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Additions to *Crassiparies* and *Neobrevicollum* (Neohendersoniaceae, Pleosporales) associated with woody hosts in Southwest China

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Abstract

During an investigation of ascomycetous fungi from decaying wood in southwest China, seven taxa with sexual morphs were found to be saprobic from terrestrial habitats. These taxa were identified based on morphology, phylogeny, and cultural characteristics. The morphology and phylogenetic evidence placed these new fungal collections in Neohendersoniaceae, distributing in the genera *Crassiparies* and *Neobrevicollum*. The phylogenetic analyses of a combined ITS, LSU, SSU, *RPB2*, and *TEF1-α* sequence dataset also confirmed their taxonomic placement. A new species, *Neobrevicollum biancaeae* is introduced to accommodate the two taxa, which have thin-walled ascomata without ostiole, and smaller asci and ascospores compared to the type species, *N. oleae*. The other five samples were identified as *Crassiparies quadrisporus* and *N. oleae* representing three and two taxa, respectively. The identification and establishment of these species are justified based on morpho-molecular analyses. In addition, the new host records of *C. quadrisporus* and *N. oleae* were reported. Detailed descriptions and illustrations are provided for collected taxa. This study contributed to the microfungal diversity in Southwest China.

Key words: 1 new species, Dothideomycetes, multi-gene, phylogeny, taxonomy

Introduction

Pleosporales was introduced by Luttrell (1955) which is abundant and distributed in terrestrial, marine, and freshwater habitats (Ramesh 2003, Kruys *et al.* 2006, Hyde *et al*. 2013, Hongsanan *et al.* 2020). This is the largest order in Dothideomycetes, which includes more than 90 families, 650 genera (Wijayawardene *et al.* 2022), and consists of 10,142 species (Bánki *et al.* 2022). Neohendersoniaceae was established by Giraldo *et al.* (2017) to accommodate a monotypic genus *Neohendersonia* typified by *N. kickxii*. The study conducted by Tanaka *et al.* (2017) accepted four genera, namely *Brevicollum*, *Crassiparies*, *Medicopsis*, and *Neohendersonia* in Neohendersoniaceae. Subsequently, *Muriformispora* and *Neomedicopsis* were assigned to this family (Crous *et al*. 2019, de Silva *et al.* 2022). The members of Neohendersoniaceae are endophytic or saprobic fungi on plants, and human pathogens (Tanaka *et al.* 2017, Hongsanan *et al.* 2020). For example, *Neohendersonia kickxii* has been reported as a specific endophyte from beech twigs in Europe (Danti *et al.* 2002, Sieber 2007), *Medicopsis romeroi* was found to be a human pathogen (de Gruyter *et al.* 2013), and *Brevicollum hyalosporum* was known as saprobic on dead twigs of *Syzygium samarangense* (*Myrtaceae*) in Japan (Tanaka *et al.* 2017). Most genera of the family have sexual morphs except for *Neohendersonia* (Wijayawardene *et al.* 2016) and *Neomedicopsis* (Crous *et al.* 2019).

Neobrevicollum was introduced by Li *et al.* (2023), with the sexual morph of *N. oleae*, collected from *Olea europaea* in China. *Neobrevicollum* is characterized by having cylindrical to obclavate asci with an elongate and cylindrical pedicel, hyaline, fusiform and 1–3-septate ascospores (Li *et al.* 2023). *Neobrevicollum* is morphologically

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distinct from *Brevicollum* and *Crassiparies* from their ascospore characters. *Neobrevicollum* has 1–3-septate and hyaline ascospores with a mucilaginous sheath, while *Brevicollum* has 3–5-septate, hyaline or brown ascospores, surrounded by a rounded mucilaginous sheath, and *Crassiparies* has hyaline ascospores without a sheath (Tanaka *et al*. 2017, Li *et al.* 2023).

In this study, we deal with seven sexual morphs taxa collected from southwest China. Based on the phylogenetic and morphological studies, they are identified as three species, which belong to *Crassiparies* and *Neobrevicollum. Crassiparies quadrisporus* and *N. oleae* were reported as the new host record and *N. biancaeae* is introduced as a new species. Detailed descriptions and illustrations along with phylogenetic analyses based on the combined LSU, SSU, ITS, *RPB2* and *TEF1-α* sequence data are provided.

Materials & Methods

Specimen collection, morphological studies, and isolation

Specimens of the dead branches were collected from terrestrial habitats in southwest China, in 2022. Samples were packed and brought to the laboratory in envelopes. Morphological observations were examined by using a Motic SMZ (Stereoscopic Zoom Microscope) 168 Series dissecting microscope (Motic, Xiamen, China) for fungal structures on a natural substrate. The fruiting bodies were collected by using a syringe needle and transferred to a drop of tap water on a clean slide. Fungal structures were examined and photographed by a Nikon E80i microscope-camera system. Measurements were made with the Tarosoft Image Frame Work v. 0.9.7 software following the procedures outlined by Liu *et al.* (2010), and images used for photo plates were processed with Adobe Photoshop CC 2022 software (Adobe Systems, San Jose, CA, USA). Pure cultures were obtained by single spore isolation following the method described by Senanayake *et al.* (2020). Incubation and cultural growth were observed at 25 ℃ for one month.

Herbarium specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China, and the herbarium of University of Electronic Science and Technology (HUEST), Chengdu, China. The isolates obtained in this study was deposited in the China General Microbiological Culture Collection Center (CGMCC) in Beijing, China, and the University of Electronic Science and Technology Culture Collection (UESTCC), Chengdu, China. The names of the new taxa were registered in MycoBank (http://www.mycobank.org/)

DNA extraction, PCR amplification and sequencing

Isolates grew in PDA medium at 25 ℃ for three weeks. Fungal mycelia were scraped off and transferred to 1.5 mL microcentrifuge tubes using a sterilized lancet for genomic DNA extraction. Fungal DNA was extracted from mycelia (about 50–100 mg) using the Trelief TM Plant Genomic DNA Kit (TsingKe Co., Beijing, China). Five gene regions were amplified by Polymerase chain reaction (PCR); the internal transcribed spacer region (ITS), the small subunit rDNA (SSU), the large subunit rDNA (LSU), translation elongation factor 1-alpha (*TEF1-α*), and the RNA polymerase II second-largest subunit (*RPB2*). The primers used were ITS5/ITS4 for ITS (White *et al.* 1990), NS1/NS4 for SSU (White *et al.* 1990), LR0R/LR5 for LSU (White *et al.* 1990), TEF1-983F/TEF1-2218R for *TEF1-α* (Rehner & Buckley 2005), and fRPB2-5F/fRPB2-7cR for *RPB2* (Liu *et al.* 1999). The amplifications were performed in a 25 μL reaction volume containing 9.5 μL of ddH_2O, 12.5 μL of 2 × Taq PCR Master Mix with blue dye (Sangon Biotech, Shanghai, China), 1 μL of DNA template, and 1 μL of each primer. The amplification condition for ITS, SSU, LSU, and *TEF1-α* consisted of initial denaturation at 94℃ for 3 min, followed by 35 cycles of 45s at 94℃, 50s at 55℃ and 1 min at 72℃, and a final extension period of 10 min at 72℃. The amplification condition for the *RPB2* gene consisted of initial denaturation at 95℃ for 5 min; followed by 37 cycles of 15s at 95℃, 50s at 56℃ and 2 min at 72℃, and a final extension period of 10 min at 72℃. The PCR product purification and sequencing were performed at Beijing Tsingke Biotechnology (Chengdu) Co., Ltd., Chengdu, China. Newly generated sequences were deposited in GenBank.

Phylogenetic analyses

The sequences used for the phylogenetic analyses were obtained from previous studies (Hyde *et al.* 2018, Li *et al.* 2023) and GenBank (Table 1). The multi-gene dataset comprised 50 taxa, with seven newly generated and 43 retrieved from GenBank. *Cyclothyriella rubronotata* (CBS 141486 and CBS 419.85) was set as an outgroup (Hyde *et al.* 2018).

Single-gene fragments were aligned with MAFFT v.7 (Katoh *et al.* 2019) and visually checked with AliView (Larsson 2014). The alignments were trimmed using trimAl v.1.2 (Capella-Gutiérrez *et al.* 2009) with minimal coverage (-cons) = 0.8 and gap threshold (-gt) = 0.6. Five single-gene alignments were combined using SequenceMatrix 1.7.8 (Vaidya *et al.* 2011). Maximum likelihood (ML), Bayesian inference (BI), and maximum parsimony (MP) analyses were employed to assess phylogenetic relationships following Dissanayake *et al.* (2020).

The ML, BI, and MP analyses were performed with RAxML-HPC2 on XSEDE 8.2.12 (Stamatakis *et al.* 2008) at the CIPRES Science Gateway (Miller *et al.* 2010). One thousand non-parametric bootstrap iterations were employed with a general time reversible (GTR) model and a discrete gamma distribution, plus estimating the proportion of invariable sites for ML analysis. The MP and Bayesian analysis were performed using PAUP v. 4.0b (Swofford *et al.* 2003), MrModeltest v.2.3 (Nylander 2004) and MrBayes v.3.2.7 (Ronquist *et al.* 2012). GTR + I + G is the best-fit model selected by AIC in MrModeltest based on each gene (ITS, SSU, LSU, *RPB2* and *TEF1-α*), and was used for Bayesian analysis. The Markov Chain Monte Carlo (MCMC) algorithm of six chains started from a random tree topology with two parallel runs for 10 million generations and trees were sampled every 100 generations, and the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. A 50% majority rule consensus tree was summarized after discarding the first 25% samples. Phylogenetic trees were visualized with Figtree v.1.4.4 (Rambaut 2014) and the layout was made with Adobe Illustrator 22.1.

TABLE 1. Taxa used in this study and their GenBank accession numbers. Newly generated sequences are indicated with * and the ex-type strains are in bold. "/" indicates the sequences are unavailable.

Taxa	Strains/Vouchers	GenBank Accession Numbers				
		ITS	SSU	LSU	*RPB2*	*TEF1-α*
Acrocalymma aquatica	**MFLUCC 11-0208**	**JX276951**	**JX276953**	**JX276952**	**/**	**/**
Acrocalymma ficus	**CBS 317.76**	**KP170619**	**/**	**KP170712**	**/**	**/**
Acrocalymma walkeri	CBS 257.93	MH862398	FJ795495	FJ795454	FJ795471	/
Alternaria alternata	**CBS 916.96**	**KF465761**	**KC584507**	**DQ678082**	**KC584375**	**DQ677927**
Amarenographium ammophilae	**MFLUCC 16-0296**	**KU848196**	**KU848198**	**KU848197**	**/**	**MG520894**
Amarenographium ammophilicola	**MFLU 17-2571**	**MN047087**	**MN017913**	**MN017847**	**/**	**MN077065**
Amarenographium solium	MFLU 12-0059	/	JX181943	JX181942	/	/
Ascocylindrica marina	MD6011	/	KT252907	KT252905	/	/
Ascocylindrica marina	MD6012	/	/	KT252906	/	/
Boeremia exigua	CBS 431.74	FJ427001	EU754084	EU754183	GU371780	GU349080
Brevicollum hyalosporum	**MAFF 243400**	**LC271242**	**LC271236**	**LC271239**	**LC271249**	**LC271245**
Brevicollum hyalosporum	MFLUCC 17-0071	MG602204	MG602202	MG602200	/	MG739516
Brevicollum versicolor	**MAFF 246251**	**LC271243**	**LC271237**	**LC271240**	**LC271250**	**LC271246**
Crassiparies octosporus	**MFLUCC 18-0304a**	**OL782147**	**/**	**OL782065**	**/**	**OL875105**
*Crassiparies quadrisporus**	UESTCC 23.0134	OR754075	OR754090	OR754082	OR855455	OR855448
*Crassiparies quadrisporus**	UESTCC 23.0143	OR754080	OR754095	OR754087	OR855456	OR855449
*Crassiparies quadrisporus**	UESTCC 23.0144	OR754081	OR754096	OR754088	OR855457	OR855450
Crassiparies quadrisporus	**MAFF 245408**	**LC100020**	**LC100017**	**LC100025**	**LC271251**	**LC271247**
Crassiparies quadrisporus	MAFF 246250	LC271244	LC271238	LC271241	LC271252	LC271248
Crassiparies yunnanensis	**KUMCC 21-0215**	**OK564664**	**OK564663**	**OK564661**	**OK562422**	**OK562423**
Crassiparies yunnanensis	KUMCC 21-0384	OL679694	OL679696	OL679695	OL689026	OL689027
Cyclothyriella rubronotata	**CBS 141486**	**KX650544**	**KX650507**	**KX650544**	**KX650574**	**KX650519**
Cyclothyriella rubronotata	CBS 419.85	/	/	GU301875	GU371728	GU349002
Didymella exigua	**CBS 183.55**	**/**	**EU754056**	**EU754155**	**GU371764**	**/**
Didymella glomerata	**CBS 528.66**	**FJ427013**	**EU754085**	**EU754184**	**GU371781**	**GU349081**
Halojulella avicenniae	BCC 18422	/	GU371831	GU371823	GU371787	GU371816
Halojulella avicenniae	BCC 20173	/	GU371830	GU371822	GU371786	GU371815
Medicopsis chiangmaiensis	**MFLUCC 17-2457**	**MG873485**	**MG873483**	**MG873481**	**/**	**/**

.....continued on the next page

TABLE 1 (Continued**)**

Taxa	Strains/Vouchers	GenBank Accession Numbers				
		ITS	**SSU**	**LSU**	***RPB2***	***TEF1-α***
Medicopsis romeroi	CBS 122784	KF366447	EU754109	EU754208	KF015707	KF015679
Medicopsis romeroi	CBS 123975	KF015657	KF015650	KF015623	KF015710	KF015681
Medicopsis romeroi	**CBS 252.60**	**KF366446**	**EU754108**	**EU754207**	**KF015708**	**KF015678**
Muriformispora magnoliae	MFLU 18-2645	OM212459	OL824795	OL813499	ON502385	ON303277
Muriformispora magnoliae	**MFLUCC 19-0036**	**OM212460**	**OL824796**	**OL813500**	**/**	**ON303278**
Neobrevicollum oleae	**CGMCC 3.25054**	**OR253105**	**OR253183**	**OR253257**	**/**	**OR251157**
Neobrevicollum oleae	UESTCC 23.0068	OR253106	OR253184	OR253258	/	OR251158
*Neobrevicollum oleae**	UESTCC 23.0145	OR754076	OR754091	OR754083	OR855458	OR855451
*Neobrevicollum oleae**	UESTCC 23.0146	OR754077	OR754092	OR754084	OR855459	OR855452
Neobrevicollum biancaeae*	**CGMCC 3.25420**	**OR754078**	**OR754093**	**OR754085**	**OR855453**	**OR855446**
*Neobrevicollum biancaeae**	UESTCC 23.0142	OR754079	OR754094	OR754086	OR855454	OR855447
Neohendersonia kickxii	**CBS 112403**	**KX820255**	**/**	**KX820266**	**/**	**/**
Neohendersonia kickxii	CBS 122938	KX820257	/	KX820268	/	/
Neohendersonia kickxil	CBS 114276	KX820256	/	KX820267	/	/
Neohendersonia kickxil	CBS 122941	KX820258	/	KX820269	/	/
Neohendersonia kickxil	CPC 24865	KX820259	/	KX820270	/	/
Neomedicopsis prunicola	**CBS 145031**	**MK442603**	**/**	**MK442539**	**MK442670**	**/**
Paradendryphiella salina	CBS 142.60	DQ411540	KF156098	KF156158	/	DQ414251
Phaeosphaeria musae	MFLU 11-0133	KM434267	KM434287	KM434277	KM434304	KM434296
Phaeosphaeria oryzae	**CBS 110110**	**MH862850**	**NG_061080**	**MH874442**	**ON419520**	**/**
Pleospora herbarum	CBS 191.86	KC584239	DQ247812	DQ247804	DQ247794	DQ471090
Stemphylium botryosum	CBS 714.68	AF071345	KC584603	KC584345	AF107804	JQ672391

Results

Phylogenetic analyses

Five gene loci ITS, LSU, SSU, *RPB2*, and *TEF1-α* were used to determine the phylogenetic placement of the new fungal collections. The dataset comprised 50 taxa with a total of 4,216 characters (ITS: 502 bp; LSU: 848 bp; SSU: 1004 bp; *RPB2*: 951 bp; *TEF1-α*: 911 bp) including gaps. The dataset used for parsimony analysis consists of 4,216 characters, 2,856 characters were constant; 1,212 (29%) characters were parsimony informative, and 148 variable characters are parsimony-uninformative. A heuristic search yield one equally most parsimonious trees (TL = 4150, CI = 0.503, RI = 0.776, RC = 0.391, HI = 0.497).

These 50 taxa representing eight families, of which Neohendersoniaceae was represented by 30 taxa including all seven genera with its type-species. Single-gene analyses were performed to compare the topologies and clade stabilities, which are generally consistent except for *Medicopsis chiangmaiensis* (MFLUCC 17-2457), which is somewhat unstable. The results showed that ML and BI were similar in topology without significant conflictions, and these results agree with previous studies (Senwanna *et al.* 2021, de Silva *et al.* 2022). The best-scoring ML tree with a final optimization likelihood value of -25040.483240, is shown in Fig. 1. The aligned matrix had 1604 distinct alignment patterns, and 25.97% completely undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.246387, C = 0.243783, G = 0.267520, T = 0.242309; substitution rates AC = 1.784317, AG = 4.347963, AT = 1.759034, CG = 1.159632, CT = 8.165366, GT = 1.0. Gamma distribution shape parameter α = 0.173803. Six simultaneous Markov chains were run for 500,200 generations and trees were sampled every 100 generations and 5002 trees were obtained. The first 1250 trees representing the burn-in phase of the analyses were discarded, while the remaining 3752 trees were used for calculating posterior probabilities in the majority rule consensus tree.

Representatives of all the genera and their type species of Neohendersoniaceae were included in our phylogenetic tree (Fig. 1). All the seven genera of Neohendersoniaceae, represented by 30 taxa and they formed well-supported

clades, Our new collections clade within two genera: *Crassiparies* and *Neobrevicollum*. Three strains (UESTCC 23.0143, UESTCC 23.0144 and UESTCC 23.0134) were clade within *Crassiparies* and four strains (UESTCC 23.0145, UESTCC 23.0146, CGMCC 3.25420 and UESTCC 23.0142) clade within *Neobrevicollum* (Fig. 1).

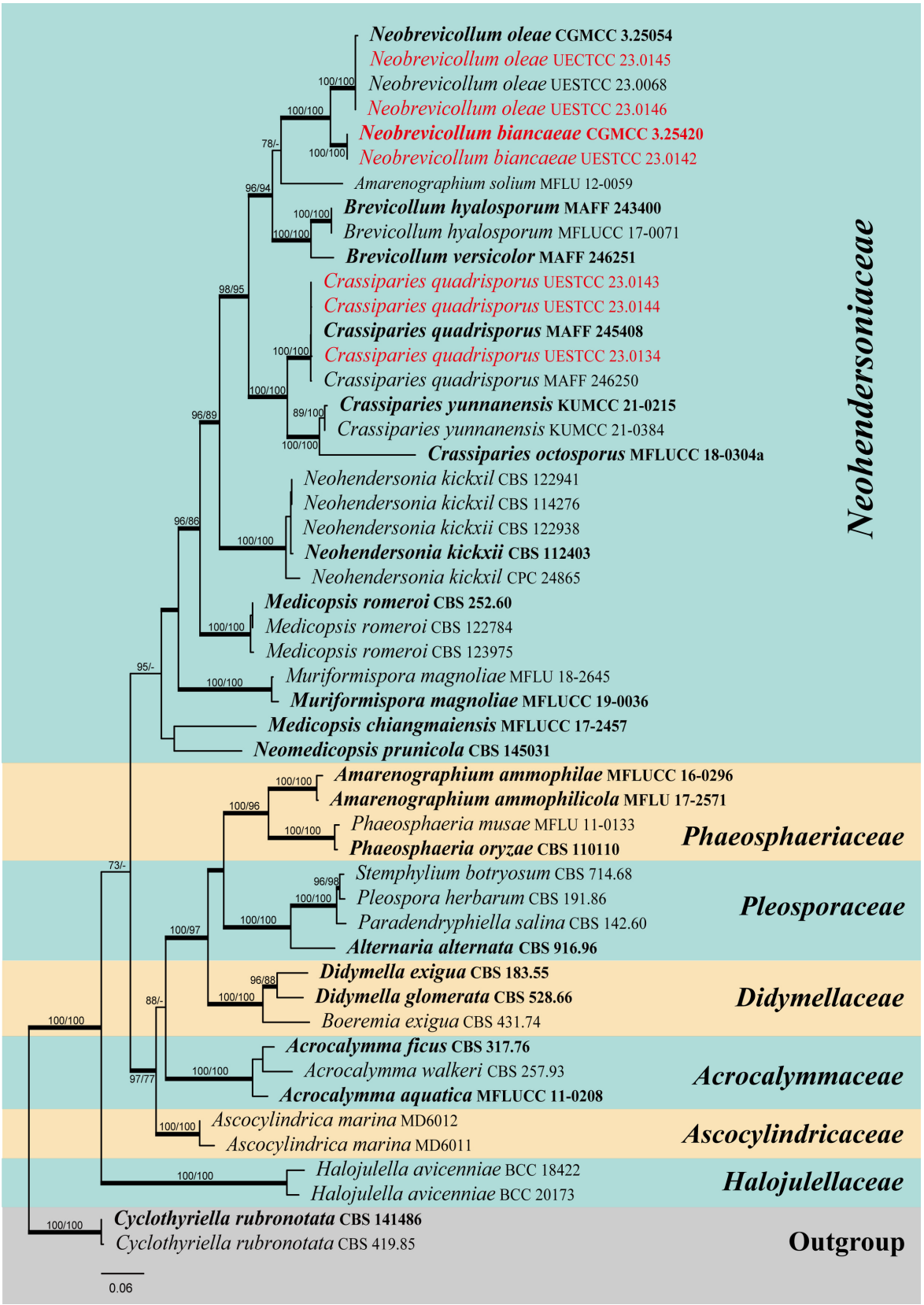

FIGURE 1. Phylogenetic tree from ML analysis based on the combined ITS, SSU, LSU, *RPB2*, and *TEF1-α* sequences data. Bootstrap values for ML and MP ≥ 75% are placed above the branches. Branches with Bayesian posterior probabilities (BYPP) ≥ 0.95 are in bold. The tree is rooted to *Cyclothyriella rubronotata* (CBS 141486 and CBS 419.85). The ex-type strains were indicated in bold, and newly generated sequences were indicated in red.

Taxonomy

Neobrevicollum biancaeae H.Z. Du, Y.H. Lu & Jian K. Liu, *sp. nov.*, Fig. 2
MycoBank: MB 851140

Etymology:—The epithet '*biancaeae*' refers to the host genus *Biancaea* on which the fungus was collected.

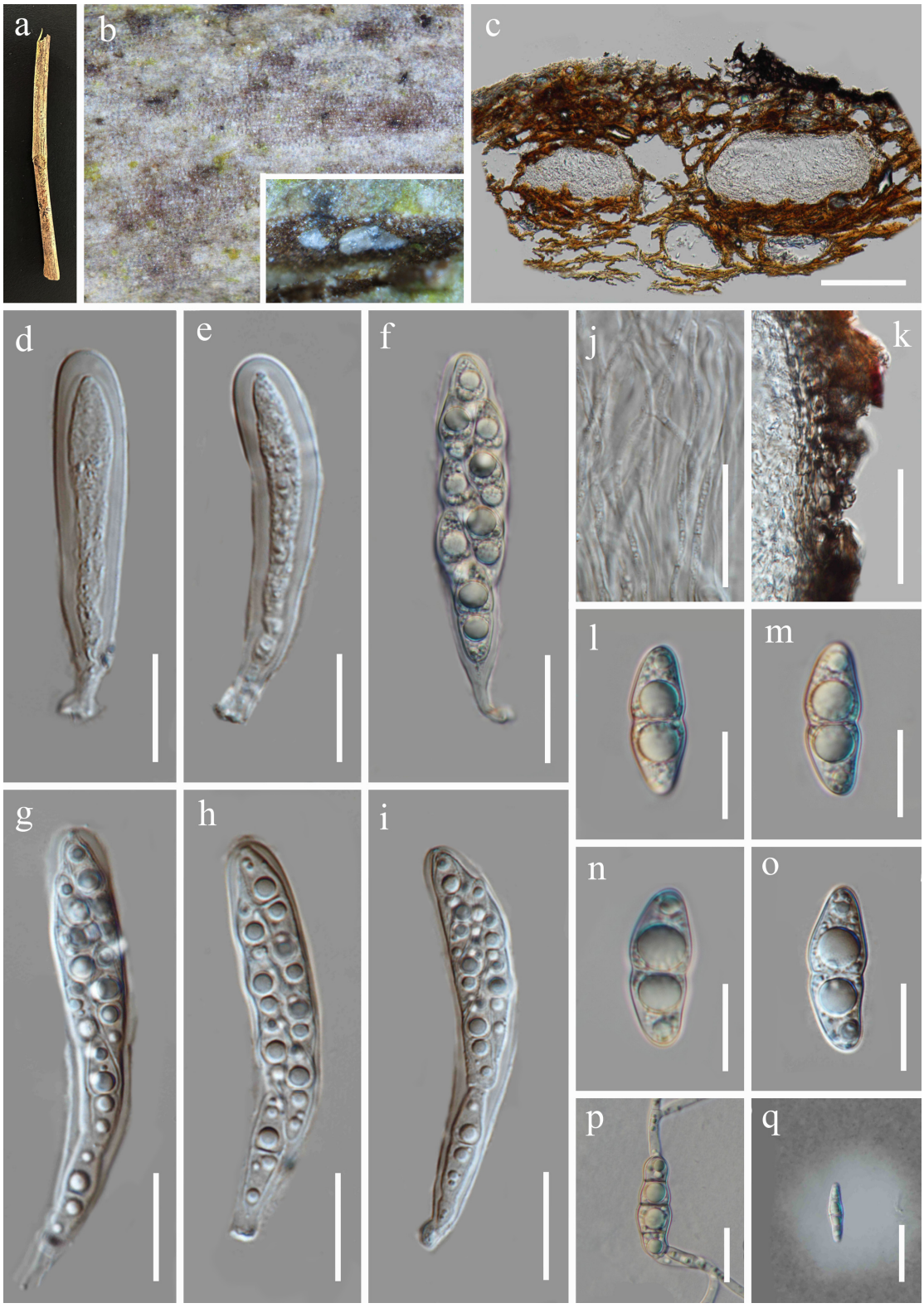

FIGURE 2. ***Neobrevicollum biancaeae*** (HKAS 130507, holotype) **a** Substrate. **b** Ascomata on host surface. **c** Vertical section of ascomata. **d–i** Asci. **j** Hamathecium. **k** Peridium. **l–o** Ascospores. **p** Germinating ascospore. **q** Ascospore stained with India ink showing the mucilaginous sheath. Scale bars: **c** = 100 µm, **d–i** = 20 µm, **j, k** = 30 µm, **l–q** = 20 µm.

***Holotype:*—**HKAS 130507

Saprobic on dead branches of *Biancaea sappan*. **Sexual morph:** *Ascomata* 109–145 µm high, 125–200 µm diam ($\overline{x}$ = 122 × 170 µm, n = 10), immersed, scattered, uniloculate, globose or subglobose, glabrous, dark brown to black, thin-walled, and white interior, without *ostiole. Peridium* 9–14 µm wide, thin, composed of several layers of brown cells of *textura angularis*. *Hamathecium* 1.7–2.5 µm wide ($\overline{x}$ = 2 µm), numerous, filamentous, cellular pseudoparaphyses, with indistinct septa. *Asci* 68–140 × 16–25 µm ($\overline{x}$ = 112 × 20 µm, n = 30), 8-spored, hyaline, bitunicate, fissitunicate, cylindrical to clavate with a short pedicel, apically rounded, with a small ocular chamber. *Ascospores* 30–35 × 9–13 µm ($\overline{x}$ = 32 × 11 µm, n = 30), 1–2-seriate, overlapping in the ascus, hyaline to pale yellowish-brown, broadly fusiform, 1-septate, constricted at the septa, obtuse at both ends, smooth-walled, surrounded by a mucilaginous sheath, guttulate. **Asexual morph:** Undetermined.

Culture characteristics:—Ascospores germinated within 24 hours on PDA. *Colonies* on PDA reaching about 34 mm after one month incubated at 25 °C, irregular, with undulate edge, dark brown to brown at the surface with white margin and brown from the centre of the colony in reverse with white margin.

***Material examined:*—**CHINA, Yunnan Province, Xishuangbanna Dai autonomous prefecture, Xishuangbanna tropical botanical garden Chinese Academy of Sciences. 101°15′6″E, 21°55′51″N, 502 m elevation, on dead branches of medicinal plant *Biancaea sappan* (L.) Tod.9 (*Fabaceae*), 9 November 2022, H.Z. Du, S578A (HKAS 130507, holotype); ex-holotype living culture CGMCC 3.25420); *ibid.*, HUEST 23.0142, isotype, ex-isotype living culture UESTCC 23.0142.

Notes:*—Neobrevicollum biancaeae* morphologically fits into the generic concept of *Neobrevicollum* by having immersed, uniloculate and globose ascomata, numerous and hyaline pseudoparaphyses, bitunicate, cylindrical to obclavate asci with a cylindrical pedicel, hyaline, overlapping, fusiform, 1-septate ascospores with a mucilaginous sheath (Li *et al.* 2023). However, *N. biancaeae* differs from *N. oleae* in having thin-walled ascomata without ostiolate, smaller ascomata (125–200 × 109–145 µm vs. 230–270 × 200–230 µm), and asci (68–140 × 16–25 µm vs. 105–135 × 18–23 µm) (Li *et al.* 2023). The results of phylogenetic analysis also indicated that *N. biancaeae* and *N. oleae* are phylogenetically distinct species and *N. biancaeae* (CGMCC 3.25420 and UESTCC 23.0142) formed separate clade with statistical support (100% ML/1.00 BYPP) (Fig. 1). Additionally, *N. biancaeae* can be distinguished from *N. oleae* based on ITS, LSU, and *TEF1-α* base pair differences; ITS = 25/433 (5.77%), LSU = 8/852 (0.94%), and *TEF1-α* = 31/909 (3.41%).

Neobrevicollum oleae W.L. Li & Jian K. Liu, in Mycosphere 14(1): 1495 (2023), Fig. 3
MycoBank: MB 849239

Saprobic on dead branches of *Acer palmatum*. **Sexual morph:** *Ascomata* 240–310 µm high, 350–550 µm diam ($\overline{x}$ = 280 × 525 µm, n = 10), immersed, solitary, scattered, black, visible as black spots on host surface, globose to subglobose, glabrous, dark brown to black, rough walled, and white interior. *Ostiole* central, dark brown, with short papillate. *Peridium* 42–98 µm wide ($\overline{x}$ = 69 µm), thick, multi-layered, comprising of 5–8 layers of light brown cells of *textura angularis*. *Hamathecium* 1.8–2.6 µm wide, numerous, filamentous, cellular pseudoparaphyses, with inconspicuous septa. *Asci* 72–168 × 14–24 µm ($\overline{x}$ = 108 × 20 µm, n = 30), 8-spored, hyaline, bitunicate, fissitunicate, cylindrical to clavate, shortly pedicellate (7.2–16.3 µm, n = 30). *Ascospores* 22.5–30 × 6.5–12 µm ($\overline{x}$ = 26 × 8.5 µm, n = 50), 1–2-seriate, partially overlapping, hyaline, broadly fusiform, obtuse at both ends, 1-septate, slightly constricted at the septa, the upper cell slightly larger than the lower cell, guttulate, smooth-walled, and surrounded by a mucilaginous sheath, 10.3–19.5 µm wide. **Asexual morph:** Undetermined.

Culture characteristic:—*Colonies* on PDA reaching about 20 mm diam. after ten days incubated at 25°C, and about 27 mm diam. after two weeks, circular, flattened, felt-like, sparse, aerial, yellowish-white and becoming grey at the center, the surface smooth with filamentous edge, reverse dark brown at the center and yellowish-white towards the margin.

***Material examined:*—**CHINA, Sichuan province, Chengdu City, Chengdu Botanical Garden, 30°45′55″N, 104°7′32″E, 530 m elevation, 21 September 2022, on dead branches of *Acer palmatum* (*Sapindaceae*) in a terrestrial habitat, Y.H. Lu & H.Z. Du, C17A (HUEST 23.0145), living culture UESTCC 23.0145; *ibid.*, HUEST 23.0146, living culture UESTCC 23.0146.

Notes:—*Neobrevicollum oleae* was introduced by Li *et al.* (2023) from *Olea europaea* in Sichuan province, China. Our two collections are morphologically similar to the holotype of *N. oleae* in having a central ostiole and cylindrical to obclavate asci with an elongate and cylindrical pedicel, and hyaline, fusiform ascospores (Li *et al.* 2023). Based on the results of phylogenetic analyses, two isolates (UESTCC 23.0145 and UESTCC 23.0146) grouped with

the type strain (CGMCC 3.24430) and the other strain (UESTCC 23.0068) of *N. oleae* with 100% ML and 1.00 BYPP. Therefore, we identified them as *N. oleae* and reported the new host record in this study.

FIGURE 3. ***Neobrevicollum oleae*** (HUEST 23.0145) **a** Substrate. **b, c** Ascomata on host surface. **d, e** Vertical section of ascomata. **f–i** Asci. **j** Peridium. **k** Hamathecium. **l–q** Ascospores. **r** Germinating ascospore. **s** Ascospore stained with India ink showing the mucilaginous sheath. **t, u** Colony on PDA. Scale bars: **d, e** = 100 µm, **f–i** = 20 µm, **j** = 20 µm, **k–r** = 10 µm, **s** = 50 µm.

Crassiparies quadrisporus Matsumura, K. Hiray. & Kaz. Tanaka, in Fungal Diversity 78: 63 (2016), Fig. 4
MycoBank: MB 815295

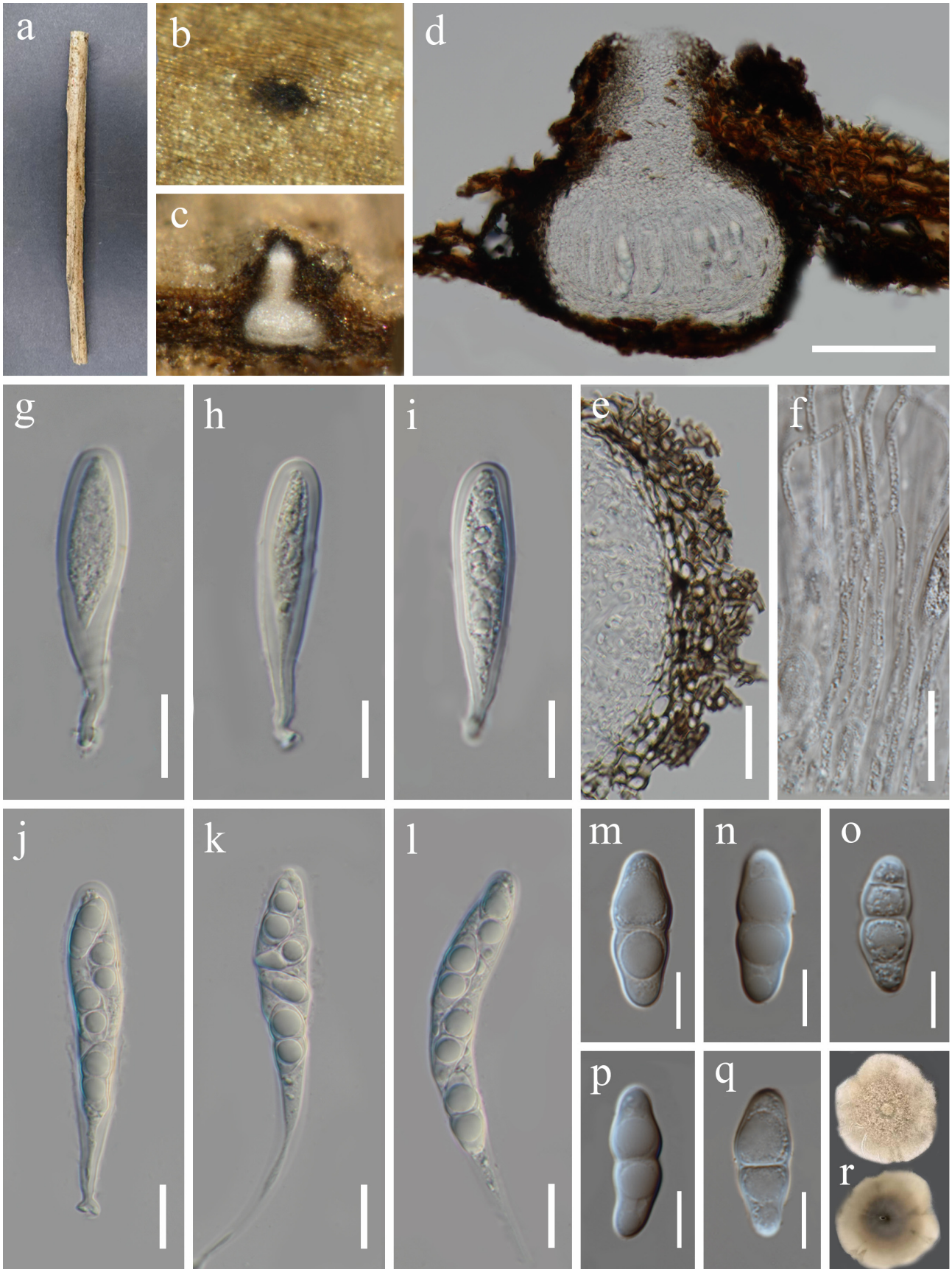

FIGURE 4. ***Crassiparies quadrisporus*** (HUEST 23.0144) **a** Substrate. **b, c** Ascomata on host surface. **d** Vertical section of ascomata. **e** Peridium. **f** Hamathecium. **g–l** Asci. **m–q** Ascospores. **r** Colony on PDA. Scale bars: **d** = 100 µm, **e** = 25 µm, **f–l** = 20 µm, **m–q** = 10 µm.

Saprobic on the branch of *Jasminum nudiflorum* and *Camellia sinensis*. **Sexual morph:** *Ascomata* 210–250 µm high, 255–270 µm diam ($\overline{x}$ = 235 × 260 µm, n = 10), scattered, sometimes in groups of 2–4, immersed, globose to subglobose, with a central ostiole. *Peridium* 15–30 µm wide, outer layers dark brown to black, inner layers thin-walled, composed of hyaline cells of *textura angularis*. *Hamathecium* 2.0–2.5 µm wide, numerous, dense, hyaline, septate, filamentous pseudoparaphyses. *Asci* 65–145 × 13–18 µm ($\overline{x}$ = 93 × 15 µm, n = 30), 4-spored, bitunicate, fissitunicate, cylindric-clavate or clavate, short pedicellate, apically rounded. *Ascospores* 24–30 × 8–12 µm ($\overline{x}$ = 26×10 µm, n = 50), hyaline, broadly fusiform, overlapping biseriate, ends rounded, 1-septate, with a septum mostly submedian, minutely echinulate, guttulate, without mucilaginous sheath. **Asexual morph:** Undetermined.

Culture characteristics:—Ascospores germinated within 24 hours on PDA, *Colonies* reaching about 25 mm diam after three weeks, irregular, with undulate edge, light brown to dark brown at the surface with white margin and brown from the centre of the colony in reverse with white margin. Mycelium 2.4–3.6 µm broad, hyaline to pale brown, septate, branched. Chlamydospores apical or intercalary, produced after 150 days of growth on PDA at 25 °C, 4.9–16.0 × 4.0–10.5 µm, ellipsoidal, thick-walled, pale brown when young, brown when mature.

Material examined:—CHINA, Sichuan province, Chengdu city, Chengdu Botanical Garden, 30°45′52″N, 104°7′35″E, 535m elevation, 21 September 2022, on branches of *Jasminum nudiflorum (Oleaceae*) in a terrestrial habitat, Y.H. Lu & H.Z. Du, C05 (HUEST 23.0134), living culture UESTCC 23.0134; *ibid.*, Yaan city, Mingshan county, Mengding Mountain, 30°4′35″N, 103°2′29″E, 1251m elevation, 16 July 2023, on branches of *Camellia sinensis* (*Theaceae*), Y.H. Lu & X.D. Liang, MD33 (HUEST 23.0144), living culture UESTCC 23.0144; *ibid.*, Guizhou province, Guiyang city, Huaxi county, 26°30′43″N, 106°39′32″E, 1155m elevation, 2 February 2023, on branches of *Camellia sinensis* (*Theaceae*), Y.H. Lu & Y.X. Yu, GY18 (HUEST 23.0143), living culture UESTCC 23.0143.

Notes:—*Crassiparies* was introduced with *C. quadrisporus* as the type by Li *et al*. (2016) and revised by Tanaka *et al*. (2017). *Crassiparies quadrisporus* occur in various plant species as saprobes and endophytes and is widely distributed in temperate and tropical regions (Hongsanan *et al.* 2020). Our collection is morphologically similar to the original description of *Crassiparies quadrisporus* in Li *et al*. (2016), which has thick-walled ascomata, 4-spored asci and hyaline, broad fusiform ascospores. Based on the phylogenetic analyses, three strains (UESTCC 23.0134, UESTCC 23.0143 and UESTCC 23.0144) were grouped with the ex-type strain (MAFF 245408) and the other strain of *C. quadrisporus* (MAFF 246250) with statistical support (100% ML/1.00 BYPP) (Fig. 1). *Crassiparies quadrisporus* has been reported on *Acer* sp., Japan (Li *et al.* 2016), *Machilus japonica*, Japan (Tanaka *et al.* 2017), *Hevea brasiliensis*, Thailand (Senwanna *et al.* 2021), *Jasminum nudiflorum* and *Camellia sinensis*, China (this study). We identified our three collections as *C. quadrisporus* and introduced it as a new geography (China) and host record (*Camellia sinensis*).

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