DNA EXTRACTION

We ordered the culture CBS:357.53, *Subbaromyces splendens* (USA: NY, Rockland Co., Pearl River, coll. C.W. Hesseltine, no. 2340; Hesseltine 1953), from the Westerdijk Fungal Biodiversity Institute and made subcultures on selective media. Samples were removed for DNA isolation by using a J-hook to remove a piece of agar with hyphal tissue or to superficially scrape off hyphal tissue. DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Valencia, California), following the manufacturer's instructions.

PCR AMPLIFICATION AND SEQUENCING

We amplified the nuclear small and large ribosomal subunits (SSU and LSU) and the internal transcribed spacer region of the ribosomal DNA (ITS). Primer combinations used were NS1/NS2 for SSU (White et al. 1990), ITS1f/ITS4A and ITS9mun/ITS4 for ITS (White et al. 1990; Gardes and Bruns 1993; Egger 1995; Larena et al. 1999), and LR0R/LR7 for LSU (Vilgalys and Hester 1990; Rehner and Samuels 1994). PCR reactions consisted of 1.0 µL of each 10 µM primer, 12.5 µL of 2× MyTaq Mix (Bioline, Swedesboro, New Jersey), 9.5 µL of ddH_2O, and 1.0 µL of DNA extract. For all amplifications, an Eppendorf Mastercycler ep gradient thermocycler was used with initial denaturation at 94 °C for 3:00 min; followed by 35 cycles of denaturing at 94 °C for 1:00 min, annealing at 50 °C for 0:45 min, extension at 72 °C for 1:30 min; and a final extension step of 72 °C for 10:00 min. PCR products that showed clear bands on agarose gel outsourced to Genewiz (South Plainfield, New Jersey) for purification and sequencing. Generated sequence reads were assembled, trimmed, and edited in Sequencher v.5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan).

DATASET AND PHYLOGENETIC ANALYSIS

We created a two-locus SSU+LSU dataset of 75 isolates (TABLE S1): 3 isolates of Lulworthiales (Sordariomycetes) as outgroup, 4 Pyxidiophorales, 11 Herpomycetales, 50 Laboulbeniales, 2 *Laboulbeniopsis termitarius*, 1 *Chantransiopsis*, 2 *Tetrameronycha*, and 2 *Subbaromyces*. All sequences were downloaded from GenBank, except for those of *Subbaromyces splendens*, which were generated in this study (2 SSU, 2 LSU). Sequences for both loci were aligned using MAFFT on XSEDE v.7.305 (Katoh and Standley 2013) as implemented on the Cipres Science Gateway v.3.3 (Miller et al. 2010) and then trimmed using the command-line version of trimAl v.1.3 (Capella-Gutiérrez et al. 2009) with gap threshold (-gt) = 0.6 and minimal coverage (-cons) = 0.5. Aligned–trimmed sequences of both loci were concatenated in MEGA7 (Kumar et al. 2016) to create a dataset of 3299 bp (SSU 1821 bp, LSU 1478 bp).

The ML tree of the two-locus dataset was inferred using the command-line version of IQ-TREE (Nguyen et al. 2015). Nucleotide substitution models were selected under the Akaike Information Criterion corrected for small sample size (AICc) with the help of ModelFinder (Kalyaanamoorthy et al. 2017). For the SSU dataset, the GTR+F+R3 model was selected (-lnL = 11273.321); for LSU, the GTR+F+R4 model (-lnL = 10069.582). The ML analysis was run under partitioned models (Chernomor et al. 2016) and ultrafast bootstrapping was implemented with 1000 replicates (Hoang et al. 2017).

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TABLE S1. Overview of isolates used in phylogenetic analysis. Sequences in bold were newly generated during this study.

Isolate	Order	Genus	Species	SSU	LSU	Reference
AFTOL-ID 747	Lulworthiales	*Lulworthia*	*grandispora*	DQ522855	DQ522856	Schoch et al. (2009)
AFTOL-ID 5013	Lulworthiales	*Lulworthia*	*lignoarenaria*	FJ176848	FJ176903	Schoch et al. (2009)
AFTOL-ID 5014	Lulworthiales	*Lulworthia*	*unispetata*	FJ176849	FJ176904	Schoch et al. (2009)
DH483e	Herpomycetales	*Herpomyces*	*chaetophilus*	MG438320	MG438351	Haelewaters et al. (2019c)
DH602b	Herpomycetales	*Herpomyces*	*chaetophilus*	KT800023	KT800009	Haelewaters et al. (2015)
DH1097b	Herpomycetales	*Herpomyces*	*chaetophilus*	MG438321	MG438352	Haelewaters et al. (2019c)
DH1097c	Herpomycetales	*Herpomyces*	*chaetophilus*	MG438322	MG438353	Haelewaters et al. (2019c)
DH1187d	Herpomycetales	*Herpomyces*	*periplanetae*	MG438331	MG438359	Haelewaters et al. (2019c)
TW437c	Herpomycetales	*Herpomyces*	*periplanetae*	MG438324	MG438355	Haelewaters et al. (2019c)
TW448b	Herpomycetales	*Herpomyces*	*periplanetae*	MG438325	MG438356	Haelewaters et al. (2019c)
DH602c	Herpomycetales	*Herpomyces*	*periplanetae*	KT800025	KT800010	Haelewaters et al. (2015)
DH602d	Herpomycetales	*Herpomyces*	*periplanetae*	MG438327	MG438357	Haelewaters et al. (2019c)
DE_HerpBL1	Herpomycetales	*Herpomyces*	*shelfordellae*	KT800026	KT800011	Haelewaters et al. (2015)
Bud_Slat	Herpomycetales	*Herpomyces*	*shelfordellae*	MG438333	MG438361	Haelewaters et al. (2019c)
DH1015d	Laboulbeniales	*Arthrorhynchus*	*nycteribiae*	MG438336	MG438363	Haelewaters et al. (2019c)
Edeleny_13.xi.2014	Laboulbeniales	*Arthrorhynchus*	*nycteribiae*	KY094496	KY094497	Haelewaters et al. (2017)

N/A	Laboulbeniales	*Autoicomyces*	*falcatus*	MG687407	N/A	Goldmann & Weir (2018)
N/A	Laboulbeniales	*Autoicomyces*	*recurvatus*	MG687409	N/A	Goldmann & Weir (2018)
N/A	Laboulbeniales	*Bordea*	sp.	MG687403	N/A	Goldmann & Weir (2018)
DH1222d	Laboulbeniales	*Camptomyces*	sp.	**MF314140**	**MF314141**	this study
N/A	Laboulbeniales	*Ceratomyces*	*mirabilis*	MG679452	N/A	Goldmann & Weir (2018)
N/A	Laboulbeniales	*Chitonomyces*	*affinis*	MG679288	N/A	Goldmann & Weir (2018)
N/A	Laboulbeniales	*Chitonomyces*	*simplex*	JN127389	N/A	Goldmann & Weir (2018)
N/A	Laboulbeniales	*Chitonomyces*	*spiniger*	JN127397	N/A	Goldmann & Weir (2018)
H73-1	Laboulbeniales	*Coreomyces*	sp.	KY523236	N/A	Sundberg et al. (2018)
H81-1	Laboulbeniales	*Coreomyces*	sp.	KY523242	N/A	Sundberg et al. (2018)
H82-1	Laboulbeniales	*Coreomyces*	sp.	KY523243	N/A	Sundberg et al. (2018)
LG260B	Laboulbeniales	*Euzodiomyces*	*lathrobii*	MG687402	N/A	Goldmann & Weir (2018)
DH1312b	Laboulbeniales	*Gloeandromyces*	*dickii*	MH040546	MH040580	Haelewaters et al. (2018b)
DH1323b	Laboulbeniales	*Gloeandromyces*	*dickii*	MG958011	MH040582	Haelewaters et al. (2018b)
DH1323c	Laboulbeniales	*Gloeandromyces*	*dickii*	MH040548	MH040583	Haelewaters et al. (2018b)
DH1319b	Laboulbeniales	*Gloeandromyces*	*nycteribiidarum*	MH040533	MH040566	Haelewaters et al. (2018b)
DH1334c	Laboulbeniales	*Gloeandromyces*	*nycteribiidarum*	MH040534	MH040567	Haelewaters et al. (2018b)
DH1306c	Laboulbeniales	*Gloeandromyces*	*streblae*	MG958012	MH040585	Haelewaters et al. (2018b)

DH1309a	Laboulbeniales	*Gloeandromyces*	*streblae*	MH040550	MH040587	Haelewaters et al. (2018b)
DH1335c	Laboulbeniales	*Gloeandromyces*	*streblae*	MH040552	MH040589	Haelewaters et al. (2018b)
DH1320b	Laboulbeniales	*Gloeandromyces*	*streblae sigmomorphus*	MH040545	MH040579	Haelewaters et al. (2018b)
DH1174a	Laboulbeniales	*Hesperomyces*	*virescens*	MG760598	MG745345	Haelewaters et al. (2018a)
DH1188g	Laboulbeniales	*Hesperomyces*	*virescens*	MG438341	MG438365	Haelewaters et al. (2019c)
DH1268b	Laboulbeniales	*Hesperomyces*	*virescens*	MG760610	MG745357	Haelewaters et al. (2018a)
DH1268d	Laboulbeniales	*Hesperomyces*	*virescens*	MG760611	MG745358	Haelewaters et al. (2018a)
LT179	Laboulbeniales	*Ilyomyces*	sp.	MG687397	N/A	Goldmann & Weir (2018)
DH1346b	Laboulbeniales	*Laboulbenia*	*bruchii*	**MN530040**	MN394843	this study, Haelewaters et al. (2019b)
DH1007a	Laboulbeniales	*Laboulbenia*	*calathi*	MG438342	N/A	Haelewaters et al. (2019c)
LG613	Laboulbeniales	*Laboulbenia*	*cristata*	MG696296	N/A	Goldmann & Weir (2018)
H84-1	Laboulbeniales	*Laboulbenia*	*pedicellata*	KY523244	KY350537	Sundberg et al. (2018)
DH971a	Laboulbeniales	*Laboulbenia*	sp. *Systena*	MG438345	N/A	Haelewaters et al. (2019c)
DH1324b	Laboulbeniales	*Nycteromyces*	*streblidinus*	MH040554	MH040590	Haelewaters et al. (2018b)
DH1324d	Laboulbeniales	*Nycteromyces*	*streblidinus*	MH040556	MH040591	Haelewaters et al. (2018b)
DH1324e	Laboulbeniales	*Nycteromyces*	*streblidinus*	MH040557	MH040592	Haelewaters et al. (2018b)
LG479	Laboulbeniales	*Peyritschiella*	*angolensis*	MG687385	N/A	Goldmann & Weir (2018)
DH313f	Laboulbeniales	*Polyandromyces*	*coptosomalis*	KT800035	KT800020	Haelewaters et al. (2015)

HNHM:107914	Laboulbeniales	*Prolixandromyces*	*triandrus*	LT158294	LT158295	Pfliegler et al. (2016)
SR4sand	Laboulbeniales	*Rickia*	*laboulbenioides*	MH040558	MH040593	Haelewaters et al. (2018b)
SR5sand	Laboulbeniales	*Rickia*	*laboulbenioides*	**MN530041**	MK500060	this study, Haelewaters et al. (2019a)
SR1sand	Laboulbeniales	*Rickia*	*pachyiuli*	MH040559	MH040594	Haelewaters et al. (2018b)
SR13sand	Laboulbeniales	*Rickia*	*pachyiuli*	**MN530042**	MK500059	this study, Haelewaters et al. (2019a)
DE_Rak4	Laboulbeniales	*Rickia*	*wasmannii*	KT800037	KT800021	Haelewaters et al. (2015)
ADK6272a	Laboulbeniales	*Rickia*	*wasmannii*	**MN530043**	MK500050	this study, Haelewaters et al. (2019a)
DH1136h	Laboulbeniales	*Stigmatomyces*	*ceratophorus*	MG958013	MH145384	Haelewaters et al. (2018b)
DH1008a	Laboulbeniales	*Stigmatomyces*	*gregarius*	MG438348	N/A	Haelewaters et al. (2019c)
LG652	Laboulbeniales	*Tettigomyces*	*africanus*	MG687405	N/A	Goldmann & Weir (2018)
MG003	Laboulbeniales	*Zodiomyces*	*vorticellarius*	KT800038	KT800022	Haelewaters et al. (2015)
AW819	Laboulbeniales	*Zodiomyces*	*vorticellarius*	AF407577	N/A	Weir & Blackwell 2001
DAOM:229465	Pyxidiophorales	*Gliocephalis*	*hyalina*	AH012810	N/A	Jacobs et al. (2005)
AFTOL-ID 1297	Pyxidiophorales	*Pyxidiophora*	*arvernensis*	FJ176839	FJ176894	Schoch et al. (2009)
MG200	Pyxidiophorales	*Pyxidiophora*	*microspora*	MG438334	MG438362	Haelewaters et al. (2019c)
IMI-1989	Pyxidiophorales	*Pyxidiophora*	sp.	AF313769	N/A	A. Weir & M. Blackwell unpubl.
LterJP02	*Laboulbeniopsis* clade	*Laboulbeniopsis*	*termitarius*	MF785100	N/A	Guswenrivo et al. (2018)
DAH 18	*Laboulbeniopsis* clade	*Laboulbeniopsis*	*termitarius*	AY212810	N/A	Henk et al. (2003)

LG589	*Chantransiopsis* clade	*Chantransiopsis*	sp.	MG687411	N/A	Goldmann & Weir (2018)
LG653	*Chantransiopsis* clade	*Tetrameronycha*	sp.	MG687412	N/A	Goldmann & Weir (2018)
LG600	*Chantransiopsis* clade	*Tetrameronycha*	sp. Labiidae	MG687410	N/A	Goldmann & Weir (2018)
CBS 357.53 AA1	*Chantransiopsis* clade	*Subbaromyces*	*splendens*	**MN526931**	**MN526934**	this study
CBS 357.53 B	*Chantransiopsis* clade	*Subbaromyces*	*splendens*	**MN526932**	**MN526935**	this study