawei Village (25.333648 °N, 107.463978 °E), 860 msl, on dead bamboo culms, 24 November 2021, Youpeng Wu, 2021MWC1-1 (GMB0122, **holotype**; ex-type GMBC0122); *ibid* (KUN-HKAS123438, **isotype**).

Etymology: Refers to the morphological similarity of the new taxon to *A. mirabilis*.

Saprobic on the surface of dead bamboo culms, visible as black raised dots on the host. **Sexual morph**: ***Stromata*** 0.64–0.83 mm diam., 0.38–0.52 mm high, superficial, hexagonal prism-shaped, flat top, blackened, carbonaceous, containing 1–2 perithecia, surface smooth or ectostroma splitting into lobes forming a star-like pattern. ***Perithecia*** 370–480 µm diam., 330–410 µm high, comprising black, fragile, carbonaceous tissue. ***Asci*** 128–156 × 8–11 µm ($\bar{x}$ = 146 × 9.3 µm, n = 30), 8-spored, unitunicate, cylindrical, with a short pedicel, persistent, apically rounded, with a J+, square-shaped apical apparatus, blue in Melzer's reagent, 2.5–4 µm ($\bar{x}$ = 3.2 µm, n = 30) high, 3–3.5 µm ($\bar{x}$= 3.3 µm, n = 30) wide. ***Ascospores*** 11–14 × 6–8 µm ($\bar{x}$= 12.4 × 7 µm, n = 30), uniseriate, ellipsoid, aseptate, brown to dark brown, guttulate, smooth-walled, with a straight germ slit slightly nearly full spore-length, lacking appendages and sheaths. **Asexual morph: coelomycetous; *Conidiomata*** globose, solitary, black. ***Conidiogenous cells*** 11.4–19.2 × 4.6–7.3 µm ($\bar{x}$= 15.6 × 6 µm, n = 10), enteroblastic, phialidic, terminal, cylindrical, hyaline, aseptate, smooth-walled. ***Conidia*** 6.4–7.6 × 4.2–4.5 µm ($\bar{x}$= 7.3 × 4.3 µm, n = 30), ellipsoidal, hyaline, smooth, with flattened bases.

Culture characteristics: Colonies on OA reaching 4–5 cm diam. after 2 weeks at 25 °C. White at first, cottony, mycelium superficial or immersed in media, circular, edge irregular, white from above, reverse similar in colour. After 6 weeks, colonies on OA covered 9 cm Petri dish, white, velvety, appressed with entire margins. Reverse uncoloured. Sporulation after 8 weeks**.**

Additional specimens examined: CHINA, Guizhou Province: Dushan County, Mawei Village (25.333686°N, 107.463988°E), 872 msl, on dead bamboo culms, 24 November 2021, Youpeng Wu, 2021MWC1-2 (GMB0123; KUN-HKAS 123439; living culture, GMBC0123); CHINA. Guizhou Province: Guiyang City, Guiyang Forest Park (26.234628°N, 106.468462°E), 1163 msl, on dead bamboo culms, 16 June 2021, Qirui Li, 2021GZ60 (GACP QR21037).

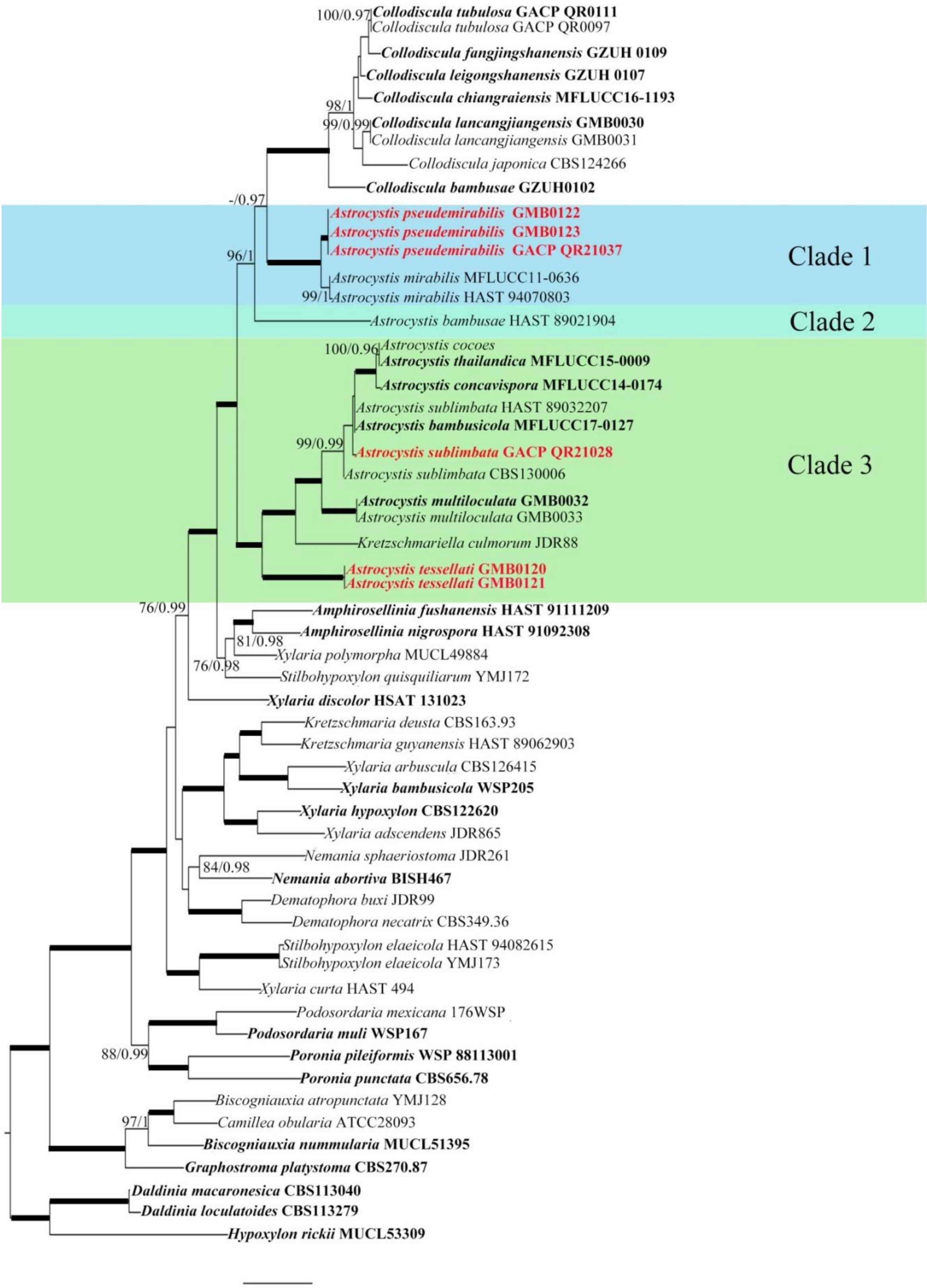

Figure 1 RAxML tree based on analysis of a combined dataset of ITS–*rpb2*–β-tubulin–α-actin sequences from selected species of *Xylariales*. Branches with 100 % ML and 1.00 BYPP are shown in black bold. Type strains are in black bold. New strains are in red bold.

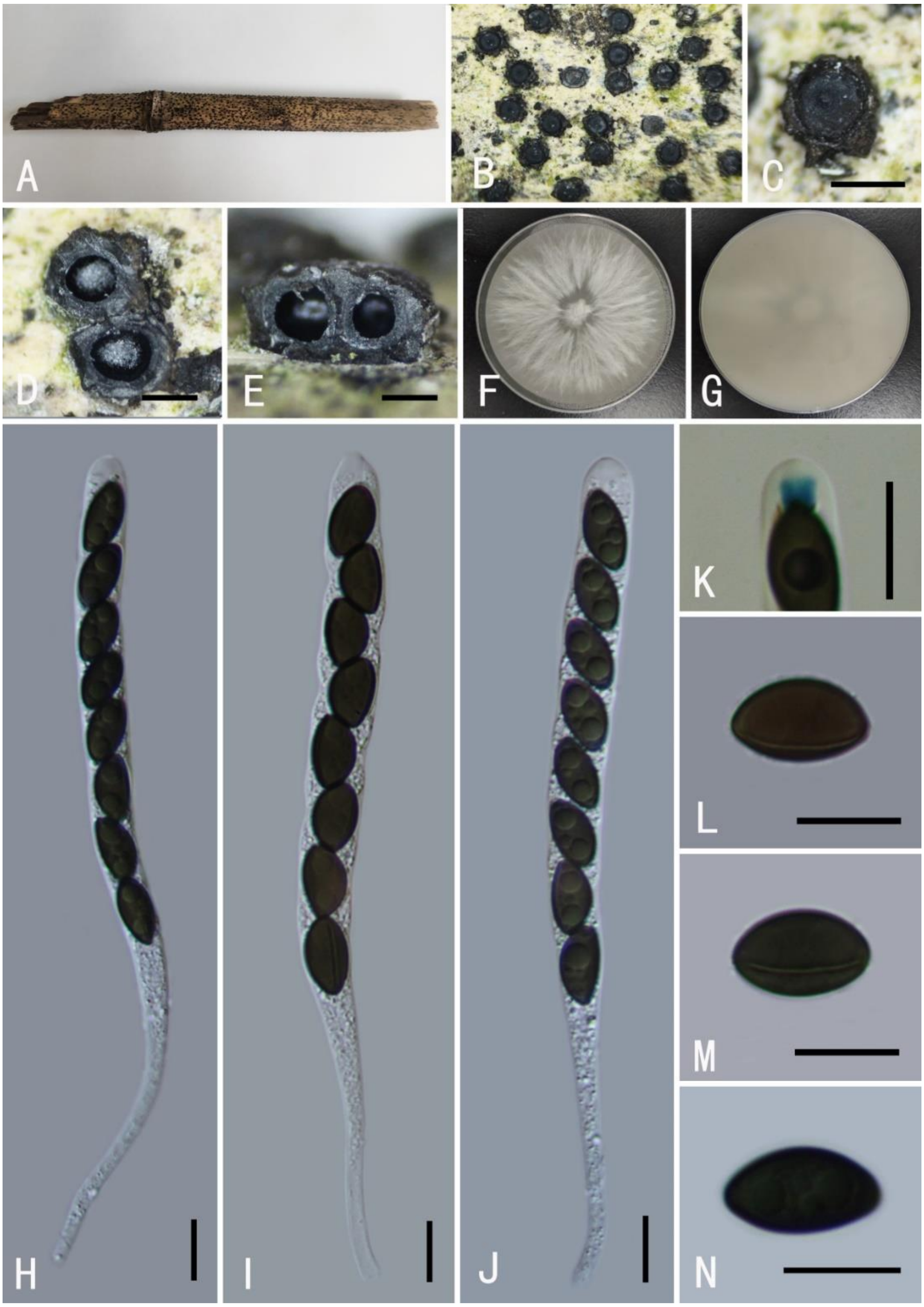

Figure 2 Sexual morph of ***Astrocystis pseudomirabilis*** (GMB0122, **holotype**). A, B. Stromata on the surface of the host. C. Close-up of a stroma surface. D. Transverse section of stromata. E. Longitudinal section of a stroma. F. Culture on OA from obverse. G. Culture on OA from reverse. H–J. Asci with ascospores. K. Ascus apex with a J+, apical apparatus (stained in Melzer's reagent). L–N. Ascospores. Scale bars: C= 200 µm, D, E = 150 µm, H–N = 10 µm.

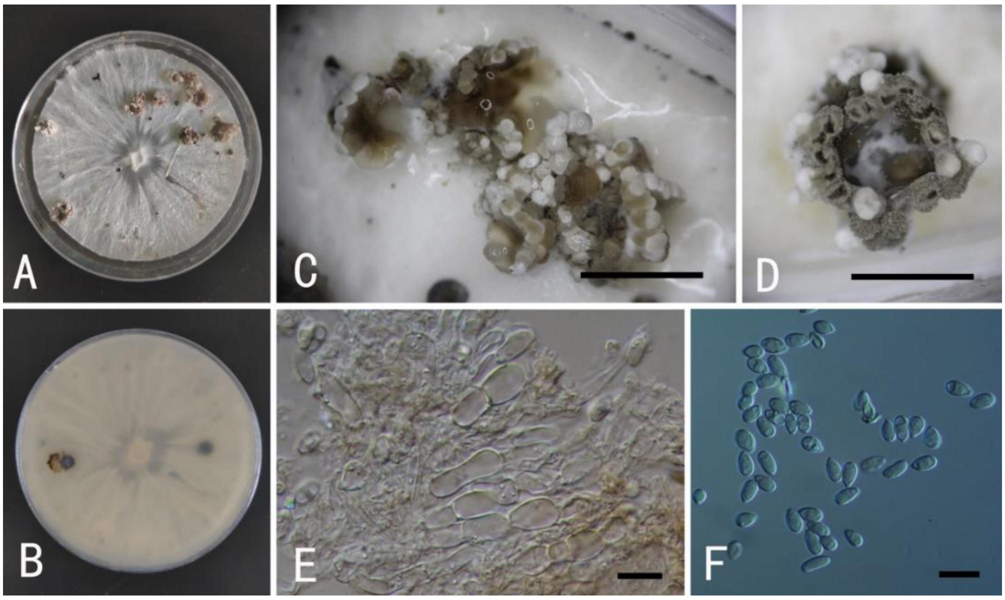

Figure 3 Asexual morph of ***Astrocystis pseudomirabilis*** (GMBC0122). A, B. Culture characters (obverse and reverse). C, D. Conidiomata produced on OA. E. Conidia attached to conidiogenous cells. F. Conidia. Scale bars: C, D = 0.5 mm, E, F = 10 µm.

Habitat/Distribution: Known to inhabit dead bamboo, Guizhou Province, China

Notes: *Astrocystis pseudomirabilis* forms a sister clade to *A. mirabilis* with high statistical support (100 % ML, 1.00 BYPP, Figure 1), while *A. pseudomirabilis* can be distinguished from *A. mirabilis* by its larger stromata (0.64–0.83 × 0.38–0.52 mm vs. 0.4–0.6 × 0.2–0.5 mm) and larger ascospores (11–14 × 6–8 µm vs. 10–12 × 4.5–6 µm). Besides, *Astrocystis bambusae* differs from *A. pseudomirabilis* by its top blunt, obviously erumpent stromata, oval, slightly curved and narrower ascospores (4–5.5 vs. 6–8 µm) (Berkeley and Broome 1873, Dai et al. 2017).

Astrocystis sublimbata (Durieu & Mont.) G.C. Hughes, Mycol. Pap. 50: 9 (1953) (Fig. 4)
Synonyms:
=*Sphaeria sublimbata* Durieu & Mont., in Durieu, Expl. Sci. Alg., Fl. Algér. 1(livr. 13): 498 (1848)
=*Rosellinia sublimbata* (Durieu & Mont.) Pass., in Thümen, Inst. Coimbra: no. 294 (1879)
=*Anthostomella sublimbata* (Durieu & Mont.) Speg., Revta Fac. Agron. Vet. Univ. nac. La Plata, Ser. 2 6(1): 38 (1910)
=*Hypoxylon sublimbatum* (Durieu & Mont.) P.M.D. Martin, Jl S. Afr. Bot. 42(1): 74 (1976)
MycoBank number: MB 293531

Saprobic on the surface of dead bamboo culms, visible as black dots by erumpent on the host. **Sexual morph**: ***Stromata*** 1–3 perithecia, 0.6–1.2 mm diam., 0.3–0.5 mm high, superficial, dome-shaped, clustered or solitary, black, smooth or slightly scurfy, with narrow discoid base and almost vertical sides, apex subconical with inconspicuous papillate ostioles. ***Perithecia*** 340–480 µm diam. and 220–260 µm high, subglobose to oblate, thin-walled, carbonaceous tissue. ***Asci*** 122–137 × 11.3–16.8 µm ($\bar{x}$= 131 × 14.5 µm, n = 30), 8-spored, cylindrical, short-pedicellate, apically rounded, with a J+, square-shaped apical apparatus, blue in Melzer's reagent, 5.5–7.5 µm ($\bar{x}$ = 6.2 µm, n = 30) high, 3.0–

5.0 µm ($\bar{x}$= 4.3 µm, n = 30) wide. ***Ascospores*** 15.7–18.4 × 7–8 µm ($\bar{x}$ = 16.5 × 7.5 µm, n = 30), fusiform-ellipsoidal and strongly inequilateral with the flattened side flat to slightly concave, occasionally slightly beaked at one end dark brown, smooth-walled, with a straight germ slit slightly nearly full spore-length, sometimes with a short gelatinous appendage at one or both ends and more rarely with a prominent gelatinous sheath. **Asexual morph**: undetermined.

Specimens examined: CHINA, Guizhou Province, Guiyang City, Guiyang Forest Park (26.234426 °N, 106.463308 °E), 1154 msl, on dead bamboo culms, 16 June 2021, Qirui Li, 2021GZ71 (GACP QR21028; no culture was obtained; DNA was extracted directly from stromata).

Habitat/Distribution: Known to inhabit Arundo (*Gramineae*, *Arundineae*); Sasa (*Bambuseae*, *Arundinariinae*); Ireland, France, Portugal, United Kingdom, Sudan, Venezuela, Guizhou Province, China.

Notes: Morphologically, *Astrocystis sublimbata* is similar to *A. madeirensis* (Henn.) Læssøe & Spooner, but the latter has larger ascospores (23.5–27 × 10.5–12.5 vs. 15.7–18.4 × 7–8 µm) (Læssøe and Spooner 1994). Phylogenetic analyses of the combined data set of ITS, *rpb2*, β-tubulin and α-actin genes show that new strain (GACP QR21028) groups with *A. sublimbata* (CBS130006) (Vu et al. 2018) with high support (99 % ML, 0.99 BYPP, Figure 1). However, *Astrocystis sublimbata* (HAST 89032207) accommodated closely to the *Astrocystis bambusicola* R.H. Perera & K. D. Hyde (MFLUCC 17-0127) (Hyde et al. 2017), while *Astrocystis bambusicola* differs from *A. sublimbata* by its superficial stromata (vs. erumpent), smaller ascospores (15.7–18.4 × 7–8 vs. 12–16 × 6.1–7 µm) (Hyde et al. 2017, 2019). This is the first report of *A. sublimbata* from China.

Astrocystis tessellati Y.P. Wu & Q.R. Li, **sp. nov**. (Fig. 5)
MycoBank number: MB 843996

Type: CHINA. Yunnan Province, Baoshan City, Lancang River Nature Reserve (25.013041 °N, 99.352222 °E), 2545 msl, on dead bamboo culms, 10 October 2019, Yinhui Pi and Lili Liu, 2019LC254 (GMB0120, **holotype**; ex-type GMBC0120); *ibid*., KUN-HKAS 123440, **isotype**).

Etymology: Refers to the host, *Indocalamus tessellatus* (Munro) Keng f., a bamboo species.

Saprobic on the surface of dead bamboo culms, visible as black raised lump on the host. **Sexual morph**: ***Stromata*** 0.3–0.7 mm diam., 0.25–0.5 mm high, erumpent, effused lump with flat top, irregular in outline, with a discoid black base, containing many perithecia (mostly more than 8, with independent ostiole), gregarious or solitary, scattered, carbonaceous, embedded on the host surface, rough. ***Perithecia*** 210–350 µm diam., 200–280 µm high, spherical to obovate, with carbonaceous stromatal tissue surrounding the individual perithecia. Ostioles at the same level as stromatal surface, with slight papillate openings. ***Asci*** 105–135 × 11–14.3 µm ($\bar{x}$= 117 × 10.6 µm, n = 30), 8-spored, unitunicate, cylindrical, short-pedicellate, persistent, apically rounded, with a J+, square-shaped apical apparatus, blue in Melzer's reagent, 3.5–5 µm ($\bar{x}$ = 4.2 µm, n = 30) high, 2.5–4.5 µm ($\bar{x}$ = 3.6 µm, n = 30) wide. ***Ascospores*** 16–19.5 × 7–9 µm ($\bar{x}$ = 17.3 × 8.2 µm, n = 30), uniseriate, dark

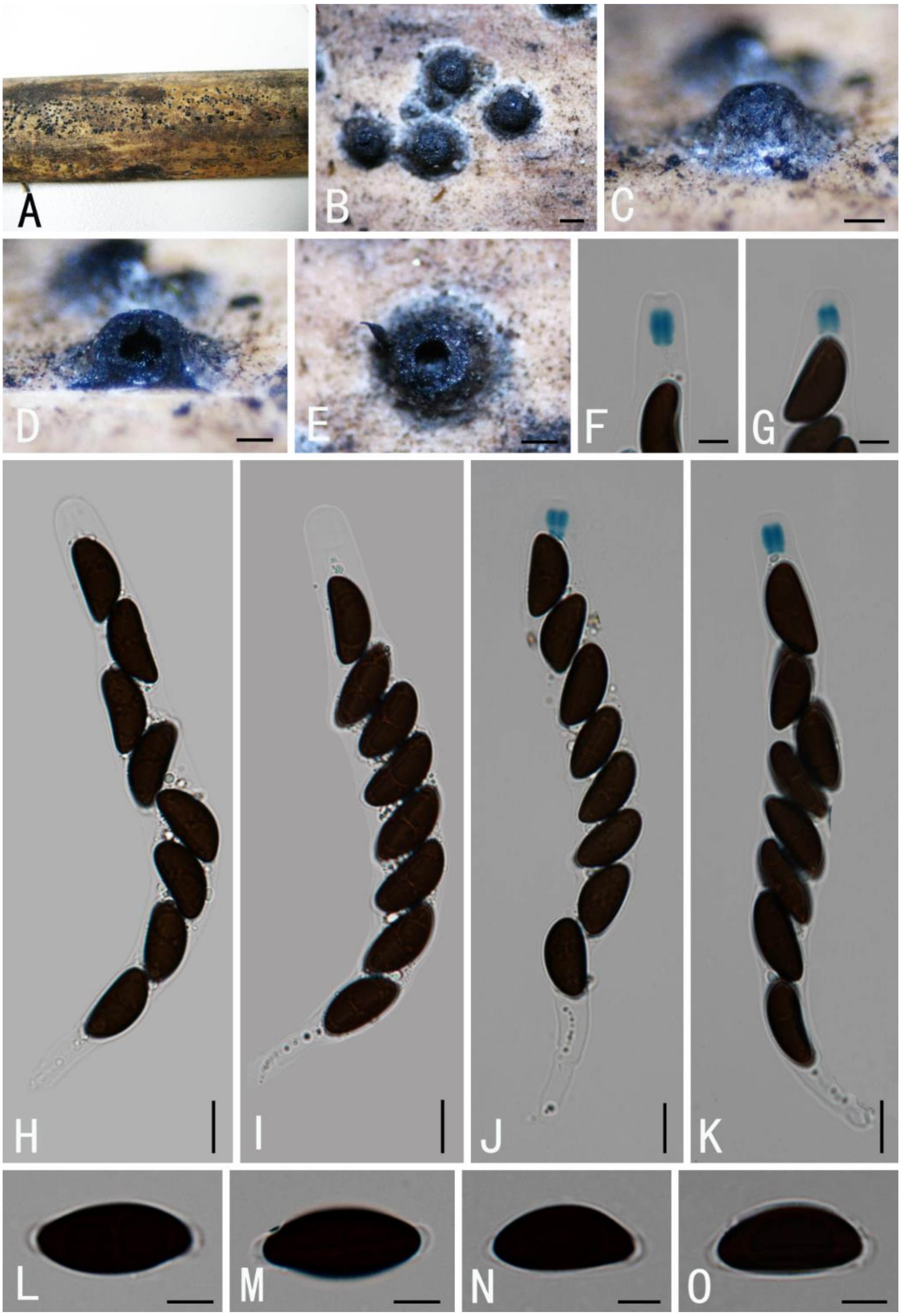

Figure 4 ***Astrocystis sublimbata*** (GACP QR21028). A, B. Stromata on the surface of the host. C. Close-up of stroma surface. D. Longitudinal section of a stroma. E. Transverse section of a stroma. F, G. Ascus apex with a J+, apical apparatus (stained in Melzer's reagent). H–K. Asci with ascospores. L–O. Ascospores. Scale bars: B = 150 µm, C, E = 200 µm, F, G, L–O = 5 µm, H–K = 10 µm.

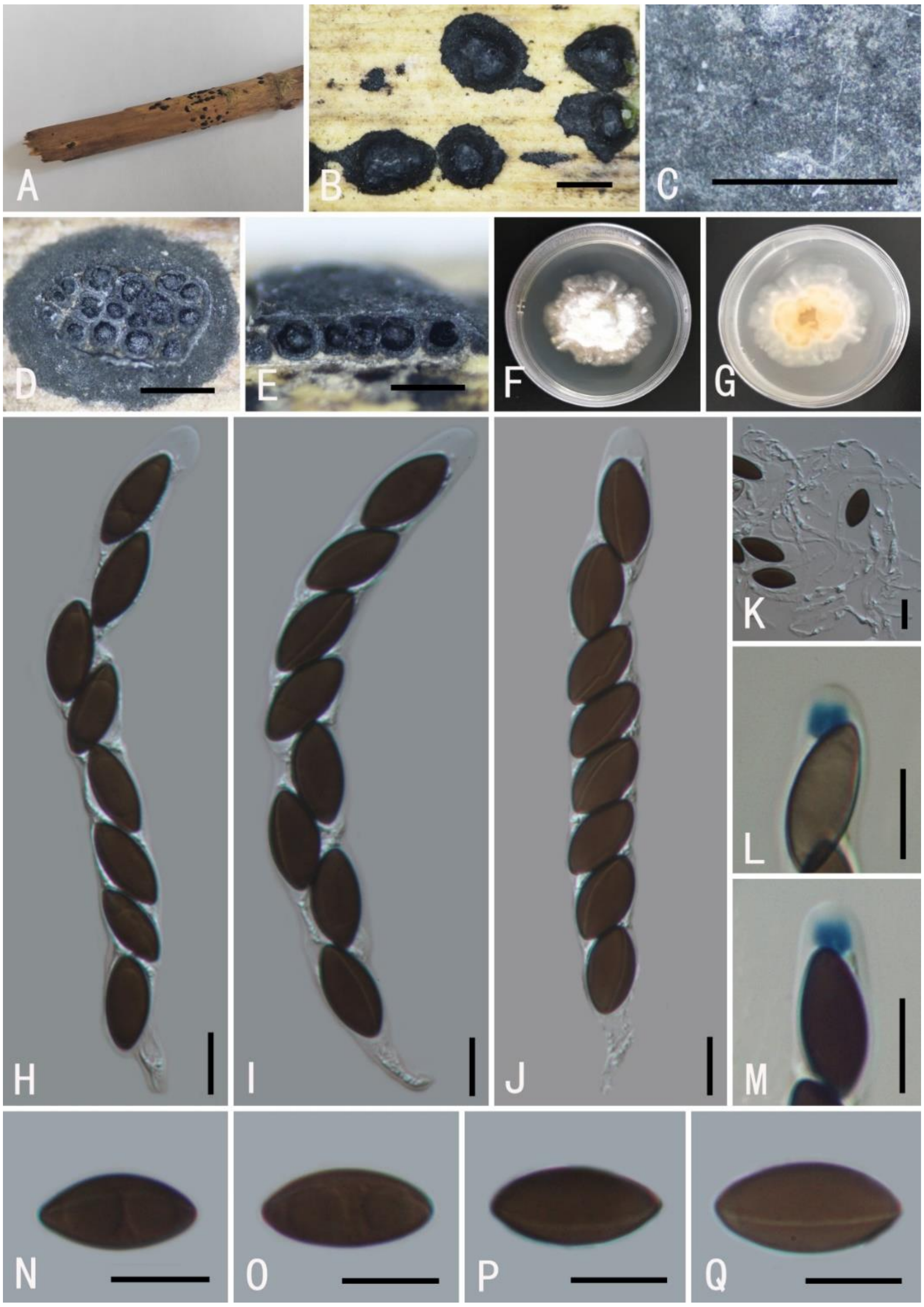

Figure 5 ***Astrocystis tessellati*** (GMB0120, **holotype**). A, B. Stromata on the surface of the host. C. Close-up of a stroma surface. D. Transverse section of a stroma. E. Longitudinal section of a stroma. F. Culture on PDA from above. G. Culture on PDA from reverse. H–J. Asci with ascospores. K. Paraphyses. L, M. Ascus apex with a J+, apical apparatus (stained in Melzer's reagent). N–Q. Ascospores. Scale bars: B = 150 µm, C = 500 µm, D, E= 200 µm, H–Q = 10 µm.

reddish brown, aseptate, equilateral ellipsoid, with rounded ends, smooth, with a straight germ slit slightly less than full-length, lacking appendages and sheaths. **Asexual morph**: undetermined.

Culture characteristics: Colonies on PDA reaching 4–5 cm diam. after 4 weeks at 25 °C, slow growth.

White at first, with irregular margins, becoming moderately radiating furrows spreading toward the edge, medium becoming milky white from central; reverse light yellow, with a white belt. After 8 weeks, colonies on PDA covered 9 cm Petri dish, white, velvety, appressed with entire margins. Reverse light brown. No conidia were observed.

Habitat/Distribution: Known to inhabit dead bamboo, Yunnan Province, China

Additional specimen examined: CHINA. Yunnan Province: Baoshan City, Lancang River Nature Reserve (25.123046°N, 99.364324°E), 2486 msl, on dead bamboo culms, 10 October 2019, Yinhui Pi and Lili Liu, 2019LC237 (GMB0121, paratype; living culture, GMBC0121)

Notes: Morphologically, *A. tessellati* shows similar stromatal characteristics with *A. multiloculata* Y.P. Wu & Q.R. Li, which contains many perithecia, but, *A. tessellati* can be distinguished from *A. multiloculata* by its smaller ascospores (16.2–19.2 × 7.2–9 µm vs. 19–25 × 7–11 µm) (Wu et al. 2021). Phylogenetic analyses based on combined ITS, *rpb2*, β-tubulin and α-actin dataset show that *A. tessellati* formed an independent branch in *Astrocystis s. str.* with a high bootstrap support (100 % ML, 1 BYPP, Figure 1). *Kretzschmariella culmorum* (Cooke) Y.M. Ju & J.D. Rogers (Ju and Rogers 1994) usually bears a cellular appendage on immature ascospores which distinguishes them from *A. tessellati*.

Discussion

In this study, we introduced two new species of *Astrocystis viz*., *A. pseudomirabilis*, *A. tessellati* based on morphological characteristics and molecular phylogeny. Besides, *A. sublimbata* is reported from China for the first time. Phylogenetic analyses based on combined data set of ITS, *rpb2*, β-tubulin and α-actin indicated that *Astrocystis* is separated into three clades (clades 1–3, Figure 1). Taxa in clade 1 display hexagonal prism-shaped, black, superficial stromata, with acuminate or rounded ascospores, 10–14 × 4.5–8 µm (Berkeley and Broome 1873, Dai et al. 2017). Taxa in clade 2 have hexagonal prism-shaped, top blunt, black, obviously erumpent stromata, oval, with slightly curved ascospores, 10.5–14 × 4–5.5 µm. Taxa in clade 3 (except *A. multiloculata*, *A. tessellati*) possess a dome-shaped stroma, inequilaterally, ellipsoid ascospores, 9–25 × 6–11 µm (Wu et al. 2021, this study).

It is worth noting that *Kretzschmariella culmorum* (JDR 88) grouped with *A. multiloculata* and *A. tessellati* with high statistical support (100 % ML, 1 BYPP, Figure 1), and this result agrees with previous studies (Johnston et al. 2016, Wu et al. 2021). Morphologically, three species with many perithecia in superficial stromata, while, *Kretzschmariella culmorum* is mainly in its possession of a cellular appendage in some mature ascospores (Fournier et al. 2018). Moreover, its anamorph has large multicellular conidia, but molecular data indicate that *K. culmorum* should be accepted in genus *Astrocystis*. Unfortunately, the holotype of *K. culmorum* was not verified; therefore, we still retain its original classification status. Currently, seven species of *Astrocystis* have been reported from China (Taylor and Hyde 2003, Hyde et al. 2019, Wu et al. 2021, this study). We believe that the diversity of *Astrocystis* in China is higher than we expect as the sub-tropical regions (Guizhou and Yunnan Provinces) of China are rich in novel taxa. It is essential to carry out more research to reveal the hidden species and we suggest that Yunnan and Guizhou Provinces are the best regions for such work.

Acknowledgments
This research was supported by the National Natural Science Foundation of China (31960005 and 32000009); the Fund of the Science and Technology Foundation of Guizhou Province ([2020]1Y059); Guizhou Province Ordinary Colleges and Universities Youth Science and Technology Talent Growth Project [2021]154. Yingqian Kang is grateful to the 111Project (D20009) and Talent Base Project of Guizhou Province, China (FCJD2018-22).

Conflict of Interest: The authors declare no conflict of interest.

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