Three noteworthy pleosporalean fungi on Southern Magnolia (*Magnolia grandiflora*) and grapevine (*Vitis* sp.) from Qujing, Yunnan, P. R. China

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Abstract

Three pleosporalean fungi were collected from Qujing, Yunnan, China. Morphological characterisation and multigene phylogenetic analyses confirmed that these fungi belong to the order *Pleosporales* but in different families. *Alternaria alternata sensu lato* (*Pleosporaceae*) is reported herein as a new geographical record from southwestern China. *Neocucurbitaria juglandicola* (*Cucurbitariaceae*) is reported from *Magnolia grandiflora* as a new host and country record. *Phragmocamarosporium qujingensis* (*Lentitheciaceae*) is reported from *Vitis* sp. for the first time. Morphological data, photographic illustrations and descriptions are provided along with the phylogenetic trees.

Keywords: DNA sequences, host-range, new records, phylogeny, taxonomy

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Introduction

Southwestern China is rich in fungal diversity, and a significant number of novel taxa are being introduced from different habitats annually. Wijayawardene et al. (2022a) emphasized the importance of collecting fungi from globally well-studied host plants in biodiversity-rich regions, such as Yunnan Province in China. The above assumption was confirmed by Dai et al. (2022) and Wijayawardene et al. (2022b) who introduced several novel taxa from bamboo plants and southern magnolia (*Magnolia grandiflora*), respectively. These host plants have been thoroughly studied in regions such as Europe, Japan, Thailand, and some parts of China (Farr and Rossman 2022). At the same time, recent studies have reported several new host and country records (Dai et al. 2022, Wijayawardene et al. 2022b). It is essential to report new host and geographical records to understand the distribution of species (i.e., biogeography), host-fungal relationships and recognize potential novel pathogens. This study reports three new records of pleosporalean fungi viz. *Alternaria alternata sensu lato*, *Neocucurbitaria juglandicola* and *Phragmocamarosporium qujingensis* on southern magnolia (*M. grandiflora*) and grapevine (*Vitis* sp.), from Qujing, Yunnan Province, China.

Neocucurbitaria was introduced by Wanasinghe et al. (2017) with *N. unguis-hominis* (Punith. & M.P. English) Wanas. et al. as the type. *Neocucurbitaria* is the third most speciose genus in

Cucurbitariaceae (*Cucurbitaria* 94 species; *Fenestella* 28 species; *Neocucurbitaria* 21 species; *Parafenestella* 14 species; *Syncarpella* 7 species; *Rhytidiella* 4 species; *Allocucurbitaria* 2 species; *Astragalicola* 2 species; *Paracucurbitaria* 2 species; *Synfenestella* 2 species; *Cucitella* 1 species; *Protofenestella* 1 species; *Seltsamia* 1 species) (Su et al. 2022, Wijayawardene et al. 2022c). Jaklitsch et al. (2020) recently re-visited the genus and provided a detailed generic description.

Alternaria was introduced by Nees (1816). The type species *A. alternata* is currently considered to be a cosmopolitan species having saprobic and pathogenic lifestyles, with a wide host range such as soil, plants, or indoor environments (Woudenberg et al. 2013, Armitage et al. 2015, Lawrence et al. 2016, Armitage et al. 2020). According to a recent study based on morphology and phylogeny, *Alternaria* contains many species with conidial succession, including plant, human and post-harvest pathogens (Sánchez et al. 2022). Most of the *Alternaria* species are saprobic and endophytic within various parts of crops including leaves, seeds, and fruits (Thomma et al. 2003, Sánchez et al. 2022). Rarely, they cause infections in humans and animals to develop toxicosis and allergic diseases (Woudenberg et al. 2015, Lawrence et al. 2016, Sánchez et al. 2022).

Phragmocamarosporium was introduced by Wijayawardene et al. (2015). Currently, the genus comprises the following species: *P. hederae*, *P. magnoliae*, *P. platani*, *P. qujingensis* and *P. rosae* (Wijayawardene et al. 2015, 2022b). *Phragmocamarosporium platani* is the type species that was reported from Guizhou Province, China. The anamorphs are characterized by pycnidial conidiomata, with a centrally located papillate ostiole. Conidiogenous cells are simple to branched at the base, phialidic and hyaline. Conidia with obtuse apex and truncate base, 2−4-transverse septate, rarely with 1 longitudinal septum (Wijayawardene et al. 2015). In this study, we provide morphological descriptions, illustrations, and phylogenetic analyses of all the new host and geographical records.

Materials and methods

Sample collection, morphological studies, and fungal isolation

Dead, aerial plant samples (leaves, stems) were collected from four sampling sites in Qujing University garden, Qujing City, Yunnan Province, China (25°52'36.75"N, 103°74'46.73"E, 1853.8 m) and returned to the laboratory using Zip-lock polythene bags and envelopes. They were maintained at room temperature (25 °C) until further processing. Fruiting bodies were examined using a Leica S8AP0 stereomicroscope with an HDMI 200C camera (Leica Corporation, Germany). Morphological characters were examined and photographed using an Olympus BX53 compound microscope (Olympus Corporation, Japan) with differential interference contrast (Olympus BX53 DIC compound microscope with an Olympus DP74 camera, Japan). The color codes appearing in this article were from https://www.colorhexa.com/ea3434, accessed on 29 December 2022.

Ascomata and conidiomata were sectioned by hand using a razor blade to obtain thin sections. These sections were transferred with a pickling needle onto a slide with a drop of distilled water (Dai et al. 2022). Measurements were made using Tarosoft (R) Image FrameWork software (http://www.tarosoft.in.th/). Adobe Photoshop CC 2018 (Adobe Systems, USA) software was used to edit and provide the photographic plates.

Single spore isolation was performed to obtain pure cultures following the methods described by Dai et al. (2017). Germinated spores were transferred to potato dextrose agar (PDA) and kept under dark at 27-28 °C. Dried specimens were deposited at the herbarium of Guizhou Medical University, Guiyang, China (GMB). Living cultures were deposited at the culture collection centre of Guizhou Medical University, Guiyang, China (GMBCC).

DNA extraction and polymerase chain reaction (PCR) amplification

Fungi were cultured on PDA medium in the dark at 28 °C for 30 days. Genomic DNA was extracted from fresh fungal cultures according to the specifications of Biospin Fungal Genomic DNA Extraction Kit (BioFlux, China). Internal transcribed spacer (ITS), small subunit rDNA (SSU) and large subunit rDNA (LSU) were PCR amplified, with ITS5 and ITS4, NS1 and NS4 (White et al. 1990), LROR and LR5 primers (Vilgalys and Hester 1990). Primers EF1-983F and EF1-2218R (Rehner 2001) were used to amplify the translation elongation factor 1-α gene region (*tef1-α*). Primers fRPB2-5f and fRPB2-7cr (Liu et al. 1999) were used to amplify RNA polymerase II second largest subunit (*rpb2*) gene region.

The final volume of PCR was prepared as described by Dai et al. (2017), including 1 µl of DNA template, 1 µl of each forward and reverse primers, 12.5 µl of 2x Master Mix (mixture of Easy Taq DNA Polymerase, dNTPs, and optimized buffer) (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, China), and 9.5 µl of double-distilled water. The PCR thermal cycle programs for the amplification of ITS, SSU, LSU, *rpb2* and *tef1-α* gene regions were as previously described by Dai et al. (2017, 2022). The PCR products were sequenced at Shanghai Majorbio Bio-Pharm Technology Co., Ltd., and BGI Tech Solutions Co., Ltd. (BGI-Tech), China. All new sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

LSU, SSU, ITS, *rpb2* and *tef1-α* sequence data were used for BLAST search in NCBI-GenBank to obtain homologous sequences. Closely related sequences were downloaded from GenBank based on BLAST sequence similarity and reference publications (Woudenberg et al. 2015, Su et al. 2022, Wijayawardene et al. 2022b). MAFFT v. 7.215 (Katoh and Standley 2013, http://mafft.cbrc.jp/alignment/server/ index.html) was used for single gene sequence alignment. BioEdit v.7.0 (Hall 2004) was used to make final improvements wherever necessary. The alignments of single LSU, SSU, ITS, *rpb2* or *tef1-α* gene regions were combined with MEGA version 6.0 (Tamura et al. 2013). Alignments were converted to PHYLIP format and loaded from the website (www.sing group.org/ALTER/).

Maximum likelihood (ML) analysis was performed via the online portal CIPRES Science Gateway v. 3.3 (Miller et al. 2010), using RAxML-HPC v.8 on XSEDE (8.2.12) tool, using the default settings but adapted: the GAMMA nucleotide substitution model and 1000 rapid bootstrap replicates. FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk software/figtree/) (Rambaut 2006) was used to view trees. Microsoft Office PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA) was used to edit the phylogram, and Adobe Photoshop CC 2018 software was used to convert it to jpg file.

Bayesian analysis was performed by MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003), and the model of evolution was estimated with MrModeltest v. 2.2 (Nylander 2004). The posterior probabilities (PP) (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) were determined by following

Markov chain Monte Carlo sampling (MCMC) in MrBayes v.3.0b4. Six simultaneous Markov chains were run for 1,000,000 generations, with trees sampled every 100th generation. The preburn was set to 0.25, and the run was automatically stopped when the mean standard deviation of the split frequency reached below 0.01.

Results

Phylogenetic analyses

Multi-gene analyses for *Neocucurbitaria sensu stricto*

The concatenated dataset of LSU, ITS, *rpb2* and *tef1*-α regions contained 35 isolates, which comprised 3682 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Astragalicola amorpha* (CBS 142999) was used as the outgroup taxon. The RAxML tree with a final likelihood value of -13544.315860 is presented in Figure 1.

The matrix had 750 distinct alignment patterns, with 12.72 % of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.242225, C = 0.257686, G = 0.266088, T = 0.234001; substitution rates AC = 1.278524, AG = 5.077650, AT = 1.529790, CG = 1.354327, CT = 9.270734, GT = 1.000000; gamma distribution shape parameter alpha = 0.102448. GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our strain (GMBCC 1115) and *Neocucurbitaria juglandicola s. str.* clustered together with high support (100 % MLBP, 1.00 BYPP). Therefore, we confirm our new strain (GMBCC 1115) as *N. juglandicola* based on phylogenetic analyses.

Multi-gene analyses for *Alternaria alternata sensu lato*

The concatenated dataset of SSU, LSU, ITS and *rpb2* regions contained 45 isolates, which comprised 3154 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Alternaria arborescens* (CBS 102605) and *A. geophila* (CBS 101.13) were used as the outgroup taxa. The RAxML analysis of the combined dataset yielded a best-scoring tree with a final ML optimization likelihood value of -4675.618227 (Figure 2).

The matrix had 54 distinct alignment patterns, with 0.97 % of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.258381, C = 0.223065, G = 0.266221, T = 0.252334; substitution rates AC = 1.017648, AG = 9.270911, AT = 2.707874, CG = 0.000100, CT = 25.589046, GT = 1.000000; gamma distribution shape parameter alpha = 0.020000. GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the multi-locus analysis of SSU, LSU, ITS, and *rpb2* loci, our new strain GMBCC 1171 clustered with 17 other strains of *Alternaria alternata* with high statistical values (88/ 0.98) (Figure 2). Hence, we conclude that our strain is a part of *A. alternata s. str.* However, the interspecific relationships of these *Alternaria* species have not received a clear phylogenetic resolution.

Multi-gene analyses for *Lentitheciaceae*

The concatenated dataset of SSU, LSU, ITS, and *tef*1-α regions contained 41 isolates, which comprised 3159 characters with gaps. Single gene analysis was carried out to compare the topology of the tree and clade stability. *Bambusicola massarinia* (MFLUCC 11-0389) and *Palmiascoma gregariascomum* (MFLUCC 11-0175) were used as the outgroup taxa.

The RAxML tree with a final likelihood value of -13374.481248 is presented in Figure 3. The matrix had 838 distinct alignment patterns, with 12.09 % of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.237433, C = 0.253387, G = 0.269982, T = 0.239197; substitution rates AC = 1.219537, AG = 2.022723, AT = 1.369739, CG = 1.287700, CT = 7.057765, GT = 1.000000; gamma distribution shape parameter alpha = 0.135741. GTR+I+G model was selected as the best model based on MrModeltest and was used for the Bayesian analysis. In the phylogenetic analysis, our strains GMBCC 1053, GMBCC 1054 and the ex-type strain of *Phragmocamarosporium qujingensis* (GMBCC 1176) clustered together with high statistical support (100 % MLBP, 1.00 BYPP). Therefore, we confirm our strain as *P. qujingensis.*

Taxonomy

Neocucurbitaria juglandicola Jaklitsch & Voglmayr, Stud. Mycol. 90: 96 (2017)
Index Fungorum Number: IF 823007

Saprobic on dead branches of *Magnolia grandiflora* (*Magnoliaceae*). **Sexual morph:** *Ascomata* 118–391 × 94–301 µm ($\bar{x}$ = 228 × 151 µm, n = 20), forming under a light black area, immersed in bark, but visible in bark fissures, scattered or aggregated in small groups, sub-globose to pyriform, with or without a rounded apical papilla, black, with verruculose to nearly smooth surface, basally and laterally surrounded by subiculum 2–5 µm wide, thick-walled brown hyphae. *Peridium* 10−15 µm wide, composed of thick-walled dark brown *textura angularis* cells becoming hyaline towards inner side. *Hamathecium* comprises 2−3 µm wide, numerous, branched and septate pseudoparaphyses. *Asci* 47−130 × 12−20 µm ($\bar{x}$ = 93 × 15 µm, n = 20), 8-spored, biseriate, oblong, bitunicate, fissitunicate, with a distinct ocular chamber, short stipe, simple or knob-like base. *Ascospores* 18−25 × 8−12 µm ($\bar{x}$ = 23 × 10 µm, n = 20), ellipsoid, straight or slightly curved, slightly constricted at the median primary septum, upper half often slightly enlarged, initially pale brown, turning medium to dark brown when mature, with 3–7 transverse and 1−2 longitudinal septa, smooth-walled. **Asexual morph:** Undetermined.

Culture characters: *Colonies* slow-growing, 5−10 mm diam. after 15 days at 28 °C, under 24h dark, globose to sub-oblong, with uneven margin, flocculent. From above: pale brown (987654), and ivory (fffff0) at the centre, from below: light brown (b5651d) to creamy white (fffdd0) margins.

Known Hosts: *Juglans regia*, *Quercus rubra* (Jaklitsch et al. 2018).

Known distribution: Austria, and China (Yunnan Province) (Jaklitsch et al. 2018).

Material examined: China, Yunnan Province, Qujing City, Qujing Normal University Park, on dead branch of *M. grandiflora* L., 7 November 2019, Mei-Lin Zhu, Lin 04, (GMB 1376), living culture (GMBCC 1115).

Table 1 Taxa used in the *Neocucurbitaria juglandicola*, *Alternaria alternata* complex and *Phragmocarosporium qujingensis* phylogenetic analyses and their corresponding GenBank numbers. The newly generated sequences are indicated in red; ex-type strains are with "T".

Taxa	Strain no.	GenBank accession no.				
		LSU	SSU	ITS	*rpb2*	*tef1-α*
Alternaria alternata	CBS 104.26	KP124450	KP124920	KP124299	KP124767	N/A
A. alternata	CBS 806.96	KP124477	KP124947	KP124325	KP124793	N/A
A. alternata	CBS 639.97	KP124479	KP124949	KP124327	KP124795	N/A
A. alternata	CBS 109455	KP124487	KP124957	KP124335	KP124803	N/A
A. alternata^{T}	CBS 115616	DQ678082	KC584507	AF347031	KC584375	N/A
A. alternata	CBS 113025	KP124497	KP124967	KP124345	KP124813	N/A
A. alternata	CBS 115069	KP124499	KP124969	KP124347	KP124815	N/A
A. alternata	CBS 115190	KP124502	KP124972	KP124350	KP124818	N/A
A. alternata	CBS 115199	KP124503	KP124973	KP124351	KP124819	N/A
A. alternata	CBS 115200	KP124504	KP124974	KP124352	KP124820	N/A
A. alternata	CBS 119115	KP124512	KP124982	KP124360	KP124828	N/A
A. alternata	CBS 154.31	KP124452	KP124922	KP124301	KP124769	N/A
A. alternata	CBS 795.72	KP124461	KP124931	KP124309	KP124778	N/A
A. alternata	CBS 125606	KP124529	KP124999	KP124375	KP124845	N/A
A. alternata	CBS 130260	KP124541	KP125011	KP124387	KP124857	N/A
A. alternata	CBS 127334	KP124534	KP125004	KP124380	KP124850	N/A
A. alternata	CBS 121456	KP124523	KP124993	KP124369	KP124839	N/A
A. alternata	CBS 126908	KP124532	KP125002	KP124378	KP124848	N/A
A. alternata	CBS 112251	KP124491	KP124961	KP124339	KP124807	N/A
A. alternata	CBS 127672	KP124536	KP125006	KP124382	KP124852	N/A
A. alternata	CBS 121455	KP124522	KP124992	KP124368	KP124838	N/A
A. alternata	CBS 121544	KP124525	KP124995	KP124371	KP124841	N/A

A. alternata	CBS 267.77	KP124463	KP124933	KP124311	KP124779	N/A
A. alternata	CBS 102.47	KP124456	KP124926	KP124304	KP124773	N/A
A. alternata	CBS 107.27	KP124451	KP124921	KP124300	KP124768	N/A
A. alternata	CBS 119543	KP124515	KP124985	KP124363	KP124831	N/A
A. alternata	CBS 121454	KP124521	KP124991	AF278836	KP124837	N/A
A. alternata	CBS 119408	KP124514	KP124984	KP124362	KP124830	N/A
A. alternata	CBS 117.44	KP124455	KP124925	KP124303	KP124772	N/A
A. alternata	CBS 107.53	KP124457	KP124927	KP124305	KP124774	N/A
A. alternata	CBS 106.34	KP124454	KP124924	Y17071	KP124771	N/A
A. alternata	CBS 106.24	KP124449	KP124919	KP124298	KP124766	N/A
A. alternata	CBS 121336	KP124517	KP124987	KJ862254	KP124833	N/A
A. alternata	CBS 194.86	KP124468	KP124938	KP124316	KP124784	N/A
A. alternata	CBS 127671	KP124535	KP125005	KP124381	KP124851	N/A
A. alternata	CBS 175.80	KP124465	KP124935	KP124313	KP124781	N/A
A. alternata	CBS 126910	KP124533	KP125003	KP124379	KP124849	N/A
A. alternata	CBS 880.95	KP124474	KP124944	KP124322	KP124790	N/A
A. alternata	CBS 965.95	KP124475	KP124945	KP124323	KP124791	N/A
A. alternata	CBS 126072	KP124531	KP125001	KP124377	KP124847	N/A
A. alternata	CBS 112252	KP124492	KP124962	KP124340	KP124808	N/A
A. alternata	CBS 121547	KP124526	KP124996	KP124372	KP124842	N/A
A. alternata	GMBCC1171	OP597506	OP597500	OP597513	OP615659	OP615655
A. arborescens^{T}	CBS 102605	KC584253	KC584509	AF347033	KC584377	N/A
A. geophila^{T}	CBS 101.13	KP124546	KP125016	KP124392	KP124862	N/A
Astragalicola amorpha^{T}	CBS 142999	MF795753	N/A	MF795753	MF795795	MF795842
Bambusicola massarinia^{T}	MFLUCC 11-0389	JX442037	JX442041	NR_121548	N/A	KP761725
Darksidea alpha^{T}	CBS 135650	NG_059126	NG_061189	NR_137619	N/A	KP184166
D. beta^{T}	CBS 135637	KP184023	KP184074	NR_137957	N/A	KP184189

D. delta[T]	CBS 135638	KP184024	KP184069	NR_137075	N/A	KP184184
Halobyssothecium obiones[T]	MFLUCC 15-0381	MH376744	MH376745	MH377060	N/A	MH376746
Katumotoa bambusicola[T]	KT 1517a	AB524595	AB524454	LC014560	N/A	AB539108
Keissleriella breviasca[T]	KT 649	AB807588	AB797298	AB811455	N/A	AB808567
K. cirsii[T]	MFLUCC 16-0454	NG_059776	KY497782	NR_155248	N/A	KY497786
K. quadriseptata[T]	KT 2292	AB807593	AB797303	AB811456	N/A	AB808572
K. quadriseptata	MFLU 19-2871	MT183478	MT214957	MT185515	N/A	MT454026
Lentithecium aquaticum[T]	CBS 123099	GU301823	GU296156	MH863276	N/A	GU349068
L. clioninum[T]	KT 1149A	AB807540	AB797250	LC014566	N/A	AB808515
L. clioninum	KT 1220	AB807541	AB797251	LC014567	N/A	AB808516
L. pseudoclioninum	KT 1111	AB807544	AB797254	AB809632	N/A	AB808520
L. pseudoclioninum[T]	KT 1113	AB807545	AB797255	AB809633	N/A	AB808521
Murilentithecium clematidis	MFLUCC 14-0561	KM408758	KM408760	KM408756	N/A	KM454444
M. clematidis[T]	MFLUCC 14-0562	KM408759	NG_061185	NR_154174	N/A	KM454445
M. lonicerae[T]	MFLUCC 18-0675	MK214373	MK214376	MK214370	N/A	MK214379
M. rosae[T]	MFLUCC 15-0044	MG829030	MG829137	MG828920	N/A	N/A
Neocucurbitaria acanthocladae[T]	CBS 142398	MF795766	N/A	MF795766	MF795808	MF795854
N. acerina	C26a	MF795767	N/A	MF795767	MF795809	MF795855
N. acerina	CBS 142403	MF795768	N/A	MF795768	MF795810	MF795856
N. aetnensis[T]	CBS 142404	MF795769	N/A	MF795769	MF795811	MF795857
N. aetnensis[T]	C270	MF795770	N/A	MF795770	MF795812	MF795858
N. aquatica[T]	CBS 297.74	EU754177	N/A	LT623221	LT623278	N/A
N. cava	CBS 115979	EU754198	N/A	AY853248	LT623273	N/A
N. cava[T]	CBS 257.68	EU754199	N/A	JF740260	LT717681	N/A
N. cinereae[T]	CBS 142406	MF795771	N/A	MF795771	MF795813	MF795859
N. cisticola[T]	CBS 142402	MF795772	N/A	MF795772	MF795814	MF795860
N. hakeae[T]	CBS 142109	KY173526	N/A	KY173436	KY173593	N/A

N. irregularis[T]	CBS 142791	LN907372	N/A	LT592916	LT593054	N/A
N. juglandicola	C316	MK356301	N/A	MK356301	MK357529	MK357573
N. juglandicola[T]	CBS 142390	MF795773	N/A	MF795773	MF795815	MF795861
N. juglandicola	GMBCC1115	OP597505	N/A	OP597512	OP615658	OP615654
N. keratinophila[T]	CBS 121759	LT623215	N/A	EU885415	LT623275	N/A
N. populi[T]	CBS 142393	MF795774	N/A	MF795774	MF795816	MF795862
N. quercina[T]	CBS 115095	GQ387619	N/A	LT623220	LT623277	N/A
N. rhamni[T]	CBS 142391	MF795775	N/A	MF795775	MF795817	MF795863
N. rhamni	C112	MF795776	N/A	MF795776	MF795818	MF795864
N. rhamni	C133	MF795777	N/A	MF795777	MF795819	MF795865
N. rhamni	C190	MF795778	N/A	MF795778	MF795820	MF795866
N. rhamni	C277	MF795779	N/A	MF795779	MF795821	MF795867
N. rhamnicola[T]	CBS 142396	MF795780	N/A	MF795780	MF795822	MF795868
N. rhamnicola	KRx	MF795781	N/A	MF795781	MF795823	MF795869
N. rhamnioides	C222	MF795783	N/A	MF795783	MF795825	MF795871
N. rhamnioides	C223	MF795784	N/A	MF795784	MF795826	MF795872
N. rhamnioides[T]	CBS 142395	MF795782	N/A	MF795782	MF795824	MF795870
N. ribicola[T]	CBS 142394	MF795785	N/A	MF795785	MF795827	MF795873
N. ribicola	C155	MF795786	N/A	MF795786	MF795828	MF795874
N. unguis-hominis	CBS 111112	GQ387623	N/A	LT623222	LT623279	N/A
N. vachelliae[T]	CBS 142397	MF795787	N/A	MF795787	MF795829	MF795875
N. sasicola[T]	KT 1706	AB524599	AB524458	LC014577	N/A	AB539111
Palmiascoma gregariascomum[T]	MFLUCC 11-0175	KP744495	KP753958	KP744452	N/A	N/A
Parafenestella ostryae	MFLU 16-0184	KY563075	N/A	KY563072	N/A	N/A
P. pittospori[T]	CPC 34462	MN567606	N/A	MN562098	N/A	N/A
P. hederae	KUMCC 18-0165	MK214372	MK214375	MK214369	N/A	MK214378
P. hederae[T]	MFLUCC 13-0552	KP842915	KP842918	N/A	N/A	N/A

P. magnoliae[T]	GMBCC1180	OM855600	OM855614	OM855591	N/A	OM857555
P. magnoliae	GMBCC1041	ON364110	ON364114	ON364112	N/A	ON375375
P. platani[T]	MFLUCC 14-1191	KP842916	KP842919	KP852526	N/A	N/A
P. qujingensis[T]	GMBCC1176	OM855599	OM855613	OM855590	N/A	OM857554
P. qujingensis	GMBCC1044	ON364109	ON364113	ON364111	N/A	ON375374
P. rosae[T]	MFLUCC 17-0797	NG_059874	MG829156	N/A	N/A	MG829225
P. qujingensis	GMBCC1053	OP597507	OP597501	OP597514	OP615656	N/A
P. qujingensis	GMBCC1054	OP597508	OP597502	OP597515	OP615657	N/A
Poaceascoma aquaticum[T]	MFLUCC 14-0048	KT324690	KT324691	N/A	N/A	N/A
P. helicoides[T]	MFLUCC 11-0136	KP998462	KP998463	KP998459	N/A	KP998461
Sclerostagonospora cycadis	CBS 291.76	N/A	N/A	KR611890	N/A	N/A
Setoseptoria arundinacea	KT 552	AB807574	AB797284	LC014594	N/A	AB808550
S. arundinacea	KT 600	AB807575	AB797285	LC014595	N/A	AB808551
S. magniarundinacea[T]	KT 1174	AB807576	AB797286	LC014596	N/A	AB808552
Tingoldiago graminicola[T]	KH 68	AB521743	AB521726	LC014598	N/A	AB808561
T. graminicola	KH 155	AB521745	AB521728	LC014599	N/A	AB808562
T. graminicola	KT 891	AB521744	AB521727	LC014600	N/A	AB808563
Towyspora aestuari[T]	MFLUCC 15-1274	NG_060798	NG_061225	NR_148095	N/A	N/A

Abbreviations: **N/A**: not available; **C**: Natural History Museum of Denmark; **CBS**: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **CPC**: Culture collection of Pedro Crous, Netherlands; **GMB**: Herbarium of Guizhou Medical University, Guiyang, China; **GMBCC**: Guizhou Medical University Culture Collection, Guiyang, China; **KH**: Korea National Arboretum; **KR**: Staatliches Museum fuer Naturkunde Karlsruhe; **KUMCC**: Kunming Institute of Botany Culture Collection, Kunming, China; **MFLU**: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **KT**: K. Tanaka.

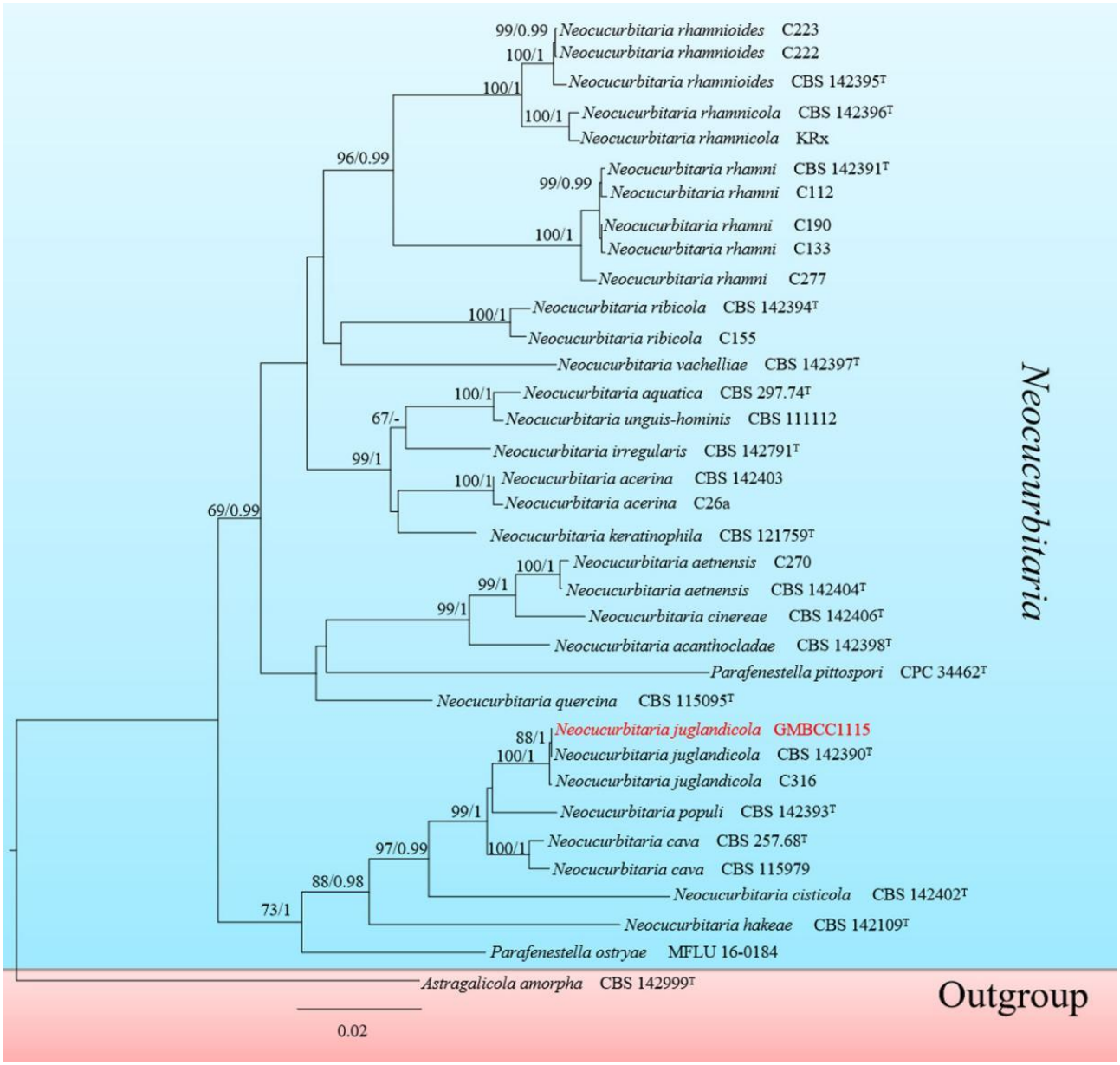

Figure 1. RAxML tree based on a combined dataset of partial LSU, ITS, *rpb2* and *tef*1-*α* DNA sequence analyses. Bootstrap values for maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) equal to or greater than 50 % and 0.95 are given at the respective branches. Hyphen (-) means a value lower than 50 % (BS) or 0.95 (PP). The tree is rooted with *Astragalicola amorpha* (CBS 142999). The type strains are indicated with "T", and new isolates are in red.

Notes: In the phylogenetic analyses (Figure 1), our strain (GMBCC 1115) grouped with the ex-type strain of *Neocucurbitaria juglandicola* (CBS 142390) with 88 % ML and 1.00 BYPP support. Our collection shares similar morphological characteristics (Ascomata becoming visible in bark fissures, with or without a rounded apical papilla. Asci with a distinct ocular chamber, short stipe. *Ascospores* with 3–7 transverse and 1−2 longitudinal septa.) with the original description of *N. juglandicola* (Jaklitsch et al. 2018). The sizes of ascomata, asci and ascospores of our collection and *Neocucurbitaria juglandicola* also compared (ascomata: 118–391 × 94–301 µm *vs*. 177–320 × 177–265 µm, asci: 47−130 × 12−20 µm *vs*. 79–133 × 12–17 µm and ascospores: 18−25 × 8−12 µm *vs*. 15–

27 × 8–11 µm). *Neocucurbitaria juglandicola* was isolated from a twig of *Juglans regia* by Jaklitsch and Voglmay et al. (2020) in Austria. Based on morphology and molecular phylogeny, we confirm that our collection represents *N. juglandicola*. This is the first host record of *M. grandiflora* and the country record from mainland China.

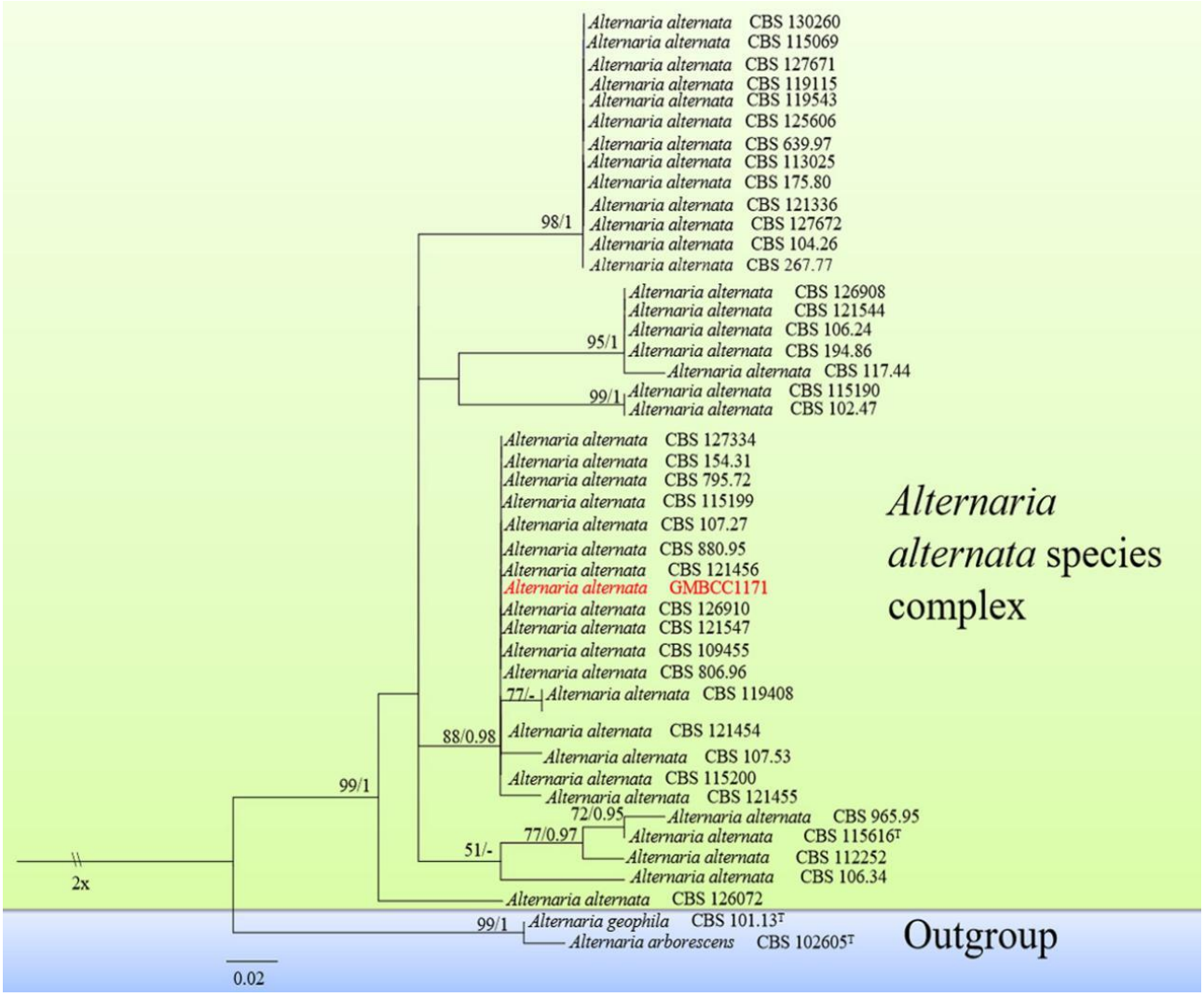

Figure 2. RAxML tree based on a combined dataset of partial SSU, LSU, ITS, and *rpb2* DNA sequence analyses. Accessions with names were synonymized by Woudenberg et al. (2015). Bootstrap values for maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) equal to or greater than 50 % and 0.95 are given at the respective branches. Hyphen (-) means a value lower than 50 % (BS) or 0.95 (PP). The tree is rooted with *Alternaria geophila* (CBS 101.13) and *A. arborescents* (CBS 102605). The type strains are indicated with "T", and new isolates are in red.

Alternaria alternata (Fr.) Keissl., Beih. bot. Zbl., Abt. 2 29: 434 (1912)
Index Fungorum Number: IF119834

Saprobic on a dead leaf of *Magnolia grandiflora*. **Sexual morph**: See Ariyawansa et al. (2015).

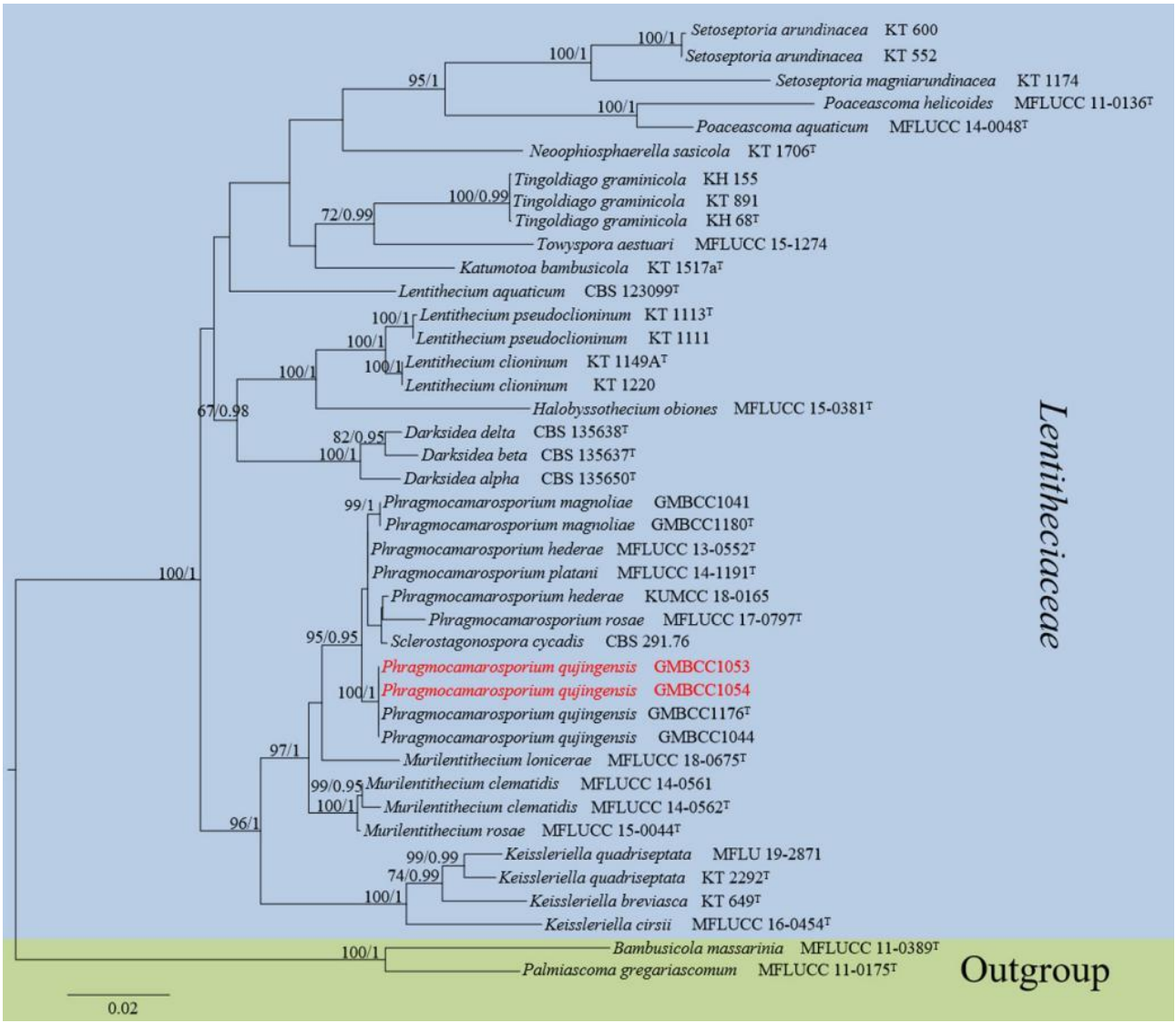

Figure 3. RAxML tree based on a combined dataset of partial SSU, LSU, ITS, and *tef* 1-α DNA sequence analyses. Bootstrap values for maximum likelihood (ML) and Bayesian posterior probabilities (BYPP) equal to or greater than 50 % and 0.95 are given at the respective branches. The tree is rooted with *Bambusicola massarinia* (MFLUCC 11-0389) and *Palmiascoma gregariascomum* (MFLUCC 11-0175). The type strains are labelled with "T", and new isolates are in red.

Asexual morph: Spots formed on the leaves. *Conidiophores* 29.8−74.5 × 3.6−6 µm, ($\bar{x}$ = 50 × 4.7 µm, n = 10), dark brown, straight or curved primary, short to long, simple or branched, with one or several apical conidiogenous loci. *Conidiogenous* cells, enteroblastic, pale brown, oblong. *Conidia* 8−12 × 18−20 µm, ($\bar{x}$ = 10 × 19 µm, n = 20), light brown to dark brown, obclavate, long ellipsoid, septate, slightly constricted near some septa, with 3−7 transverse and 1−2 longitudinal septa, smooth-walled, simple or branched chains. The conidium body can narrow gradually into a tapered beak or secondary conidiophore. Secondary conidiophores can be formed apically or laterally with one or a few conidiogenous loci.

Culture characters: Conidia germinating on PDA within 24h and germ tubes produced from sides.

Colonies slow growing, 15 mm diam. after 15 days at 28 °C, under 24h dark, globose to sub-oblong, with even margin, flocculent, and pale brown (987654).

Known Hosts: Air, *Allium* sp., *Anemone occidentalis*, *Arachis hypogaea*, *Arbutus unedo*, *Artemisia brevifolia*, *Astragalus bisulcatus*, *Brassica oleracea*, *Broussonetia papyrifera*, *Capsicum annuum*, *Citrus*, *Cyperaceae*, *Cucumis melo*, *Cucumis sativus*, *Cuscuta gronovii*, *Daucus carota*, Desert sand, *Dianthus chinensis*, *Euphorbia esula*, *Fragaria vesca*, *Godetia* sp., *Helianthus annuus*, Human, *Juncus mertensianus*, *Linum usitatissimum*, *Lolium* sp., *Magnolia grandiflora*, *Malus*, *Malva* sp., *Minneola*, *Nicotiana tabacum*, *Plantago aristida*, *Platycodon grandiflflorus*, *Prunus* sp., *Psychotria serpens*, *Punica granatum*, *Pyrus*, *Quercus* sp., *Sanguisorba offificinalis*, *Senecio cineraria*, Soil, *Solanum lycopersicum*, *Stanleya pinnata*, *Staphylea trifolia*, *Triticum* sp., *Vaccinium* sp. (Woudenberg et al. 2015).

Known distribution: Australia, Canada, China, Belgium, Denmark, Egypt, Germany, Greece, Israel, India, Italy, Japan, Kuwait, Morocco, Namibia, Netherlands, Papua New Guinea, Sahara, South Africa, UK, and the USA (Woudenberg et al. 2015).

Material examined: China, Yunnan Province, Qujing City, Qujing Normal University Park, on dead leaf of *M. grandiflora* L., 7 November 2019, Mei-lin Zhu, lin12, (GMB 1377), living culture (GMBCC 1171).

Notes: Phylogenetic analysis showed that our collection (GMBCC 1171) groups with *Alternaria alternata* species complex. *Alternaria alternata* has been reported from a wide range of hosts, such as soil, the atmosphere, plants, and fruits (Woudenberg et al. 2013, Armitage et al. 2015, 2019, Lawrence et al. 2016, Sánchez et al. 2022). While Liu et al. (2019) reported the isolation of the pathogenic fungus *A. alternata* from leaf spots of *M. grandiflora* collected from Jiangsu Province, southeastern China. Herein, we identify our collection as *A. alternata* based on phylogenetic analyses along with morphological comparison. This is the first record of *A. alternata* as a saprophytic fungus on *M. grandiflora* litter from Yunnan Province, southwestern China.

Phragmocamarosporium qujingensis D.Q. Dai, Wanas. & Wijayaw., Frontiers in Microbiology: 10.3389/fmicb.2022.954680, 20 (2022)
Index Fungorum Number: IF 555251

Saprobic on dead stem of grapevine (*Vitis vinifera*). **Sexual morph**: Undetermined. **Asexual morph**: *Conidiomata* 93–130 µm high, 138–187 µm diam ($\overline{x}$ = 156 × 107 µm, n = 5), pycnidial, immersed to semi-immersed, black, gregarious to solitary, unilocular, globose to sub-globose, and with a centrally located papillate ostiole. *Pycnidial* wall with outer 3–4 layers of dark brown cells of *textura angularis* and with inner layer of thin hyaline cells. *Conidiophores* reduced to *conidiogenous* cells. *Conidiogenous* cells 4.3–9.1 × 2–4.8 µm ($\overline{x}$ = 7.3 × 3.5µm, n = 20), smooth, long, phialidic, hyaline. *Conidia* 15–20 × 7.7–9.3 µm ($\overline{x}$ = 17.8 × 8.2 µm, n = 20), medium brown, clavate or ellipsoid to subcylindrical, with obtuse apex and truncate base, straight to curved, 3-transverse septate, guttulate or eguttulate, constricted at the septa.

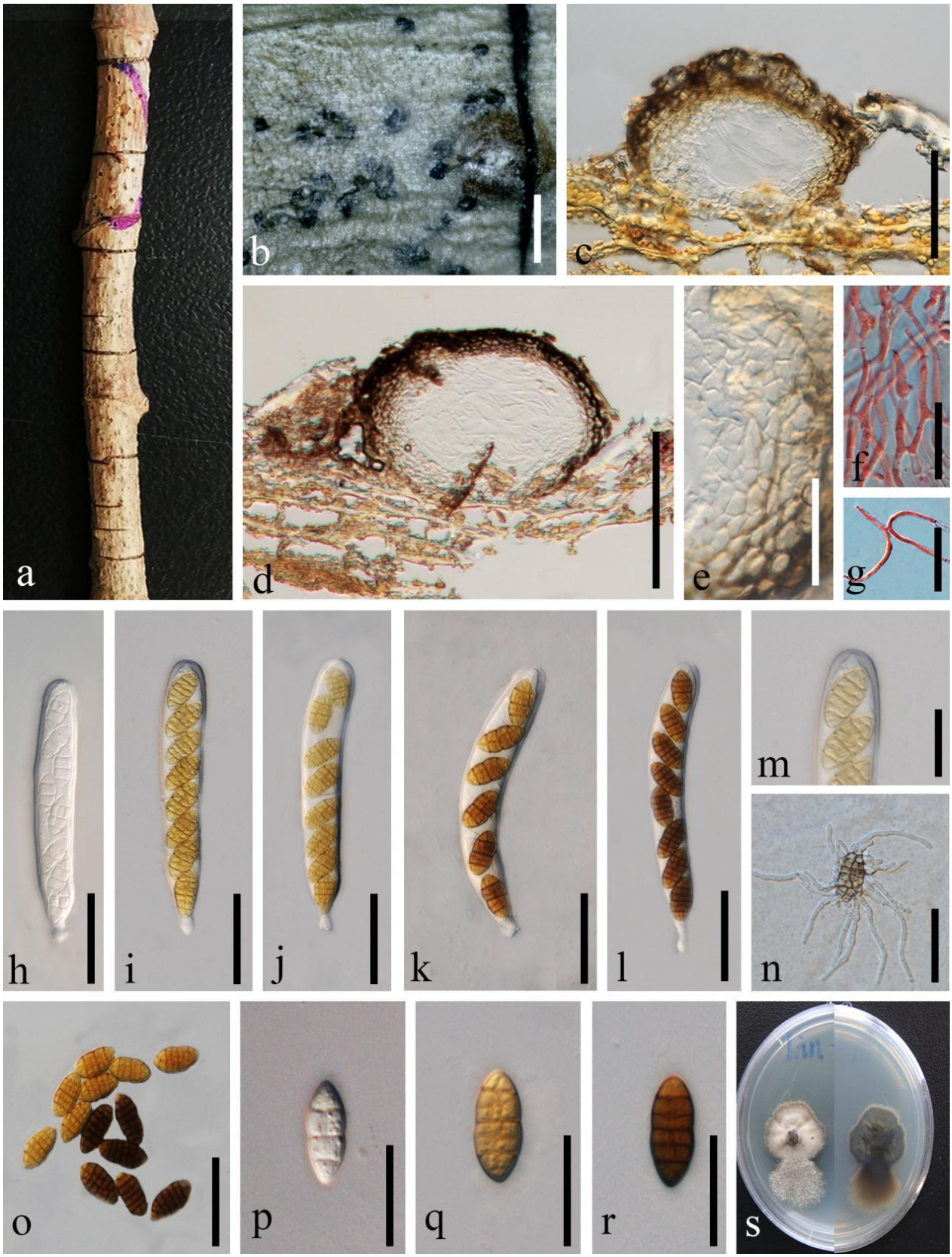

Figure 4 *Neocucurbitaria juglandicola* (GMB 1376, new host and country record). a. *Magnolia* branch. b. Immersed ascomata on the host. c, d. Vertical sections of ascoma. e. Peridium. f, g. Pseudoparaphyses. h–l. Asci. m. Ocular chamber. n. Germinating ascospore. o–r. Ascospores. s. Cultures on PDA (Obverse and reverse). Scale bars: b = 200 µm, c, d = 100 µm, f, g = 1 µm, h–l = 10 µm, m = 20 µm, n, o = 15 µm, p–r = 25 µm.

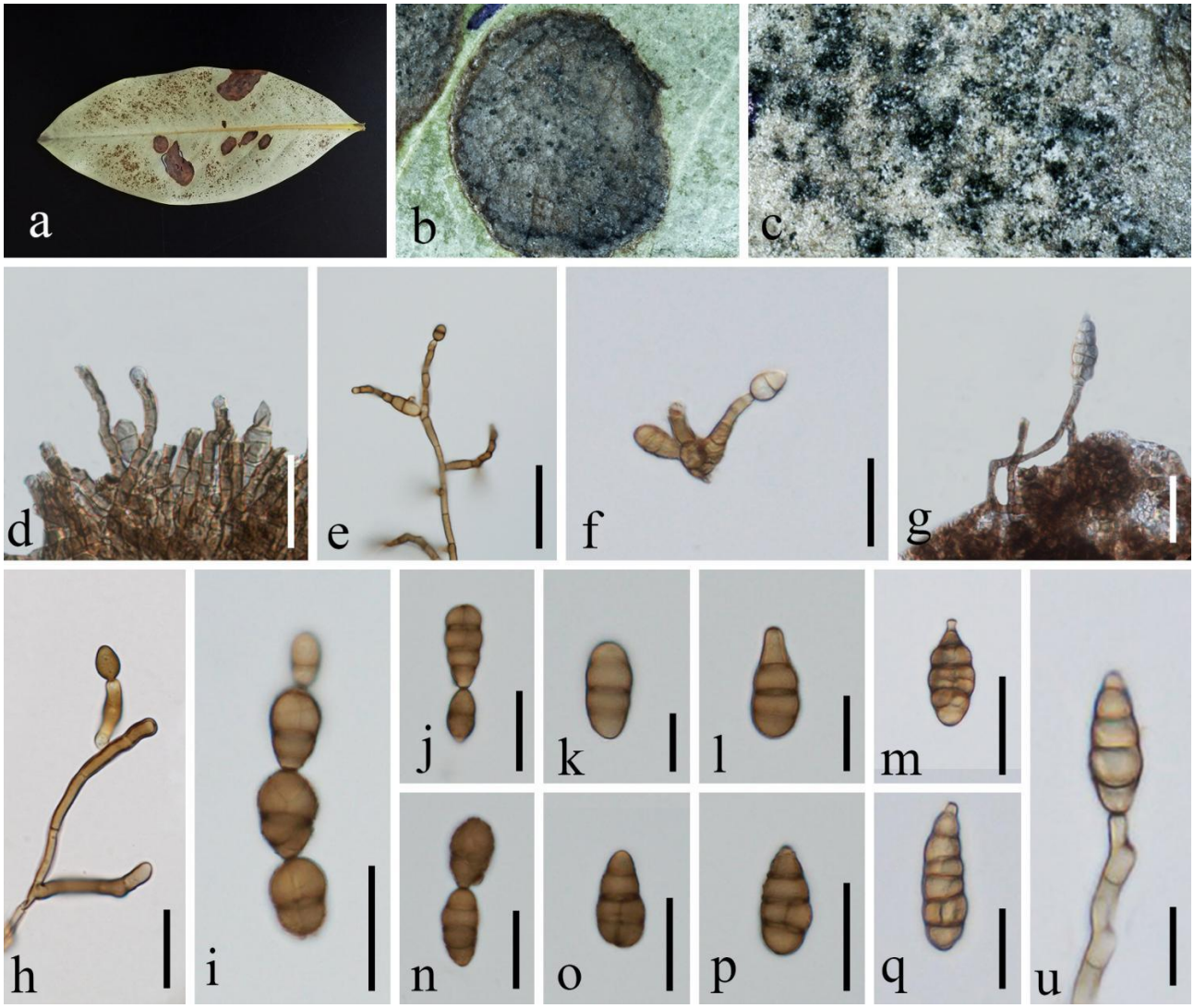

Figure 5. *Alternaria alternata* (GMB 1377, new geographical record). a. A leaf of *Magnolia*. b, c. Leaf spots form on the leaf. d–h. Conidiogenous cells with developing conidia. i–r. Conidia. Scale bars: d–f = 1 µm, g, i–r = 5 µm, h = 3 µm.

Culture characteristics: *Conidia* germinating on PDA within 24 h and germ tubes produced from both sides. *Colonies* growing slowly on PDA, reaching 5 cm in 1 week at 28 °C, dense, irregular, uneven margin, white in the first week, gray (808080) from above, light yellow (ffffed) at the margin and dark gray (a9a9a9) at the center, and with light gray (d3d3d3) in the middle from below.

Known Hosts: *Magnolia grandiflora*, *Vitis* sp. (Wijayawardene et al. 2022b).

Known distribution: Yunnan, China (Wijayawardene et al. 2022b).

Material examined: China, Yunnan Province, Qujing City, Xuanwei, on a dead grapevine (*Vitis vinifera*), 01 October 2021, Dong-Qin Dai and Gui-Qing Zhang, P9, (GMB 1194), living culture (GMBCC 1054). *ibid.* P1, (GMB 1193), living culture (GMBCC 1053).

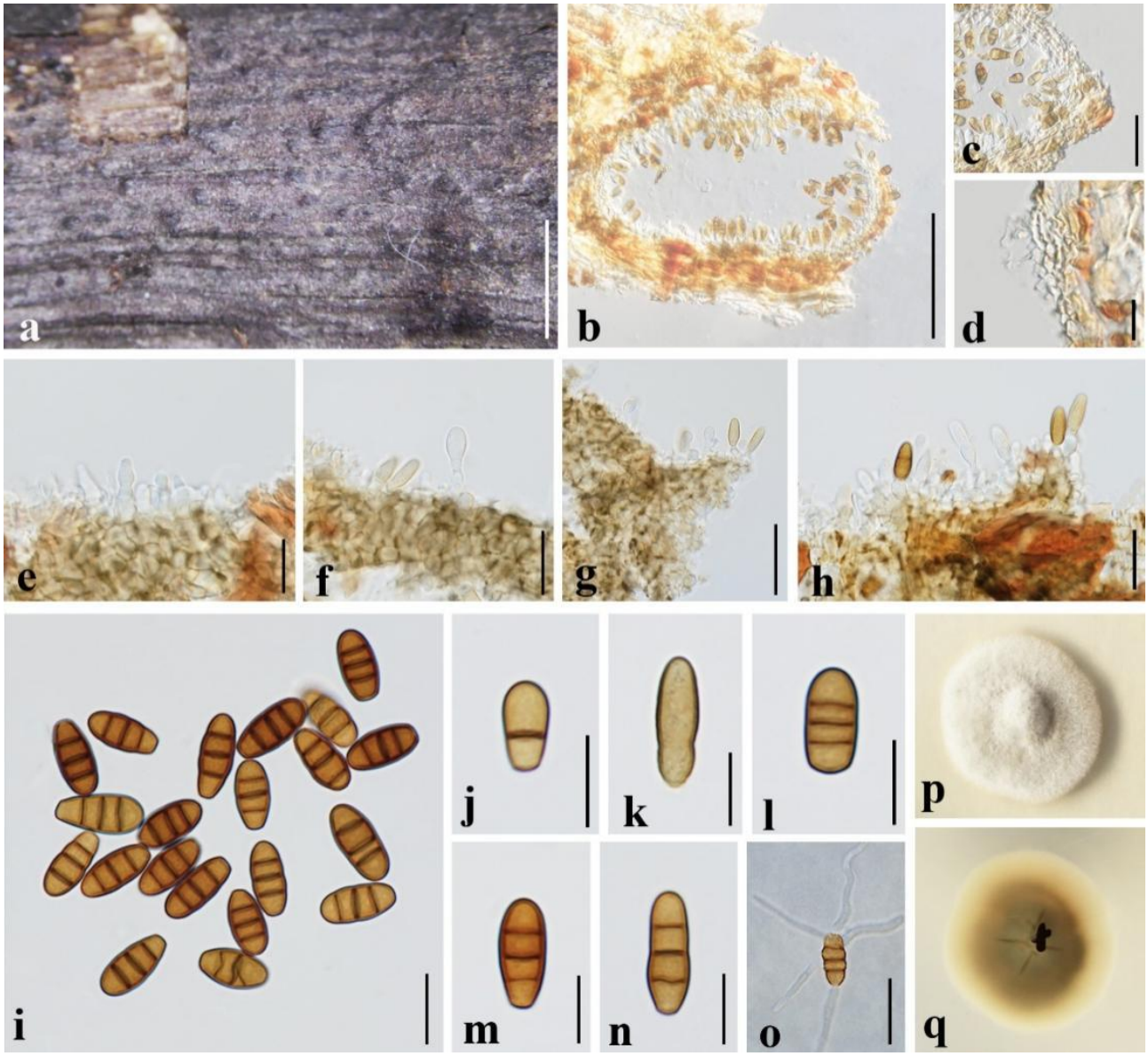

Figure 6. *Phragmocamarosporium qujingensis* (GMB 1193, GMB 1194, new host record). a. Conidiomata on host. b. Vertical section of ascoma. c, d. Pycnidial wall. e–h. Immature and mature conidia attached to conidiogenous cells. i–n. Conidia. o. Germinating conidium. p, q. Cultures on PDA (Obverse and reverse). Scale bars: a = 30 µm, b = 350 µm, c = 10 µm, d, j = 1 µm, e, f, h = 2 µm, i–o = 4 µm.

Notes: Our collection (GMBCC 1053, GMBCC 1054) is morphologically similar (conidiomata with a centrally located papillate ostiole, conidiophores reduced to conidiogenous cells, conidiogenous cells simple to simple branch at the base, smooth, long, phialidic. Conidia with obtuse apex and truncate base, 3−4 transverse septate) to the original protologue of the holotype (GMB1384) of *Phragmocamarosporium qujingensis* (Wijayawardene et al. 2022b). In molecular analyses (Figure 1), our new strains group with the ex-type (GMBCC 1176) and ex-paratype (GMBCC 1044) of *P. qujingensis*, respectively, reported by Wijayawardene et al. (2022b). Wijayawardene al. (2022b) reported that *P. qujingensis* is saprobic on the dead branches of *M. grandiflora* in Yunnan. Nevertheless, to our knowledge, this is the first record of *P. qujingensis* on the grapevine from China.

Discussion

Southwest China is the largest, most comprehensive and typical karst region and comprises three provinces i.e., Guizhou, Yunnan and Sichuan. Yunnan is located in the heart of it, with a complex topography and marked ecological differences, which nurtures rich floral, faunal and microbial resources; and plays a very important role in maintaining the ecological balance of the karst region (Wijayawardene et al. 2021). Moreover, Wijayawardene et al. (2021) mentioned that Yungui Plateau is an important region for discovering new fungal taxa.

Magnolia grandiflora is a popular evergreen tree that is widely used as ornamental for landscaping (Liu et al. 2019). More than 1,000 fungal taxa have been reported from this plant genus (Farr and Rossman 2022). Seventy-two records were reported from various substrates of *Magnolia alba*, *M. delavayi*, *M. denudate*, and *M. grandiflora* in China (Wijayawardene et al. 2022b).

The grape is one of the oldest cultivated fruit trees in the world and has been cultivated in China for over 2000 years. The grapevine (*Vitis vinifera*) serves as a substrate for the complex and diverse microorganisms naturally, such as bacteria, filamentous fungi, and yeasts (Stefanini and Cavalieri 2018), which has a significant impact on vine health, growth, and productivity (Müller et al. 2016).

During an ongoing study on fungi associated with agricultural and garden (or horticulture) plants, we isolated three pleosporalean fungi from *M. grandiflora* and grapevine. *Magnolia grandiflora* is widely planted as a gardening plant, while grape is an important economic crop in Yunnan Province. Among these three new records, two were reported from *M. grandiflora* (*A. alternata* and *N. juglandicola*). *Phragmocamarosporium qujingensis* was originally reported from *M. grandiflora*, but in this study, we also report it from the grapevine. These new records will be important for future studies to understand the distribution of known taxa, speciation due to geographical isolation, and for host-jumping phenomena (Wijayawardene et al. 2022a).

Southwestern China is rich in biodiversity thus many species are yet to be discovered. Apparently, these taxa would be new to the science or new geographic or host records to China. Hence, it is essential to concentrate on collecting fungi from rarely studied host genera along with commonly studied host plants.

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Conflict of interest

Authors declare no conflict of interest.

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