



Neoostropa castaneae gen. et. sp. nov. (*Stictidaceae*, *Ostropales*) and a new report of *Fitzroyomyces cyperacearum* from China

Yang YY^{1,2,3}, Wei DP^{1,2,4}, Zhao Q², Gomes de Farias AR¹, Hyde KD^{1,3,5*}

¹Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

²CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Panlong District, Kunming, Yunnan, P. R. 650201, China

³School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁴Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Mueang Chiang Mai, Chiang Mai 50200, Thailand

⁵Innovative Institute for Plant Health, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, People's Republic of China

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Abstract

Stictidaceae species are drought resistant and have diverse lifestyles. However, because their fruiting bodies are small and not easy to observe, they are rarely collected. Our knowledge of this family is very limited, and many new species remain to be discovered, even in places where samples are often collected. During a field survey in Yunnan Province, China, specimens of saprobic *Stictidaceae* were collected and characterized. Here, a new genus, *Neoostropa*, is introduced to accommodate a novel species related to *Ostropomyces*. Maximum likelihood and Bayesian phylogenetic inference of ITS, LSU, and mtSSU sequence data, together with morphological evidence, support the taxonomic affinity of the new taxa.

Keywords – 1 new species – *Fagaceae* – Morphology – Novel species – Phylogeny

Introduction

Ostropales Nannf. was introduced by Nannfeldt in 1932 to accommodate the non-lichenized family *Stictidaceae* (= *Ostropaceae*) (Winka et al. 1998, Ekanayaka et al. 2018). This order includes a single family, *Stictidaceae*, erected by Fries (1849) and is typified by *Stictis radiata* Fr. It includes species not easily noticeable. Their exposed discs, ascomata containing a crystalline substance, long and unbranched paraphyses, and cylindrical asci surrounded by its long paraphyses and filiform ascospores characterize this family. According to Wijayawardene et al. (2022) and Thiyagaraja et al. (2021), 32 genera are accepted in *Stictidaceae* (*Absoconditella*, *Acarosporina*, *Biostictis*, *Carestiella*, *Conotremopsis*, *Cryptodiscus*, *Cyanodermella*, *Dendroseptoria*, *Delpontia*, *Eriospora*, *Fitzroyomyces*, *Geisleria*, *Glomerobolus*, *Hormodochis*, *Ingvariella*, *Karstenia*, *Lillicoa*, *Nanostictis*, *Neofitzroyomyces*, *Neostictis*, *Ostropa*, *Ostropomyces*, *Propoliopsis*, *Phacidiella*, *Robergea*, *Schizoxylon*, *Sphaeropezia*, *Stictis*, *Stictophacidium*, *Topelia*, *Trinathotrema*, and *Xyloschistes*), of which most comprise single or few species. An updated phylogeny by Wei et al. (2021) indicated that the family taxonomy is quite well resolved, but some genera seem problematic.

It is thought that the present saprotrophic lineages of *Ostropales* resulted in several losses of lichenization from their common lichenized ancestor (Baloch et al. 2010, Lutzoni et al. 2001). Species in this family present different lifestyles, including lichens, parasites, and endophytes (Gilenstam 1974, Wedin et al. 2004, Fernández-Brime et al. 2011, Baloch et al. 2009, 2013, Aptroot & Moon 2014, Jahn et al. 2017), with the predominance of saprotrophs and lichenicolous (Wedin et al. 2004, Baloch et al. 2009, 2013). They have also been recorded as facultative lichenized and saprobes *Discomycetes* (Baloch et al. 2009, 2013), with some species developing either as lichens or saprotrophs, depending on the substrate (Konoreva et al. 2016). However, some species of *Absoconditella* have been recorded as pathogens on bryophytes (Lücking et al. 2013), and species of *Acarosporina* were reported as pathogens of *Quercus* and *Fagus* (Sherwood 1977), and *Cyanodermella asteris* has been recorded as endophytic (Stenroos et al. 2010). According to Thiyyagaraja et al. (2021), species of *Stictidaceae* can switch lifestyles, which may drive their evolution and speciation. However, Wei et al. (2021) showed that the different life modes are widely dispersed throughout the evolutionary tree. Nevertheless, the plasticity of lifestyles is frequent and may represent the fungal adaptability to different habitats (Wedin et al. 2004).

Fagaceae is a large angiosperm family comprising more than 900 species belonging to up to ten genera and spread throughout the Northern Hemisphere, from the tropical to the boreal regions (Kremer et al. 2012). In China, *Fagaceae* species comprise seven genera and about 294 species, widely distributed, with some woody species representing important uses in the food and silk industry (http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10338). This study introduces a new genus of a *Stictidaceae* species associated with *Fagaceae* litter fungi in Kunming in Yunnan Province, China. Comprehensive morphological and multigene-based phylogenetic analyses are provided.

Materials & Methods

Sample collection and Phenotypic Analysis

Litter samples were collected, placed in plastic bags, and brought to the laboratory. Freehand sections of the ascomata were mounted with water, and the micro-morphological characteristics such as peridium, asci, and ascospore were photographed using a Nikon ECLIPSE Ni compound microscope fitted with an IMG SC2000C camera. The measurements were taken with the Tarosoft(R) Image Frame Work program, and the images were edited with Adobe Photoshop 2019. Pure isolates were obtained by the single spore isolation method, as described in Senanayake et al. (2020). The germinated spores were observed using a stereo microscope, transferred to a potato dextrose agar (PDA) plate, and incubated at 20 °C for one month. Living cultures were deposited in the Kunming Institute of Botany Academia Sinica (KUN) and the dry cultures at the Kunming Institute of Botany Culture Collection (KUNCC), respectively. Faces of Fungi (FoF) (Jayasiri et al. 2015) and Index Fungorum (<http://www.indexfungorum.org>) numbers were registered. All entries are deposited in the Greater Mekong Subregion database (Chaiwan et al. 2021).

DNA Extraction, PCR Amplification, and Gene Sequencing

Genomic DNA was extracted from fresh fungal mycelia grown on PDA media for 43 days and from the mycelia using TreliefTM Plant Genomic DNA Kit (BioFlux, Beijing, China) following the manufacturer's instructions. Polymerase chain reaction (PCR) was carried out for three partial gene fragments, viz., internal transcribed spacers (ITS), large subunit ribosomal rDNA (LSU), and mitochondrial small subunit ribosomal rDNA(mtSSU), with primers ITS4/ITS1(White et al. 1990), LROR/LR5 (Vilgalys & Hester 1990), and mrSSU1/mrSSU3R (Zoller et al. 1999), respectively. The PCR amplification conditions of ITS and LSU consisted of an initial denaturation step of 5 min at 98 °C, followed by 35 cycles of denaturation at 98 °C for 30 seconds, annealing at 54 °C for 40 seconds, and elongation at 72 °C for 50 seconds and a final extension step of 7 minutes at 72 °C. For mtSSU it was used an initial denaturation step of 3 min at 98 °C, followed by the 35 cycles of denaturation at 98 °C for 1 min, annealing at 52 °C for 1 min, and elongation at 72 °C for 1 min,

and final extension step of 10 min at 72 °C. Amplified PCR products were purified and sequenced by the Tsingke Biotechnology Co., Ltd. (Kunming, China).

Phylogenetic Analyses and Species Recognition

The obtained sequences were subjected to the basic local alignment search tool (BLAST) search engine of the National Center for Biotechnology Information (NCBI) to select the closely related taxa (Table 1). Reference isolates were retrieved from recent publications (Lu et al. 2021, Thiyagaraja et al. 2021, Wei et al. 2021), and the nucleotide sequences were downloaded from the NCBI GenBank database. Sequence alignments of the individual datasets of the ITS, LSU, and mtSSU partial sequences were performed by the MAFFT online service (<https://mafft.cbrc.jp/alignment/server/index.html>, Katoh et al. 2019) with default settings and automatically using trimAl v1.2rev59 (Castresana 2000, Talavera & Castresana 2007). Evolutionary models for each barcode were determined using jModelTest v.2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012) under the Akaike information criterion (AIC), and the best-fit model Rannala used for the maximum likelihood and Bayesian phylogenetic inferences. Bayesian analyses were conducted with MrBayes v. 3.2.7 (Ronquist et al. 2012) to evaluate Bayesian posterior probabilities (BYPP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) by Markov Chain Monte Carlo sampling (MCMC), with four simultaneous BMCMC and 1,000,000 generations, with trees sampled every 100th generation. The first 25% of generated trees were discarded as burn-in, and the remaining was used to calculate the BYPP of the majority rule consensus tree (Hua et al. 2006). Maximum likelihood trees were generated using RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2012) using the best-fit models for each molecular marker calculated by jModelTest. Phylograms were visualized with FigTree v1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree>) and edited in Inkscape v.1.2 (<https://inkscape.org>). Newly generated nucleotide sequences were deposited in GenBank.

Table 1 Species, strain codes, and GenBank accession numbers of the taxa used in the present phylogenetic analyses.

Species	Strain/Voucher	GenBank accession numbers		
		LSU	ITS	mtSSU
<i>Absconditella sphagnorum</i>	M24	EU940095	–	EU940247
<i>A. sphagnorum</i>	taxon:232774	AY300824	–	AY300872
<i>Carestiella socia</i>	GG2437a	AY661682	AY661682	AY661678
<i>C. socia</i>	GG2410	AY661687	AY661687	AY661677
<i>Cryptodiscus foveolaris</i>	EB88	FJ904671	–	FJ904693
<i>C. incolor</i>	EB164	–	FJ904675	FJ904697
<i>C. gloeocapsa</i>	EB93	–	FJ904674	FJ904696
<i>C. pallidus</i>	EB60	–	FJ904678	FJ904700
<i>C. pallidus</i>	EB40	–	FJ904677	FJ904699
<i>C. muriformis</i>	H.B. 6773	MG281963	MG281963	MG281973
<i>C. muriformis</i>	UPS F-647154	MG281962	MG281962	MG281972
<i>C. pini</i>	EB82	FJ904682	FJ904682	FJ904704
<i>C. pini</i>	EB178	FJ904683	FJ904683	FJ904705
<i>C. rhopaloides</i>	EB100	–	FJ904685	FJ904707
<i>C. tabularum</i>	CO205	FJ904690	FJ904690	FJ904712
<i>C. tabularum</i>	EB169	FJ904689	FJ904689	FJ904711
<i>Cyanodermella banksiae</i>	CPC:32105 ^T	NG 064548	MH327814	–
<i>C. viridula</i>	EB146	HM244763	MG281964	HM244739
<i>Fitzroyomyces cyperacearum</i>	CPC:32209 ^T	NG058513	NR156387	–
<i>F. cyperacearum</i>	MFLU 19–2725	MW293933	MW293953	–
<i>F. cyperacearum</i>	MFLU 18–0695b	MK499361	MK499349	–

Table 1 Continued.

Species	Strain/Voucher	GenBank accession numbers		
		LSU	ITS	mtSSU
<i>F. cyperacearum</i>	HKAS 124386	OP739481	OP727993	OP739485
<i>F. cyperacearum</i>	HKAS 124387	OP739480	OP727992	OP739484
<i>F. hyaloseptisporus</i>	MFLUCC 21–0111 ^T	MZ868921	MZ868916	MZ868911
<i>F. pandanicola</i>	HKAS 96206 ^T	MH260319	MH275085	–
<i>F. yunnanensis</i>	KUMCC 21 0002 ^T	MZ781317	MZ781316	MZ781329
<i>Glomerobolus gelineus</i>	AFTOL–ID 1349	DQ247803	DQ247782	DQ247784
<i>G. gelineus</i>	JK 5584C	DQ247798	–	DQ247783
<i>Hormodochis aggregata</i>	CPC 37499	MN317288	MN313807	–
<i>H. aggregata</i>	CPC 30683 ^T	NG070476	NR166307	–
<i>H. eucalypti</i>	CBS 120255 ^T	EF110617	EF110617	–
<i>H. melanochlora</i>	CBS 138861 ^T	NG070381	NR165507	–
<i>Ingvariella bispora</i>	DUKE 1444446	–	–	HQ659175
<i>I. bispora</i>	MALich 15288	HQ659184	–	HQ659173
<i>Neofitzroyomyces nerii</i>	CBS:145088 ^T	NG068278	NR161144	–
<i>Neostictis nigricans</i>	MFLU 18–1380 ^T	MT214610	MT310654	MT226721
<i>Neoostropa castaneae</i>	HKAS 124384 ^T	OP739476	OP739482	OP739478
<i>N. castaneae</i>	HKAS 124385	OP739477	OP739483	OP739479
<i>Ostropa barbara</i>	SFB1108	MG281965	MG281965	MG281974
<i>O. barbara</i>	EB85	HM244773	HM244773	HM244752
<i>Ostropomyces pruinosellus</i>	MFLU 20–0538 ^T	MW400966	MW400964	–
<i>Ost. pruinosellus</i>	MFLUCC 21–0112	MZ868917	MZ868912	MZ868907
<i>Ost. pruinosellus</i>	MJ-C5	MZ781304	MZ781300	MZ781306
<i>Ost. thailandicus</i>	MFLU 21–0116	MZ868920	MZ868915	MZ868910
<i>Ost. thailandicus</i>	MFLUCC 21–0113	MZ868919	MZ868914	MZ868909
<i>Phacidiella alsophilae</i>	CPC:37041 ^T	MT373344	MT373361	–
<i>P. podocarpi</i>	CBS:138904	KP004481	KP004453	–
<i>Schizoxylon albescens</i>	GG236	AY661689	AY661689	AY661680
<i>S. albescens</i>	GG2696a ^T	DQ401144	DQ401144	DQ401142
<i>S. berkeleyanum</i>	S F209682	MG281966	MG281966	MG281975
<i>S. gilenslamii</i>	S F300892 ^T	MG281968	MG281968	MG281977
<i>S. gilenslamii</i>	Wedin 9496	MG281969	MG281969	MG281978
<i>Sphaeropezia lyckselensis</i>	EB36	JX266158	–	JX266156
<i>S. lyckselensis</i>	Gilenslam 2659	HM244771	–	HM244750
<i>S. mycoblasti</i>	MWE61	JX266159	–	JX266157
<i>S. ochrolechia</i>	taxon:1238959	JX266160	–	–
<i>S. shangrilaensis</i>	MFLU:20–0537 ^T	NG074506	NR172005	MW400962
<i>S. capreae</i>	GG2560	AY661684	–	AY661674
<i>S. capreae</i>	taxon:909612	HM244772	–	HM244751
<i>Stictis brunnescens</i>	GG2359	AY661688	AY661688	AY661679
<i>S. brunnescens</i>	SFB1100	–	MG281970	MG281981
<i>S. mollis</i>	GG2445a	AY527318	AY527318	AY527347
<i>S. mollis</i>	GG2458b	AY527316	AY527316	AY527345
<i>S. confusa</i>	AN3222	AY527336	AY527336	AY527365
<i>S. confusa</i>	GG2609a	AY527324	AY527324	AY527353
<i>S. populorum</i>	GG2610a	AY527327	AY527327	AY527356
<i>S. populorum</i>	MW7301	AY527334	AY527334	AY527363
<i>S. radiata</i>	MW6493	AY527309	AY527309	AY527338
<i>S. radiata</i>	GG2449a	AY527308	AY527308	AY340532
<i>Thelopsis byssoidea</i>	Ertz 17384 (BR)	MT831007	–	MT831496
<i>T. rubella</i>	Sanderson 2186 (BM)	MT831014	–	MT831499

Table 1 Continued.

Species	Strain/Voucher	GenBank accession numbers		
		LSU	ITS	mtSSU
<i>T. rubella</i>	Ertz 20377 (BR)	MT831013	–	MT831498
<i>Trinathotrema stictideum</i>	F Luecking 17541b	–	–	GU380288
<i>T. stictideum</i>	F Luecking 28093	–	–	GU380287
<i>Xyloschistes platytropa</i>	AFTOL-ID 4891	KJ766680	–	KJ766517
<i>Orceolina kerguelensis</i>	Søchting 9398(C)	AY212830	AY212814	AY212853
<i>Trapelia placodioides</i>	KS163	KU844623	KU844758	KU844568
<i>Placopatia perrugosa</i>	KS137	KU844613	KU844737	KU844549

Note: The newly generated sequences are denoted in bold, and type strains are indicated with ^T

Results

Phylogenetic Analyses

The best-fit models for the alignment of the 114 isolates were SYM + I + G (ITS, 293 alignment patterns), GTR + I + G (LSU, 291 alignment patterns), and GTR + I + G (mtSSU, 413 alignment patterns). The final alignment comprising 1,618 had 997 distinct patterns, with 30.71% undetermined characters or gaps, resulting in an ML phylogenetic tree with the final ML optimization likelihood value of -19726.668399 (Fig. 1).

Taxonomy

Neoostropa, Y.Y. Yang, A.R. Gomes de Farias & K.D. Hyde, gen. nov.

Index Fungorum number: IF900059 Facesoffungi number: FoF 13373

Etymology – Referring to characteristics similar to *Ostropa*.

Saprobic on withered branches. Asexual morph: Undetermined. Sexual morph: *Ascomata* solitary, multilocular, immersed, and perithecial. *Ostiole* is covered with crystals. *Paraphyses* aseptate, branched, hyaline, filamentous. *Asci* cylindrical, long, with thickened caps. *Ascospores* hyaline, aseptate, guttulate.

Type genus – *Neoostropa castaneae*, Y. Y. Yang, A.R. Gomes de Farias & K.D. Hyde

Notes – *Neoostropa* is introduced to accommodate one newly discovered species, *Neoostropa castaneae*, collected from a forest in Yunnan Province, China. *Neoostropa* forms a distinct clade nested between *Ostropomyces* and *Ostropa* with significant support (92% ML/0.99 BYPP). However, *Neoostropa* is morphologically distinct from the genera mentioned above by having multilocular ascomata, aseptate, and guttulate ascospores. Therefore, we introduce *Neoostropa* as a new genus in *Stictidaceae*.

Neoostropa castaneae, Y. Y. Yang, A.R. Gomes de Farias & K.D. Hyde, sp. nov.

Index Fungorum number: IF900060 Facesoffungi number: FoF 13349

Etymology – Referring to the plant host *Castanea henryi* (Skan) Rehder & E.H. Wilson

Holotype – HKAS 124384

Saprobic on the dead stem of *Castanea henryi*. Surface of the substrate where the ascomata are formed white beige. Asexual morph: Undetermined. Sexual morph: *Ascomata* 450–600 × 485–600 μm (\bar{x} = 522 × 253, n = 5), perithecial, subglobose, multilocular 74–185 × 121–226 μm (\bar{x} 120 × 168, n = 10), gregarious, immersed at first and raising the substrates into small pustules and later opening by entire pores, exposing the discs, discs white, convex, inner mass hyaline. *Peridium* 32–37 μm (\bar{x} = 34, n = 5) wide, grey, and a litter brown at the base and both sides, covered by a thick white crystalline powder substance in the upper part. *Paraphyses* 165–340 μm (\bar{x} = 250, n = 20) long, anastomosing, filiform, aseptate, branched, numerous. *Asci* 205–385 × 2.5–4.5 μm (\bar{x} = 323 × 3.6, n = 25), cylindrical, unitunicate, 8-spored, thick-walled, and smooth-walled, with a thickened

cap. *Ascospores* 155–295 × 1–1.6 μm (\bar{x} = 204 × 1.4, n = 25), hyaline, filiform, easily breaking into different sizes, smooth-walled, guttulate, aseptate.

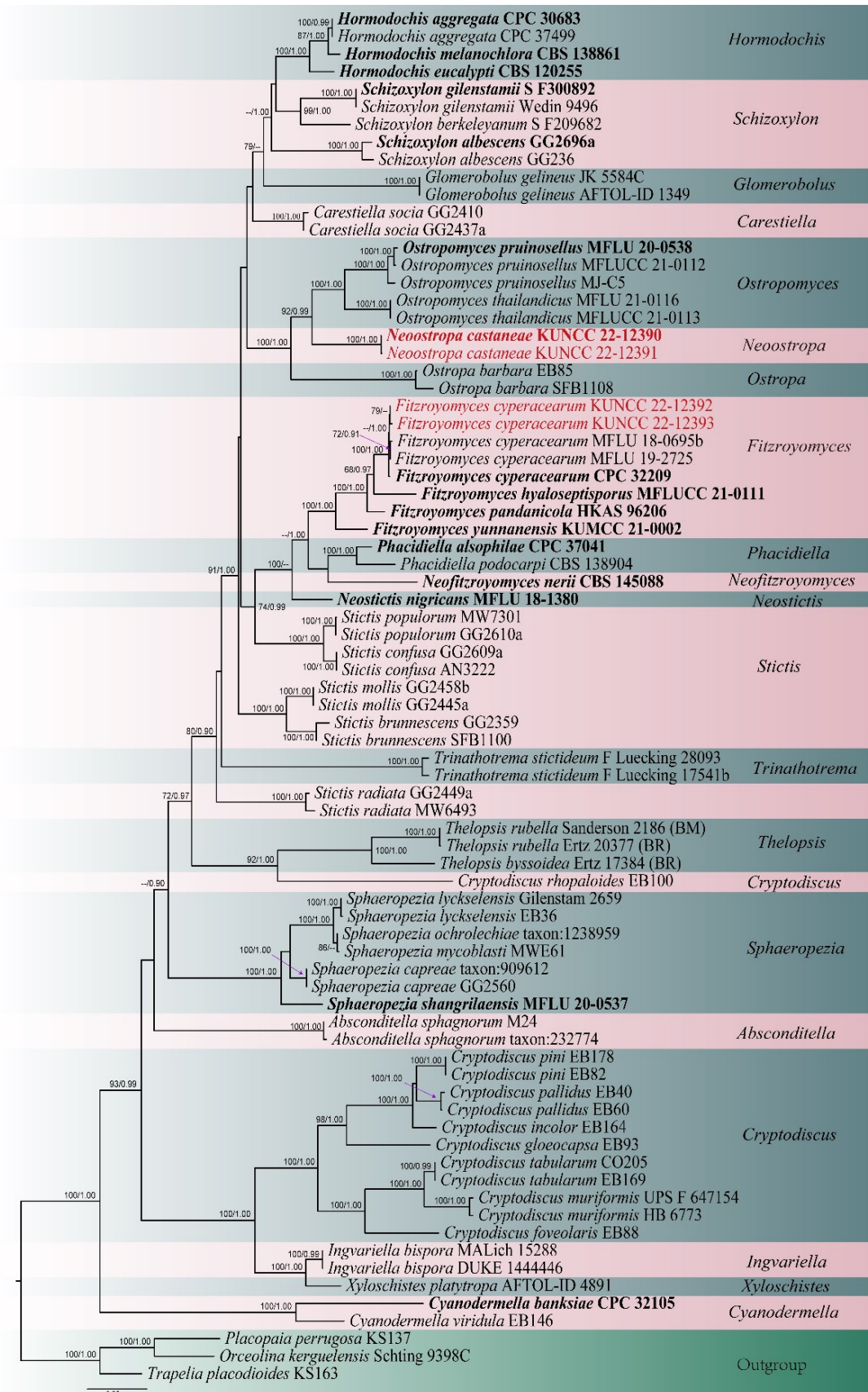


Fig. 1 – Phylogenetic tree of *Stictidaceae* species based on maximum likelihood analysis of a combined multi-sequence alignment (ITS, LSU, and mtSSU). Bootstrap support values for

maximum likelihood higher than 70% and Bayesian posterior probabilities higher than 0.95 are indicated at the node. *Placopaia perrugosa*, *Orceolina kerguelensis*, and *Trapelia placodioides* were used as the outgroup. Ex-type strains are in bold font; the newly generated sequences are in red.

Culture characteristics – culture was established from germinating ascospore. Colony slowly growing on PDA media, reaching 2 cm after one month at 18 °C, sterile, white, irregularly shaped, raised, edge undulate, mycelia dense, reverse yellowish-brown.

Material examined – China, Yunnan Province, Kunming City, Kunming Institute of Botany, on a dead stem of *Castanea henryi*. 23 September 2021, Yanyan Yang, YYY48 (holotype: HKAS 124384); ex-type living culture KUNCC 22–0112.

Notes – BLASTn searches of the newly described species (*N. castaneae*), the ITS sequence showed a similarity of 91% with *O. pruinoseus* (MFLU 21–0112 and MFLUCC 21–0115); the LSU sequence was 93% similar to *O. thailandicus* (MFLU 21–0113 and MFLU 21–0116); and the mtSSU was 91% similar to *O. pruinoseus* (MFLU 21–0112 and MFLUCC 21–0115). The multigene phylogenetic results of the combined dataset indicated that *N. castanea* (KUNCC 22–12390 and KUNCC 22–12391) formed a distinct clade within *Stictidaceae* separated from *O. thailandicus* with strong statistical support (Fig. 1). The morphological characters of our collection are different from *O. pruinoseus* and *O. thailandicus* by its aseptate and guttulate ascospores, and ascomata appearance. Besides, *N. castaneae* is saprotrophic and non-lichenized. However, on the surface of the branch, the ascomata are not easy to see, emerge and have a pruinose appearance.

Fitzroyomyces cyperacearum Crous [as ‘cype ri’], in Crous et al., *Persoonia* 39: 389 (2017).

Fig. 3

Facesoffungi number: FoF 058071

Type species – *Fitzroyomyces cyperacearum* Crous (2017).

Saprobic on the dead stem of a dicotyledonous plant. Asexual morph: Undetermined. Sexual morph: *Apothecia* 230–310 × 260–350 μm (\bar{x} = 280 × 310 μm, n = 10), arising singly or in small groups, subimmersed, unilocular, cupulate. *Discs* 230–310 μm wide (\bar{x} = 270 μm, n = 10), whitish to yellow, white margins. *Hymenium* hyaline, enclosed in a thick gelatinous matrix, epithecium absent. *Excipulum* 9–75 μm wide, composed of hyaline cells of *textura intricata*. *Paraphyses* 0.8–1.4 μm wide (\bar{x} = 1.1 μm, n = 30), numerous, filiform, aseptate, unbranched. *Asci* 180–200 × 5–20 μm (\bar{x} = 200 × 10 μm, n = 20), cylindrical, rounded at apex, broaden at the middle, attenuated toward the base, thick-walled and smooth-walled. *Ascospores* 150–210 × 2.5–3.5 μm, hyaline, fasciculate, filiform, multiseptate, slightly constricted at the septa, easily breaking into small septate part-spores.

Culture characteristics – Culture was established from germinating ascospore. Colony slowly growing on PDA media, reaching 1.3 cm after incubation for 17 days at 25 °C, sterile, canary, roughly circular, dense, umbonate, undulate, and reverse yellow-orange.

Material examined – China, Yunnan Province, Kunming City, Panlong District, on a dead stem of an unidentified dicotyledonous plant, 11 December 2021, De-Ping Wei, SHB1229 (HKAS 124387), living culture KUNCC 22–12392.

Notes – Our isolate resembles *Fitzroyomyces cyperacearum* in having cupulate apothecia, crystalline discs, and numerous and unbranched paraphyses. Asci are broad cylindrical with ascospores spiraled in the ascus, and ascospores easily disarticulate into part-spores when mature. Phylogenetic analyses supported the close affinity of our isolates to *F. cyperacearum* with great support in Bayesian analysis. *F. cyperacearum* is an asexual coelomycete genus by Crous et al. (2017) first report. In addition, the sexual form was reported in the United Kingdom (Ekanayaka et al. 2019) and Taiwan (Tennakoon et al. 2021). Thus, we report this collection as a new record of *F. cyperacearum* from Yunnan Province, China, and provide additional molecular and morphological photoplates for this species.

Discussion

This study used sequences from 71 *Stictidaceae* species belonging to 20 genera, plus four new collections and three outgroups, to infer the placement of the newly collected specimen (Table 1, Fig. 1). The remaining genera were not sampled due to the lack of sequence data in the databases. The first comprehensive taxonomy of *Stictidaceae* was provided by Sherwood, based on the excipular structure and ascospore type (Sherwood 1977). However, molecular tools have played an important role in establishing intrageneric boundaries and the concept of species and have been used as one of the bases for species identification in *Stictidaceae* (Fernández-Brime et al. 2018) and have revealed the taxonomy and evolution of this fungal group (Thiyagaraja et al. 2021, Wei et al. 2021, Phukhamsakda et al. 2022).

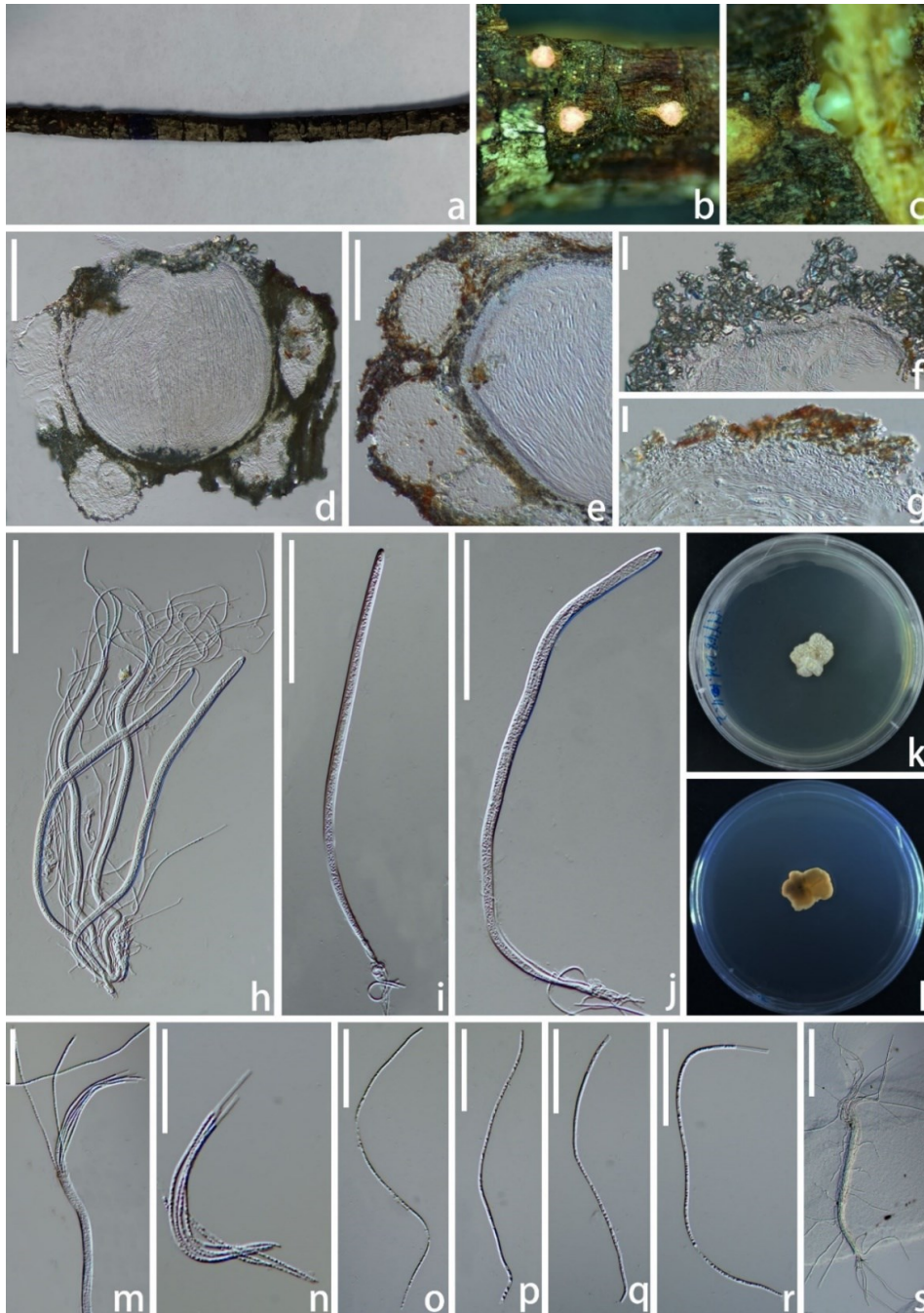


Fig. 2 – *Neoostropa castaneae*. a Substrate. b, c Ascomata on host. d, e Vertical sections through ascomata. f, g Excipulum. h–j, m Asci. k, l Upper and lower view of culture on PDA media after incubation for one month. n–r Ascospores. s Germinating ascospore. Scale bars: d, e = 200 μ m, h, i, j, s = 100 μ m, f, m–r = 50 μ m, g = 20 μ m.

In this study, we introduce *Neoostropa* to accommodate *N. castanea*, and a new record of *F. cyperacearum*, a saprobe on branches of *C. henryi*, from Yunnan Province, China, in *Stictidaceae*, based on multilocus phylogenetic analysis (Fig. 1) and morphological characters (Fig. 2). *Neoostropa* is phylogenetically related to *Ostropomyces* and *Ostropa* (Fig. 1). Morphologically, *Neoostropa* differs from *Ostropomyces* by the size of asci and spores and the morphology of paraphyses and ascospores (Table 2, Fig. 2). The value of genera has been reviewed by Liu et al. (2016). Therefore, based on the species boundary polyphasic approach delimitation (Chethana et al. 2021, Maharachchikumbura et al. 2021, Jayawardena et al. 2021), we introduce *Neoostropa* as a new genus, typified with *N. castanea*.

Table 2 Comparative morphological characters between *Neoostropa castanea*, *Ostropomyces pruinosellus*, and *Ostropa barbara*.

Species	Asci (µm)	Paraphyses	Ascospores	Reference
<i>Ostropa barbara</i>	205–385 × 2.5–4.5, 8-spored, cylindrical, unitunicate, thick-walled and smooth-walled, with a thickened cap octosporous, spores arranged parallel	branched, aseptate, filiform, numerous	155–295 × 1–1.6, hyaline, filiform, easily breaking into different sizes, smooth-walled, guttulate, aseptate	This study
<i>Ostropomyces pruinosellus</i>	165–245 × 7–11, bitunicate, cylindrical, four-spored, apical wall thickened to 2.2–3.2 µm to 2.2–3.2 µm	septate, branched, hyaline, 0.5–1.3 µm thick, generally exceeding the length of asci exceeding the length of asci	160–180 × 2–3, hyaline, transversely multi-septate, each cells almost of equal size, each locus 2–4 µm long, deeply constricted at each septum, easily breaking apart into small, aseptate, part-spores	Thiyagaraja et al. (2021)
<i>N. castanea</i>	300–400 × 6, filiform, smooth, hyaline, multiseptated, octosporous, spores arranged parallel	filiform and forked several times at the top	180–250 × 2, hyaline, filiform, regularly multiseptate	https://www.mycob.fr/fiche.php?genre=Ostropa&espece=barbara

The *Fitzroyomyces* collection grouped with *F. cyperacearum*, *F. hyaloseptisporus*, *F. yunnanensis*, and *F. pandanicola*, with the closest association with *F. cyperacearum* (Fig. 3), which allowed to assign to this species. *Fitzroyomyces* was introduced by Crous et al. (2017) and comprises a single species, *F. cyperacearum*, first isolated in association with *Cyperaceae* in Australia (Crous et al. 2017). Later, it was reported from *Epilobium angustifolium* in the United Kingdom (Ekanayaka et al. 2019) and *Ficus septica* in Taiwan (Tennakoon et al. 2021).

Stictidaceae has been neglected because the specimens were rarely collected, characterizing a poorly studied group (Wei et al. 2021). However, continued exploration of new environments will result in undescribed taxa, contributing to the descriptive fungal number curve (Hyde et al. 2020, 2018, Bhunjun et al. 2022, Phukhamsakda et al. 2022). In this study, the collection of new debris plants has resulted in the discovery of fungi that did not fit in any described genus. New sampling, descriptions of species, and incorporation of molecular data of *Stictidaceae* are needed to enrich the knowledge about the lifestyle and evolution of this family.

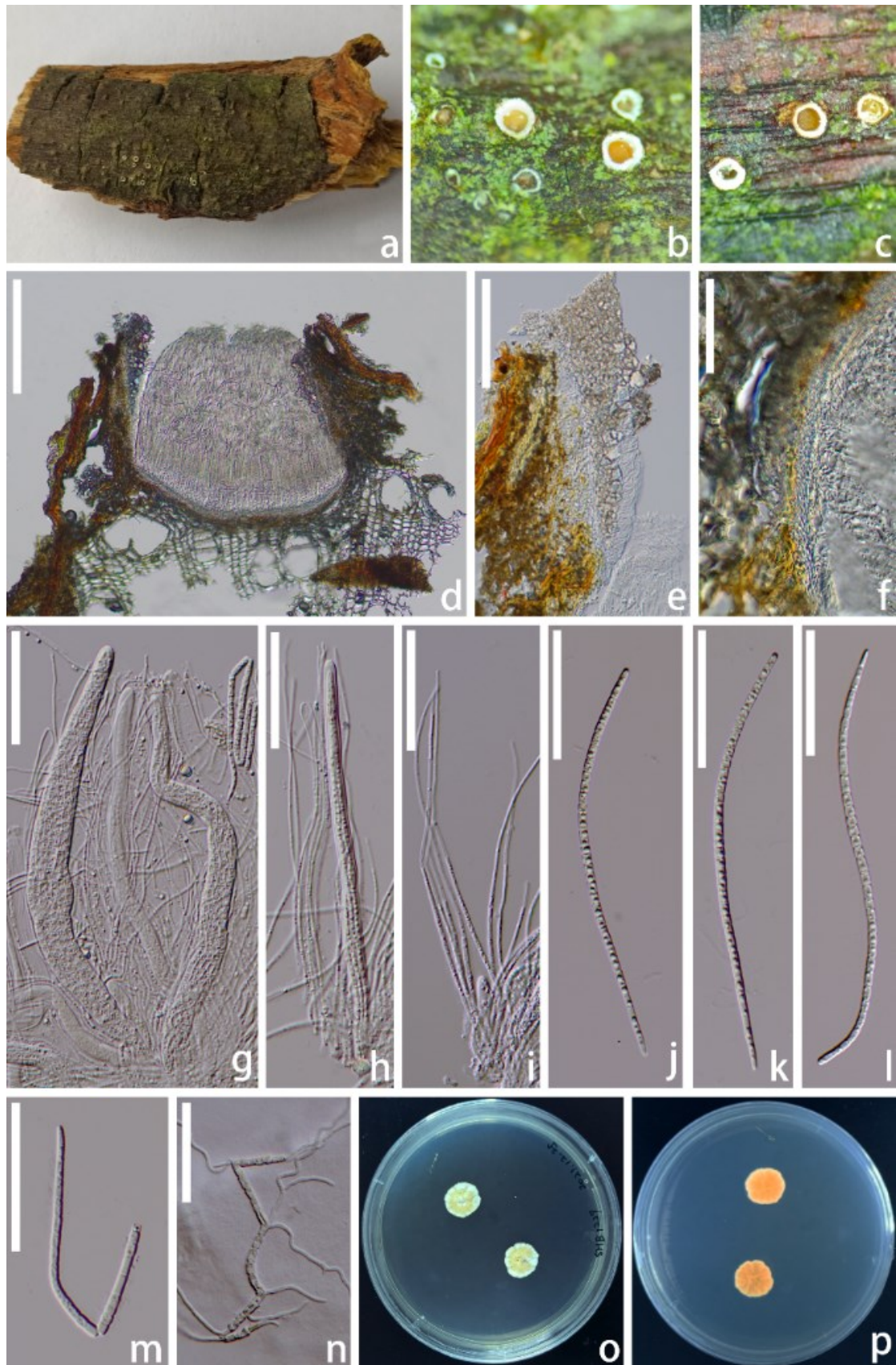


Fig. 3 – *Fitzroyomyces cyperacearum*. a Substrate with ascomata. b, c Ascomata on host. d Vertical sections through ascomata. e, f Excipulum. g, h Asci. i Paraphyses. j–m Ascospores. n Germinating ascospore. o, p Upper and lower view of culture on PDA media after incubation for 20 days. Scale bars: d = 200 μ m, e = 100 μ m, f–n = 50 μ m.

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