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Diversity of *Akanthomyces* on moths (Lepidoptera) in Thailand

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Abstract

Akanthomyces is a genus of invertebrate-pathogenic fungi from the family Cordycipitaceae (Ascomycota, Hypocreales). Its species occurs on two different types of hosts, spiders and insects, and in the latter case specifically Lepidoptera adults. Three new species of *Akanthomyces*, *A. noctuidarum*, *A. pyralidarum*, and *A. tortricidarum* occurring on adult moths from Thailand are proposed based on the differences of their morphological characteristics and molecular data. Phylogenetic analyses using a combined dataset, including the internal transcribed spacer regions, the large subunit of the ribosomal DNA, translation elongation factor 1-α, the largest subunit of RNA polymerase II, and the second largest subunit of RNA polymerase II, support the delimitation of these new species in *Akanthomyces*.

Keywords

Akanthomyces, entomopathogenic fungi, fungal taxonomy, multilocus phylogeny

Introduction

Cordycipitaceae is one of the families of the order Hypocreales with entomogenous nutritional habit. Many of the species in this family have been originally isolated from dead insects and spiders that are buried in the soil, leaf litter, or attached to the undersides or upper sides of a leaf. Some species, especially in *Beauveria*, could be found in the soil (Rehner and Buckley 2005) or as endophytes (Mantzoukas and Lagogiannis 2019; Afandhi et al. 2019). Cordycipitaceae is validated based on the type of *Cordyceps*, *Cordyceps militaris*, and it has initially included pyrenomycetes that possess pallid to brightly colored, fleshy stromata (Kepler et al. 2017). It is also characterized by producing superficial to completely immersed perithecia, cylindrical asci with thickened apex, and multi-septate filiform ascospores that disarticulate into part-spores or remain intact at maturity (Sung et al. 2007). Well-known for its use in traditional Chinese medicine, *C. militaris* produces some polysaccharides and cordycepin that have been used for anti-inflammatory, antioxidant, anti-tumor, anti-metastatic, and immunomodulatory functions (Das et al. 2010). The recent study of *C. militaris* shows that this fungus has an anti-hypertension and neuroprotective effect to delayed neural death (Takakura et al. 2017; Kim et al. 2018).The most popular anamorph in this family is *Beauveria*, notably with its type species, *Beauveria bassiana*, which has been used globally as a mycoinsecticide since the 1960s (Vega et al. 2012). Spider pathogens are mostly found within Cordycipitaceae (Shrestha et al. 2019). Their anamorph are found in *Akanthomyces*, *Gibellula*, or *Hevansia* (Kepler et al. 2017).

Akanthomyces was established by Lebert (1858) with *Akanthomyces aculeatus*, the type species, found on a moth in Europe (Mains 1950). *Gibellula* differs from *Akanthomyces* in the production of aspergillus-like conidiophores and the host range (Kepler et al. 2017). *Gibellula* is only found on spiders, while *Akanthomyces* can be found on both, spiders and insects. *Akanthomyces* was known attacking some insect orders such as Hemiptera (*Akanthomyces lecanii*), Coleoptera (*Akanthomyces neocoleopterorum*), Lepidoptera (*Akanthomyces pistillariiformis*), and Orthoptera (*Akanthomyces fragilis*) (Hodge et al. 2003; Mongkolsamrit et al. 2018; Chen et al. 2020). In general, the host range of *Akanthomyces* for both, teleomorph and anamorph are similar. The genus includes *Cordyceps tuberculata* found on adult moths, which is linked to the anamorph *Akanthomyces pistillariiformis. Akanthomyces* has taxonomic priority by date over *Lecanicillium*, one of the anamorphs in Cordycipitaceae with verticillium-like morphologies (Gams and Zare 2001; Kepler et al. 2017). The type species of *Lecanicillium*, *L. lecanii* (*Cephalosporium lecanii*, now regarded as *Akanthomyces lecanii*) is found on lice and scale insects and is known as the anamorph of *Cordyceps confragosa*. On the basis of previous studies on *Akanthomyces* in Thailand, Mongkolsamrit et al. (2018) proposed four new species of *Akanthomyces* on spiders, namely, *A. kanyawimiae*, *A. sulphureus*, *A. thailandicus*, and *A. waltergamsii*. Here, we describe three new *Akanthomyces* species found on adult moths (Lepidoptera) from Thailand based on morphological and molecular studies.

Species complexes or cryptic species are common in the kingdom Fungi. Given the simplicity of the phenotypic characters and the overlap of the size and shapes of

important diagnostic features, species in many genera cannot be easily classified and identified. Cryptic species refers to taxa that are morphologically similar, yet evidence has shown that they are on different evolutionary paths as revealed by molecular phylogenetic methods and can only be recognized by their DNA sequences. Entomopathogenic fungi from Thailand are commonly encountered in the forests and constitute a huge number in our collections (Kobmoo et al. 2012; Luangsa-ard et al. 2018; Mongkolsamrit et al. 2018; Tasanathai et al. 2019).

In surveys of entomopathogenic fungi in national parks and community forests, collections of pathogens on adult moths were found on the underside of leaves of dicotyledonous forest plants. The phenotypic characters of the collections in having cylindrical to narrowly clavate synnemata and superficial perithecia scattered on the body and wings of the moth identify them primarily to be members of *Akanthomyces* in Cordycipitaceae, mostly as *Akanthomyces* cf. *tuberculatus*. The aims of this study were (1) to elucidate the relationships of these collections to known members of Cordycipitaceae, (2) to uncover hidden species in *A. tuberculatus* species complex, and (3) to describe new taxa to accommodate species diversity in *Akanthomyces*.

Materials and methods

Fungal materials and isolation

The specimens used in this study were obtained from BIOTEC Culture Collection (BCC) and BIOTEC Bangkok Herbarium (BBH), Thailand. Fungal specimens were collected from several national parks in Thailand. Soil from the forest floor, leaf litter, undersides, and upper sides of the leaves were scanned for fungal growth on dead insects. Collected specimens were stored in plastic boxes, returned to the laboratory, and examined under a stereo microscope (Olympus SZ61). Isolation from the teleomorphs followed the method described by Luangsa-ard et al. (2018).

Isolation from the anamorphs was carried out using a sterilized inoculation needle to pick the conidia out from sporulating structures and then transfer them on to a PDA plate. These plates were stored in a plastic box chamber at room temperature, left overnight until the conidia germinated, and treated the same way as described in Luangsa-ard et al. (2018).

Colony growth and morphology

Fungal structures of both, anamorph and teleomorph, such as perithecia, asci, ascospores, synnemata, phialides, and conidia were mounted on glass slides with a drop of lactophenol cotton blue solution. Microscopic measurements of 50 individual fungal structures were obtained using a light microscope (Olympus CX31). Variability was provided as the mean ± standard deviation with absolute minima and maxima

in parentheses. Detailed colony descriptions and morphological comparisons of some fungal structures were determined from cultures grown on PDA and OA for 14 days at 25 °C (Mongkolsamrit et al. 2018). The colors of specimens and cultures incubated were described and codified following the Online Auction Color Chart (www.boletales.com/2011/01/new-colour-chart-for-mycologists; abbreviated "OAC" herein). For DNA extraction purposes, starter cultures were grown on PDA for 2 weeks at 25 °C.

DNA extraction

Genomic DNA was extracted from fungal cultures on PDA using a modified CTAB method (Sung et al. 2001). About 600 µL of CTAB buffer was added to the microcentrifuge tube that contained fungal mycelium, which was ground with pestles and incubated at 65 °C for 1 h. Once the suspension had cooled down, 600 µL of chloroform:isoamyl alcohol (24:1) was added. The supernatant was gently mixed until an emulsion was obtained and centrifuged at 12,000 rpm for 20 min. The aqueous phase was transferred to a new sterile microcentrifuge tube. About 300 µL of cold isopropanol alcohol was added to precipitate DNA and left at -20 °C for 1 h. DNA was then separated from the solution by centrifugation at 4 °C and 12,000 rpm for 20 min. The pellet was washed in 200 µL of 70% cold ethanol and air-dried at room temperature. The DNA pellet was then dissolved in 50 µL of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA pH 8.0) (Læssøe et al. 2013). The extracted DNA was stored at -20 °C before amplification (Chen et al. 2018).

PCR amplification and sequencing

Five nuclear loci regions, namely, internal transcribed spacers 1 and 2 along with the 5.8S rDNA (ITS), large subunit of the ribosomal DNA (LSU), translation elongation factor 1-α (*TEF*), the largest subunit of RNA polymerase II (*RPB1*), and the second largest subunit of RNA polymerase II (*RPB2*), were amplified and sequenced. PCR amplifications were conducted in a 25 µL volume consisting of 1× PCR buffer, 0.4 M betaine, 200 µM of each of the four dNTPs, 1 U Taq DNA polymerase (Thermo Scientific, USA), and 0.2 µM of each primer. The primer pairs used in this study were ITS5 and ITS 4 for ITS (White et al. 1990), LROR and LR5 for LSU (Vilgalys et al. 1994), 983F and 2218R for *TEF* (Rehner and Buckley 2005), CRPB1 and RPB1Cr for *RPB1* (Castlebury et al. 2004), and 5F2 and 7cR for *RPB2* (Liu et al. 1999). PCR amplifications were performed using a BioRad T100 thermal cycler following the procedure described in Luangsa-ard et al. (2005) for ITS and Sung et al. (2001) for the other gene regions. PCR products were visualized by ethidium bromide staining after gel electrophoresis of 4 µL of the product in 1% agarose gel (Luangsa-ard et al. 2004). The PCR products were quantified using a standard DNA marker of known size and weight.

Sequence alignment and phylogenetic analysis

Each DNA sequence was checked for ambiguous bases and assembled in BioEdit v.7.0.5.3 (Hall 2005). Additional sequences from previous studies (Kepler et al. 2017; Mongkolsamrit et al. 2018) were used as a dataset of taxa in Cordycipitaceae. Multiple sequence alignment was conducted with MUSCLE 3.6 software (Edgar 2004) and manually adjusted. The DNA sequences were compared to sequences in the GenBank database by BLAST search to determine the closest matches with *Akanthomyces*. The final sequence alignment of the combined dataset was used for analyses using maximum parsimony (MP), Bayesian inference, and maximum likelihood to infer their phylogenetic relationships.

MP analysis used PAUP4.0a116 (Swofford 2019), and heuristic searches were performed with 100 replicates of random sequence addition and tree bisection reconnection swapping algorithm. Bootstrap analysis was performed using the MP criterion with 1000 replications. MrModeltest 2.2 (Nylander 2004) was used to choose the best model of DNA substitution that fit the data. MrBayes (Ronquist and Huelsenbeck 2003) was used to determine the Bayesian phylogenetic inference with a general time-reversible plus proportion-invariant plus gamma (GTR+I+G) model of DNA substitution as the best model. Maximum likelihood analysis was performed with RAxML-HPC2 on XSEDE in CIPRES Science Gateway 3.3 (https://www.phylo.org/) using a GTRCAT model of evolution with 1000 bootstrap replicates (Stamatakis 2014).

Results

Multilocus phylogeny

A total of 55 new sequences from 11 specimens were obtained in this study (Table 1). ITS sequences were used in a preliminary study to select 11 specimens that represent new species. The combined dataset included 101 taxa and four loci consisting of 3511 bp (LSU 850 bp, *TEF* 1041 bp, *RPB1* 732 bp, and *RPB2* 888 bp). *Purpureocillium lilacinum* in Ophiocordycipitaceae was used as the outgroup for this dataset.

The phylogenetic analyses were run using a combined dataset comprising four loci: LSU, *TEF, RPB1*, and *RPB2*. The combined dataset included 3511 characters, of which 2053 characters were constant, 231 were parsimony-uninformative, and 1227 were parsimony-informative. Gaps were treated as missing data. The maximum parsimony analyses resulted in 31 equally most parsimonious trees, of which one is shown in Figure 1 (tree length = 6438 steps; consistency index [CI] = 0.3458; retention index [RI] = 0.7410; homoplasy index [HI] = 0.6542). The result of MrModeltest selected the general time-reversible (GTR) model with proportion in invariable sites (I) and gamma distribution (G) (GTR+I +G) (Lanave et al. 1984) as the best-fit model by the Akaike Information Criterion (AIC) in MrModeltest 2.2. The parameters included base frequencies A = 0.4768, C = 0.7426, G = 1.000, T = 1.2195 and the rate matrix for the substitution model: [A–C] = 0.2693, [A–G] = 0.2507, [A–T] = 0.2694,

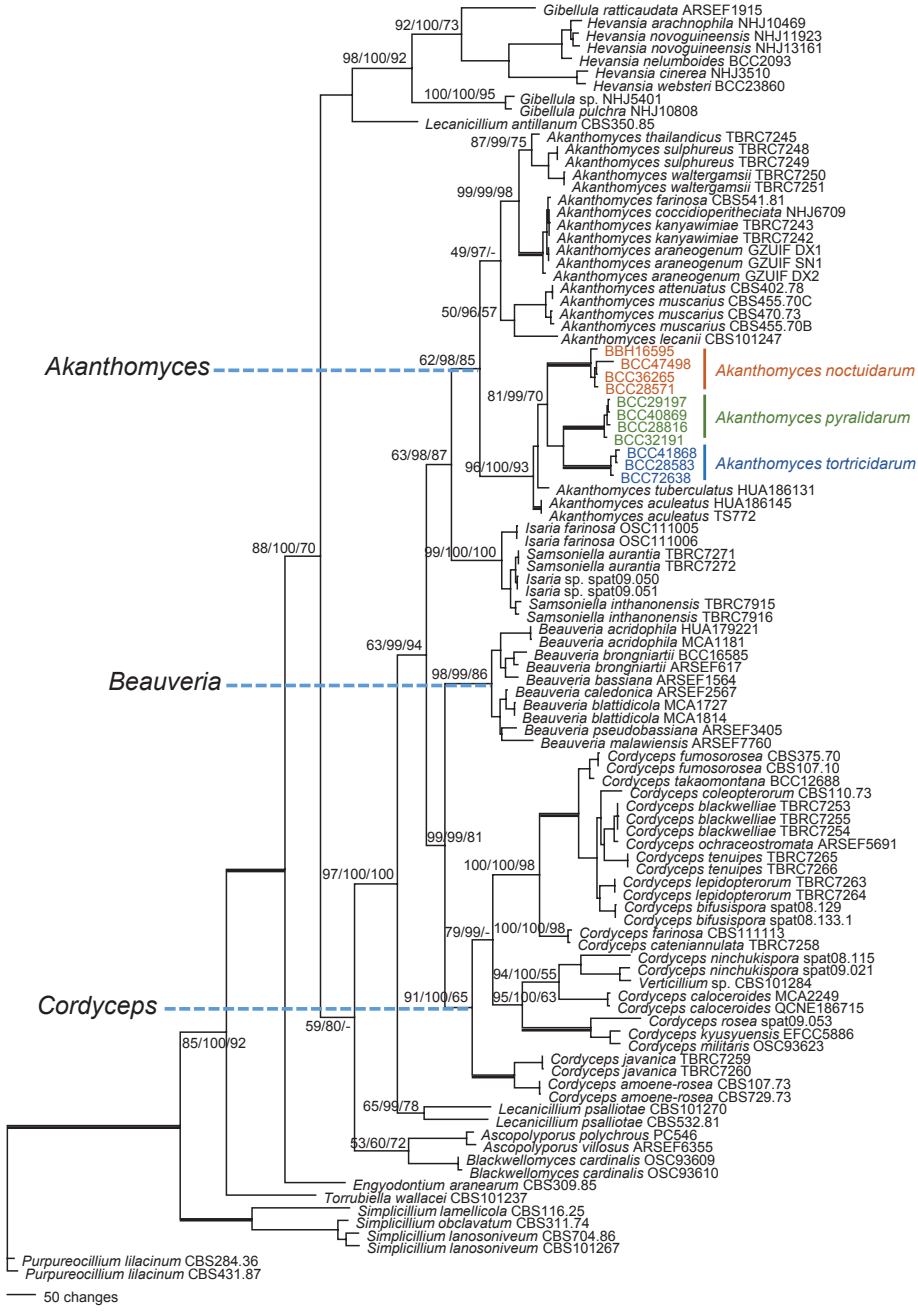

Figure 1. Phylogenetic tree based on combined dataset of LSU, *TEF*, *RPB1* and *RPB2*, sequences showing the relationship of *Akanthomyces* from Thailand with other species of Cordycipitaceae. Numbers above lines at significant nodes represent Maximum likelihood bootstrap values, Bayesian posterior probabilities, and MP bootstrap values. Bold lines mean support for the three analyses were 100%.

Table 1. List of species and GenBank accession numbers of sequences used in this study.

Species	Strain	Host	GenBank accession numbers				
			ITS	LSU	*TEF*	*RPB1*	*RPB2*
Akanthomyces aculeatus	HUA186145	–	–	MF416520[f]	MF416465[f]	–	–
	TS772	Lepidoptera; Sphingidae	KC519371[g]	KC519370[g]	KC519366[g]	–	–
Akanthomyces araneogenum	GZUIFDX2 T	*Araneus* sp.	KU893153[j]	MH978179[j]	MH978187[j]	MH978182[j]	MH978185[j]
	GZUIFDX1	*Araneus* sp.	KU893152[j]	MH978178[j]	–	MH978181[j]	MH978184[j]
	GZUIFSN1	*Araneus* sp.	MH978177[j]	MH978180[j]	MH978188[j]	MH978183[j]	MH978186[j]
Akanthomyces attenuatus	CBS402.78	Leaf litter; *Acer saccharum*	AJ292434[f]	AF339565[f]	EF468782[f]	EF468888[f]	EF468935[f]
Akanthomyces coccidioperitheciata	NHJ6709	Araneae; spider	JN049865[f]	EU369042[f]	EU369025[f]	EU369067[f]	EU369086[f]
Akanthomyces farinosa	CBS541.81	–	–	AY6241807[b]	–	JQ4256867[b]	–
Akanthomyces kanyawimiae	TBRC7242	Araneae; spider	MF140751[i]	MF140718[i]	MF140838[i]	MF140784[i]	MF140808[i]
	TBRC7243	Unidentified	MF140750[i]	MF140717[i]	MF140837[i]	MF140783[i]	MF140807[i]
Akanthomyces lecanii	CBS101247	Hemiptera; *Coccus viridis*	JN049836[f]	AF339555[f]	DQ522359[f]	DQ522407[f]	DQ522466[f]
Akanthomyces muscarius	CBS470.73	–	–	MH878385[k]	–	–	–
	CBS455.70B	–	–	MH871560[k]	–	–	–
	CBS455.70C	–	–	MH871561[k]	–	–	–
Akanthomyces noctuidarum	BCC36265 T	Lepidoptera; Noctuidae	**MT356072**	**MT356084**	**MT477978**	**MT477994**	**MT477987**
	BBH16595		**MT356073**	**MT356085**	**MT477979**	**MT477995**	**MT478005**
	BCC47498		**MT356074**	**MT356086**	**MT477980**	**MT477996**	**MT477988**
	BCC28571		**MT356075**	**MT356087**	**MT477981**	**MT478009**	**MT478006**
Akanthomyces pyralidarum	BCC28816 T	Lepidoptera; Pyralidae	**MT356080**	**MT356091**	**MT477982**	**MT478000**	**MT478007**
	BCC32191		**MT356081**	**MT356092**	**MT477983**	**MT478001**	**MT477989**
	BCC40869		**MT356082**	**MT356093**	**MT477984**	**MT478002**	**MT477990**
	BCC29197		**MT356083**	**MT305694**	**MT508840**	**MT478003**	**MT477991**
Akanthomyces sulphureus	TBRC7248 T	Araneae; spider	MF140758[i]	MF140722[i]	MF140843[i]	MF140787[i]	MF140812[i]
	TBRC7249	Araneae; spider	MF140757[i]	MF140721[i]	MF140842[i]	MF140786[i]	MF140734[i]
Akanthomyces thailandicus	TBRC7245 T	Araneae; spider	MF140754[i]	–	MF140839[i]	–	MF140809[i]
Akanthomyces tortricidarum	BCC72638 T	Lepidoptera; Tortricidae	**MT356076**	**MT356088**	**MT478004**	**MT477997**	**MT477992**
	BCC41868		**MT356077**	**MT356089**	**MT477985**	**MT477998**	**MT478008**
	BCC28583		**MT356079**	**MT356090**	**MT477986**	**MT477999**	**MT477993**
Akanthomyces tuberculatus	HUA186131	Lepidoptera (adult moth)	–	MF416521[h]	MF416466[h]	–	–
Akanthomyces waltergamsii	TBRC7250	Araneae; spider	MF140749[i]	MF140715[i]	MF140835[i]	–	–
	TBRC7251	Araneae; spider	MF140747[i]	MF140713[i]	MF140833[i]	MF140781[i]	MF140805[i]
Ascopolyporus polychrous	PC 546	Plant	–	DQ118737[a]	DQ118745[a]	DQ127236[a]	–
Ascopolyporus villosus	ARSEF6355	Plant	–	AY886544[a]	DQ118750[a]	DQ127241[a]	–
Beauveria acridophilla	HUA179221	–	–	JQ895537[g]	JQ958615[g]	JX003853[g]	JX003843[g]
	MCA1181	Romaleidae; *Tropidacris cristata*	JQ958607[g]	JQ895542[g]	–	JX003856[g]	–
Beauveria bassiana	ARSEF1564	Lepidoptera; Arctiidae	HQ880761[e]	–	HQ880974[e]	HQ880833[e]	HQ880905[e]
Beauveria blattidicola	MCA1727	–	–	MF416539[h]	MF416483[h]	MF416640[h]	–
	MCA1814	–	–	MF416540[h]	MF416484[h]	MF416641[h]	–
Beauveria brongniartii	BCC16585	Coleoptera; *Anomala cuprea* (larva)	JN049867[f]	JF415967[f]	JF4160092[f]	JN049885[f]	JF415991[f]
	ARSEF617	Coleoptera; Scarabaeidae	HQ880782[e]	–	HQ880991[e]	HQ880854[e]	HQ880926[e]
Beauveria caledonica	ARSEF2567	Soil	HQ880817[e]	AF339520[g]	EF469057[g]	EF469086[g]	HQ880961[g]
Beauveria malawiensis	ARSEF7760	Coleoptera; Cerambycidae	–	–	DQ376246[g]	HQ880897[g]	HQ880969[g]

Species	Strain	Host	GenBank accession numbers				
			ITS	LSU	*TEF*	*RPB1*	*RPB2*
Beauveria pseudobassiana	ARSEF3405	Lepidoptera: Tortricidae	AY532022[e]	–	AY531931[e]	HQ880864[e]	HQ880936[e]
Blackwellomyces cardinalis	OSC93609	Lepidoptera; Tineidae (larva)	–	AY184962[g]	DQ522325[g]	DQ522370[g]	DQ522422[g]
	OSC93610		JN049843[f]	AY184963[f]	EF469059[f]	EF469088[f]	EF469106[f]
Cordyceps amoene-rosea	CBS107.73	Coleoptera (pupa)	AY624168[h]	MG665224[i]	–	–	MG665234[i]
	CBS729.73	Coleoptera; Nitidulidae	AY624169[h]	MG665225[i]	HM161732[i]	–	MG665235[i]
Cordyceps bifusispora	spat08.129	–	–	MF416523[h]	MF416468[h]	MF416630[h]	–
	spat08.133.1	–	–	MF416524[h]	MF416469[h]	MF416631[h]	MF416434[h]
Cordyceps blackwelliae	TBRC7253	Lepidoptera	MF140739[i]	MF140705[i]	MF140825[i]	MF140774[i]	MF140798[i]
	TBRC7254	Lepidoptera	MF140738[i]	MF140704[i]	MF140824[i]	MF140773[i]	MF140797[i]
	TBRC7255	Lepidoptera	MF140737[i]	MF140703[i]	MF140823[i]	MF140772[i]	MF140796[i]
Cordyceps caloceroides	MCA2249	–	–	MF416525[h]	MF416470[h]	MF416632[h]	–
	QCNE186715	–	–	MF416526[h]	–	–	–
Cordyceps cateniannulata	TBRC7258	Araneae; spider	MF140753[i]	MF140729[i]	MF140850[i]	MF140767[i]	–
Cordyceps coleopterorum	CBS110.73	Coleoptera (larva)	AY624177[f]	JF415988[f]	JF416028[f]	JN049903[f]	JF416006[f]
Cordyceps farinosa	CBS111113	–	AY624181[h]	FJ765253[i]	GQ250022[i]	–	GU979973[i]
Cordyceps fumosorosea	CBS375.70	Food	AY624183[h]	MG665229[i]	HM161736[i]	–	MG665238[i]
	CBS107.10	–	AY624184[h]	MG665227[i]	HM161735[i]	–	MG665237[i]
Cordyceps javanica	TBRC7259	Lepidoptera	MF140745[i]	MF140711[i]	MF140831[i]	MF140780[i]	MF140804[i]
	TBRC7260	Lepidoptera	MF140744[i]	MF140710[i]	MF140830[i]	MF140779[i]	MF140803[i]
Cordyceps kyusyuensis	EFCC5886	Lepidoptera (pupa)	–	EF468813[c]	EF468754[c]	EF468863[c]	EF468917[c]
Cordyceps lepidopterorum	TBRC7263	Lepidoptera (larva)	MF140765[i]	MF140699[i]	MF140819[i]	MF140768[i]	MF140792[i]
	TBRC7264		MF140766[i]	MF140700[i]	MF140820[i]	MF140769[i]	MF140793[i]
Cordyceps militaris	OSC93623	Lepidoptera (pupa)	–	EF468821[h]	EF468762[h]	EF468869[h]	–
Cordyceps ochraceostromata	ARSEF5691	Lepidoptera	–	EF468819[h]	EF468759[h]	EF468867[h]	EF468921[h]
Cordyceps ninchukispora	spat08.115	–	–	MF416532[h]	MF416476[h]	MF416635[h]	MF416439[h]
	spat09.021	–	–	MF416533[h]	MF416477[h]	MF416636[h]	–
Cordyceps rosea	spat09.053	–	–	MF416536[h]	MF416480[h]	MF416637[h]	MF416442[h]
Cordyceps takaomontana	BCC12688	Lepidoptera	EU807996[j]	–	–	–	–
Cordyceps tenuipes	TBRC7265	Lepidoptera (pupa)	MF140741[i]	MF140707[i]	MF140827[i]	MF140776[i]	MF140800[i]
	TBRC7266		MF140742[i]	MF140708[i]	MF140828[i]	MF140777[i]	MF140801[i]
Engyodontium aranearum	CBS309.85	Araneae; spider	–	AF339526[c]	DQ522341[c]	DQ522387[c]	DQ522439[c]
Gibellula pulchra	NHJ10808	Araneae; spider	–	EU369035[d]	EU369018[d]	EU369056[d]	EU369076[d]
Gibellula ratticaudata	ARSEF1915	Araneae; spider	–	DQ518777[d]	DQ522360[d]	DQ522408[d]	DQ522467[d]
Gibellula sp.	NHJ5401	Araneae; spider	–	–	–	EU369059[d]	EU369097[d]
Hevansia arachnophila	NHJ10469	Araneae; spider	–	EU369031[d]	EU369008[d]	EU369047[d]	–
Hevansia cinerea	NHJ3510	Araneae; spider	–	–	EU369009[d]	EU369048[d]	EU369070[d]
Hevansia nelumboides	BCC2093	–	–	MF416530[h]	MF416473[h]	–	MF416437[h]
Hevansia novoguineensis	NHJ11923	Araneae; spider	–	EU369032[d]	EU369013[d]	EU369052[d]	EU369072[d]
	NHJ13161	Araneae; spider	–	–	EU369011[d]	EU369050[d]	–
Hevansia websteri	BCC23860	–	–	–	GQ250030[j]	–	–
Isaria farinosa	OSC111005	–	–	DQ518772[h]	DQ522348[h]	DQ522394[h]	–
	OSC111006	–	–	EF469080[h]	EF469065[h]	EF469094[h]	–
Isaria sp.	spat09.050	–	–	MF416559[h]	MF416506[h]	MF416663[h]	MF416457[h]
	spat09.051	–	–	MF416560[h]	MF416507[h]	MF416664[h]	MF416458[h]
Lecanicillium antillanum	CBS350.85 T	Fungi; agaric (Hymenomycetes)	–	AF339536[d]	DQ22350[d]	DQ522396[d]	DQ522450[d]

Species	Strain	Host	GenBank accession numbers				
			ITS	**LSU**	***TEF***	***RPB1***	***RPB2***
Lecanicillium psalliotae	CBS101270	Soil	–	EF469081[c]	EF469066[c]	EF469095[c]	EF469113[c]
	CBS532.81		–	AF339560[c]	EF469067[c]	EF469096[c]	EF469112[c]
Purpureocillium lilacinum	CBS284.36	Soil	AY624189[b]	FR775484[c]	EF468792[c]	EF468898[c]	EF468941[c]
	CBS431.87	Nematoda; *Meloidogyne* sp.	AY624188[f]	EF468844[f]	EF468791[f]	EF468897[f]	EF468940[f]
Samsoniella aurantia	TBRC7271	Lepidoptera	MF140764[i]	MF140728[i]	MF140846[i]	MF140791[i]	MF140818[i]
	TBRC7272		MF140763[i]	MF140727[i]	MF140845[i]	–	MF140817[i]
Samsoniella inthanonensis	TBRC7915	Lepidoptera (pupa)	MF140761[i]	MF140725[i]	MF140849[i]	MF140790[i]	MF140815[i]
	TBRC7916		MF140760[i]	MF140724[i]	MF140848[i]	MF140789[i]	MF140814[i]
Simplicillium lamellicola	CBS116.25	Soil	AJ292393[f]	AF339552[f]	DQ522356[f]	DQ522404[f]	DQ522462[f]
Simplicillium lanosoniveum	CBS704.86	Fungi; *Hemileia vastatrix*	–	AF339553[c]	DQ522358[c]	DQ522406[c]	DQ522464[c]
	CBS101267		AJ292395[f]	AF339554[f]	DQ522357[f]	DQ522405[f]	DQ522463[f]
Simplicillium obclavatum	CBS311.74	Air above sugarcane field	–	AF339517[c]	EF468798[c]	–	–
Torrubiella wallacei	CBS101237	Lepidoptera	–	AY184967[c]	EF469073[c]	EF469102[c]	EF469119[c]
Verticillium sp.	CBS102184	–	–	AF339564[h]	EF468803[h]	EF468907[h]	EF468948[h]

Note. The accession numbers in bold font refer to sequences generated in this study. Strain numbers with T are type species.
References. [a]Chaverri et al. (2005), [b]Luangsa-ard et al. (2005), [c]Sung et al. (2007), [d]Johnson et al. (2009), [e]Rehner et al. (2011), [f]Kepler et al. (2012), [g]Sanjuan et al. (2014), [h]Kepler et al. (2017), [i]Mongkolsamrit et al. (2018), [j]Chen et al. (2018), [k]Kuephadungphan et al. (2018), [l]Vu et al. (2019).

[C–G] = 0.2159, [C–T] = 1.1151, [G–T] = 1.000. For among-site variation, the proportion of invariable sites (I) was 0.3370 and the gamma distribution shape parameter (G) was 0.5036. This model was used in MrBayes and RAxML. MP and RAxML trees are provided as Suppl. materials 1, 2.

Taxonomy

Akanthomyces noctuidarum **Aini, Luangsa-ard, Mongkolsamrit & Thanakitpipattana, sp. nov.**
MycoBank No: 835652
Figure 2

Type. Thailand. Narathiwat Province, Hala Bala Wildlife Sanctuary, Headquarter Nature Trail; 5°928'N, 101°883'E; on adult moth; 3 Mar 2009; K. Tasanathai (KT), P. Puyngain (PP), T. Chohmee (TC) (holotype BBH 26019 dried culture; ex-type living culture BCC 36265). GenBank: ITS = MT356072, LSU = MT356084, *TEF* = MT477978, *RPB1* = MT477994, *RPB2* = MT477987.

Etymology. Referring to the host (Noctuidae, Lepidoptera) where the fungus was found.

Description. Teleomorph: Adult moth attached to the midrib of monocotyledonous leaf or undersides of dicotyledonous leaf covered by white to cream mycelium (OAC816). Stroma arising from host body and wing veins, white to cream, cylindrical, length ca. 5 mm. Perithecia superficial, orange to light brown (OAC825), few to numerous, crowded at the tip of the stroma or growing directly from mycelium in host

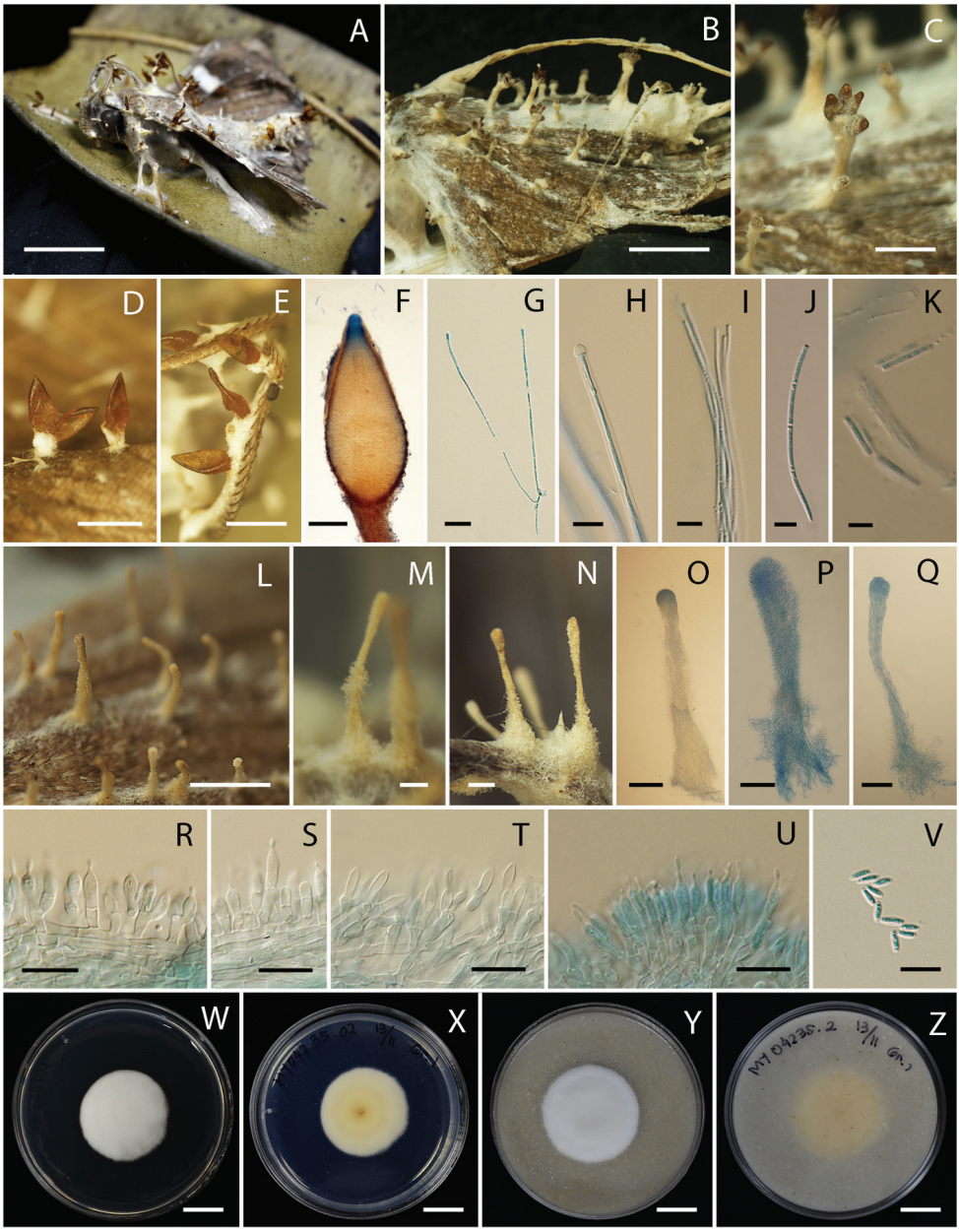

Figure 2. *Akanthomyces noctuidarum* (BBH 26019, BCC 36265) **A, B** fungus on adult moth **C–F** perithecia **G** asci **H** tip of ascus **I** ascus with ascospores **J** ascospores with clear septae **K** ascospores break into part-spores **L–Q** synnemata **R–T** phialides through the length of synnema **U** phialides at the tip of synnema **V** conidia **W, X** culture on PDA 14 days **X** reverse **Y, Z** culture on OA 14 days **Z** reverse. Scale bars: 1 cm (**A, B, W, X, Y, Z**); 5 mm (**C, I, J, K**); 1 mm (**D, E, L**); 200 μm (**F, M, N, O, P, Q**); 50 μm (**G**); 10 μm (**H, R, S, T, U, V**).

body and wing veins, ovoid, (530–)623–993(–1000) × (290–)308–413(–425) µm. *Asci* cylindrical, hyaline, (170–)196–423(–550) × (2–)2.7–3.8(–4) µm. Ascospores cylindrical, filiform, hyaline, multi-septate, breaking into one-celled fragments at maturity, (6–)7–10.7(–13) × 1 µm.

Anamorph: Synnemata arising from moth body and wing veins, white to cream (OAC816), erect, simple, cylindrical to clavate, (650–)668–1191(–1500) × (50–)53.4–102(–120) µm. Conidiogenous cells produced along the synnemata, monophialidic or polyphialidic. Phialides cylindrical with papillate end, hyaline, (5–)6.8–9(–10) × (1.8–)2–2.4(–3) µm. Conidia cylindrical with round end, hyaline, (3–)3.5–4.5(–6) × 1 µm.

Culture characters. Colony on PDA growing with a diameter of 20–24 mm in 14 days, circular, flat to raised, entire edges, white (OAC909) and fluffy mycelium. Colony reverse cream (OAC814). Colony on OA growing with a diameter of 20–25 mm in 14 days, circular, flat to raised, entire, white (OAC 909) and fluffy mycelium. Colony reverse uncolored. Conidia and reproductive structures not observed on both, PDA and OA in 14 days.

Distribution. Thailand, known from various national parks throughout the country.

Ecology. All specimens were found on the underside of leaves of plants.

Additional specimens examined. Thailand. Nakhon Ratchasima Province, Khao Yai National Park, Km.29; 14°711'N, 101°421'E; on adult moth; 24 Jan 2006; KT, W. Chaygate (WC), S. Sivichai, Le Tan Hung (BBH16595). Narathiwat Province, Hala Bala Wildlife Sanctuary, Headquarter Nature Trail; 5°928'N, 101°883'E; on adult moth; 19 Feb 2011; KT (BBH30267, BCC 47498). Kamphaeng Phet Province, Khlong Lan National Park, Saphan Ton Nature Trail; 16°203'N, 99°321'E; on adult moth; 6 Nov 2007; BT, KT, WC, S. Mongkolsamrit (SM), P. Srikitikulchai (PS), R. Ridkaew (RR), A. Khonsanit (AK) (BBH22738, BCC 28571).

Notes. This species produced both, anamorph and teleomorph. The type strain of this species, BBH 26019/ BCC 36265, consisted of both, anamorph and teleomorph. The other strains produced only one morph on the insect, either anamorph or teleomorph.

***Akanthomyces pyralidarum* Aini, Luangsa-ard, Mongkolsamrit & Thanakitpipattana, sp. nov.**

MycoBank No: 835653

Figure 3

Type. Thailand. Kanchanaburi Province, Thung Yai Naresuan Wildlife Sanctuary, Krathon Ruesi Nature Trail; 14°746'N, 98°625'E; on adult moth; 11 Dec 2007; KT, SM, RR, B. Thongnuch (BT) (holotype BBH23823 dried culture, ex-type living culture BCC 28816). GenBank: ITS = MT356080, LSU = MT356091, *TEF* = MT477982, *RPB1* = MT478000, *RPB2* = MT478007.

Etymology. Refers to the host (Pyralidae, Lepidoptera) of the fungus.

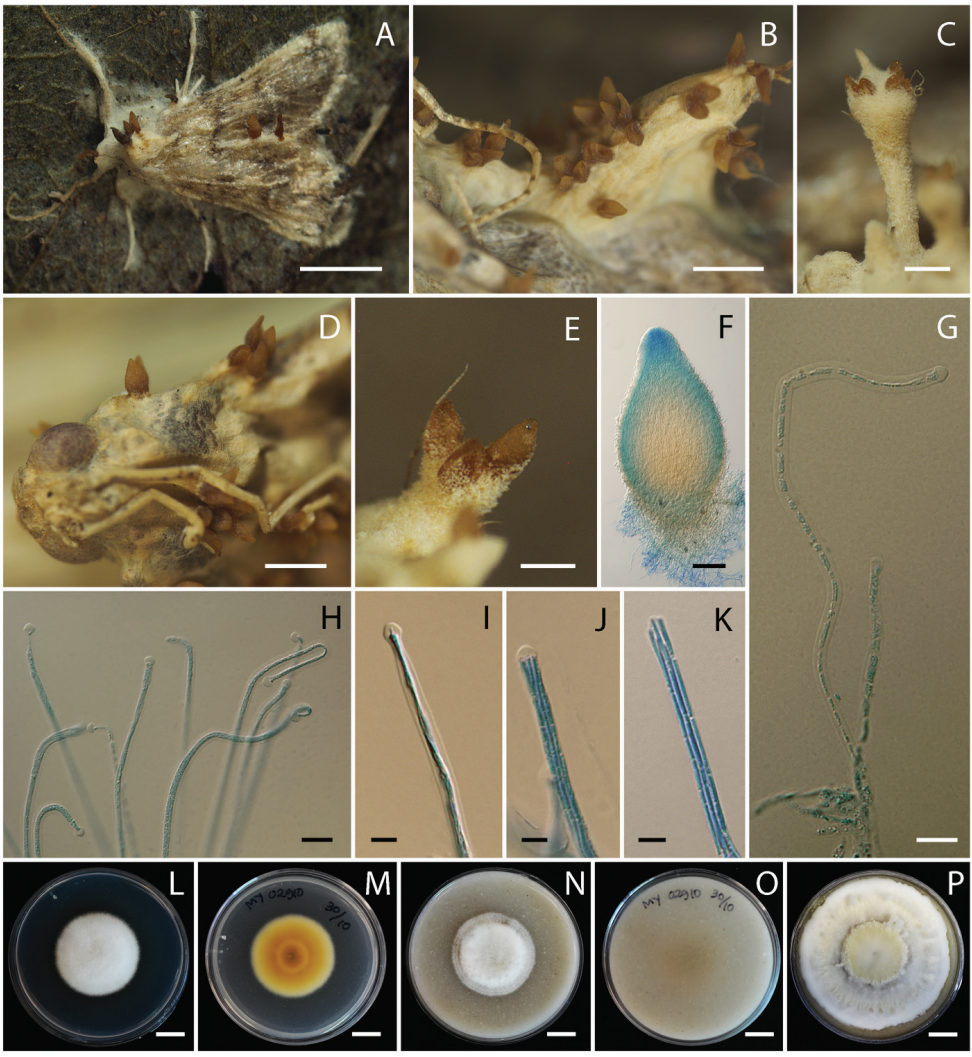

Figure 3. *Akanthomyces pyralidarum* (BBH 23823, BCC 28816) **A** fungus on adult moth **B–F** perithecia **G, H** asci **I** tip of ascus with immature ascospore **J** tip of ascus with mature ascospores **K** ascospores **L, M** culture on PDA 14 days **M** reverse **N, O** culture on OA 14 days **O** reverse **P** culture on OA 28 days. Scale bars: 1 cm (**A, L, M, N, O, P**); 1 mm (**B**); 500 µm (**C, D, E**); 100 µm (**F**); 10 µm (**G, H**); 5 µm (**I, J, K**).

Description. Teleomorph: Adult moth attached on the undersides of dicotyledonous leaf covered by white to cream mycelium (OAC816). Stroma arising from host body and wings, white to cream (OAC816), cylindrical. Perithecia superficial, crowded at the tip of stroma or growing directly from mycelium that covers the host body, few to numerous, ovoid to obpyriform, (290–)342–580(–650) × (150–)186–291(–340) µm. Asci cylindrical, the bottom of asci thicker than the middle part, (170–)222–

329(–360) × (2–)2.5–3.3(–4) µm. Ascospores hyaline, filiform, multi-septate, discharged into part-spores, (5–)5.9–9.4(–12) × 1 µm.

Culture characters. Colonies on PDA growing with a diameter of 23–28 mm in 14 days, white (OAC909), circular, flat, entire. Colony reverse pale yellow (OAC856) at the center. Conidia and reproductive structures not observed. Colonies on OA growing with a diameter of 27–30 mm in 14 days, white (OAC909), circular, flat, entire. Colony reverse uncolored. Conidia and reproductive structures not observed.

Distribution. Thailand, known from various national parks throughout the country.

Ecology. All specimens are found on the underside of leaves of plants.

Additional specimens examined. Thailand. Chiang Mai Province, Huai Nam Dang National Park, Pong Dueat Pa Pae Geyser; 19°121'N, 98°943'E; on adult moth; 5 Sep 2008; KT, WC, PS, AK, SM (BBH 24623, BCC 32191). Phetchabun Province, Nam Nao National Park, Headquarter Nature Trail; 16°768'N, 101°671'E; on adult moth; 24 Nov 2009; KT, TC, AK (BBH 27293, BCC 40869). Kanchanaburi Province, Thung Yai Naresuan Wildlife Sanctuary, Thi Khong Protect Forest Unit; 14°746'N, 98°625'E; on adult moth; 12 Dec 2007; KT, SM, RR, BT (BBH 23778, BCC 29197).

Notes. *Akanthomyces pyralidarum* is found only in its teleomorph state. This species differs from *Akanthomyces noctuidarum* by having smaller perithecia (290–650 × 150–340 µm) than *A. noctuidarum* (530–1000 × 290–425 µm).

***Akanthomyces tortricidarum* Aini, Luangsa-ard, Mongkolsamrit & Thanakitpipattana, sp. nov.**

MycoBank No: 835654

Figure 4

Type. Thailand. Nakhon Ratchasima Province, Khao Yai National Park, Mo Sing To Nature Trail; 14°711'N, 101°421'E; on adult moth; 6 Jun 2014; W. Noisripoom, PS, TC, S. Sommai, R. Somnuk (holotype BBH 38669 dried culture, ex-type living culture BCC 72638). GenBank: ITS = MT356076, LSU = MT356088, *TEF* = MT478004, *RPB1* = MT477997, *RPB2* = MT477992.

Etymology. Refers to the host (Tortricidae, Lepidoptera) of the fungus.

Description. Anamorph: Specimens examined in this study can be found on the underside of dicotyledonous leaves and palm leaf. The hosts were adult moths, ca. 4–9 × 1–2 mm. Two types of synnemata were produced on insect hosts. Several long synnemata arose at the head and in the middle of the host body, white to cream, up to 5 mm long and ca. 120–150 µm wide, rarely branched, cylindrical to clavate with acute or blunt end. Conidiogenous cells produced along synnemata, monophialidic or polyphialidic. Phialides (5–)6–8(–10) × (1.8–)2–2.7(–3) µm, cylindrical to ellipsoidal with papillate end. Conidia smooth-walled, hyaline, single-celled, fusoid, (2–)2.5–3(–3.2) × (0.8–)1–1.4(–2) µm. Several short synnemata arose on moth body, wings, and legs, white to cream, (197–)200–267(–300) × (15–)17.7–31.6(–40) µm,

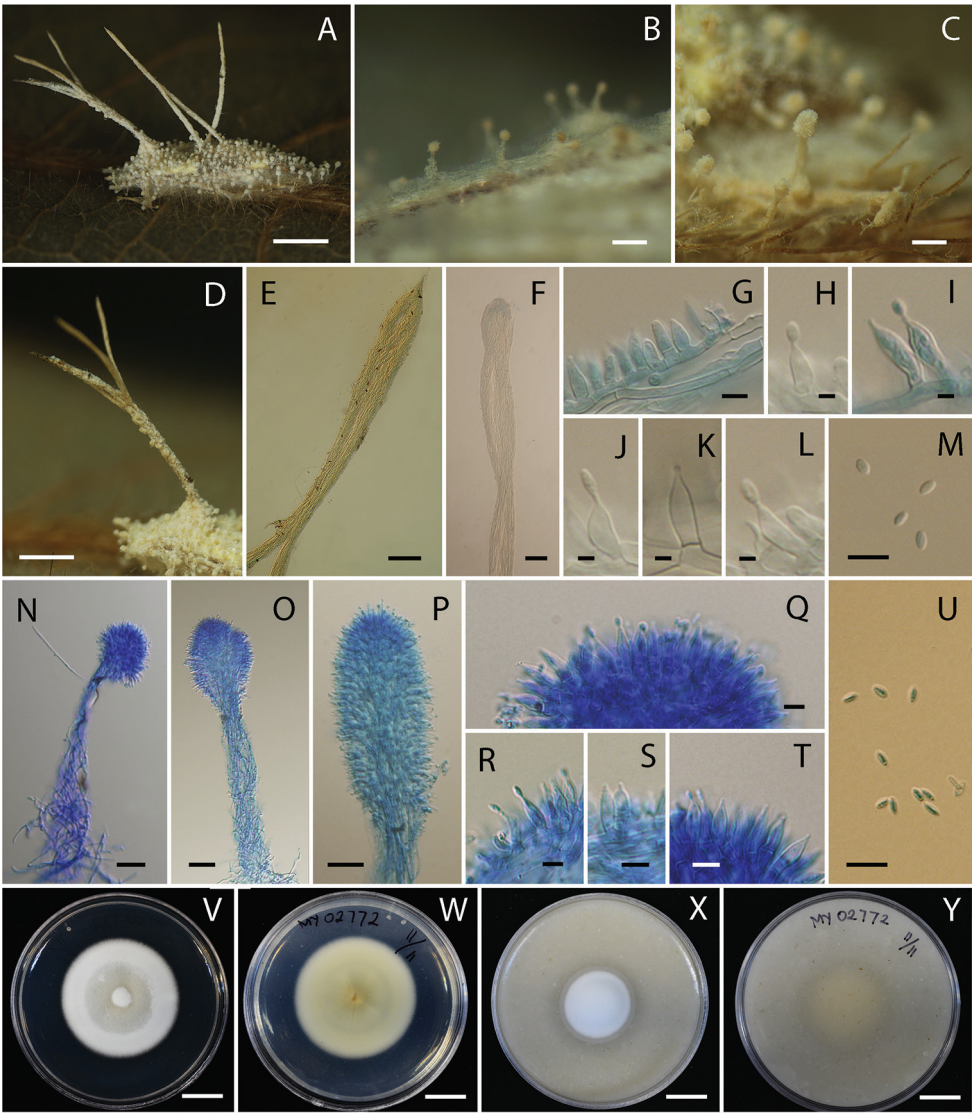

Figure 4. *Akanthomyces tortricidarum* (BBH 38669, BCC 72638) **A** fungus on adult moth **B, C, N–P** short synnemata **D–F** long synnemata **G–L** phialides from long synnema **M** conidia from long synnema **Q–T** phialides from short synnema **U** conidia from short synnema **V,W** culture on PDA 14 days **W** reverse **X,Y** culture on OA 14 days **Y** reverse. Scale bars: 2 mm (**A**); 200 µm (**B, E**); 100 µm (**C, F**); 500 µm (**D**); 5 µm (**G, M, Q, R, S, T, U**); 2 µm (**H, I, J, K, L**); 30 µm (**N, O, P**); 1 cm (**V,W, X,Y**).

with diameter of the tip (43–)51.5–73(–75) µm, cylindrical with subglobose or oblong end. Conidiogenous cells produced at the end of synnemata, monophialidic or polyphialidic. Phialides (5–)6.2–8.3(–10) × (1.8–)2–2.5(–3) µm, cylindrical to ellipsoidal with papillate end. Conidia smooth-walled, hyaline, single-celled, fusoid,

(1–)1.8–2.7(–3) × 1–2 µm. Phialides and conidia from both long and short synnemata were on the same size range.

Culture characters. Colonies on PDA growing with a diameter of 25–31 mm in 14 days, white (OAC909), circular, flat, entire, reverse pale yellow (OAC858). Mycelium smooth, septate, hyaline. Colonies on OA growing with a diameter of 18–25 mm in 14 days, circular, flat, entire, white (OAC909), reverse brownish yellow (OAC812). Mycelium smooth, septate, hyaline. Conidia and reproductive structures not produced on both, PDA and OA in 14 days.

Distribution. Thailand, known from various national parks throughout the country.

Ecology. All specimens are found on the underside of leaves of plants.

Additional specimens examined. Thailand. Nakhon Ratchasima Province, Khao Yai National Park, Mo Sing to Nature Trail; 14°711'N, 101°421'E; on adult moth; 7 Apr 2010; KT, SM, TC, AA, RR (BBH 28530, BCC 41868). Nakhon Ratchasima Province, Khao Yai National Park, Mo Sing to Nature Trail; 14°711'N, 101°421'E; on adult moth; 11 Nov 2009; KT, SM, TC, RR, M. Sudhadham, AK (BBH 27283, BCC 40005). Kamphaeng Phet Province, Khlong Lan National Park, Saphan Ton Nature Trail; 16°203'N, 99°321'E; on adult moth; 6 Nov 2007; KT, SM, PS, BT, RR, AK, WC (BBH 23097, BCC 28583).

Notes. *Akanthomyces tortricidarum* is found only in its anamorph state. This species differs from *A. noctuidarum* by having smaller conidia (2–3 × 1 µm) than *A. noctuidarum* (3–6 × 1 µm). Furthermore, the shape of conidia of *A. tortricidarum* is fusoid, while conidia of *A. noctuidarum* is cylindrical with a round end.

Discussion

The genus *Akanthomyces* established by Lebert (1858) was revised by Mains (1950). This genus is characterized by cylindrical synnemata covered by a hymenium-like layer of phialides producing single-celled catenulate conidia (Samson 1974). Presently, 20 *Akanthomyces* species have been formally described (Kepler et al. 2017; Mongkolsamrit et al. 2018), while eight species of *Akanthomyces* on spiders were transferred to the genus *Hevansia*. *Hevansia* includes the type species *Hevansia novoguineensis* (previously described as *Akanthomyces novoguineensis*), which differs from *Akanthomyces* by the immersed perithecia in a disk sitting at the top of a well-formed stipe. However, now it has to be an akanthomyces-like teleomorph (Kepler et al. 2017). *Akanthomyces* is considered as a synonym of *Lecanicillium*, an anamorph within Cordycipitaceae with verticillium-like morphologies (Gams and Zare 2001). *Lecanicillium* does not form a single monophyletic clade and species within this genus are distributed throughout Cordycipitaceae (Sukarno et al. 2009). Based on the molecular analyses from five nuclear genes (SSU, LSU, *TEF*, *RPB1*, and *RPB2*), Kepler et al. (2017) proposed that *Lecanicillium* should be rejected and *Akanthomyces* has priority by date over this genus. The type species of *Lecanicillium*, *L. lecanii* as well as some other species (*L. attenuatum*, *L. muscarium*, and *L. sabanense*) have phylogenetic affinities to *Akanthomyces* (Chiriví-Salomón et al. 2015)

The type species of *Akanthomyces*, *A. aculeatus* and another *Akanthomyces* species on moth, *A. pistillariiformis* (= *A. tuberculatus*), were the closest related species to the three new species described here. Two of three new species were found in their anamorph state. Fortunately, in *A. noctuidarum* both, teleomorph and anamorph are present in the same specimen. The anamorph comparison between some species within *Akanthomyces* is shown in Table 2. The conidia of *A. noctuidarum* and *A. aculeatus* are almost in the same size (*A. noctuidarum*; 3–6 × 1 μm, *A. aculeatus*; 3–6 × 2–3 μm). However, the conidial shape of *A. noctuidarum* is cylindrical with a round end while *A. aculeatus* is ellipsoid or obovoid. *Akanthomyces noctuidarum* has the smallest synnemata compared to all the others (*A. noctuidarum*; 650–1500 μm, *A. aculeatus*; 1–8 × 0.1–0.5 mm, *A. tuberculatus*; 1–6 mm × 50–300 μm). *Akanthomyces noctuidarum* also has smaller phialides than both aforementioned species (5–10 × 2–3 μm, *A. aculeatus*; 6–16 × 2.5–4 μm, *A. tuberculatus*; 7–10.5 × 2.7–3.5 μm) with cylindrical shape and papillate at the end.

Akanthomyces tortricidarum was distinguished from the others species by having two different types of synnemata. The long synnemata of *A. tortricidarum* are cylindrical to clavate with acute or blunt ends. The hyphae diverged in the upper portion of the synnema and repeatedly branched more or less dichotomously, whereas the phialides were terminal on the branches. At the lower portion of synnema, the phialides were produced either as lateral cells or frequently as terminal cells of short lateral branches produced along the entire length of the outer hyphae of the synnema. The production of phialides was abundant at the upper portion of the synnema, resulting in a compact hymenial layer, whereas the phialides at the lower portion of the synnema were scattered and well separated from each other. Unlike the long synnemata, the hymenium-like layer of phialides on the short synnemata was limited to its upper part and the lower portion was sterile, forming a stipe. In the upper portion of the short synnema, the hyphae diverged and repeatedly branched more or less dichotomously and terminated with phialides. However, at the lower portion, the outer longitudinal hyphae did not produce any lateral phialides or lateral branches bearing phialides. This character was similar to the genus *Insecticola* proposed by Mains (1950). However, Samson and Evans (1974) transferred all members of this genus to *Akanthomyces* because variations in these characters did not support the distinction. The shape of synnemata and arrangement of phialides from *A. noctuidarum* and long synnemata from *A. tortricidarum* were similar. Nevertheless, *A. tortricidarum* differs from *A. noctuidarum* by having smaller conidia (2–3 × 1 μm) than *A. noctuidarum* (3–6 × 1 μm). Furthermore, the shape of conidia in *A. tortricidarum* is fusoid, while the conidia of *A. noctuidarum* is cylindrical with rounded ends.

The teleomorph comparison between some species within *Akanthomyces* is shown in Table 3. *Akanthomyces noctuidarum* and *A. pyralidarum* differed from *A. tuberculatus* by the size of ascospores, asci, and perithecia. *Akanthomyces tuberculatus* has smaller ascospores measuring 2–6 × 0.5–1 μm, whereas *A. noctuidarum* and *A. pyralidarum* have larger ascospores at 6–13 × 1 μm and 5–12 × 1 μm, respectively. However, all three of them have the same shape of ascospore and asci. *Akanthomyces pyralidarum* has the smallest size of asci (*A. pyralidarum*; 170–360 × 2–4 μm, *A. noctuidarum*; 170–550 × 2–4 μm, and *A. tuberculatus*; 300–600 × 4–5 μm). The shape of perithecia from

Table 2. Morphological comparisons between anamorph of closely related *Akanthomyces* species used in this study.

Species	Host	Synnemata	Phialides	Conidia
Akanthomyces aculeatus[2]	Moth (Lepidoptera)	Yellowish, cylindrical, narrowing upward, 1–8 mm long and 0.1–0.5 mm wide	Subcylindric to narrowly ellipsoidal, 6–16 × 2.5–4 µm	Ellipsoidal or obovoid, 3–6 × 2–3 µm
Akanthomyces angustispora[2]	Coleoptera larva	Flesh colored, simple or branched, 8–13 mm long and 0.2–0.6 mm wide	Oblong or narrowly ellipsoidal, 6–14 × 3–4 µm	Narrowly clavate, 4.5–6 × 1.2–1.4 µm
Akanthomyces arachnophilus[4]	Spider (Araneae)	Creamish yellow to pale brown, simple or branched, cylindrical, 2.5–5 mm × 50–75 µm	Globose, 3.2–4.3 × 6.5–8.5 µm	Fusiform, 4.5–5.5(–6) × 1.5–3 µm
Akanthomyces araneogenum[5]	Spider	Conidiophores mononematous or synnematous, 21.6–48 × 1.2–2.2 µm, penicillium-like from hyphae directly	Cylindrical, somewhat inflated base, tapering to a thin neck, 4.3–17.3 × 0.9–3.1 µm	Globose, 1.3–2.4 µm in diam, or ellipsoidal, 2.1–3.3 × 1.1–1.6 µm
Akanthomyces gracilis[4]	Hymenoptera, Coleoptera, Lepidoptera (moth larvae) Heteroptera, Homoptera	White to yellow-brown, simple, rarely branched, cylindrical, usually 0.7–2 mm × 100–400 µm, occasionally up to 30 mm long and 0.5 mm wide	Cylindrical, 7–10 × 1.5–2.5 µm	Ellipsoidal, fusiform, 2.5–3 × 1–1.6 µm
Akanthomyces kanyawimiae[3]	Spider (Araneae)	Up to 1.5 mm long, up to 400 µm wide; loosely covered by dense white to cream mycelia	Cylindrical to ellipsoidal, (7–)8–10.5(–12) × 2–3 µm	Fusiform or lemon-shaped, (2–)2.5–3.5(–4) × 1–2 µm
Akanthomyces noctuidarum[1]	Lepidoptera; Noctuidae	White to cream (OAC816), simple, cylindrical to clavate, (650–)668–1191(–1500) × (50–)53–102(–120) µm	Cylindrical with papillate end, (5–)6.8–9(–10) × (1.8–)2–2.4(–3) µm	Cylindrical with round end, (3–)3.5–4.7(–6) × 1 µm
Akanthomyces pistillariiformis[4] (= *A. tuberculatus*)	Moth (Lepidoptera)	White to creamish, simple, occasionally branched, cylindrical to clavate and stipitate, 1–6 mm long and 50–300 µm wide	Cylindrical, 7–10.5 × 2.7–3.5 µm	Cylindrical to narrowly fusiform, 4.5–6 × 1.2–1.5 µm
Akanthomyces suphureus[3]	Spider (Araneae)	–	Cylindrical, (5–)7.5–11(–12) × 2–2.5 µm	Cylindrical to ellipsoidal, (3–)4(–5) × (1–)1.5(–2) µm
Akanthomyces tortricidarum[1]	Lepidoptera; Tortricidae	**Long** synnemata white to cream, rarely branched, cylindrical to clavate with acute or blunt end, up to 5 mm long and wide ca. 120–150 µm.	Cylindrical to ellipsoidal with papillate end, (5–)6–8(–10) × (1.8–)2–2.7(–3) µm	Fusoid, (2–)2.5–3(–3.2) × 1–2 µm
		Short synnemata white to cream, cylindrical with subglobose or oblong at the end, (197–)200–267(–300) × (15–)17.7–31.6(–40) µm, with diameter of the tip (43–)51.5–73(–75) µm	Cylindrical to ellipsoidal with papillate end, (5–)6.2–8.3(–10) × (1.8–)2–2.5(–3) µm	Fusoid, (1–)1.8–2.7(–3) × 1–2 µm
Akanthomyces waltergamsii[3]	Spider (Araneae)	White to cream synnemata up to 1.5 mm long and ca. 100–120 µm wide	Cylindrical to ellipsoidal, (7–)8.5–11(–12) × 2.5–3 µm	Ellipsoidal, fusiform, (3–)4–5.5(–6) × 1.5–2 µm

Notes. [1]Current study, [2]Mains (1950), [3]Mongkolsamrit et al. (2018), [4]Samson and Evans (1974), [5]Chen et al. (2018).

A. noctuidarum is ovoid, while *A. pyralidarum* is ovoid to obpyriform and *A. tuberculata* is narrowly ovoid or conoid. *Akanthomyes pyralidarum* also has the smallest perithecia compared to the other species in the genus (*A. pyralidarum*; 290–650 × 150–340 µm,

Table 3. Morphological comparisons between teleomorph of closely related *Akanthomyces* species used in this study.

Species	Host	Perithecia	Asci	Ascospores
Akanthomyces noctuidarum[1]	Lepidoptera; Noctuidae	Superficial, orange to light brown, ovoid, (530–)623–993(–1000) × (290–)308–413(–425) µm	Cylindrical, (170–)196–423(–550) × (2–)2.7–3.8(–4) µm	Cylindrical, filiform, multi-septate, part-spores, (6–)7–10.7(–13) × 1 µm
Akanthomyces pyralidarum[1]	Lepidoptera; Pyralidae	Superficial, ovoid to obpyriform, (290–)342–580(–650) × (150–)186–291(–340) µm	Cylindrical, (170–)222–329(–360) × (2–)2.5–3.3(–4) µm	Filiform, multi-septate, part-spores, (5–)5.9–9.4(–12) × 1 µm
Akanthomyces suphureus[2]	Spider (Araneae)	Superficial, ovoid, (650–)676(–680) × (240–)324.5(–330) µm	Cylindrical, up to 500 µm long, 2–3 µm wide	Whole, filiform, (300–)336(–450) × 1–1.5 µm
Akanthomyces thailandicus[2]	Spider (Araneae)	Superficial, narrowly ovoid, (700–)752–838(–850) × (300–)305–375(–400) µm	Cylindrical, up to 550 µm long, 5–7 µm wide	Cylindrical, multi-septate, part-spores, 4–6 × 1–1.5 µm
Akanthomyces tuberculatus[3] (= *C. tuberculata*)	Moth (Lepidoptera)	Superficial, narrowly ovoid or conoid, dark brown, 420–900 × 180–370 µm	Cylindrical, 300–600 × 4–5 µm with a 4 µm thick cap	Filiform, multi-septate, part-spores, 2–6 × 0.5–1 µm

Notes. [1]Current study, [2]Mongkolsamrit et al. (2018), [3]Mains (1958).

A. tuberculatus; 420–900 × 180–370 µm, *A. sulphureus*; 650–680 × 240–330 µm, *A. thailandicus*; 700–850 × 300–400 µm, and *A. noctuidarum*; 530–1000 × 290–425 µm). Moreover, *A. sulphureus* and *A. thailandicus* are found on spiders (Araneae) while the others were found on moths.

All strains from these species did not produce conidia or reproductive structures when grown on PDA and OA for 14 days at 25 °C. Nevertheless, one strain from *A. pyralidarum* (BCC 29197) started to produce a synnemata-like structure on OA after 28 days. However, this synnemata-like structure was sterile and did not produce any phialides or conidia. Overall, fungal growth was faster in OA than in PDA.

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Supplementary material 1

MP tree

Authors: Arifah Nur Aini, Suchada Mongkolsamrit, Wijanarka Wijanarka, Donnaya Thanakitpipattana, J. Jennifer Luangsa-ard, Anto Budiharjo

Data type: phylogenetic tree

Explanation note: Branches showing Maximum Parsimony bootstrap values.

Link: https://doi.org/10.3897/mycokeys.71.55126.suppl1

Supplementary material 2

RAxML tree

Authors: Arifah Nur Aini, Suchada Mongkolsamrit, Wijanarka Wijanarka, Donnaya Thanakitpipattana, J. Jennifer Luangsa-ard, Anto Budiharjo

Data type: phylogenetic tree

Explanation note: Branches showing Maximum Likelihood bootstrap values from RAxML.

Link: https://doi.org/10.3897/mycokeys.71.55126.suppl2

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Checklist of thallus-forming Laboulbeniomycetes from Belgium and the Netherlands, including *Hesperomyces halyziae* and *Laboulbenia quarantenae* spp. nov.

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Abstract

In this paper we present an updated checklist of thallus-forming Laboulbeniomycetes (Ascomycota, Pezizomycotina), that is, the orders Herpomycetales and Laboulbeniales, from Belgium and the Netherlands. Two species are newly described based on morphology, molecular data (ITS, LSU ribosomal DNA) and ecology (host association). These are *Hesperomyces halyziae* on *Halyzia sedecimguttata* (Coleoptera, Coccinellidae) from both countries and *Laboulbenia quarantenae* on *Bembidion biguttatum* (Coleoptera, Carabidae) from Belgium. In addition, nine new country records are presented. For Belgium: *Laboulbenia aubryi* on *Amara aranea* (Coleoptera, Carabidae) and *Rhachomyces spinosus* on *Syntomus foveatus* (Coleoptera, Carabidae). For the Netherlands: *Chitonomyces melanurus* on *Laccophilus minutus* (Coleoptera, Dytiscidae), *Euphoriomyces agathidii* on *Agathidium laevigatum* (Coleoptera, Leiodidae), *Laboulbenia fasciculata* on *Omophron limbatum* (Coleoptera, Carabidae), *Laboulbenia metableti* on *Syntomus foveatus* and *S. truncatellus* (Coleoptera, Carabidae), *Laboulbenia pseudomasei* on *Pterostichus melanarius* (Coleoptera, Carabidae), *Rhachomyces canariensis* on *Trechus obtusus* (Coleoptera, Carabidae), and *Stigmatomyces hydrelliae* on *Hydrellia albilabris* (Diptera, Ephydridae). Finally, an identification key to 140 species of thallus-forming Laboulbeniomycetes in Belgium and the Netherlands is provided. Based on the combined data, we are able to identify mutual gaps that need to be filled as well as weigh the impact of chosen strategies (fieldwork, museum collections) and techniques in these neighboring countries. The aim of this work is to serve as a reference for studying Laboulbeniomycetes fungi in Europe.

Keywords

2 new taxa, arthropod-associated fungi, Ascomycota, Herpomycetales, integrative taxonomy, key, Laboulbeniales

Introduction

Herpomycetales and Laboulbeniales are two orders within the class Laboulbeniomycetes (Ascomycota, Pezizomycotina), consisting of arthropod-associated biotrophs. Both orders are unique among related fungi in that they do not form hyphae; instead, *thalli* are produced by mitotic divisions from a two-celled ascospore. Herpomycetales was recently described and includes a single genus, *Herpomyces* Thaxt., with 27 described species–all associated with cockroaches (Blattodea) (Haelewaters et al. 2019b; Gutierrez et al. 2020). The Laboulbeniales order, on the other hand, successfully radiated on a wide range of hosts. Representatives of this order can be found in three arthropod subphyla, including mites and harvestmen (in subphylum Chelicerata), millipedes (in subphylum Myriapoda), and many orders of true insects (in subphylum Hexapoda). The vast majority of about 2,325 described species (Kirk 2019) are known from beetles (order Coleoptera), hence the common name once introduced for the group, "beetle hangers" (Cooke 1892). The early taxonomic history of these fungi is fraught with confusion (Blackwell et al. 2020), but the incorporation of sequence data has led to a conclusive placement of these fungi within Ascomycota (Blackwell 1994; Weir and Blackwell 2001; Schoch et al. 2009).

Early studies on Laboulbeniales (including *Herpomyces* at that time) in Belgium and the Netherlands are scarce. In Belgium, Collart (1945, 1947) and Rammeloo (1986) made noteworthy contributions, followed by multiple publications by De Kesel and colleagues (1989–present). The Laboulbeniomycetes from Belgium were for the first time summarized by De Kesel and Rammeloo (1992), who reported 1 species of *Herpomyces* and 47 species of Laboulbeniales. De Kesel et al. (2020) provided an updated – and illustrated – *Catalogue of the Laboulbeniomycetes of Belgium*, with a total of 115 species (3 Herpomycetales, 112 Laboulbeniales) from 222 host species. For more details regarding the study of Herpomycetales and Laboulbeniales in Belgium, we refer to De Kesel and Rammeloo (1992) and De Kesel et al. (2020). In the Netherlands, thus far, no effort has been made to publish a checklist.

The study of Laboulbeniales in the Netherlands started during a meeting of the Dutch Entomological Society in 1906, triggered by a question from Dr. Johannes P. Lotsy, then director of the "Rijksherbarium" (Leiden). In response, Prof. Dr. De Meijere remembered that he once observed an infected *Drosophila funebris* (Fabricius, 1787) fly, collected at the ARTIS Amsterdam Royal Zoo in 1904, but had not thought it worthy of mention at the time. Recent infected material of *D. funebris* from nature reserve De Kaaistoep has thus far always been associated with *Stigmatomyces entomophilus* (Peck) Thaxt. (Haelewaters et al. 2015b) and hence it is likely that *S. entomophilus* represents the very first report of Laboulbeniales from the Netherlands. The first published account was a developmental study of *Stigmatomyces baeri* H. Karst. by Boedijn (1923). The fungus was found on an atypical host – *Fannia canicularis* (Linnaeus, 1761); this fly is the only reported host for *Fanniomyces ceratophorus* (Whisler) T. Majewski, which is morphologically different from Boedijn's (1923) drawings. We agree with Thaxter (1931) that the fungus was probably correctly identified by Boedijn, but perhaps the host was not.

Next, in the 1930s, only two species of Laboulbeniales were reported in the Netherlands: *Laboulbenia cristata* Thaxt. from *Paederus riparius* (Linnaeus, 1758) (Kossen 1936, 1938) and *Laboulbenia flagellata* Peyr. from *Platynus* spp. (Zaneveld 1938). It was not until Abraham Middelhoek (1906–1968) that the number of reported species of Laboulbeniales in the Netherlands would increase by 25 (Middelhoek 1941, 1942, 1943a, b, c, d, 1945, 1947a, b, 1949). Middelhoek was first an artist who, among other things, made stained glass windows. Only after World War II, he studied biology and raised an interest in fungi, particularly the Laboulbeniales. After Middelhoek, Laboulbeniales were forgotten about in the Netherlands except for a single paper by Meijer (1975), who proposed to use Laboulbeniales fungi as "biological tags" to trace migration patterns. Since 2012, Haelewaters and colleagues have published several papers dealing with Laboulbeniales in the Netherlands, which together have more than doubled the number of reported species in this country (De Kesel and Gerstmans 2012; Haelewaters 2012, 2013; Haelewaters et al. 2012a, b, 2014, 2015a, b, 2020; De Kesel et al. 2013; Haelewaters and De Kesel 2013; De Kesel and Haelewaters 2014, 2019; Haelewaters and van Wielink 2016). To date, 79 species of Laboulbeniales are reported from the Netherlands.

In this contribution we compile all available data from Belgium and the Netherlands. Keeping in mind that both countries show some geographical differences, especially due to specific soils and increasing altitude in the southern part of Belgium, we think a combined checklist makes sense at this point. This is mainly because the sampling effort for Laboulbeniomycetes in the southern part of Belgium has been much lower compared to the northern and central areas of the country (De Kesel et al. 2020). As a result, the bulk of Belgian and Dutch records come from biogeographically comparable regions. The here presented checklist is useful to illustrate where mutual gaps need to be filled and what the impact has been of the chosen strategies (fieldwork, museum collections) and trapping techniques. In combination with the recently published Belgian catalogue (De Kesel et al. 2020) presenting illustrations and identification keys to 115 taxa, this checklist will serve as a reference for mycologists, students, and scholars studying Laboulbeniomycetes fungi. In addition, this work is an appropriate starting point for an updated checklist of thallus-forming Laboulbeniomycetes from Europe–an ongoing project that needs to be updated, three decades after the massive undertaking of Santamaría et al. (1991).

Materials and methods

Specimen collection and morphological study

Insects were collected in Belgium and the Netherlands using pitfall traps and on an illuminated white screen at night. Specimens were preserved in 96–99% ethanol until they were screened for presence of thalli of Laboulbeniomycetes at 20–50× magnification. Thalli were removed from the host at the foot and mounted in Amann solution

following the methods in De Kesel et al. (2020). Drawings and measurements were made using a BX51 light microscope (Olympus, Tokyo, Japan) with drawing tube, digital camera, and AnalySIS software (Soft Imaging System GmbH, Münster, Germany); or an an Olympus BH2 bright field compound microscope with SC30 camera and cellSens 1.18 imaging software.

Infected hosts found in Belgium and the Netherlands are preserved at Meise Botanic Garden (BR) and the Brabant Museum of Nature, Tilburg (NNKN), respectively. Microscope slides of Laboulbeniales are deposited at BR, FH, GENT, and NMBT (Thiers continuously updated).

DNA extraction, PCR amplification, sequencing

Three thalli of *Laboulbenia quarantenae* sp. nov. were used for DNA isolation using the REPLI-g Single Cell Kit (Qiagen, Stanford, California) with modifications (Haelewaters et al. 2019b). The DNA extract was stored at -20 °C until PCR amplification. Recent studies found that even though the internal transcribed spacer (ITS) region is a good marker for species delimitation in Laboulbeniomycetes, it is difficult to amplify in this group. Instead, the large subunit (LSU) of the ribosomal RNA gene has been put forward as a secondary barcode because it is easy to amplify and provides high discriminative resolution at species-level (e.g., Haelewaters et al. 2018; Sundberg et al. 2018b; Walker et al. 2018; Liu et al. 2020). The partial LSU was amplified using primers LIC15R (Miadlikowska et al. 2002) and LR6 (Vilgalys and Hester 1990). Sequencing was outsourced to Macrogen Europe (Amsterdam, the Netherlands) with the same PCR primers and an additional reverse primer, LR3 (Vilgalys and Hester 1990). Resulting forward and both reverse sequence reads were assembled and edited with Sequencher version 5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan).

For *Hesperomyces halyziae*, molecular work had been done previously (Haelewaters et al. 2018). DNA was extracted using the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St. Louis, Missouri) (methods in Haelewaters et al. 2015c). Seven thalli were placed in a 1.5 mL tube with 40 µL of Extraction Solution and sterilized sand. The tube was then placed in a FastPrep FP120 Cell Disrupter (Thermo Fisher Scientific, Waltham, Massachusetts) to mechanically crush fungal material at 5.5 m/s for 20 sec, and then on a heating block to incubate at 95 °C for 10 min. Finally, a total of 120 µL Dilution Solution was added to the mixture. Because we needed to define "*H. virescens* sensu stricto", additional extractions from single *Hesperomyces* thalli removed from *Chilocorus stigma* (Say, 1835) were performed using the REPLI-g Single Cell Kit with modifications. Amplification of the ITS was done using primers ITS1f (Gardes and Bruns 1993) and ITS4 (White et al. 1990) as well as *Hesperomyces*-specific primers ITShespL and ITShespR (Haelewaters et al. 2019b). Purification and sequencing (same primers) of these PCR products were outsourced to Genewiz (Plainfield, New Jersey).

Phylogenetic analyses

Methods for both datasets – ITS for *Hesperomyces*, LSU for *Laboulbenia* – were largely identical. Sequences were downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and supplemented with sequences that were generated during this study. Sequences were aligned using MUSCLE version 3.7 (Edgar 2004), which is available on the CIPRES Science Gateway V. 3.3 (Miller et al. 2010). After alignment of the ITS dataset, partial SSU and partial LSU were removed by looking for the motifs 5'-ATCATTA-3' (3' end of SSU) and 5'-TGACCT-3' (5' start of LSU), and deleting downstream and upstream sequence data, respectively (Baral et al. 2018). For the LSU dataset, we unsuccessfully searched for the 5'-TGACCT-3' motif. We then looked for the motif following 5'-TGACCT-3' in a *Hesperomyces* sequence (GenBank acc. no. MG757513), which is 5'-CGGAT-3', found this motif in the *Laboulbenia* dataset, and then realized that the 5' start of LSU in *Laboulbenia* includes one nucleotide substitution compared to the conventional motif: 5'-TGGCCT-3'. We deleted the downstream sequence data to remove partial ITS. Next, ambiguously aligned regions and uninformative positions were removed using the command line version of trimAl v1.2 (Capella-Gutiérrez et al. 2009) with gap threshold = 0.6 and minimal coverage = 0.5. Models of nucleotide substitution were selected by considering the Akaike Information Criterion corrected for small samples (AICc) with ModelFinder Plus (Kalyaanamoorthy et al. 2017). Maximum likelihood (ML) was inferred for each dataset under the selected model with IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016). Ultrafast bootstrap (BS) analysis with 1000 replicates estimated branch support in the ML trees (Hoang et al. 2018).

Bayesian analyses were done using a Markov chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.8.4 (Drummond et al. 2012), with a strict clock assuming a constant rate of evolution across the tree, a Yule Speciation tree prior (Yule 1925; Gernhard 2008), and the nucleotide substitution model as selected by jModelTest 2.1 (Darriba et al. 2012) under the AICc criterion. For each dataset, four runs were performed from a random starting tree for 10 million generations with a sampling frequency of 1000. All settings were entered in BEAUti 1.8.4 to generate an XML file, which was run in BEAST on the CIPRES Science Gateway (Miller et al. 2010). Resulting log files were entered in Tracer version 1.6 (Rambaut et al. 2014) to check MCMC trace plots for convergence and to assess effective sample sizes (ESS). A standard 10% burn-in was used resulting in overall ESS values of well above 200 for all sampled parameters. After removal of 10% burn-in, trees files were combined in LogCombiner 1.8.4. TreeAnnotator 1.8.4 was used to generate consensus trees with 0% burn-in and to infer the Maximum Clade Credibility tree with highest product of individual clade posterior probabilities (pp) for both datasets.

Trees with ML BS and Bayesian pp were visualized in FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator 2020 version 24.1.1 (San Jose, California).

Checklist

For the checklist of thallus-forming Laboulbeniomycetes from Belgium and the Netherlands, we used De Kesel et al. (2020) for Belgium and all available published papers (since 1938 up to 2020) for the Netherlands. Laboulbeniomycetes and their hosts are listed alphabetically, starting with Herpomycetales, followed by Laboulbeniales. Fungal species are numbered throughout (1–140), authority and reference to the protologue are presented. For each fungus, hosts are presented alphabetically, with classification (order, family) and country in which the association has been reported: "Be" for Belgium, "Nl" for the Netherlands. No detailed collection information is shown except for new country records. In several instances, taxonomic notes are provided. Hosts are according to Vorst (2010) and Beccaloni et al. (2014). Names of fungi correspond to Index Fungorum (2020).

Identification key

The key to species of Laboulbeniomycetes in Belgium and the Netherlands is based on diagnostic characters referring to morphology and/or host taxa. It requires microscope equipment and morphological study as described in Benjamin (1971), Huldén (1983), Majewski (1994), Santamaría (1998), and De Kesel et al. (2020). Terminology follows Tavares (1985), Santamaría (1998, 2003), and De Kesel et al. (2020).

Results

The ITS dataset consisted of 31 *Hesperomyces* sequences (Table 1) and 724 characters, of which 462 were constant and 198 were parsimony-informative. The selected nucleotide substitution model under AICc was TVM+F+G4 (-lnL = 2790.545, ModelFinder Plus) and TVM+G (-lnL = 2786.8769, jModelTest 2). The *Hesperomyces virescens* sensu lato (Haelewaters et al. 2018) clade has maximum support from both ML and Bayesian analyses (Figure 1). Each of the nine clades within *H. virescens* s.l. consists of isolates from thalli removed from a single host species, except for the *Adalia* clade, which includes isolates from both *A. bipunctata* and *A. decempunctata*. One of the clades consists of isolates from *Chilocorus stigma*, the host on which *H. virescens* was originally described (Thaxter 1891). This clade, representative of *Hesperomyces virescens* sensu stricto, receives maximum support. The single isolate of *Hesperomyces halyziae*, from *Halyzia sedecimguttata*, is placed as sister to *H. virescens* s.l. from *Harmonia axyridis* (Pallas, 1773) (pp = 0.8).

The LSU dataset consisted of 24 *Laboulbenia* sequences (Table 2) and 682 characters, of which 558 were constant and 63 were parsimony-informative. The selected

Table 1. *Hesperomyces* sequences used in phylogenetic analysis of the ITS dataset. Asterisks (*) indicate sequences that were generated during the course of this study.

Species	Host	Isolate	GenBank (ITS)	Reference
Hesperomyces coleomegillae	*Coleomegilla maculata*	632A	KF192888	Goldmann et al. (2013)
	Coleomegilla maculate	635D	KF192906	Goldmann et al. (2013)
Hesperomyces halyziae	*Halyzia sedecimguttata*	D. Haelew. 955b	MG757813	Haelewaters et al. (2018)
Hesperomyces virescens s.s.	*Chilocorus stigma*	D. Haelew. 1444a	MT373697*	This paper
	Chilocorus stigma	D. Haelew. 1444b	MT373698*	This paper
Hesperomyces virescens s.l.	*Adalia bipunctata*	D. Haelew. 1193g	MG757817	Haelewaters et al. (2018)
	Adalia bipunctata	D. Haelew. 1231a	MG757821	Haelewaters et al. (2018)
	Adalia bipunctata	D. Haelew. 1232a	MG757822	Haelewaters et al. (2018)
	Adalia decempunctata	D. Haelew. 1248b	MG757823	Haelewaters et al. (2018)
	Azya orbigera	D. Haelew. 928g	MG745343	Haelewaters et al. (2018)
	Cheilomenes propinqua	D. Haelew. 655c	MG757804	Haelewaters et al. (2018)
	Cheilomenes propinqua	D. Haelew. 659b	MG757805	Haelewaters et al. (2018)
	Cheilomenes propinqua	D. Haelew. 1259a	MG757828	Haelewaters et al. (2018)
	Cycloneda sanguinea	D. Haelew. 924a	MG757808	Haelewaters et al. (2018)
	Cycloneda sanguinea	D. Haelew. 1374a	MG757831	Haelewaters et al. (2018)
	Harmonia axyridis	352B	KF192916	Goldmann et al. (2013)
	Harmonia axyridis	D. Haelew. 361a	MG757801	Haelewaters et al. (2018)
	Harmonia axyridis	D. Haelew. 486c	KT800044	Haelewaters et al. (2015c)
	Harmonia axyridis	D. Haelew. 669a	MG757807	Haelewaters et al. (2018)
	Harmonia axyridis	D. Haelew. 1188g	MG438317	Haelewaters et al. (2019b)
	Harmonia axyridis	D. Haelew. 1268d	MG757830	Haelewaters et al. (2018)
	Harmonia axyridis	DH1	KF192920	Goldmann et al. (2013)
	Harmonia axyridis	LT1	KF192910	Goldmann et al. (2013)
	Harmonia axyridis	MT001	KT800048	Haelewaters et al. (2015c)
	Olla v-nigrum	D. Haelew. 954e	MG757812	Haelewaters et al. (2018)
	Olla v-nigrum	D. Haelew. 1200h	MG757819	Haelewaters et al. (2018)
	Olla v-nigrum	JP353b	MG757799	Haelewaters et al. (2018)
	Olla v-nigrum	JP354b	MG757800	Haelewaters et al. (2018)
	Psyllobora vigintimaculata	D. Haelew. 1250b	MG757825	Haelewaters et al. (2018)
	Psyllobora vigintimaculata	D. Haelew. 1250c	MG757826	Haelewaters et al. (2018)
	Psyllobora vigintimaculata	D. Haelew. 1251b	MG757827	Haelewaters et al. (2018)

nucleotide substitution model under AICc was TN+F+G4 (-lnL = 1876.681, ModelFinder Plus) and TrN+G (-lnL = 1872.4616, jModelTest 2). Our phylogenetic analyses show nine distinct species, which are all supported. The relationships among species are unresolved in different places, but this is not unsurprising because of extremely limited taxon sampling. *Laboulbenia quarantenae* holds an unresolved position in the tree but is clearly separated from both *L. flagellata* and the morphologically similar *L. vulgaris*, confirming its status as a separate species. *Laboulbenia vulgaris* isolates E10T2 and E11T6, which originated from *Bembidion tetracolum*, are placed among isolates of the same species removed from *Ocys harpaloides*. Interestingly, and in accordance with De Weggheleire (2019) and Haelewaters et al. (2019a), *L. flagellata* falls apart in three species. However, only ten isolates are included, originating from six host species, none of which were reported in the protologue (Peyritsch 1873). As a result, it is too early to make taxonomic decisions *within* this problematic taxon.

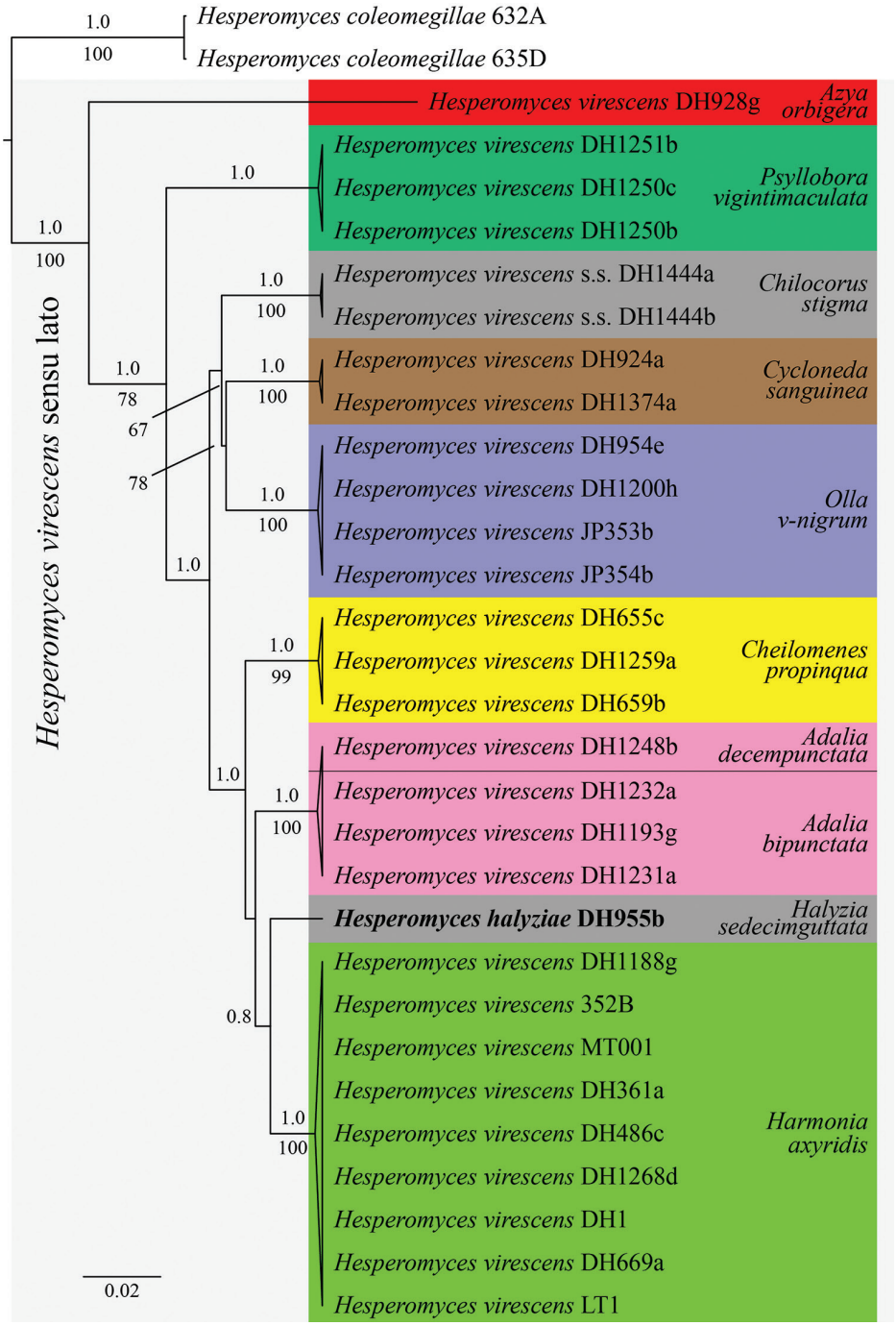

Figure 1. Maximum clade creditability tree of *Hesperomyces* isolates reconstructed from an ITS dataset, with *H. coleomegillae* as outgroup. The topology is the result of Bayesian inference performed with BEAST. For each node, ML BS (≥ 65) and Bayesian pp (≥ 0.7) are presented above/below the branch leading to that node. *Hesperomyces virescens* sensu lato is highlighted with light gray shading, isolates are color-coded by host; *H. virescens* sensu stricto and *H. halyziae* sp. nov. are highlighted with dark gray shading.

Table 2. *Laboulbenia* sequences used in phylogenetic analysis of the LSU dataset. Asterisks (*) indicate sequences that were generated during the course of this study.

Species	Host	Isolate	GenBank (LSU)	Reference
Laboulbenia bruchii	*Neolema adunata*	D. Haelew. 1346b	MN394843	Haelewaters et al. (2019a)
Laboulbenia collae	*Paranchus albipes*	D. Haelew. 1456a	MN394844	Haelewaters et al. (2019a)
	Paranchus albipes	D. Haelew. 1456b	MN394845	Haelewaters et al. (2019a)
	Paranchus albipes	D. Haelew. 1461b	MN397131	Haelewaters et al. (2019a)
Laboulbenia quarantenae	*Bembidion biguttatum*, ADK6448	E13T12	MT371368*	This paper
Laboulbenia flagellata	*Agonum emarginatum*, ADK6428	E13T1	MT703825*	This paper
	Agonum micans, ADK6332	D. Haelew. 1457a	MN394851	Haelewaters et al. (2019a)
	Agonum micans, ADK6332	D. Haelew. 1457b	MN394852	Haelewaters et al. (2019a)
	Agonum micans, ADK6332	D. Haelew. 1457c	MN394853	Haelewaters et al. (2019a)
	Agonum nigrum, ADK6445	E13T11	MT703826*	This paper
	Limodromus assimilis, ADK6329-1	D. Haelew. 1454a	MN394849	Haelewaters et al. (2019a)
	Limodromus assimilis, ADK6329-1	D. Haelew. 1454b	MN394850	Haelewaters et al. (2019a)
	Limodromus assimilis, ADK6329-2	D. Haelew. 1458a	MN394854	Haelewaters et al. (2019a)
	Loricera pilicornis	H85-1	KY350538	Sundberg et al. (2018a)
	Oxypselaphus obscurus, ADK6374	E11T11	MT703824*	This paper
Laboulbenia pedicellata	*Dyschirius globosus*	H84-1	KY350537	Sundberg et al. (2018a)
Laboulbenia systenae	*Disonycha procera*	D. Haelew. 1342b	MN394858	Haelewaters et al. (2019a)
Laboulbenia vulgaris	*Bembidion tetracolum*, ADK6420	E10T2	MT703822*	This paper
	Bembidion tetracolum, ADK5557	E11E6	MT703823*	This paper
	Ocys harpaloides, ADK6330-1	D. Haelew. 1455a	MN397135	Haelewaters et al. (2019a)
	Ocys harpaloides, ADK6330-1	D. Haelew. 1455b	MN397136	Haelewaters et al. (2019a)
	Ocys harpaloides, ADK6330-2	D. Haelew. 1459a	MN397137	Haelewaters et al. (2019a)
	Ocys harpaloides, ADK6330-3	D. Haelew. 1460a	MN397138	Haelewaters et al. (2019a)
	Ocys harpaloides, ADK6353-1	E0T6	MT703821*	This paper

Taxonomy

Hesperomyces halyziae **Haelew. & De Kesel, sp. nov.**
MycoBank No: 835489
Figure 3

Etymology. Referring to the host genus, *Halyzia*.

Diagnosis. Morphologically very similar to other taxa within *H. virescens* sensu lato, but forming a distinct species supported by ITS data. The ITS sequence shares 95.8–97.9% identity with *H. virescens* s.l. from *Harmonia axyridis*, and 96.5–95.4% with *H. virescens* s.l. from *Adalia bipunctata*/*A. decempunctata*. Unique molecular synapomorphies in the ITS at positions 478, 517, 652.

Types. ***Holotype*****:** The Netherlands, Noord Brabant Province, Tilburg, nature reserve De Kaaistoep, 51.5333333N, 5.0166667E, 11 Aug. 2015, *leg*. H. Spijkers & P. van Wielink, on female *Halyzia sedecimguttata* (Linnaeus, 1758) (Coleoptera, Coccinellidae) (NNKN), slide D. Haelew. 955a (FH, 4 juvenile and 3 mature thalli, left elytron), reported as *Hesperomyces virescens* in Haelewaters and van Wielink (2016). ***Paratypes*****:** Belgium, Province Vlaams-Brabant, Meise, Domein van Bouchout, 50.927925N, 4.333069E, 28 Mar. 2019, *leg*. C. Gerstmans, on *H. sedecimguttata* (BR, CG437–CG440), slides BR5020212155379V, BR5020212156406V, BR5020212157434V, and BR5020212158462V; reported as *Hesperomyces virescens* sensu lato in De Kesel et al. (2020). *Ibid.*, 1 Apr. 2019, *leg*. C. Gerstmans, on *H. sedecimguttata* (BR, CG441–

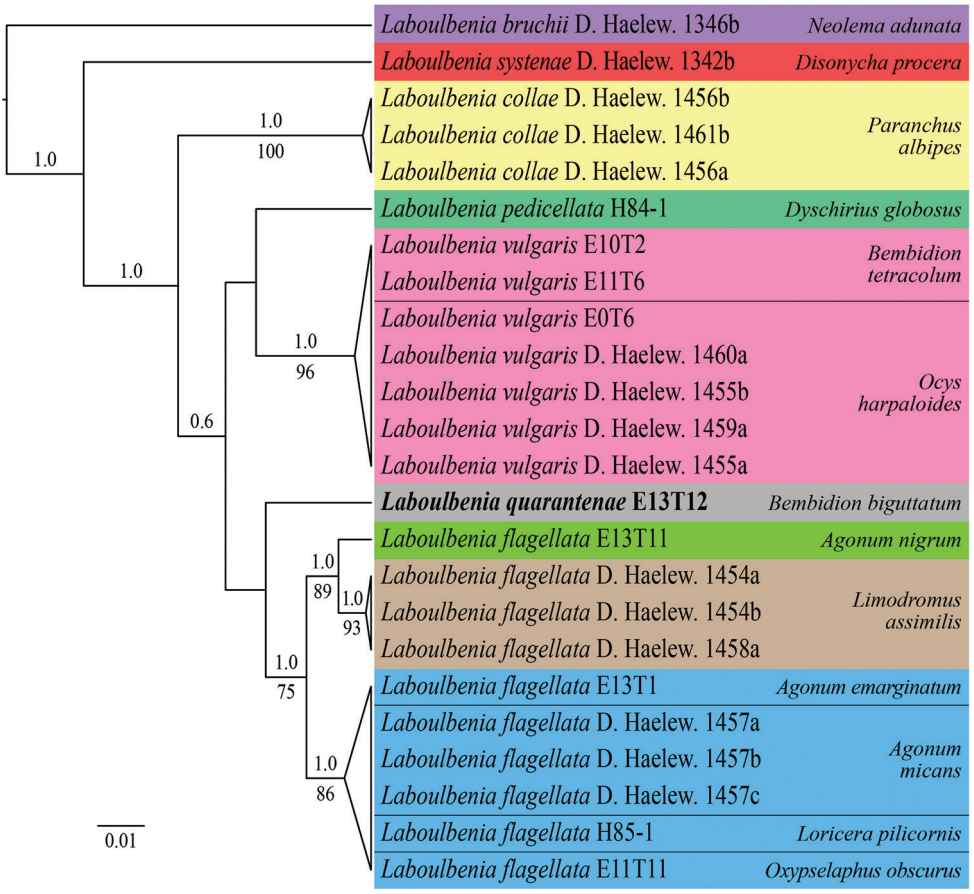

Figure 2. Maximum clade creditability tree of *Laboulbenia* isolates reconstructed from an LSU dataset, with *L. bruchii* as outgroup. The topology is the result of Bayesian inference performed with BEAST. For each node, ML BS (≥ 65) and Bayesian pp (≥ 0.7) are presented above/below the branch leading to that node. Isolates are color-coded by host; *L. quarantenae* sp. nov. is highlighted with gray shading.

442), slides BR5020212159490V and BR5020212160236V; reported as *Hesperomyces virescens* sensu lato in De Kesel et al. (2020).

Description. ***Thallus*** 335–453 µm long from foot to perithecial apex; colored yellow except for a somewhat darker region right above the foot. ***Cell I*** obtriangular, 2.0–2.5× longer than broad, broadening distally, with very oblique septum I–II. ***Cell II*** longer than broad, 23–28 × 16–21 µm, subtrapezoidal in section. ***Cell III*** always smaller than cell II, 14–20 × 14–19 µm, with inflated dorsal cell wall. ***Primary appendage*** consisting of 4 superposed cells, 61–67 µm long; in the same axis as cells I and III, separated from the latter by the constricted primary septum; its basal cell somewhat longer than broad, longer than each of the remaining cells of the appendage; second to fourth cells carrying a single antherium externally, the fourth cell also carrying a second upwardly directed antherium. ***Antheridia*** flask-shaped,

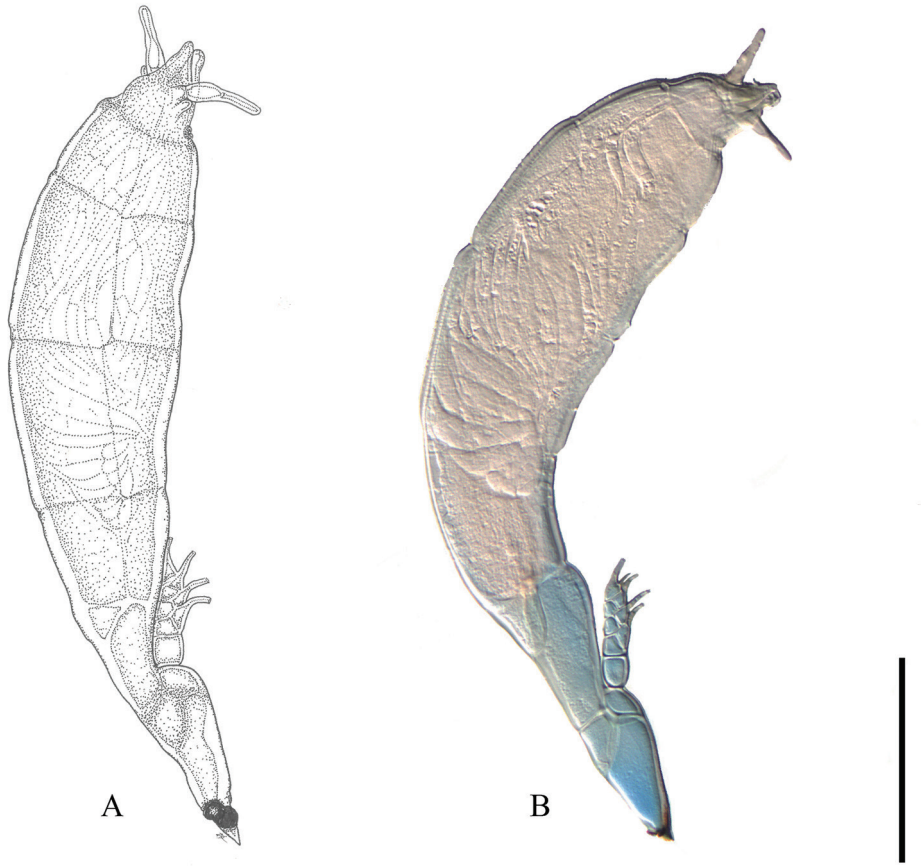

Figure 3. *Hesperomyces halyziae* Haelew. & De Kesel from *Halyzia sedecimguttata* **A** mature thallus from slide D. Haelew. 955a, holotype **B** mature thallus from slide BR5020212156406V. Scale bar: 100 µm.

with slightly (dorsally and/or basally) curved efferent necks, the upper antheridium carrying at its dorsal side a pointed process, which represents the original ascospore apex. ***Cell VI*** with subparallel margins to broadening distally, 33–70 × 23–33 µm. ***Perithecium*** 194–291 × 62–86 µm (not including basal cells), symmetric or with the anterior margin convex and the posterior one almost straight or concave; broadest near the upper third, then gradually tapering towards the apex; apex complex with 2 short lower lobes, 2 upper (terminal) lobes, and 2 prominent lips surrounding the ostiole; lower lobes tapering to a rounded tip, the ventral lobe outwardly directed; terminal lobes unicellular, elongated, 29–42 µm in length, curved upwards and outwardly; ostiole with two lips, 25–29 µm in length, one lip triangular, the other slightly shorter, blunt or rounded, basally carrying the remainder of the trichogyne. ***Ascospores*** 70–85 µm long, with conspicuous slime sheath only surrounding the larger cell.

Material sequenced. The Netherlands, Noord Brabant Province, Tilburg, nature reserve De Kaaistoep, 51.5333333N, 5.0166667E, 11 Aug. 2015, *leg*. H. Spijkers & P. van Wielink, on female *Halyzia sedecimguttata* (Coleoptera, Coccinellidae) (NNKN), isolate D. Haelew. 955b (7 thalli, elytra, ITS: MG757813).

Hosts and distribution. On *Halyzia sedecimguttata* from Belgium and the Netherlands. Previously reported as *H. virescens* (Haelewaters and van Wielink 2016, Haelewaters et al. 2017) and *H. virescens* sensu lato (De Kesel et al. 2020). One unverified record is available from France (Justamond 2019).

Notes. Supported by multi-locus phylogenetic analyses and sequence-based species delimitation methods, Haelewaters et al. (2018) showed that *H. virescens* Thaxt. is a complex of multiple species, segregated by host. The authors proposed to "restrict *H. virescens* sensu stricto to those thalli found on *Chilocorus stigma*, the host species on which the fungus was originally described" (Thaxter 1891). Here, we included two isolates from *C. stigma* (Say, 1835), and found the clade representative of *H. virescens* sensu stricto. Based on this analysis and previous work (Haelewaters et al. 2018), we can start describing the individual clades as distinct species. A monographic work with formal descriptions for the seven other species within *H. virescens* s.l. is in preparation, but in the light of this checklist we decided to describe *H. halyziae*, which was only known from a single collection in the Netherlands until we recently collected it in Belgium (Mar.–Apr. 2019).

Haelewaters and van Wielink (2016) reported an infected specimen of *Halyzia sedecimguttata* from nature reserve De Kaaistoep in the Netherlands. In 1997–2015, 476 individuals of *H. sedecimguttata* were collected on a lighted white sheet and screened for presence of Laboulbeniales, only resulting in one individual (parasite prevalence 0.2%). In Belgium, a population of infected *H. sedecimguttata* was found at the Meise Botanic Garden. Specimens were collected in spring 2019 while they were leaving their overwintering place–deep cracks in the woodwork of a small forest chapel. Screening of 46 specimens of *H. sedecimguttata* revealed nine infected ones (parasite prevalence 19.5%). This ladybird species seems to overwinter singly or in small congregations in narrow overwintering places, including in leaf litter, under foliage on stone walls, on trunks and branches (Majerus and Williams 1989). This congregation behavior is beneficial for transmission of the fungus and is also observed in *Harmonia axyridis* (Haelewaters et al. 2017).

Morphologically, *H. halyziae* is very similar to what we have thus far accepted as *H. virescens*. Within the Kingdom Fungi, there is an incredible diversity that cannot be perceived through morphology. Cryptic species are being uncovered in Agaricomycetes (e.g., Stefani et al. 2014; Sánchez-García et al. 2016), Lecanoromycetes (e.g., Singh et al. 2015), Leotiomyces (e.g., Grünig et al. 2008), Pucciniomycetes (Bennett et al. 2011), Ustilaginomycetes (e.g., Li et al. 2017), and other major clades. And while the Laboulbeniales has been the subject of a large-scale study to estimate the global species richness of the group (Weir and Hammond 1997), cryptic diversity was not part of the equation. In other words, the number of estimated species of Laboulbeniales, between 15,000 and 75,000, is likely to be corrected to include cryptic species. We note that the recognition of *H. halyziae* is only possible through molecular data and host associa-

tion. Our current understanding is that, within this species complex, there is a strict parasite-host association, with one parasite found only on one host. We think that this host specificity exists at the genus level, given the *Adalia* clade (Figure 1), which includes isolates from thalli removed from two host species within the same genus.

Laboulbenia quarantenae **De Kesel & Haelew., sp. nov.**
MycoBank No: 835490
Figure 4

Diagnosis. Morphologically similar to *Laboulbenia vulgaris* Peyr., but the insertion cell is attached to the lower fifth of the posterior margin of the perithecial wall and the outer appendage is composed of 4–6(–8) branches resulting from successive dichotomies starting at the suprabasal cell, which is poorly pigmented or nearly hyaline. The LSU sequence shares 89.7–98.0% identity with other sequenced taxa of *Laboulbenia*, 97.4% with *L. flagellata* from *Agonum nigrum*, 97.5–98.0% with *L. flagellata* from *Limodromus assimilis*, 97.0–98.0% with *L. flagellata* from *Agonum emarginatum*/*A. micans*/*Loricera pilicornis*/*Oxypselaphus obscurus*, and 97.0–97.7% with *L. vulgaris* from *Bembidion tetracolum*/*Ocys harpaloides*. Unique molecular synapomorphies in the LSU at positions 503, 545.

Types. ***Holotype*****:** Belgium, Province Vlaams Brabant, Meise, Domein van Bouchout, 50.9267056N, 4.3220028E, 30 m a.s.l., 26 Apr. 2019, *leg*. A. De Kesel, rivulet-associated grassland, on *Bembidion* (*Philochtus*) *biguttatum* (Fabricius, 1779) (Coleoptera, Carabidae), ADK6448 (BR), slide BR5020212163329V (1 mature thallus, prothorax). Isotypes: *ibid.*, slides BR5020212162292V (2 mature thalli, right mesofemur), BR5020212161264V (6 mature thalli, right protibia), BR5020212166412V (5 immature thalli, mesothorax), BR5020212165385V (1 mature thallus, right protibia), and BR5020212164357V (1 mature thallus, right mesofemur). ***Paratype*****:** Belgium, Province Vlaams-Brabant, Meise, Domein van Bouchout, 50.92745N, 4.323917E, 32 m a.s.l., 30 Apr. 2020, *leg*. A. De Kesel, rivulet-associated grassland, on *B.* (*P.*) *biguttatum*, ADK6523 (BR), slide BR5020195033527V (2 mature thalli, mesosternum).

Etymology. From *quarantena*, which was used in 14th–15th century Venetian language for a forty-day isolation period. The new species was described during the 2020 quarantine period imposed to curb the spread of the COVID-19 virus.

Description. ***Thallus*** 300–465 µm long from foot to perithecial tip; colored hyaline at the lower receptacular cells (I and II) and the inner appendage, otherwise pigmented light to dark brown; especially the upper receptacular cells (III, IV and V), cell VI, and the perithecium darkening with age. ***Cell I*** elongated, usually straight, 56–107 × 22–33 µm; sometimes bent and then wider at the upper end. ***Cell II*** slender, mostly with parallel margins, longer than cell I, 73–160 × 29–40 µm, anterior margin shorter than posterior. ***Cells III and VI*** side by side, with septum II–III always much shorter than septum II–VI. Cell III with a narrow base, 29–43 µm long, widening upwards and then 22–29 µm wide at the apex. Cell VI more or less rectangu-

Figure 4. A–I *Laboulbenia quarantena* De Kesel & Haelew. from *Bembidion biguttatum*, specimen ADK6448: **A** mature thallus from prothorax, slide BR5020212163329V, holotype **B** mature thallus from prothorax with less pigmented perithecium **C** mature thallus from the right mesofemur **D–F** mature thalli from the right protibia **G** immature thallus from the prothorax **H** mature thallus from the right mesofemur **I** ascospores **J–K** laboulbenia vulgaris Peyr: **J** mature thallus from prothorax of *Bembidion tetracolum*, specimen ADK5557 **K** mature thallus from mesothorax of *Ocys harpaloides*, specimen ADK6353. One of the diagnostic characteristics of the new species–the positioning of the insertion cell–is shown in a mature thallus of *L. quarantenae* (**E**) and one of *L. vulgaris* (**J**). Scale bar: 100 µm.

lar, 30–34 × 23–30 µm. ***Cell IV*** more or less rectangular, slightly broader than long, 20–32 × 25–30 µm. ***Cell V*** small, triangular, situated in the inner-upper corner of cell IV, 9–14 × 7–14 µm, as pigmented as surrounding cells. ***Insertion cell*** brownish black, flattened, barely marking a constriction on the posterior margin of the thallus, attached to the lower fifth of the posterior margin of the perithecial wall, 18–25 µm wide and 90–128 µm from the perithecial tip. ***Inner appendage*** hyaline, composed of 2–4(–6) short branches, rarely exceeding the perithecial tip, 88–150 µm long, resulting from successive dichotomies starting at the basal cell, the latter 9–14 × 6–12 µm. ***Antheridia*** short, flask-shaped, few in number, usually on the young inner appendage and arising laterally from its suprabasal cell. ***Outer appendage*** up to 250–335 µm long, extending beyond the perithecial tip, often entirely light brown, composed of 4–6(–8) branches, resulting from successive dichotomies starting at the suprabasal cell; the basal cell longer than broad, 23–32 × 15–21 µm, almost entirely hyaline. ***Perithecium*** ellipsoid, venter only very slightly asymmetrical, anterior and posterior margins almost equally convex, 109–157 × 43–64 µm, length/width ratio 1.9–2.5, widest in the middle; perithecial tip asymmetrical, with prominent and rounded posterior margin; preostiolar spots black, in older thalli merging into a pre-apical ring, always with distinctly paler zone under the posterior spot. ***Ascospores*** two-celled, hyaline, 59–65 × 4.2–5.5 µm, with slime sheath.

Material sequenced. Belgium, Province Vlaams Brabant, Meise, Domein van Bouchout, 50.9267056N, 4.3220028E, 30 m a.s.l., 26 Apr. 2019, *leg*. A. De Kesel, rivulet associated grassland, on *Bembidion biguttatum* (Coleoptera, Carabidae), ADK6448 (BR), isolate E13T12 (3 mature thalli, prothorax, LSU: MT371368).

Hosts and distribution. Thus far only known on *Bembidion biguttatum* from the type locality in Belgium. Reported as *Laboulbenia* sp. nov. in De Weggheleire (2019).

Notes. Morphologically, *L. quarantenae* mostly resembles *L. vulgaris* Peyr., but it differs from it by the very low position of the insertion cell (regardless of the origin of the thallus), the successive dichotomous branching of the outer appendage, the poorly pigmented to nearly hyaline basal cell of the outer appendage, and the slender habitus. Although these characters may vary to some extent, eventually resulting in specimens that are morphologically close to *L. vulgaris*, our LSU phylogeny (Figure 2) shows that sequences of typical *L. vulgaris* obtained from Carabidae known to host *L. vulgaris*–*Bembidion tetracolum* Say, 1823 and *Ocys harpaloides* (Audinet-Serville, 1821) (Santamaría et al. 1991; Majewski 1994; Haelewaters et al. 2019a; De Kesel et al. 2020)–fall in a monophyletic clade separated from *L. quarantenae*. The two isolates of *L. vulgaris* from *B. tetracolum* were collected in Belgium (isolate E10T2) and Latvia (isolate E11T6), from populations that are 1,550 km apart, but they were placed together among isolates from *O. harpaloides* (all from Belgium). *Laboulbenia quarantenae*, on the other hand, was collected between <1 and 21 km distance from where hosts of *L. vulgaris* were collected.

Phylogenetically, *L. quarantenae* may be more closely related to *L. flagellata* than to *L. vulgaris*. *Laboulb quarantenae* and *L. flagellata* (sensu lato) were retrieved as sister taxa in our phylogeny, although no statistical support was retrieved for this sister relationship. Whereas species boundaries are evident based on our phylogeny, it goes with-

out saying that both taxon sampling and sequence data need to be greatly expanded upon to resolve relationships among species of *Laboulbenia*. The new species is apparently very rare and was never found in combination with *L. vulgaris*, the more common parasite from *Bembidion biguttatum* in Belgium (De Kesel 1998; De Kesel et al. 2020).

In Europe, many species of *Laboulbenia* have been reported on *Bembidion* Latreille, 1802 (Santamaría et al. 1991). Of those, *L. pedicellata* Thaxt. and *L. vulgaris* Peyr. are among the most reported ones. *Bembidion biguttatum* belongs to subfamily Trechinae. To our knowledge, this species is infected by either *L. murmanica* Huldén (S. Santamaría pers. comm.), *L. pedicellata* (Scheloske 1969; Majewski 1994), or *L. vulgaris* (Majewski 1994; De Kesel et al. 2020). Based on the position of its insertion cell as well as the morphology of both the outer appendage and the androstichum (cells II, IV, and V), *L. quarantenae* is fundamentally different from these three species. The outer appendage of *L. quarantenae* is reminiscent of the one from *L. flagellata*, which, however, is a more robust species reported from 80 genera of Carabidae belonging to Anthiinae, Brachininae, Elaphrinae, Harpalinae, Loricerinae, Nebriinae, and Patrobinae (but not Trechinae) (Santamaría et al. 1991; Santamaría 1998; Haelewaters et al. 2019a).

Bembidion biguttatum, the host for *L. quarantenae*, belongs to the subgenus *Philochtus.* Representatives of *Laboulbenia* reported from *Bembidion* subgenus *Philochtus* are few and include two species only: *L. pedicellata* and *L. vulgaris*. Two thalli of *Laboulbenia* "sp. similar to *L. vulgaris*" from *Bembidion bruxellense* Wesmael, 1835 [as *B. rupestre* (Linnaeus, 1767) are illustrated in Majewski (1994: Pl. 53, Figs 1, 2). Their morphology comes close to *L. quarantenae* but cell V is much larger and the insertion cell is not situated low enough along the posterior margin of the perithecial wall. Also *L. parvula* is reported on subgenus *Philochtus* in Santamaría et al. (1991), but this species is much smaller (180–190 μm total length) compared to *L. quarantenae*, it has a deeply pigmented basal cell of the outer appendage, the inner and outer appendage each carry 4–8 very slender branches, and its perithecial tip is rather squarish.

As we explore patterns of speciation of taxa in both Herpomycetales and Laboulbeniales using integrative taxonomy, we can start linking some of these patterns to morphological or life history traits. One candidate trait is the haustorium–a rhizoidal structure that penetrates the host's integument to make contact with the haemocoel, increasing surface area for nutrient uptake and providing holdfast. We hypothesize that – due to the invasive nature of their haustorium – Herpomycetales and haustorial Laboulbeniales, such as species of *Hesperomyces*, maintain close interactions with their hosts, possibly involving adaptations to the hosts' defense systems and leading to escape-and-radiate coevolution (Ehrlich and Raven 1964). These developments result in an evolutionary arms race, with specialization and leading to speciation (One Host One Parasite model, Figure 1). While all 27 species of *Herpomyces* form multiple haustoria, not all Laboulbeniales penetrate their host. Recently, Tragust et al. (2016) presented evidence for four species of Laboulbeniales to be superficially attached to their host, and also *L. flagellata* and *L. vulgaris* do not seem to perforate their hosts. There are no strict developmental barriers for non-penetrating species and their ascospores

may develop on multiple arthropods given that they co-occur in a given microhabitat, resulting in parasite species with more than one host (e.g., *L. vulgaris* in Figure 2), in contrast to the host-specific species of *Hesperomyces*. Undoubtedly, other factors come into play; more studies of speciation and species limits, specificity, host shifting, and transmission patterns are needed to test said hypothesis.

Alphabetical checklist of thallus-forming Laboulbeniomycetes in Belgium and the Netherlands

Herpomycetales

1. *Herpomyces ectobiae* Thaxt., Proc. Am. Acad. Arts Sci. 38(2): 20 (1902) [1903]

- *Blattella germanica* (Linnaeus, 1767) (Blattodea, Ectobiidae) ..... Be

2. *Herpomyces periplanetae* Thaxt., Proc. Am. Acad. Arts Sci. 38(2): 13 (1902) [1903]

- *Blatta orientalis* Linnaeus, 1758 (Blattodea, Blattidae) ..... Be
- *Periplaneta americana* (Linnaeus, 1758) (Blattodea, Blattidae) ..... Be

3. *Herpomyces stylopygae* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 29: 551 (1917)

- *Blatta orientalis* Linnaeus, 1758 (Blattodea, Blattidae) ..... Be

Laboulbeniales

4. *Aphanandromyces audisioi* W. Rossi, Mycologia 74: 522 (1982)

- *Brachypterus urticae* (Fabricius, 1792) (Coleoptera, Kateretidae)..... Be

5. *Asaphomyces tubanticus* (Middelh. & Boelens) Scheloske, Parasitol. Schriftenr. 19: 92 (1969)

- *Catops fuliginosus* Erichson, 1837 (Coleoptera, Leiodidae) ..... Nl
- *Catops fuscus* (Panzer, 1794) ..... Be, Nl
- *Catops longulus* Kellner, 1846..... Be
- *Catops nigricans* (Spence, 1813) ..... Be, Nl[a]
- *Catops* sp. ..... Be
- *Choleva* sp. (Coleoptera, Leiodidae) ..... Nl

[a] Fungus as *Barbariella tubantica* Middelh. & Boelens ex Middelh. in Middelhoek (1949).

6. *Bordea denotata* Haelew. & De Kesel, Nova Hedwig. 98: 114 (2014)

- *Bibloporus bicolor* (Denny, 1825) (Coleoptera, Staphylinidae) ..... Nl

7. *Botryandromyces heteroceri* (Thaxt.) I.I. Tav. & T. Majewski, Mycotaxon 3: 195 (1976)

- *Heterocerus fenestratus* (Thunberg, 1784) (Coleoptera, Heteroceridae) ..... Be
- *Heterocerus flexuosus* Stephens, 1828..... Be
- *Heterocerus hispidulus* Kiesenwetter, 1843..... Be
- *Heterocerus obsoletus* Curtis, 1828..... Nl

8. *Cantharomyces denigratus* Thaxt., Mem. Am. Acad. Arts Sci. 16: 27 (1931)

- *Dryops luridus* (Erichson, 1847) (Coleoptera, Dryopidae) ..... Be

9. *Cantharomyces elongatus* Haelew. & De Kesel, Mycotaxon 123: 468 (2013)

- *Syntomium aeneum* (Müller, 1821) (Coleoptera, Staphylinidae) ..... Nl

10. *Cantharomyces italicus* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 27: 42 (1915)

- *Dryops luridus* (Erichson, 1847) (Coleoptera, Dryopidae) ..... Be

11. *Cantharomyces orientalis* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 27: 43 (1915)

- *Carpelimus corticinus* (Gravenhorst, 1806) (Coleoptera, Staphylinidae)..... Be, Nl[a]
- *Carpelimus foveolatus* (Sahlberg, 1832) ..... Be
- *Carpelimus* sp. ..... Be
- *Diglotta mersa* (Haliday, 1837) (Coleoptera, Staphylinidae) ..... Be

[a] Host as *Troglophloeus corticinus* (Gravenhorst, 1806), fungus as *Cantharomyces thaxteri* Maire in Middelhoek (1949).

12. *Cantharomyces platystethi* Thaxt., Proc. Am. Acad. Arts Sci. 35: 415 (1900)

- *Platystethus* sp. (Coleoptera, Staphylinidae) ..... Be

13. *Cantharomyces robustus* T. Majewski, Acta Mycol. 23: 99 (1990) [1987]

- *Carpelimus bilineatus* Stephens, 1834 (Coleoptera, Staphylinidae) ..... Be
- *Carpelimus corticinus* (Gravenhorst, 1806) ..... Be
- *Carpelimus rivularis* (Motschulsky, 1860)..... Be, Nl

- *Carpelimus* sp. ..... Be
- *Gnypeta rubrior* Tottenham, 1939 (Coleoptera, Staphylinidae) ..... Be

14. *Chaetarthriomyces crassiappendicatus* Scheloske

- *Chaetarthria seminulum* (Herbst, 1797) (Coleoptera, Hydrophilidae) ..... Nl

15. *Chitonomyces aculeifer* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 27: 44 (1915)

- *Graptodytes pictus* (Fabricius, 1787) (Coleoptera, Dytiscidae) ..... Be
- *Haliplus* sp. (Coleoptera, Haliplidae) ..... Be

16. *Chitonomyces bidessarius* (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. 12: 292 (1902)

- *Hygrotus impressopunctatus* (Schaller, 1783) (Coleoptera, Dytiscidae) ..... Nl

17. *Chitonomyces italicus* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 27: 46 (1915)

- *Laccophilus hyalinus* (De Geer, 1774) (Coleoptera, Dytiscidae) ..... Be

18. *Chitonomyces melanurus* Peyr., Sitzber. Akad. Wiss. Wien Math.-Naturw. Kl. 68: 250 (1873)

- *Laccophilus hyalinus* (De Geer, 1774) (Coleoptera, Dytiscidae) ..... Be
- *Laccophilus minutus* (Linnaeus, 1758) ..... Nl[a]

[a] New record: Utrecht Province, Soest, Soesterveen, 17 Oct. 1924, *leg*. F.C. Drescher, on *Laccophilus minutus* [as *Laccophilus obscurus* (Panzer, 1795)] (Naturalis Biodiversity Center), slide D. Haelew. 075a (BR-MYCO, 5 thalli, margin of left elytron).

19. *Chitonomyces paradoxus* (Peyr.) Thaxt., Mem. Am. Acad. Arts Sci. 12: 287 (1902)

- *Laccophilus hyalinus* (De Geer, 1774) (Coleoptera, Dytiscidae) ..... Be
- *Laccophilus minutus* (Linnaeus, 1758) ..... Nl

20. *Compsomyces lestevae* Thaxt., Proc. Am. Acad. Arts Sci. 35: 439 (1900)

- *Lesteva longoelytrata* (Goeze, 1777) (Coleoptera, Staphylinidae) ..... Be
- *Lesteva pubescens* Mannerheim, 1830 ..... Be
- *Lesteva sicula* subsp. *heeri* Fauvel, 1871..... Be, Nl
- *Lesteva* sp. ..... Be

21. *Coreomyces arcuatus* Thaxt., Mem. Am. Acad. Arts Sci. 16: 324 (1931)

- *Sigara striata* (Linnaeus, 1758) (Hemiptera, Corixidae)..... Be

22. *Corethromyces henrotii* Balazuc [as 'henroti'], Bull. Mens. Soc. Linn. Lyon 42: 283 (1973)

- *Choleva cisteloides* (Frölich, 1799) (Coleoptera, Leiodidae)..... Be
- *Choleva fagniezi* Jeannel, 1922 ..... Nl
- *Choleva jeanneli* Britten, 1922..... Nl
- *Choleva oblonga* Latreille, 1708 ..... Nl

23. *Corethromyces stilici* Thaxt., Proc. Am. Acad. Arts Sci. 37: 42 (1901)

- *Rugilus* (*Rugilus*) *rufipes* Germar, 1836 (Coleoptera, Staphylinidae)..... Be, Nl[a]
- *Rugilus* (*Rugilus*) *similis* (Erichson, 1839) ..... Be
- *Rugilus* sp. ..... Be

[a] Host as *Stilicus rufipes* (Germar, 1836) in Middelhoek (1943a, 1945).

24. *Cryptandromyces bibloplecti* T. Majewski, Acta Mycol. 25: 43 (1990)

- Pselaphinae gen et sp. indet. (Coleoptera, Staphylinidae)..... Be

25. *Cryptandromyces elegans* (Maire) W. Rossi & D. Castaldo, Pl. Biosystems 138: 264 (2004)

- *Brachygluta fossulata* (Reichenbach, 1816) (Coleoptera, Staphylinidae) ..... Nl
- *Brachygluta xanthoptera* Reichenbach, 1816 ..... Be

26. *Cryptandromyces euplecti* Santam., Nova Hedwig. 72: 384 (2001)

- *Euplectus sanguineus* Denny, 1825 (Coleoptera, Staphylinidae) ..... Be

27. *Dimorphomyces myrmedoniae* Thaxt., Proc. Am. Acad. Arts Sci. 36: 409 (1900) [1901]

- *Gnypeta rubrior* Tottenham, 1939 (Coleoptera, Staphylinidae)..... Be

28. *Diphymyces kaaistoepi* Haelew. & De Kesel, Sterbeeckia 35: 63 (2019)

- *Choleva cisteloides* (Frölich, 1799) (Coleoptera, Leiodidae)..... Be
- *Choleva fagniezi* Jeannel, 1922 ..... Nl

29. *Distolomyces forficulae* (T. Majewski) I.I. Tav., Mycol. Mem. 9: 207 (1985)

- *Forficula auricularia* Linnaeus, 1758 (Dermaptera, Forficulidae) ..... Be, Nl

30. *Ecteinomyces trichopterophilus* Thaxt., Proc. Am. Acad. Arts Sci. 38: 26 (1902) [1903]

- *Acrotrichis fascicularis* (Herbst, 1793) (Coleoptera, Ptiliidae) ..... Be
- *Acrotrichis grandicollis* (Mannerheim, 1844) ..... Nl
- *Acrotrichis intermedia* (Gillmeister, 1845) ..... Be
- *Acrotrichis* sp. ..... Be

31. *Eucantharomyces stammeri* Scheloske, Parasitol. Schriftenr. 19: 108 (1969)

- *Calathus melanocephalus* (Linnaeus, 1758) (Coleoptera, Carabidae) ..... Be

32. *Euphoriomyces agathidii* (Maire) I.I. Tav., Mycol. Mem. 9: 218 (1985)

- *Agathidium laevigatum* Erichson, 1845 (Coleoptera, Leiodidae) ..... Nl[a]

[a] New record: Noord Brabant Province, Tilburg, nature reserve De Kaaistoep, 51.540672 N 5.013867 E, 3–17 Jun. 2000, *leg*. Working Group Insects of the Royal Dutch Natural History Association (KNNV), pitfall trap, ±2.5 m S of *Quercus robur* #2, on *Agathidium laevigatum* (NNKN), slides D. Haelew. 1064a (FH, 1 submature and 2 mature thalli, tip of left elytron) and D. Haelew. 1064b (NMBT, 1 juvenile and 2 mature thalli, tip of right elytron).

33. *Euzodiomyces lathrobii* Thaxt., Proc. Am. Acad. Arts Sci. 35: 449 (1900)

- *Lathrobium brunnipes* (Fabricius, 1793) (Coleoptera, Staphylinidae) ..... Be
- *Lathrobium elongatum* (Linnaeus, 1767) ..... Be, Nl
- *Lathrobium geminum* Kraatz, 1857 ..... Be, Nl
- *Lathrobium laevipenne* Heer, 1839 ..... Nl
- *Lathrobium* sp. ..... Be
- *Lobrathium multipunctum* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) .... Be
- *Patrobus atrorufus* (Stroem, 1768) (Coleoptera, Carabidae) ..... Be
- *Pterostichus strenuus* (Panzer, 1796) (Coleoptera, Carabidae) ..... Be

34. *Fanniomyces burdigalensis* Balazuc, Revue Mycol. 43: 402 (1979)

- *Copromyza stercoraria* (Meigen, 1830) (Diptera, Sphaeroceridae) .....Be[a]
- *Crumomyia pedestris* (Meigen, 1830) (Diptera, Sphaeroceridae) .....Be[a]

[a] Fungus as *Stigmatomyces burdigalensis* (Balazuc) A. Weir & W. Rossi in De Kesel et al. (2020).

35. *Fanniomyces ceratophorus* (Whisler) T. Majewski, Acta Mycol. 8: 230 (1972)

- *Fannia canicularis* (Linnaeus, 1761) (Diptera, Fanniidae) ..... Nl[a]

[a] Fungus described as *Stigmatomyces ceratophorus* Whisler, and later recombined in *Fanniomyces* T. Majewski by Majewski (1972), based on the branching pattern of the primary appendage. Weir and Rossi (1995), in turn, found no valid rationale to maintain *Fanniomyces* as a separate genus and considered it a junior synonym of *Stigmatomyces*, stating that "the structure of the antheridial appendage is particularly variable". However, based on an SSU–LSU ribosomal DNA dataset, Haelewaters et al. (in press) found that 1) *Stigmatomyces* as currently circumscribed is paraphyletic and 2) *Fanniomyces* is supported as a stand-alone genus with two species, *F. burdigalensis* and *F. ceratophorus*.

36. *Haplomyces texanus* Thaxt., Proc. Am. Acad. Arts Sci. 28: 160 (1893)

- *Bledius gallicus* (Gravenhorst, 1806) (Coleoptera, Staphylinidae) ..... Nl[a]

[a] Host as *Bledius fracticornis* (Paykull, 1790) in Middelhoek (1943a).

37. *Helodiomyces elegans* F. Picard, Bull. Soc. Mycol. Fr. 29: 557 (1913)

- *Dryops anglicanus* Edwards, 1909 (Coleoptera, Dryopidae) ..... Nl
- *Dryops auriculatus* (Geoffroy, 1785) ..... Nl
- *Dryops luridus* (Erichson, 1847) ..... Be, Nl

38. *Hesperomyces coccinelloides* Thaxt., Mem. Am. Acad. Arts Sci. 16: 110 (1931)

- *Stethorus punctillum* (Weise, 1891) (Coleoptera, Coccinellidae) ..... Be

39. *Hesperomyces halyziae* Haelew. & De Kesel, sp. nov.

- *Halyzia sedecimguttata* (Linnaeus, 1758) (Coleoptera, Coccinellidae)..... Be[a], Nl[b]

[a] Fungus as *Hesperomyces virescens* Thaxt. sensu lato in De Kesel et al. (2020).
[b] Fungus as *Hesperomyces virescens* Thaxt. in Haelewaters and van Wielink (2016) and Haelewaters et al. (2017).

40. *Hesperomyces virescens* Thaxt., Proc. Am. Acad. Arts Sci. 25: 264 (1891), sensu lato

- *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae) ..... Be, Nl

- *Tytthaspis sedecimpunctata* (Linnaeus, 1761) (Coleoptera, Coccinellidae) ..... Be

41. *Hydraeomyces halipli* (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. 12: 294 (1902)

- *Haliplus flavicollis* Sturm, 1834 (Coleoptera, Haliplidae) ..... Nl
- *Haliplus immaculatus* Gerhardt, 1877 ..... Be
- *Haliplus lineatocollis* (Marsham, 1802) ..... Be
- *Haliplus lineolatus* Mannerheim, 1844 ..... Be
- *Haliplus ruficollis* (De Geer, 1774) ..... Be, Nl
- *Haliplus* sp. ..... Be

42. *Hydrophilomyces* cf. *gracilis* T. Majewski, Acta Mycol. 10: 272 (1974)

- *Cercyon marinus* Thomson, 1853 (Coleoptera, Hydrophilidae) ..... Be
- *Cercyon* sp. ..... Be

43. *Hydrophilomyces* cf. *hamatus* T. Majewski, Acta Mycol. 10: 274 (1974)

- *Cercyon marinus* Thomson, 1853 (Coleoptera, Hydrophilidae) ..... Be

44. *Idiomyces peyritschii* Thaxt., Proc. Am. Acad. Arts Sci. 28: 162 (1893)

- *Deleaster dichrous* Gravenhorst, 1802 (Coleoptera, Staphylinidae)..... Be, Nl

45. *Kainomyces rehmanii* T. Majewski, Polish Bot. Stud. 1: 121 (1990)

- *Acrotrichis dispar* (Matthews, 1865) (Coleoptera, Ptiliidae) ..... Nl
- *Acrotrichis* sp. ..... Be

46. *Laboulbenia acupalpi* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 26: 458 (1915)

- *Acupalpus parvulus* (Sturm, 1825) (Coleoptera, Carabidae)..... Nl

47. *Laboulbenia anoplogenii* Thaxt., Proc. Am. Acad. Arts Sci. 35: 156 (1899) [1899–1900]

- *Stenolophus mixtus* (Herbst, 1784) (Coleoptera, Carabidae)..... Be, Nl
- *Stenolophus teutonus* (Schrank, 1781) ..... Be

48. *Laboulbenia argutoris* Cépède & F. Picard, Bull. Biol. Fr. Belg. 42: 260 (1909)

- *Pterostichus diligens* (Sturm, 1824) (Coleoptera, Carabidae) ..... Be
- *Pterostichus strenuus* (Panzer, 1796) ..... Be, Nl
- *Pterostichus vernalis* (Panzer, 1796) ..... Nl

49. *Laboulbenia atlantica* Thaxt., Mem. Am. Acad. Arts Sci. 12: 336 (1902)

- *Lobrathium multipunctum* (Gravenhorst, 1802) (Coleoptera, Staphylinidae).... Be

50. *Laboulbenia aubryi* Balazuc, Revue Mycol. 43: 393 (1979)

- *Amara aenea* (De Geer, 1774) (Coleoptera, Carabidae).....Be[a]

[a] New record: Belgium, Province Vlaams Brabant, Meise, Domein van Bouchout, 50.9274389 N 4.323925 E, ca. 25 m a.s.l., 6 Apr. 2020, leg. A. De Kesel, wet meadow, on *Amara aenea*, ADK6520 (BR), slides ADK6520a (BR-MYCO, 1 mature thallus, elytra) and ADK6520b (BR-MYCO, 2 immature and 4 mature thalli, elytra).

51. *Laboulbenia barbara* Middelh. & Boelens, Ned. Kruidk. Arch. 53: 99 (1943a)

- *Philonthus punctus* (Gravenhorst, 1802) (Coleoptera, Staphylinidae)..... Nl

52. *Laboulbenia benjaminii* Balazuc ex Santam., Fl. Mycol. Iber. 4: 45 (1998)

- *Badister bullatus* (Schrank, 1798) (Coleoptera, Carabidae)..... Be, Nl[a]
- *Badister lacertosus* Sturm, 1815..... Be
- *Badister sodalis* (Duftschmid, 1812) ..... Be, Nl[b]
- *Badister unipustulatus* Bonelli, 1813..... Be

[a] Host as *Badister bipustulatus* (Fabricius, 1792), fungus as *Laboulbenia polyphaga* Thaxt. in Middelhoek (1949) and Meijer (1975).
[b] Fungus as *Laboulbenia polyphaga* Thaxt. in Meijer (1975).

53. *Laboulbenia calathi* T. Majewski, Polish Bot. Stud. 7: 89 (1994)

- *Calathus erratus* (Sahlberg, 1827) (Coleoptera, Carabidae)..... Be
- *Calathus fuscipes* (Goeze, 1777)..... Nl
- *Calathus melanocephalus* (Linnaeus, 1758) ..... Be, Nl

54. *Laboulbenia clivinalis* Thaxt., Proc. Am. Acad. Arts Sci. 35: 155 (1899) [1899–1900]

- *Clivina collaris* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be
- *Clivina fossor* (Linnaeus, 1758) ..... Be, Nl

55. *Laboulbenia collae* T. Majewski, Polish Bot. Stud. 7: 104 (1994)

- *Agonum micans* (Nicolai, 1822) (Coleoptera, Carabidae) ..... Be
- *Paranchus albipes* (Fabricius, 1796) (Coleoptera, Carabidae) ..... Be, Nl

56. *Laboulbenia coneglianensis* Speg., Redia 10: 47 (1914)

- *Harpalus affinis* (Schrank, 1781) (Coleoptera, Carabidae) ..... Be, Nl[a]
- *Harpalus atratus* Latreille, 1804 ..... Be
- *Harpalus attenuatus* Stephens, 1828 ..... Be
- *Harpalus griseus* (Panzer, 1796) ..... Be, Nl[b]
- *Harpalus rufipes* (De Geer, 1774) ..... Be
- *Harpalus tardus* (Panzer, 1796) ..... Be, Nl
- *Harpalus* sp. ..... Be
- *Ophonus rufibarbis* (Fabricius, 1792) (Coleoptera, Carabidae) ..... Be
- *Parophonus maculicornis* (Duftschmid, 1812) (Coleoptera, Carabidae) ..... Nl[c]

[a] Host as *Harpalus aeneus* (Fabricius, 1775), fungus as *Laboulbenia elongata* Thaxt. in Middelhoek (1949).
[b] Host as *Pseudophonus griseus* (Panzer, 1796), fungus as *Laboulbenia elongata* Thaxt. in Middelhoek (1949).
[c] Fungus as *Laboulbenia melanaria* Thaxt. in Haelewaters et al. (2012a).

57. *Laboulbenia cristata* Thaxt., Proc. Am. Acad. Arts Sci. 29: 174 (1893)

- *Paederus fuscipes* Curtis, 1826 (Coleoptera, Staphylinidae) ..... Nl
- *Paederus littoralis* Gravenhorst, 1802 ..... Be
- *Paederus riparius* (Linnaeus, 1758) ..... Be, Nl
- *Paederus* sp. ..... Be

58. *Laboulbenia dubia* Thaxt., Proc. Am. Acad. Arts Sci. 38: 35 (1902) [1903]

- *Philonthus cognatus* Stephens, 1832 (Coleoptera, Staphylinidae) ..... Be

59. *Laboulbenia egens* Speg., Anal. Soc. Cient. Argent. 85: 323 (1918)

- *Elaphropus parvulus* (Dejean, 1831) (Coleoptera, Carabidae) ..... Be
- *Paratachys micros* (Fischer von Waldheim, 1828) (Coleoptera, Carabidae) ..... Be

60. *Laboulbenia elaphri* Speg., Anal. Mus. Nac. B. Aires 26: 64 (1915)

- *Elaphrus cupreus* Duftschmid, 1812 (Coleoptera, Carabidae) ..... Be
- *Elaphrus riparius* (Linnaeus, 1758) ..... Be

61. *Laboulbenia eubradycelli* Huldén, Karstenia 25: 4 (1985)

- *Bradycellus harpalinus* (Audinet-Serville, 1821) (Coleoptera, Carabidae) .... Be, Nl
- *Bradycellus ruficollis* (Stephens, 1828) ..... Be
- *Bradycellus verbasci* (Duftschmid, 1812) ..... Be, Nl

- *Trichocellus placidus* (Gyllenhal, 1827) (Coleoptera, Carabidae) ..... Be

62. *Laboulbenia fasciculata* Peyr., Sitzber. Akad. Wiss. Wien Math.-naturw. Kl. 68: 248 (1873)

- *Nebria brevicollis* (Fabricius, 1792) (Coleoptera, Carabidae)..... Be
- *Omophron limbatum* (Fabricius, 1777) (Coleoptera, Carabidae)..... Be, Nl[a]
- *Patrobus atrorufus* (Stroem, 1768) (Coleoptera, Carabidae) ..... Be
- *Pterostichus nigrita* (Paykull, 1790) (Coleoptera, Carabidae)..... Be

[a] New record: No locality, no date, on *Omophron limbatum* (Naturalis Biodiversity Center), slide D. Haelew. 074a (BR-MYCO, 3 thalli, left metatibia).

63. *Laboulbenia fennica* Huldén, Karstenia 23: 54 (1983)

- *Gyrinus marinus* Gyllenhal, 1808 (Coleoptera, Gyrinidae) ..... Nl
- *Gyrinus substriatus* Stephens, 1829 ..... Be, Nl

64. *Laboulbenia filifera* Thaxt., Proc. Am. Acad. Arts Sci. 28: 165 (1893)

- *Harpalus affinis* (Schrank, 1781) (Coleoptera, Carabidae) ..... Nl[a]

[a] Host as *Harpalus aeneus* (Fabricius, 1775) in Middelhoek (1949). The microscope slide from the collection of W.J. Kossen was reported to be in very poor condition; as a result, no illustrations could be made (Middelhoek 1949). For the time being, we retain the identification of the fungus. *Laboulbenia filifera* was described on a species of *Anisodactylus* Dejean, 1829 (Coleoptera, Carabidae) in the USA, and it is possible that European records of *L. filifera* belong in *L. flagellata* (Majewski 1994, Haelewaters et al. 2019a). The species is not included in the identification key.

65. *Laboulbenia flagellata* Peyr., Sitzber. Akad. Wiss. Wien Math.-naturw. Kl. 68: 247 (1873), sensu lato

- *Agonum emarginatum* (Gyllenhal, 1827) (Coleoptera, Carabidae)..... Be
- *Acupalpus flavicollis* (Sturm, 1825) ..... Nl[a]
- *Agonum fuliginosum* (Panzer, 1809)..... Be, Nl[b]
- *Agonum marginatum* (Linnaeus, 1758)..... Be, Nl
- *Agonum micans* (Nicolai, 1822)..... Be
- *Agonum moestum* (Duftschmid, 1812) ..... Be, Nl[a]
- *Agonum muelleri* (Herbst, 1784) ..... Be, Nl
- *Agonum nigrum* Dejean, 1828..... Be
- *Agonum thoreyi* Dejean, 1828..... Be, Nl
- *Agonum viduum* (Panzer, 1796)..... Nl

- *Agonum viridicupreum* Goeze, 1777 ..... Be
- *Anchomenus dorsalis* (Pontoppidan, 1763) (Coleoptera, Carabidae) ..... Nl[c]
- *Anisodactylus binotatus* (Fabricius, 1787) (Coleoptera, Carabidae) ..... Be
- *Laemostenus terricola* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be
- *Limodromus assimilis* (Paykull, 1790) (Coleoptera, Carabidae) ..... Be, Nl[d]
- *Loricera pilicornis* (Fabricius, 1775) (Coleoptera, Carabidae) ..... Be
- *Nebria brevicollis* (Fabricius, 1792) (Coleoptera, Carabidae) ..... Be
- *Oxypselaphus obscurus* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be
- *Paranchus albipes* (Fabricius, 1796) (Coleoptera, Carabidae) ..... Be, Nl[e]
- *Parophonus maculicornis* (Duftschmid, 1812) ..... Be
- *Pterostichus vernalis* (Panzer, 1796) ..... Be
- *Trichotichnus laevicollis* (Duftschmid, 1812) (Coleoptera, Carabidae) ..... Be

[a] Fungus as *Laboulbenia elongata* Thaxt. in Middelhoek (1949).
[b] Host as *Europhilus fuliginosus* (Panzer, 1809), fungus as *Laboulbenia elongata* Thaxt. in Middelhoek (1949).
[c] Host as *Platynus dorsalis* (Pontoppidan, 1763) in Zaneveld (1938), as *Agonum dorsale* (Pontoppidan, 1763) in Meijer (1975).
[d] Host as *Platynus assimilis* (Paykull, 1790) in Zaneveld (1938).
[e] Host as *Platynus ruficornis* (Goeze, 1777) in Zaneveld (1938).

66. *Laboulbenia giardii* Cépède & F. Picard, Bull. Sci. Fr. Belg. 42: 258 (1908)

- *Dicheirotrichus gustavii* Crotch, 1871 (Coleoptera, Carabidae) ..... Be, Nl[a]
- *Dicheirotrichus obsoletus* (Dejean, 1829) ..... Be

[a] Host as *Dicheirotrichus pubescens* (Paykull, 1790) in Meijer (1975).

67. *Laboulbenia gyrinicola* Speg., Redia 10: 34 (1914)

- *Gyrinus marinus* Gyllenhal, 1808 (Coleoptera, Gyrinidae) ..... Be, Nl
- *Gyrinus natator* (Linnaeus, 1758) ..... Be
- *Gyrinus substriatus* Stephens, 1829 ..... Nl

68. *Laboulbenia hyalopoda* De Kesel, Sterbeeckia 18: 17 (1998)

- *Paradromius linearis* (Olivier, 1795) (Coleoptera, Carabidae) ..... Be

69. *Laboulbenia inflata* Thaxt., Proc. Am. Acad. Arts Sci. 27: 41 (1892)

- *Acupalpus dubius* Schilsky, 1888 (Coleoptera, Carabidae) ..... Be, Nl
- *Acupalpus exiguus* Dejean, 1829 ..... Be
- *Acupalpus parvulus* (Sturm, 1825) ..... Nl
- *Stenolophus mixtus* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be

70. *Laboulbenia kajanensis* Huldén, Karstenia 23: 56 (1983)

- *Pterostichus diligens* (Sturm, 1824) (Coleoptera, Carabidae) ..... Be
- *Pterostichus strenuus* (Panzer, 1796) ..... Be

71. *Laboulbenia lecoareri* (Balazuc) Huldén, Karstenia 25: 6 (1985)

- *Trechoblemus micros* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be

72. *Laboulbenia leisti* J. Siemaszko & Siemaszko, Polsk. Pism. Entomol. 6: 203 (1928) [1927]

- *Agonum muelleri* (Herbst, 1784) (Coleoptera, Carabidae) ..... Be
- *Leistus ferrugineus* (Linnaeus, 1758) (Coleoptera, Carabidae) ..... Be, Nl

73. *Laboulbenia lichtensteinii* F. Picard, Bull. Sci. Fr. Belg. 50: 449 (1917) [1916–1917]

- *Cillenus lateralis* Samouelle, 1819 (Coleoptera, Carabidae) ..... Nl

74. *Laboulbenia littoralis* De Kesel & Haelew., Mycologia 106: 408 (2014)

- *Cafius xantholoma* (Gravenhorst, 1806) (Coleoptera, Staphylinidae) ..... Be, Nl

75. *Laboulbenia luxurians* Peyr., Sitzber. Akad. Wiss. Wien Math.-naturw. Kl. 68: 248 (1873)

- *Bembidion dentellum* (Thunberg, 1787) (Coleoptera, Carabidae) ..... Nl

76. *Laboulbenia metableti* Scheloske, Parasitol. Schriftenr. 19: 124 (1969)

- *Syntomus foveatus* (Geoffroy, 1785) (Coleoptera, Carabidae) ..... Be, Nl[a]
- *Syntomus truncatellus* (Linnaeus, 1760) ..... Be, Nl[a]

[a] New records: Noord-Holland Province, Zuid-Kennemerland National Park, 31 Oct. 2016, *leg*. M. Boeken, pitfall trap, on *Syntomus truncatellus*, slide D. Haelew. 1236b (GENT, 2 juvenile thalli, pronotum). *Ibid.*, 5 Jun. 2017, *leg*. M. Boeken, pitfall trap, on *Syntomus truncatellus*, slide D. Haelew. 1378a (GENT, 2 mature thalli, posterior margin of right elytron). *Ibid.*, 5 Jun. 2017, *leg*. M. Boeken, pitfall trap, on *Syntomus foveatus*, slide D. Haelew. 1387a (GENT, 1 mature thallus, left elytron). *Ibid.*, 5 Jun. 2017, *leg*. M. Boeken, pitfall trap, on *Syntomus foveatus*, slides D. Haelew. 1391a (FH, 5 mature thalli, right elytron), D. Haelew. 1391b (FH, 1 mature thallus, left metatrochanter), and D. Haelew. 1391c (FH, 1 submature and 2 mature thalli, mesocoxae). *Ibid.*, 17 Jul. 2017,

leg. M. Boeken, pitfall trap, on *Syntomus truncatellus*, slide D. Haelew. 1379a (GENT, 2 juvenile thalli, left elytron).

77. *Laboulbenia murmanica* Huldén, Karstenia 23: 57 (1983)

- *Bembidion assimile* Gyllenhal, 1810 (Coleoptera, Carabidae) ..... Be

78. *Laboulbenia notiophili* Cépède & F. Picard, Bull. Biol. Fr. Belg. 42: 259 (1909)

- *Demetrias atricapillus* (Linnaeus, 1758) (Coleoptera, Carabidae)..... Be
- *Demetrias imperialis* (Germar, 1824)..... Be
- *Demetrias monostigma* Leach, 1819 ..... Be
- *Notiophilus biguttatus* (Fabricius, 1779) (Coleoptera, Carabidae) ..... Be, Nl
- *Notiophilus rufipes* Curtis, 1829..... Be
- *Notiophilus substriatus* Waterhouse, 1833 ..... Nl
- *Notiophilus* sp..... Be
- *Paradromius linearis* (Olivier, 1795) (Coleoptera, Carabidae) ..... Be, Nl[a]
- *Philorhizus melanocephalus* (Dejean, 1825) (Coleoptera, Carabidae)..... Nl

[a] Fungus as *Laboulbenia casnoniae* Thaxt. in Haelewaters et al. (2012a).

79. *Laboulbenia ophoni* Thaxt., Proc. Am. Acad. Arts Sci. 35: 190 (1899) [1899–1900]

- *Harpalus rubripes* (Duftschmid, 1812) (Coleoptera, Carabidae)..... Be
- *Ophonus rufibarbis* (Fabricius, 1792) (Coleoptera, Carabidae)..... Be

80. *Laboulbenia pedicellata* Thaxt., Proc. Am. Acad. Arts Sci. 29: 109 (1893)

- *Bembidion aeneum* Germar, 1824 (Coleoptera, Carabidae) ..... Be, Nl
- *Bembidion articulatum* (Panzer, 1796)..... Nl
- *Bembidion biguttatum* (Fabricius, 1779)..... Nl
- *Bembidion gilvipes* Sturm, 1825..... Be
- *Bembidion guttula* (Fabricius, 1792)..... Be, Nl
- *Bembidion iricolor* Bedel, 1879..... Be, Nl
- *Bembidion lunulatum* (Geoffroy, 1785) ..... Be, Nl
- *Bembidion minimum* (Fabricius, 1792) ..... Be, Nl
- *Bembidion normannum* Dejean, 1831 ..... Be, Nl
- *Bembidion obtusum* Audinet-Serville, 1821 ..... Be
- *Bembidion quadrimaculatum* (Linnaeus, 1760) ..... Be, Nl
- *Bembidion ustulatum* (Linnaeus, 1758) ..... Nl
- *Bembidion varium* (Olivier, 1795)..... Be, Nl
- *Dyschirius globosus* (Herbst, 1784) (Coleoptera, Carabidae)..... Nl
- *Dyschirius salinus* Schaum, 1843 ..... Nl

- *Dyschirius thoracicus* (P. Rossi, 1790) ..... Nl[a]
- *Dyschirius tristis* Stephens, 1827 ..... Be
- *Dyschirius* sp. ..... Nl
- *Pogonus chalceus* (Marsham, 1802) (Coleoptera, Carabidae) ..... Be, Nl

[a] Host as *Dyschirius arenosus* Stephens, 1827 in Middelhoek (1943a).

81. *Laboulbenia philonthi* Thaxt., Proc. Am. Acad. Arts Sci. 28: 174 (1893)

- *Philonthus micans* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Nl
- *Philonthus rubripennis* Stephens, 1832 (Coleoptera, Staphylinidae) ..... Be
- *Philonthus* sp. ..... Be

82. *Laboulbenia pseudomasei* Thaxt., Proc. Am. Acad. Arts Sci. 35: 196 (1899)

- *Loricera pilicornis* (Fabricius, 1775) (Coleoptera, Carabidae) ..... Be
- *Nebria brevicollis* (Fabricius, 1792) (Coleoptera, Carabidae) ..... Be
- *Pterostichus anthracinus* (Panzer, 1795) (Coleoptera, Carabidae) ..... Be
- *Pterostichus melanarius* (Illiger, 1798) ..... Nl[a]
- *Pterostichus minor* (Gyllenhal, 1827) ..... Be
- *Pterostichus nigrita* (Paykull, 1790) ..... Be
- *Pterostichus strenuus* (Panzer, 1796) ..... Be
- *Stomis pumicatus* (Panzer, 1796) (Coleoptera, Carabidae) ..... Be

[a] New record: Drenthe Province, Oude Willem, 52.897438 N 6.323432 E, 2 Jun. 2014, *leg*. A.J. Dees, on *Pterostichus melanarius* (NNKN), slides D. Haelew. 1013a (FH, 1 juvenile thallus, right elytron) and D. Haelew. 1013b (FH, 1 submature thallus, prosternum).

83. *Laboulbenia quarantenae* De Kesel & Haelew, sp. nov.

- *Bembidion* (*Philochtus*) *biguttatum* (Fabricius, 1779) (Coleoptera, Carabidae) ... Be

84. *Laboulbenia rougetii* Mont. & C.P. Robin, in Robin, *Histoire Naturelle des végétaux parasites qui croissent sur l'homme et sur les animaux vivants* (Paris): 622 (1853)

- *Brachinus crepitans* (Linnaeus, 1758) (Coleoptera, Carabidae) ..... Be

85. *Laboulbenia slackensis* Cépède & F. Picard, Compt. Rend. Assoc. Franç. Avancem. Sci. 35: 775 (1907)

- *Pogonus chalceus* (Marsham, 1802) (Coleoptera, Carabidae) ..... Be, Nl

86. *Laboulbenia stilicicola* Speg., Redia 10: 41 (1914)

- *Rugilus orbiculatus* (Paykull, 1789) (Coleoptera, Staphylinidae) ..... Be, Nl[a]
- *Rugilus rufipes* Germar, 1836 (Coleoptera, Staphylinidae) ..... Be, Nl[b]

[a] Host as *Stilicus orbiculatus* (Paykull, 1789), fungus as *Laboulbenia subterranea* Thaxt. in Middelhoek (1943a, 1947a).
[b] Host as *Stilicus rufipes* Germar, 1836, fungus as *Laboulbenia subterranea* Thaxt. in Middelhoek (1943a, 1945).

87. *Laboulbenia thaxteri* Cépède & F. Picard, Bull. Biol. Fr. Belg. 42: 260 (1909)

- *Asaphidion flavipes* (Linnaeus, 1760) (Coleoptera, Carabidae)..... Be

88. *Laboulbenia vulgaris* Peyr., Sitzber. Akad. Wiss. Wien Math.-naturw. Kl. 68: 248 (1873)

- *Asaphidion flavipes* (Linnaeus, 1760) (Coleoptera, Carabidae)..... Nl
- *Bembidion assimile* Gyllenhal, 1810 (Coleoptera, Carabidae) ..... Nl
- *Bembidion biguttatum* (Fabricius, 1779)..... Be, Nl
- *Bembidion bruxellense* Wesmael, 1835 ..... Nl[a]
- *Bembidion dentellum* (Thunberg, 1787) ..... Be, Nl
- *Bembidion elongatum* Dejean, 1831 ..... Be
- *Bembidion femoratum* Sturm, 1825 ..... Be, Nl
- *Bembidion iricolor* Bedel, 1879..... Nl
- *Bembidion mannerheimi* Sahlberg, 1827..... Be
- *Bembidion minimum* (Fabricius, 1792) ..... Nl
- *Bembidion normannum* Dejean, 1831 ..... Nl
- *Bembidion pallidipenne* (Illiger, 1802) ..... Nl
- *Bembidion properans* (Stephens, 1828) ..... Be, Nl
- *Bembidion stephensii* Crotch, 1866..... Be
- *Bembidion testaceum* (Duftschmid, 1812) ..... Nl
- *Bembidion tetracolum* Say, 1823 ..... Be, Nl
- *Bembidion tibiale* (Duftschmid, 1812) ..... Be
- *Bembidion ustulatum* (Linnaeus, 1758) ..... Nl
- *Bembidion* sp..... Be
- *Dyschirius globosus* (Herbst, 1784) (Coleoptera, Carabidae)..... Nl
- *Dyschirius salinus* Schaum, 1843 ..... Nl
- *Ocys harpaloides* (Audinet-Serville, 1821) (Coleoptera, Carabidae)..... Be
- *Trechus quadristriatus* (Schrank, 1781) (Coleoptera, Carabidae)..... Be
- *Trechus rubens* (Fabricius, 1792)..... Be

[a] Host as *Bembidion rupestre* (Linnaeus, 1767) in Meijer (1975).

89. *Mimeomyces zeelandicus* Middelh. & Boelens, Ned. Kruidk. Arch. 53: 102 (1943)

- *Heterothops binotatus* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Nl

90. *Misgomyces dyschirii* Thaxt., Proc. Am. Acad. Arts Sci. 35: 443 (1900)

- *Dyschirius aeneus* (Dejean, 1825) (Coleoptera, Carabidae) ..... Be, Nl
- *Dyschirius globosus* (Herbst, 1784) ..... Be, Nl
- *Dyschirius intermedius* Putzeys, 1846 ..... Be
- *Dyschirius politus* (Dejean, 1825) ..... Nl
- *Dyschirius salinus* Schaum, 1843 ..... Nl
- *Dyschirius tristis* Stephens, 1827 ..... Be, Nl[a]

[a] Host as *Dyschirius luedersi* Wagner, 1915 in Middelhoek (1943a).

91. *Monoicomyces bolitocharae* T. Majewski, Polish Bot. Stud. 7: 193 (1994)

- *Bolitochara obliqua* Erichson, 1837 (Coleoptera, Staphylinidae) ..... Be

92. *Monoicomyces britannicus* Thaxt., Proc. Am. Acad. Arts Sci. 35: 412 (1900)

- *Acrotona fungi* (Gravenhorst, 1806) (Coleoptera, Staphylinidae) .....Be[a]
- *Acrotona orbata* (Erichson, 1837) ..... Be[b]
- *Acrotona pseudotenera* (Cameron, 1933) ..... Nl
- *Atheta* sp. (Coleoptera, Staphylinidae) ..... Be

[a] Host as *Atheta* (*Mocyta*) *fungi* (Gravenhorst, 1806) in De Kesel et al. (2020).
[b] Host as *Atheta* (*Mocyta*) *orbata* (Erichson, 1837) in De Kesel et al. (2020).

93. *Monoicomyces californicus* (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. 16: 38 (1931)

- *Anotylus sculpturatus* (Gravenhorst, 1806) (Coleoptera, Staphylinidae) ..... Be, Nl[a]

[a] Host as *Oxytelus sculpturatus* Gravenhorst, 1806 in Middelhoek (1943a).

94. *Monoicomyces fragilis* Scheloske, Parasitol. Schriftenr. 19: 138 (1969)

- *Ocalea picata* (Stephens, 1832) (Coleoptera, Staphylinidae) ..... Be

95. *Monoicomyces homalotae* Thaxt., Proc. Am. Acad. Arts Sci. 35: 412 (1900)

- *Atheta aeneicollis* (Sharp, 1869) (Coleoptera, Staphylinidae) ..... Nl

- *Atheta amicula* (Stephens, 1832) ..... Nl
- *Atheta crassicornis* (Fabricius, 1792) ..... Nl
- *Atheta gagatina* (Baudi, 1848) ..... Nl
- *Atheta longicornis* (Gravenhorst, 1802) ..... Be
- *Atheta triangulum* (Kraatz, 1856) ..... Be, Nl
- *Atheta vestita* (Gravenhorst, 1806) ..... Be
- *Atheta xanthopus* (Thomson, 1856) ..... Nl
- *Atheta* sp. ..... Be

96. *Monoicomyces invisibilis* Thaxt., Proc. Am. Acad. Arts Sci. 36: 414 (1900) [1901]

- *Anotylus sculpturatus* (Gravenhorst, 1806) (Coleoptera, Staphylinidae) ..... Be
- *Anotylus* sp. ..... Be
- *Oxytelus laqueatus* (Marsham, 1802) (Coleoptera, Staphylinidae) ..... Be
- *Oxytelus* sp. ..... Be
- *Platystethus arenarius* (Geoffroy, 1785) (Coleoptera, Staphylinidae) ..... Be

97. *Monoicomyces matthiatis* T. Majewski, Acta Mycol. 25: 49 (1990) [1989]

- *Platystethus* cf. *arenarius* (Geoffroy, 1785) (Coleoptera, Staphylinidae) ..... Be

98. *Monoicomyces myllaenae* Santam., Nova Hedwig. 82: 358 (2006)

- *Myllaena elongata* (Matthews, 1838) (Coleoptera, Staphylinidae) ..... Nl

99. *Monoicomyces nigrescens* Thaxt., Proc. Am. Acad. Arts Sci. 35: 412 (1900)

- *Atheta atramentaria* (Gyllenhal, 1810) (Coleoptera, Staphylinidae) ..... Nl
- *Atheta* sp. ..... Be
- *Brundinia marina* (Mulsant & Rey, 1853) (Coleoptera, Staphylinidae) .....Be[a]
- *Dilacra luteipes* (Erichson, 1837) (Coleoptera, Staphylinidae) ..... Nl[b]

[a] Host as *Atheta* (*Actophylla*) *marina* (Mulsant & Rey, 1853) in De Kesel et al. (2020).
[b] Host as *Atheta luteipes* (Erichson, 1837) in Middelhoek (1943a).

100. *Peyritschiella biformis* (Thaxt.) I.I. Tav., Mycol. Mem. 9: 270 (1985)

- *Philonthus umbratilis* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Be

101. *Peyritschiella dubia* (Thaxt.) I.I. Tav., Mycol. Mem. 9: 270 (1985)

- *Philonthus politus* (Linnaeus, 1758) (Coleoptera, Staphylinidae) ..... Be

102. *Peyritschiella furcifera* (Thaxt.) I.I. Tav., Mycol. Mem. 9: 270 (1985)

- *Philonthus albipes* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Nl[a]
- *Philonthus rectangulus* Sharp, 1874 ..... Nl[a]

[a] Fungus as *Dichomyces furciferus* Thaxt. in Middelhoek (1943a).

103. *Peyritschiella heinemanniana* De Kesel, Belg. J. Bot. 131: 177 (1999) [1998]

- *Xantholinus longiventris* Heer, 1839 (Coleoptera, Staphylinidae) ..... Be

104. *Peyritschiella princeps* (Thaxt.) I.I. Tav., Mycol. Mem. 9: 270 (1985)

- *Bisnius cephalotes* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Be, Nl[a]
- *Bisnius sordidus* (Gravenhorst, 1802) ..... Be, Nl[b]
- *Bisnius subuliformis* (Gravenhorst, 1802) ..... Nl
- *Philonthus politus* (Linnaeus, 1758) (Coleoptera, Staphylinidae) ..... Be
- *Philonthus varians* (Paykull, 1789) ..... Nl[c]
- *Philonthus* sp. ..... Be

[a] Host as *Philonthus cephalotes* (Gravenhorst, 1802), fungus as *Dichomyces vulgatus* Thaxt. in Middelhoek (1943a, 1947a).

[b] Host as *Philonthus sordidus* (Gravenhorst, 1802), fungus as *Dichomyces princeps* Thaxt. in Middelhoek (1941, 1943a, 1943b, 1943c), fungus also as *Dichomyces vulgatus* Thaxt. (variety *sensu* Thaxter 1908: 252) in Middelhoek (1943a, 1943b).

[c] Fungus as *Dichomyces princeps* Thaxt. in Middelhoek (1941).

105. *Peyritschiella protea* Thaxt., Proc. Am. Acad. Arts Sci. 35: 427 (1900)

- *Anotylus insecatus* Gravenhorst, 1806 (Coleoptera, Staphylinidae) ..... Be
- *Anotylus rugosus* (Fabricius, 1775) ..... Be, Nl[a]
- *Anotylus* sp. ..... Be
- Staphylinidae sp. indet. (Coleoptera, Staphylinidae) ..... Be

[a] Host as *Oxytelus rugosus* (Fabricius, 1775) in Middelhoek (1943a, 1947a).

106. *Peyritschiella vulgata* (Thaxt.) I.I. Tav., Mycol. Mem. 9: 271 (1985)

- *Philonthus albipes* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Nl[a]

[a] Fungus as *Dichomyces vulgatus* Thaxt. in Middelhoek (1943b, 1943c).

107. *Phaulomyces simplocariae* De Kesel, Mycotaxon 50: 192 (1994)

- *Simplocaria semistriata* Fabricius, 1794 (Coleoptera, Byrrhidae) ..... Be

108. *Rhachomyces canariensis* Thaxt., Proc. Am. Acad. Arts Sci. 35: 436 (1900)

- *Trechus obtusus* Erichson, 1837 (Coleoptera, Carabidae) ..... Be, Nl[a]
- *Trechus quadristriatus* (Schrank, 1781) ..... Be
- *Trechus* sp. ..... Be

[a] New record: Noord-Holland Province, Zuid-Kennemerland National Park, 17 Oct. 2016, *leg*. M. Boeken, pitfall trap, on *Trechus obtusus* Erichson, 1837 (Coleoptera, Carabidae), slides D. Haelew. 1242a (GENT, 9 thalli, right margin of pronotum) and D. Haelew. 1242b (GENT, 3 juvenile thalli, elytra). *Ibid.*, 5 Jun. 2017, *leg*. M. Boeken, pitfall trap, on *Trechus obtusus*, slide D. Haelew. 1388a (GENT, 1 submature thallus, tip of left elytron).

109. *Rhachomyces furcatus* (Thaxt.) Thaxt., Proc. Am. Acad. Arts Sci. 30: 467 (1895) [1894]

- *Othius punctulatus* (Goeze, 1777) (Coleoptera, Staphylinidae) ..... Be
- *Othius subuliformis* Stephens, 1833 ..... Be[a], Nl

[a] Host as *Othius myrmecophilus* Kiesenwetter, 1843 in De Kesel et al. (2020).

110. *Rhachomyces lasiophorus* (Thaxt.) Thaxt., Proc. Am. Acad. Arts Sci. 30: 468 (1895) [1894]

- *Acupalpus dubius* Schilsky, 1888 (Coleoptera, Carabidae) ..... Be
- *Acupalpus exiguus* Dejean, 1829 ..... Be, Nl
- *Anthracus consputus* (Duftschmid, 1812) (Coleoptera, Carabidae) ..... Nl

111. *Rhachomyces philonthinus* Thaxt., Proc. Am. Acad. Arts Sci. 35: 435 (1900)

- *Bisnius fimetarius* (Gravenhorst, 1802) (Coleoptera, Staphylinidae) ..... Be, Nl[a]
- *Philonthus cruentatus* (Gmelin, 1790) (Coleoptera, Staphylinidae) ..... Nl[b]
- *Philonthus fumarius* (Gravenhorst, 1806) ..... Be
- *Philonthus marginatus* (Müller, 1764) ..... Be, Nl
- *Philonthus rectangulus* Sharp, 1874 ..... Be
- *Philonthus varians* (Paykull, 1789) ..... Be, Nl[b]
- *Philonthus* sp. ..... Be

[a] Host as *Philonthus fimetarius* (Gravenhorst, 1802) in Middelhoek (1943a, 1943d).
[b] Fungus as *Rhachomyces* '*philonthi*' Thaxt. in Middelhoek (1943b).

112. *Rhachomyces pilosellus* (C.P. Robin) Thaxt., Proc. Am. Acad. Arts Sci. 30: 467 (1895) [1894]

- *Lathrobium fulvipenne* (Gravenhorst, 1806) (Coleoptera, Staphylinidae)..... Be
- *Lathrobium geminum* Kraatz, 1857 ..... Be

113. *Rhachomyces spinosus* Santam. & A.D. Cuesta-Segura, Nova Hedwig. 110: 362 (2020)

- *Syntomus foveatus* (Geoffroy, 1785) (Coleoptera, Carabidae) .....Be[a]

[a] Fungus as *Rhachomyces sciakyi* W. Rossi in De Kesel et al. (2020)

114. *Rhachomyces tenenbaumii* J. Siemaszko & Siemaszko, Polsk. Pism. Entomol. 6: 205 (1928)

- *Thalassophilus longicornis* (Sturm, 1825) (Coleoptera, Carabidae)..... Be

115. *Rhadinomyces cristatus* Thaxt., Proc. Am. Acad. Arts Sci. 28: 180 (1893)

- *Lathrobium brunnipes* (Fabricius, 1793) (Coleoptera, Staphylinidae)..... Be
- *Lathrobium castaneipenne* Kolenati, 1846..... Be
- *Lathrobium elongatum* (Linnaeus, 1767) ..... Be
- *Lathrobium fulvipenne* (Gravenhorst, 1806) ..... Be
- *Lathrobium geminum* Kraatz, 1857 ..... Be
- *Lathrobium* sp. ..... Be

116. *Rhadinomyces pallidus* Thaxt., Proc. Am. Acad. Arts Sci. 28: 180 (1893)

- *Lathrobium elongatum* (Linnaeus, 1767) (Coleoptera, Staphylinidae)..... Nl

117. *Rhynchophoromyces anacaenae* Scheloske, Parasitol. Schriftenr. 19: 143 (1969)

- *Anacaena lutescens* (Stephens, 1829) (Coleoptera, Hydrophilidae)..... Be

118. *Rickia dendroiuli* W. Rossi, Rev. Mycol. 41: 531 (1977)

- Julida sp. indet. ..... Be

119. *Rickia laboulbenioides* De Kesel, Sterbeeckia 32: 6 (2013)

- *Cylindroiulus latestriatus* (Curtis, 1845) (Julida, Julidae)..... Be, Nl

- *Cylindroiulus punctatus* Leach, 1814 ..... Be
- Julida sp. indet. ..... Be

120. *Rickia peyerimhoffii* Maire, Bull. Sci. Fr. Belg. 7: 290 (1916)

- *Scaphisoma* sp. (Coleoptera, Staphylinidae) ..... Be

121. *Rickia proteini* T. Majewski, Acta Mycol. 19: 191 (1985)

- *Proteinus* sp. (Coleoptera, Staphylinidae) ..... Be

122. *Rickia wasmannii* Cavara, Malpighia 13: 182 (1899)

- *Myrmica ruginodis* Nylander, 1846 (Hymenoptera, Formicidae) ..... Nl
- *Myrmica sabuleti* Meinert, 1861 (Hymenoptera, Formicidae)..... Be, Nl[a]
- *Myrmica scabrinodis* Nylander, 1846 ..... Nl

[a] Host as *Myrmica scabrinodis* Nylander, 1846 in Haelewaters (2012).

123. *Siemaszkoa fennica* Huldén, Karstenia 23: 63 (1983)

- *Ptenidium formicetorum* Kraatz, 1851 (Coleoptera, Ptiliidae) ..... Nl

124. *Siemaszkoa ptenidii* (Scheloske) I.I. Tav. & T. Majewski, Mycotaxon 3: 204 (1976)

- *Ptenidium* sp. (Coleoptera, Ptiliidae)..... Be

125. *Stichomyces conosomatis* Thaxt., Proc. Am. Acad. Arts Sci. 37: 38 (1901)

- *Sepedophilus marshami* (Stephens, 1832) (Coleoptera, Staphylinidae)..... Be
- *Sepedophilus nigripennis* (Stephens, 1832) ..... Be, Nl
- *Sepedophilus pedicularius* (Gravenhorst, 1802)..... Be
- *Sepedophilus testaceus* (Fabricius, 1792)..... Nl
- *Sepedophilus* sp. ..... Be

126. *Stigmatomyces baeri* H. Karst., Chemismus Pfl.-Zelle: 78 (1869)

- "*Fannia canicularis*" (Linnaeus, 1761) (Diptera, Fanniidae) ..... Nl[a]

[a] Host as *Homalomyia canicularis* (Linnaeus, 1761) in Boedijn (1923). The host identification may have been incorrect; *Fannia canicularis* is typically associated with *Fanniomyces ceratophorus* Whisler, whereas *S. baeri* is typically found on *Musca domestica* Linnaeus, 1758 (Diptera, Muscidae).

127. *Stigmatomyces crassicollis* Thaxt., Proc. Am. Acad. Arts Sci. 52: 661 (1917)

- *Leptocera caenosa* (Rondani, 1880) (Diptera, Sphaeroceridae)..... Be
- *Leptocera fontinalis* (Fallén, 1826) ..... Be
- *Leptocera lutosoidea* (Duda, 1938) ..... Be
- *Opacifrons humida* (Haliday, 1836) (Diptera, Sphaeroceridae) ..... Be
- *Spelobia rufilabris* (Stenhammar, 1855) (Diptera, Sphaeroceridae) ..... Be
- Sphaeroceridae sp. indet. (Diptera) ..... Be

128. *Stigmatomyces divergatus* Thaxt., Mem. Am. Acad. Arts Sci. 16: 122 (1931)

- *Apteromyia claviventris* (Strobl, 1909) (Diptera, Sphaeroceridae) ..... Be
- *Spelobia parapusio* (Dahl, 1909) (Diptera, Sphaeroceridae)..... Be
- *Spelobia* sp. ..... Be

129. *Stigmatomyces entomophilus* (Peck) Thaxt., Proc. Am. Acad. Arts Sci. 36: 398 (1900) [1901]

- *Drosophila funebris* (Fabricius, 1787) (Diptera, Drosophilidae) ..... Nl

130. *Stigmatomyces hydrelliae* Thaxt., Proc. Am. Acad. Arts Sci. 36: 404 (1900) [1901]

- *Hydrellia albilabris* (Meigen, 1830) (Diptera, Ephydridae) ..... Nl[a]

[a] New record: Noord-Brabant Province, Udenhout, nature reserve De Brand, 51.631777 N 5.132998 E, 14–21 Jun. 1990, *leg*. Working Group Insects of the Royal Dutch Natural History Association (KNNV), malaise trap (van Zuijlen et al. 1996), on *Hydrellia albilabris* (Meigen, 1830) (Diptera, Ephydridae), slide WR1746 (will be deposited at FI, Herbarium Universitatis Florentinae, Florence, Italy), *det.* W. Rossi, *comm.* J.W.A. van Zuijlen.

131. *Stigmatomyces limosinae* Thaxt., Proc. Am. Acad. Arts Sci. 36: 406 (1900) [1901]

- *Spelobia clunipes* (Meigen, 1830) (Diptera, Sphaeroceridae) ..... Be
- *Spelobia talparum* (Richards, 1927)..... Nl

132. *Stigmatomyces majewskii* H.L. Dainat, Manier & Balazuc, Bull. Trimest. Soc. Mycol. Fr. 90: 171 (1974)

- *Drosophila subobscura* Collin, 1936 (Diptera, Drosophilidae)..... Nl

133. *Stigmatomyces minilimosinae* T. Majewski, Polish Bot. Stud. 1: 122 (1990)

- *Minilimosina parvula* (Stenhammar, 1855) (Diptera, Sphaeroceridae)..... Be

134. *Stigmatomyces platensis* Speg., Anal. Mus. Nac. Hist. Nat. B. Aires 29: 676 (1917)

- *Paralimosina fucata* (Rondani, 1880) (Diptera, Sphaeroceridae) ..... Be
- *Paralimosina subcribrata* (Rohacek, 1977) ..... Be

135. *Symplectromyces vulgaris* (Thaxt.) Thaxt., Mem. Am. Acad. Arts Sci. 13: 315 (1908)

- *Philonthus* sp. (Coleoptera, Staphylinidae) ..... Be
- *Quedius curtipennis* Bernhauer, 1908 (Coleoptera, Staphylinidae) ..... Be
- *Quedius fuliginosus* (Gravenhorst, 1802)..... Be
- *Quedius fumatus* (Stephens, 1833)..... Be
- *Quedius lateralis* (Gravenhorst, 1802)..... Nl
- *Quedius levicollis* (Brullé, 1832) .....Be[a]
- *Quedius maurorufus* (Gravenhorst, 1806) ..... Nl
- *Quedius mesomelinus* (Marsham, 1802) ..... Be, Nl
- *Quedius* sp. ..... Be

[a] Host as *Quedius tristis* (Gravenhorst, 1802) in De Kesel et al. (2020).

136. *Teratomyces actobii* Thaxt. Proc. Am. Acad. Arts Sci. 29: 98 (1894)

- *Gabrius nigritulus* (Gravenhorst, 1802) (Coleoptera, Staphylinidae)..... Be
- *Gabrius* sp. ..... Be

137. *Teratomyces philonthi* Thaxt., Proc. Am. Acad. Arts Sci. 35: 432 (1901)

- *Gabrius nigritulus* (Gravenhorst, 1802) (Coleoptera, Staphylinidae)..... Be
- *Gabrius trossulus* (Nordmann, 1837) ..... Nl[a]
- *Gabrius* sp. ..... Be
- *Quedius nitipennis* (Stephens, 1833) (Coleoptera, Staphylinidae) ..... Be
- *Quedius* sp. ..... Be

[a] Host as *Philonthus trossulus* Nordmann, 1837 in Middelhoek (1943a).

138. *Troglomyces manfrediae* S. Colla [as 'manfredii'], Nuovo G. Bot. Ital. 39: 451 (1932)

- Julida sp. indet. ..... Be

139. *Troglomyces triandrus* Santam. & Enghoff, Organ. Divers. Evol. 15: 253 (2015)

- *Archiboreoiulus pallidus* (Brade-Birks, 1920) (Julida, Blaniulidae)..... Be

140. *Zodiomyces vorticellarius* Thaxt., Proc. Am. Acad. Arts Sci. 25: 263 (1891)

- *Helochares punctatus* (Sharp, 1869) (Coleoptera, Hydrophilidae)..... Nl
- *Helochares* sp. ..... Be

Doubtful records and combinations

Laboulbenia elegans Thaxt. on *Harpalus affinis* (Schrank, 1781) (Coleoptera, Carabidae) [as *Harpalus aeneus* (Fabricius, 1775)] (Middelhoek 1949). This material could not be verified since the Middelhoek collection is currently untraceable, but it likely represents *L. coneglianensis*. *Laboulbenia coneglianensis* is reported from species of *Harpalus* Latreille, 1802 and *Ophonus* Dejean, 1821 in Europe, whereas *L. elegans* is thus far only confirmed from New England, USA (Thaxter 1890, 1896).

Laboulbenia flagellata [as *Laboulbenia elongata* Thaxt.] on *Calathus erratus* (Sahlberg, 1827) (Coleoptera, Carabidae) (Middelhoek 1947b). The material is incomplete and impossible to verify. Given the host, it is doubtful that this report represents *L. flagellata*. Possibly it is *L. calathi* T. Majewski, which is already known from the Netherlands (Haelewaters et al. 2012b).

Laboulbenia flagellata on *Pterostichus nigrita* (Paykull, 1790) (Coleoptera, Carabidae) (Meijer 1975). This record possibly represents *L. pseudomasei* Thaxt. but we cannot verify because the material of Meijer is untraceable. *Pterostichus nigrita* is routinely reported as host to *L. pseudomasei*, not *L. flagellata* (Thaxter 1899; Scheloske 1969; Majewski 1994; Santamaría 1998; De Kesel et al. 2020). Both species are easily distinguished with morphological characters.

Laboulbenia pedicellata on *Trechus quadristriatus* (Schrank, 1781) (Coleoptera, Carabidae) (Meijer 1975). This would be the only worldwide record of *L. pedicellata* on a species of *Trechus* Clairville, 1806 and thus is likely incorrect. *Laboulbenia pedicellata* is generally reported on species of *Bembidion* Latreille, 1802 sensu lato (Coleoptera, Carabidae) and *Dyschirius* Bonelli, 1810 (Coleoptera, Carabidae) (Haelewaters et al. 2019a).

Discussion

New species and new records

In this paper, we describe two new species of Laboulbeniales based on the combination of molecular data, morphology, and ecology (host association). These are *Hesperomyces halyziae* on *Halyzia sedecimguttata* in Belgium and the Netherlands, and *Laboulbenia quarantenae* on *Bembidion biguttatum* in Belgium. Additionally, *Laboulbenia aubryi* and *Rhachomyces spinosus* are newly reported from Belgium. Seven previously described species of Laboulbeniales are reported for the first time from the Netherlands: *Chitonomyces melanurus*, *Euphoriomyces agathidii*, *Laboulbenia fasciculata*, *Laboulbenia metableti*, *Laboulbenia pseudomasei*, *Rhachomyces canariensis*, and *Stigmatomyces hydrelliae*.

The report of *L. aubryi* from Belgium is only the third one from Europe. *Laboulbenia aubryi* was thus far only recorded from India, Nepal, Poland, and Spain (type). Reported hosts are species in *Amara* Bonelli, 1810 (= *Bradytus* Stephens, 1827, = *Leironotus* Ganglbauer, 1892) (Santamaría et al. 1991; Santamaría 1998; Majewski 1999), a diverse genus that is only exceptionally reported with Laboulbeniales (Santamaría et al. 1991). Scheloske (1969) mentioned *L. flagellata* on *Amara plebeja* (Gyllenhal, 1810), but considered it an accidental host ("Zufallswirt"). Moreover, based on its simple outer appendage, *L. aubryi* can easily be separated from *L. flagellata*. The closest related species, morphologically speaking, is *L. argutoris* Cépède & F. Picard, but *L. aubryi* can be separated from it by the insertion cell that is free from the perithecium wall and by the structure of its inner appendage (Santamaría 1998).

Rhachomyces spinosus was recently described from Spain (Santamaría et al. 2020). The most characteristic feature of this species is the spinous process on the second cell of the primary appendage, absent in similar species *R. lavagnei* (F. Picard) W. Rossi and *R. sciakyi* W. Rossi. The reported host for *R. spinosus* in both Belgium and Spain is *Syntomus foveatus* (Coleoptera, Carabidae). *Rhachomyces lavagnei* is found on *Microlestes* spp. and *R. sciakyi* on *Pseudomesolestes* sp. All these hosts are placed in the subtribe Dromiusina (Harpalinae, Lebiini); it is possible that these species of *Rhachomyces* have a high degree of host specificity, which will only come to light as more material will be collected.

Chitonomyces melanurus is easily recognized from other congeneric species by the apically hooked, dark brown to blackish basal cell of its primary appendage. Nine species of *Chitonomyces* Peyr. occur in Europe, all of them occupying a specific position of the host integument. *Chitonomyces melanurus* grows almost exclusively on the upper margin of the left elytron of *Laccophilus* Leach, 1815 water beetles (Coleoptera, Dytiscidae). It has thus far has been reported in Europe from Austria (type), Belgium, Croatia, Finland, France, Germany, Hungary, Italy, Poland, Spain, Ukraine, United Kingdom; also found in Asia and Africa (Bánhegyi 1960; Huldén 1983; Santamaría et al. 1991; Majewski 1994; De Kesel and Werbrouck 2008; Rossi 2018).

The Dutch report of *E. agathidii* is found on *Agathidium laevigatum*, the host species from which the type was described (Maire 1920). *Euphoriomyces agathidii* is thus far found on members of *Agathidium* Panzer, 1796, *Amphicyllis* Erichson, 1845, and *Cyrtusa* Erichson, 1842 (Coleoptera, Leiodidae) in Bulgaria, Germany, Italy, Morocco (type), Poland, South Korea, Spain, and Sweden (Huldén 1983; Majewski 1994; Lee et al. 2007; Rossi et al. 2018). Our material is consistent with *E. agathidii*, with two mature perithecia at one side and a third, immature perithecium at the other side of the receptacular axis.

Laboulbenia fasciculata is recognized by the receptacular cell V, which proliferates upwards in a series of 4–8 superposed cells V' gradually decreasing in size. Each of these cells V' gives rise to a small trapezoidal cell that carries an appendage consisting of cells separated by dark and constricted septa. This species is very widespread, with reports across Europe, in Africa, Asia, and North and South America. Hosts are members of Carabidae, often *Chlaenius* Bonelli, 1810 (subfamily Harpalinae) and *Patrobus* Dejean, 1821 (subfamily Trechinae), but also several other genera in subfamilies Cicindelinae,

Brachininae, Harpalinae, Nebriinae, Omophroninae, Patrobinae, and Trechinae (Santamaría et al. 1991). The reports on *Omophron* spp. are sometimes considered a form of *L. fasciculata* but this is not accepted by all (Spegazzini 1914; Majewski 1994; but Santamaría 1998).

The status of *L. metableti* as a separate species has been disputed. Formally synonymized with *L. notiophili* by Rossi and Santamaría (2006), De Kesel et al. (2020) reinstated *L. metableti* as a separate species based on characteristics of the appendage system. This species has a European distribution, with reports in Andorra, Austria, Belgium, Finland, Germany (type), Hungary, Italy, Poland, Russia, and the United Kingdom (reviewed in Rossi and Santamaría 2006). Hosts are species of *Syntomus* Hope, 1838 (= *Metabletus* Schmidt-Goebel, 1846) (Coleoptera, Carabidae, Harpalinae, Lebiini). We propose using molecular characters to resolve the debate given the taxonomic confusion of species of *Laboulbenia* on European hosts in the Lebiini tribe: *L. baetica* Balazuc, *L. blanchardii* Cépède, *L. cymindicola* Speg., *L. metableti*, *L. notiophili*, and *L. pulchella* Speg.

Laboulbenia pseudomasei is recognized by cell V that has an internal convex margin and is separated from the perithecium (Villarreal et al. 2010). Cell V sometimes proliferates into a simple or divided branch that grows upwards between the perithecium and insertion cell (Majewski 1994; Santamaría 1998). Rossi and Weir (1997) illustrated that *L. pseudomasei* can be morphologically highly variable even on a single host insect. Also in the newly reported material from the Netherlands, *L. pseudomasei* was variable, with the thallus from the right elytron without proliferation of cell V, and the thallus from the prosternum with proliferation of cell V. The geographic distribution of *L. pseudomasei* is problematic; many old records are unillustrated and the specimens are not preserved (Rossi and Weir 1997).

Rhachomyces canariensis was described from Tenerife (Thaxter 1900) and has since been reported from several countries in Europe and North Africa, Madeira, and the Canary Islands, always associated with species of *Trechus* Clairville, 1806 (Coleoptera, Carabidae) (Arndt and Santamaría 2004). Majewski (1994) noted the variability of this species and Tavares (1985) suggested material from large geographic distances to the type locality be segregated into a separate taxon.

The only species of Laboulbeniales found on *Hydrellia* Robineau-Desvoidy, 1830 flies (Diptera, Ephydridae) is *S. hydrelliae*. Thaxter (1901) described it from Kittery Point in Maine, USA (Thaxter 1901) and it has since then been reported in Finland, France, Italy, Poland, Portugal, Russia, the United Kingdom (Santamaría and Rossi 1993, Weir and Rossi 1995), and New Zealand (Hughes et al. 2004). The new report from the Netherlands is the first one on the European continent in 25 years. *Stigmatomyces hydrelliae* is recognized by its straight appendage with sterile basal cell and stout antheridia, the spiralled cell walls of the perithecium, and the rounded perithecial apex with one of the lip cells forming a slender, bluntly pointed projection. Hughes et al. (2004) noted that *S. hydrelliae* thalli from New Zealand are different in their perithecial wall cells not being spiralled and lacking apical projections at the perithecial apex.

Checklist

The current list of thallus-forming Laboulbeniomycetes from Belgium and the Netherlands includes 140 species. Sixty-three species have been found in both countries. A total of 118 species are found in Belgium, and 85 species in the Netherlands. Of the 140 species in the checklist, 55 have not (yet) been reported from the Netherlands, and 22 species have not (yet) been reported from Belgium. Laboulbeniales research in both Belgium and the Netherlands has also resulted in the discovery of new taxa; over the years, 16 species have been described based on material from Belgium and/or the Netherlands (Table 3). It is remarkable that we keep finding undescribed species in two of the most urbanized countries in the world. The reason for this can be found in the fact that Laboulbeniomycetes are severely understudied; only a handful of researchers work on these fungi. In addition, some of the most recently described species are the result of previously unavailable molecular data, long-term study of humid habitats, and focus on unexplored niches.

This checklist is based on fungal records obtained from at least 283 host species (including only those identified to species level). To increase the number of thallus-forming Laboulbeniomycetes known from Belgium and the Netherlands, future research should focus on screening Acari (with *Rickia*), Blattodea (*Herpomyces*–especially in the Netherlands), Coleoptera (many genera), Corixidae (*Coreomyces*), Diplopoda (*Diplopodomyces*, *Troglomyces*), Diptera (*Stigmatomyces*), Hebridae (*Tavaresiella*, *Triceromyces*), and Mallophaga (*Trenomyces*). Within Coleoptera, the beetles, aquatic and semi-aquatic hosts, such as Dytiscidae (*Chitonomyces*), Hydraenidae (*Autoicomyces*, *Hydrophilomyces*, *Thripomyces*), and Hydrophilidae (*Chaetarthriomyces*, *Eusynaptomyces*) deserve special attention. More genera of Laboulbeniales that are currently not yet

Table 3. Species of Laboulbeniales described based on type material from Belgium (Be) and the Netherlands (Nl).

Country	Laboulbeniales species	Host species	Host classification	Reference
Nl	*Asaphomyces tubanticus* [as *Barbariella tubantica*]	*Catops nigricans*	Coleoptera, Leiodidae	Middelhoek (1949)
Nl	*Cantharomyces elongatus*	*Syntomium aeneum*	Coleoptera, Staphylinidae	Haelewaters and De Kesel (2013)
Nl	*Bordea denotata*	*Bibloporus bicolor*	Coleoptera, Staphylinidae	Haelewaters et al. (2014)
Be	*Cryptandromyces euplecti*	*Euplectus sanguineus*	Coleoptera, Staphylinidae	Santamaría (2001)
Be, NL	*Diphymyces kaaistoepi*	*Choleva cisteloides*, *C. fagniezi*	Coleoptera, Leiodidae	De Kesel and Haelewaters (2019)
Be, NL	*Hesperomyces halyziae*	*Halyzia sedecimguttata*	Coleoptera, Coccinellidae	This paper
Nl	*Laboulbenia barbara*	*Philonthus punctus*	Coleoptera, Staphylinidae	Middelhoek (1943a)
Be	*Laboulbenia quarantenae*	*Bembidion biguttatum*	Coleoptera, Carabidae	This paper
Be	*Laboulbenia elaphri*	*Elaphrus cupreus*	Coleoptera, Carabidae	Spegazzini (1915)
Be	*Laboulbenia hyalopoda*	*Paradromius linearis*	Coleoptera, Carabidae	De Kesel (1998)
Be, NL	*Laboulbenia littoralis*	*Cafius xantholoma*	Coleoptera, Staphylinidae	De Kesel and Haelewaters (2014)
Nl	*Mimeomyces zeelandicus*	*Heterothops binotatus*	Coleoptera, Staphylinidae	Middelhoek (1943a)
Be	*Peyritschiella heinemanniana*	*Xantholinus longiventris*	Coleoptera, Staphylinidae	De Kesel (1999)
Be	*Phaulomyces simplocariae*	*Simplocaria semistriata*	Coleoptera, Byrrhidae	De Kesel (1994)
Be, NL	*Rickia laboulbenioides*	*Cylindroiulus latestriatus*	Julida, Julidae	De Kesel et al. (2013)
Be	*Troglomyces triandrus*	*Archiboreoiulus pallidus*	Julida, Blaniulidae	Enghoff and Santamaría (2015)

recorded from either Belgium or the Netherlands, could be discovered on Anthicidae (*Dioicomyces*), Ptiliidae (*Siemaszkoa*), Silvanidae (*Cucujomyces*), Staphylinidae (*Amorphomyces*, *Diplomyces*, *Dipodomyces*, *Haplomyces*, *Mimeomyces*, *Sphaleromyces*), and Tenebrionidae (*Dimeromyces*).

As Laboulbeniomycetes research progresses, lesser known host groups will need to be incorporated into our studies. This will eventually require intensified collaborations with specialist entomologists, as well as screening museum insect collections and the use of different collecting methods. That different sampling techniques have an impact on Laboulbeniales studies may be illustrated by our work with *Rickia wasmannii* Cavara. Based on pitfall trapping, Haelewaters et al. (2015a) reported *R. wasmannii* from three host species: *Myrmica sabuleti* Meinert, 1861 (parasite prevalence 38%), *M. scabrinodis* Nylander, 1846 (11%), and *M. ruginodis* Nylander, 1846 (0.55%). Direct sampling from a *M. scabrinodis* nest at the same locality in the Netherlands, however, resulted in a 100% prevalence (De Kesel et al. 2016).

Finally, undersampled habitats have been cited repeatedly as one of the main sources to find undescribed fungi (e.g., Blackwell 2011, Hawksworth and Lücking 2017, Wijayawardene et al. 2020). This is especially true for the Laboulbeniomycetes. Sampling from dung, fresh and brackish water, animal nests, caves, carcasses, and rotting plant debris has greatly contributed to discoveries in this field of research, not only adding to numbers of described species but also building on our understanding of the ecology of these minute fungi. For example, Pfliegler et al. (2016) sampled ants and their associates from ant nests and, for the first time since its description (Cavara 1899), *R. wasmannii* was observed on hosts other than *Myrmica*, including inquiline mites and a fly larva. A survey of Laboulbeniales from coprophilic beetles on Galloway dung in Belgium resulted in two reports of species that until then had only been found in Poland, thus representing a large geographical range expansion (De Kesel 2010). And signal crayfish traps in nature reserve 'De Kaaistoep' have thus far revealed an undescribed species of *Diphymyces* (De Kesel and Haelewaters 2019) and more material is awaiting detailed study.

Key to Laboulbeniomycetes from Belgium and the Netherlands

1 Dioecious; on Blattodea (cockroaches) ..... **50 (*Herpomyces*)**
– Thalli mostly monoecious; on other host groups **2**
2 Perithecial wall cells numerous, subequal, always ≥ 6 cells per vertical row **3**
– Perithecial wall with < 6 cells per vertical row **7**
3 Receptacle uniseriate, composed of numerous superposed cells **5**
– Receptacle multiseriate, often massive **4**
4 Receptacle turbinate, with apical depression holding numerous sterile appendages, stalked perithecia and antheridial branchlets; on Hydrophilidae ..... ***Zodiomyces vorticellarius***
– Receptacle not turbinate, bearing numerous perithecia and appendages laterally; on Carabidae and Staphylinidae ..... ***Euzodiomyces lathrobii***

5 Perithecium with an apical, darkened rostrum; receptacle with 4–5 cells; on Ptiliidae ..... ***Kainomyces rehmanii***

– Perithecium without a rostrum; receptacle with > 5 cells..... **6**

6 Perithecium long-necked, without lobes or fine appendages on the perithecial wall; on Hydrophilidae ..... ***Rhynchophoromyces anacaenae***

– Perithecium without long neck, ostiolum with 4 fine ligulae, lower wall bearing slender ramified appendices; on Dryopidae..... ***Helodiomyces elegans***

7 Antheridia simple, flask shaped; release of spermatia through small necks **8**

– Antheridia grouped into a compound structure with wall **44**

8 Sterile appendages unicellular with black basal septum; antheridia small, always with black basal septum; receptacle formed by 3 vertical tiers of cells (not always clear), at least one tier partly or entirely flanking the perithecium ..... **52 (*Rickia*)**

– Not this combination **9**

9 Suprabasal cell of the receptacle (cell II) produces multi-celled secondary appendages; the latter supporting a perithecium (with cell VI) at their base .... ***Compsomyces lestevae***

– Cell II not producing secondary appendages..... **10**

10 Perithecial wall with an elongated accessory cell along its outer venter; unicellular outgrowths are formed above the foot; on *Cercyon* (Hydrophilidae).... **11**

– Perithecium without accessory cell; no such outgrowths above the foot **12**

11 Lower receptacular cells isodiametric; perithecium neck more or les straight ..... ***Hydrophilomyces* cf. *gracilis***

– Lower receptacular cells flattened; perithecium neck strongly curved .... ***Hydrophilomyces* cf. *hamatus***

12 Cell VII and basal cells of the perithecium clearly visible in mature perithecia..... **13**

– Cell VII and basal cells of the perithecium not visible in mature perithecia.... **40**

13 Receptacle produces longitudinal septa, leading to a suprabasal complex with numerous secondary appendices **14**

– Receptacle stays a series of superposed cells, rarely forming longitudinal septa, not forming a suprabasal complex or secondary appendices **20**

14 Receptacle composed of a series of superposed cells (4–5 or more), each forming on one side a basal cell with numerous, fairly large, pigmented and multicellular appendages; thalli usually with only one perithecium .... **56 (*Rhachomyces*)**

– Not with these features **15**

15 Thallus hyaline; appendages not in bunches; On Cholevinae (Leiodidae)..... ..... ***Asaphomyces tubanticus***

– Thallus moderately to deeply pigmented in some parts; appendages appear in bunches on the receptacle **16**

16 Receptacle asymmetrical **17**

– Receptacle mostly symmetrical..... **18**

17 Antheridia in lateral series on fertile appendages; dorsal and ventral cell of the triangular receptacle supporting a series of appendages and their basal cells; perithecium stalked by elongated cells VI and VII ..... ***Idiomyces peyritschii***

– Antheridia never organized in lateral series; appendages not in series; receptacle 5-celled; cells VI and VII relatively short..... **62 (*Laboulbenia*)**

18 Appendages with pointed-curved tips, darkened septa; antheridia terminal, flask shaped, not forming ramifications with age.....**19 (*Teratomyces*)**

– Appendages with rounded tips, with series of intercalary antheridia, the latter ramifying into new appendages with age..... ***Symplectromyces vulgaris***

19 Cells I and II from receptacle becoming brown with age; basal cells of appendages with laterally aligned antheridia/septa..... ***Teratomyces philonthi***

– Cell I hyaline, contrasting with a deep blackened cell II; basal cells of appendages without such laterally aligned septa..... ***Teratomyces actobii***

20 Primary appendage bicellular, both cells separated by a dark constricted septum; antheridium below the primary appendage; on aquatic Coleoptera....**21**

– Primary appendage more developed.....**22**

21 All 4 vertical tiers of the perithecial wall have 4 cells each ....**109 (*Chitonomyces*)**

– Only 2 vertical tiers of the perithecial wall have 4 cells, the others have 6 cells; on Haliplidae ..... ***Hydraeomyces halipli***

22 Receptacle composed of ≥ 4 cells **23**

– Receptacle composed of ≤ 3 cells **26**

23 Primary receptacle composed of a chain of cells (≥ 3).....**24**

– Primary receptacle composed of cells I and II, entire receptacle with five cells..... **62 (*Laboulbenia*)**

24 Perithecium with obtuse apex and inconspicuous neck ....***Misgomyces dyschirii***

– Perithecium with long neck and differentiated venter **25**

25 Antheridia sessile, develop as corner cells of the primary appendage; Receptacle cells flattened, broadening upwards ..... ***Ecteinomyces trichopterophilus***

– Antheridia not sessile but formed on lateral branchlets; receptacle cells elongate..... ***Botryandromyces heteroceri***

26 Cell III flattened and entirely appressed against the perithecium; on Julida... .....**113 (*Troglomyces*)**

– Cell III different; on Hexapoda.....**27**

27 On Coleoptera.....**29**

– Not on Coleoptera.....**28**

28 Basal cell of appendage dark; perithecial apex with outgrowths; on *Forficula* (Dermaptera, Forficulidae)..... ***Distolomyces forficulae***

– On Diptera..... **114 (*Fanniomyces* & *Stigmatomyces*)**

29 Primary appendage easily breaking off at its strongly narrowed basal cell; on Kateretidae..... ***Aphanandromyces audisioi***

– Primary appendage persistent.....**30**

30 Receptacle cells (I, II, III) more or less superposed **31**
– Receptacle cells not superposed (cell I and III touching) **39**
31 Distal cell of primary appendage is a simple antheridium, with efferent neck and spine ..... ***Bordea denotata***
– Primary appendage without such a single and terminal antheridium..... **32**
32 Antheridial structures are born on corner cells of appendage axis cells..... **33**
– Antheridial branches not born from corner cells **36**
33 Basal (m, n, n') and stalk cells (VI, VII) of the perithecium small (together < 25 µm long); on Hydrophilidae .... ***Chaetarthriomyces crassiappendicatus***
– Basal (m, n, n') and stalk cells (VI, VII) ≥ 25 µm long; on Staphylinidae **34**
34 Cell III mostly without antheridial branches, with or without perithecium; cells VI and VII of similar length ..... ***Stichomyces conosomatis***
– Cell III always with antheridial branches, never with perithecium; cell VI much taller than cell VII **35**
35 Thallus forms perithecia and corner cells on one side (anterior) ..... ***Rhadinomyces pallidus***
– Thallus forms perithecia and corner cells on both sides (anterior and posterior) ..... ***Rhadinomyces cristatus***
36 Primary appendage simple, composed of numerous similar superposed cells..... **124 (*Cryptandromyces*)**
– Primary appendage branched **37**
37 Cell VI adnate to cell II; exclusively on Cholevinae (Leiodidae) .... ***Diphymyces kaaistoepi***
– Cell VI supported by cell II; mostly on Staphylinidae, rarely on Cholevinae (Leiodidae) **38**
38 Cell I tall and elongated, cell II flattened..... ***Mimeomyces zeelandicus***
– Cell I very short, cell II not flattened (isodiametric) .... **126 (*Corethromyces*)**
39 Perithecial tip with prominent ostiolar lips and lobes; appendage short, with sessile lateral antheridia on each cell; fresh thalli often greenish-yellow; on Coccinellidae ..... **127 (*Hesperomyces*)**
– Perithecial tip without such lobes; appendage long, with lateral antheridia on few cells; not on Coccinellidae ..... **124 (*Cryptandromyces*)**
40 Receptacle between foot and cell VI with ≥ 3 cells. **41**
– Receptacle between foot and cell VI with 2 cells; foot entirely black .... ***Phaulomyces simplocariae***
41 Receptacle a series of superposed cells, many of which laterally producing perithecia and/or appendages..... ***Euphoriomyces agathidii***
– Receptacle a series of superposed cells without lateral cells bearing perithecia and appendages..... **42**
42 Receptacle with flattened and finely appendiculate cells above cell III; Foot entirely black; on Corixidae (Hemiptera) ..... ***Coreomyces arcuatus***
– Receptacle without such flattened cells above cell III; foot with a small blackish dot; on Ptiliidae..... **43**

43 The appendage is a prolongation of the receptacle axis, the perithecium is lateral ..... ***Siemaszkoa ptenidii***

– The perithecium is often terminal and in continuation with the receptacular axis ..... ***Siemaszkoa fennica***

44 Cell I laterally extending and supporting a series of cells derived from cell II; thallus dioecious ..... ***Dimorphomyces myrmedoniae***

– Cell I not laterally extending; thallus monoecious **45**

45 Primary receptacle composed of a chain of ≥ 3 cells .... ***Misgomyces dyschirii***

– Primary receptacle not a chain of cells **46**

46 Primary appendage fertile, with a compound antheridium **47**

– Primary appendage sterile or absent **49**

47 Compound antheridium with efferent neck and tall cell on the outer side; on Carabidae ..... ***Eucantharomyces stammeri***

– Compound antheridium different, never with efferent neck; on Staphylinidae and Dryopidae (= Parnidae) **48**

48 Primary appendage is entirely transformed into a compound antheridium, with spine ..... ***Haplomyces texanus***

– Compound antheridium is an intercalary structure of the primary appendage ..... **129 (*Cantharomyces*)**

49 Receptacle formed by 3 horizontal tiers of cells; antheridia compound, sessile, often on the median series; sterile appendages unicellular .... **134 (*Peyritschiella*)**

– Receptacle differently organized; sterile appendages multicellular; antheridial structure stalked, large ..... **140 (*Monoicomyces*)**

50 Secondary receptacle (female thallus) without concentrically organized cells. ..... ***Herpomyces ectobiae***

– Secondary receptacle a series of concentrically organized and flattened cells... ..... **51**

51 Secondary receptacle ≤ 80 µm long, with rounded apex and partially darkened cells ..... ***Herpomyces stylopygae***

– Secondary receptacle ≥ 100 µm long, with pointed apex and without dark pigmentations ..... ***Herpomyces periplanetae***

52 Perithecium almost entirely embedded in the receptacle .... ***Rickia peyerimhoffii***

– Anterior part of the perithecium free **53**

53 On Diplopoda **54**

– On other arthropods **55**

54 Dorsal margin of the perithecium free in its upper third; anterior series of receptacle consisting of 2(–3) cells ..... ***Rickia laboulbenioides***

– Dorsal margin of the perithecium only free at the apex; Anterior series of receptacle consisting of > 2 cells ..... ***Rickia dendroiuli***

55 Cell I 12–18 µm long; on Staphylinidae ..... ***Rickia proteini***

– Cell I 60–90 µm long; on *Myrmica* (Hymenoptera, Formicidae) .... ***Rickia wasmannii***

56 Primary appendage hyaline, 3-celled, different from other appendages; On *Syntomus* (Carabidae) ..... ***Rhachomyces spinosus***
– Primary appendage pigmented, identical to secondary appendages **57**
57 Receptaculum between cells I and VI usually with < 6 cells, sterile appendages very long; on *Lathrobium* (Staphylinidae) ..... ***Rhachomyces pilosellus***
– Receptaculum between cells I and VI composed of ≥ 6 cells; sterile appendages do not exceed perithecial apex **58**
58 Cells of the B-appendages of unequal length **59**
– Cells of the B-appendages of similar to equal length **60**
59 B-appendages elongate, slender, tapering upwards; On *Philonthus* (Staphylinidae) ..... ***Rhachomyces philonthinus***
– B-appendages short, stout, width broad rounded apex; On *Thalassophilus* (Carabidae) ..... ***Rhachomyces tenenbaumii***
60 Cell VI elongate and situated in the median to subapical part of the (secondary) receptacle; On *Othius* (Staphylinidae) ..... ***Rhachomyces furcatus***
– Cell VI short, distally on the (secondary) receptacle; on Carabidae **61**
61 Perithecial apex with black spots; terminal cell of the B-appendages widest in the middle; on *Acupalpus* (Carabidae) ..... ***Rhachomyces lasiophorus***
– Perithecial apex hyaline; terminal cell of B-appendages cylindrical, usually proliferating; on *Trechus* (Carabidae) ..... ***Rhachomyces canariensis***
62 Insertion cell absent **63**
– Insertion cell present **65**
63 Appendages with large basal cells and dark septa; on Carabidae ..... ***Laboulbenia fasciculata***
– Appendages filiform, with fine basal cells and dark septal on Gyrinidae **64**
64 Perithecium with two hyaline apical outgrowths, one straight one hooked .... ***Laboulbenia gyrinicola***
– Both perithecial outgrowths with black spots, irregularly shaped ..... ***Laboulbenia fennica***
65 On Carabidae **66**
– On Staphylinidae **103**
66 Insertion cell free **67**
– Insertion cell attached to the posterior margin of the perithecium (not free) **72**
67 Foot almost hyaline with only a small black dot ..... ***Laboulbenia hyalopoda***
– Foot entirely black **68**
68 Cell V as tall as cell IV ..... ***Laboulbenia clivinalis***
– Cell V smaller than cell IV **69**
69 Outer appendage not branched **70**
– Outer appendage branched ..... ***Laboulbenia pseudomasei***
70 Inner appendage hardly branched, with a single antheridium ..... ***Laboulbenia lecoareri***
– Inner appendage branched, with multiple antheridia **71**

71 Lower 4–5 cells of outer appendage deeply pigmented in their middle; ostiolar papillae not conspicuous; on *Syntomus* (Carabidae) ..... ***Laboulbenia metableti***

– Lower 4–5 cells of outer appendage evenly pigmented; ostiolar papillae conspicuous; on *Amara* (Carabidae) ..... ***Laboulbenia aubryi***

72 Cell V as tall as cell IV, or almost so **73**

– Cell V smaller than cell IV **78**

73 Outer wall of perithecium with knobs ..... ***Laboulbenia egens***

– Outer wall of the perithecium without knobs **74**

74 Outer appendage without dark septum, growing beyond the perithecium .... ***Laboulbenia ophoni***

– Outer appendage with at least one dark septum, not growing beyond the perithecium **75**

75 Cells IV and V flattened, broader than long; On *Cillenus* (Carabidae) ..... ***Laboulbenia lichtensteinii***

– Cells IV and V isodiametric or longer than broad **76**

76 Thallus and receptaculum poorly pigmented (yellow-amber); basal cell of outer appendage inflated; on *Pogonus* (Staphylinidae) .... ***Laboulbenia slackensis***

– Thallus and receptaculum strongly pigmented; basal cell of outer appendage not so inflated 77

77 Cell III flattened and oblique; posterior margin of cell IV longer than the one from cell III; insertion cell extremely flat and opaque.... ***Laboulbenia luxurians***

– Cell III not flattened; posterior margin of cell IV equal or shorter than the one from cell III; insertion cell well-formed and black.... ***Laboulbenia pedicellata***

78 Outer appendage not growing beyond the perithecium ..... ***Laboulbenia murmanica***

– Outer appendage growing beyond the perithecium **79**

79 Outer appendage branched **80**

– Outer appendage not branched **90**

80 Cell IV very long, often with a conspicuous dorso-apical bump, sometimes divided **81**

– Cell IV not so long, never divided, without dorso-apical bump **82**

81 Outer appendage with > 2 branches; on *Stenolophus* (Carabidae) ..... ***Laboulbenia anoplogenii***

– Outer appendage consisting of 2 branches; on *Acupalpus* (Carabidae) ..... ***Laboulbenia acupalpi***

82 Insertion cell on or above the middle of the perithecium; inner appendage less developed than outer appendage **83**

– Insertion cell below the middle of the perithecium **84**

83 Thallus and receptaculum pale; septa from basal cells of outer appendage not darkened; on *Paranchus albipes* (Carabidae) ..... ***Laboulbenia collae***

– Thallus and receptaculum strongly pigmented; septa from basal cells of outer appendage darkened ..... ***Laboulbenia vulgaris***

84 Outer side of the base of outer appendage strongly darkened **85**
– Outer side of the base of outer appendage not or only very slightly darkened **87**
85 Thallus pale brown; appendages numerous, with tapering and pointed apices; on *Dicheirotrichus* (Carabidae) ..... ***Laboulbenia giardii***
– Thallus deep brown; appendages not so numerous, not tapering, with rounded apices **86**
86 Septum II/III clearly shorter than septum II/VI; cell V clearer than surrounding structures; on *Harpalus* and *Ophonus* (Carabidae) ..... ***Laboulbenia coneglianensis***
– Septum II/III nearly as long as septum II/VI; cell V not much paler than surrounding structures; on *Brachinus* (Carabidae) ..... ***Laboulbenia rougetii***
87 Thallus often bent, anterior side of the thallus concave **89**
– Thallus not so bent, anterior side of the thallus fairly straight **88**
88 Insertion cell near the base of the perithecium; outer appendage often composed of 4–6(–8) branches, resulting from successive dichotomies starting at the suprabasal cell ..... ***Laboulbenia quarantenae***
– Insertion cell not so deep; outer appendage branched once or twice, not as dichotomies ..... ***Laboulbenia flagellata* sensu lato**
89 Cell V quite small, less than half the length of cell IV; perithecium very slender, subcylindrical (not a stable feature); on *Harpalus* (Carabidae) ..... ***Laboulbenia coneglianensis***
– Cell V longer, usually more than half the length of cell IV; perithecium more ovate; on *Elaphrus* (Carabidae) ..... ***Laboulbenia elaphri***
90 Insertion cell located at or above the middle of the perithecium; adaxial side of the perithecium half free **91**
– Insertion cell located well below the middle of the perithecium; adaxial side of the perithecium more than half free **95**
91 Basal cells of outer appendage with darkened septa **93**
– Basal cells of outer appendage with normal septa **92**
92 Cell VI broader than long ..... ***Laboulbenia benjaminii***
– Cell VI longer than broad ..... ***Laboulbenia argutoris***
93 Basal cell of outer appendage inflated; inner appendage growing beyond the perithecium ..... ***Laboulbenia inflata***
– Basal cell of outer appendage normal; inner appendage never beyond perithecium **94**
94 Inner appendage composed of a single antheridium supported by one basal cell; on *Asaphidion* (Carabidae) ..... ***Laboulbenia thaxteri***
– Inner appendage with ≥ 2 cells each supporting one or more antheridia ..... ***Laboulbenia vulgaris***
95 Outer appendage rotated relative to the perithecium; on *Pterostichus diligens* (Carabidae) ..... ***Laboulbenia kajanensis***
– Outer appendage not rotated **96**

96 Inner appendage growing far beyond the perithecium **97**
– Inner appendage not or hardly beyond the perithecium **98**
97 Cell V clearly paler than surrounding cells and perithecium (III, IV and VI) **98**
– Cell V not paler than its surrounding cells ..... ***Laboulbenia leisti***
98 Cell IV (and cell III) evenly and deeply pigmented **100**
– Cell IV (and cell III) hyaline or pigmented, their outer margins distinctly more pigmented than inner margins **99**
99 Cell VI longer than broad; thallus ≥ 230 µm long; on *Calathus* (Carabidae) ... . ***Laboulbenia calathi***
– Cell VI isodiametric; thallus smaller; on *Demetrias*, *Notiophilus* and *Paradromius* (Carabidae) ..... ***Laboulbenia notiophili*** **sensu lato**
100 Inner appendage hardly branched, with a single antheridium; cell IV longer than broad ..... ***Laboulbenia lecoareri***
– Inner appendage branched, with multiple antheridia; cell IV isodiametrical **101**
101 Cell V minute; upper margin of cell IV 4–6× the width of cell V **102**
– Cell V larger; upper margin of cell IV only 1–2× the width of cell V .... ***Laboulbenia eubradycelli***
102 Lower 4–5 cells of outer appendage deeply pigmented in their middle; lower 3–4 cells of both branches of the inner appendage each producing a short straight branch ..... ***Laboulbenia metableti***
– Lower 4–5 cells of outer appendage evenly pigmented; inner appendage differently constructed ..... ***Laboulbenia notiophili*** **sensu lato**
103 Cell V as long as cell IV, or almost so **106**
– Cell V smaller than cell IV **104**
104 Insertion cell free from the perithecium; outer appendage branched .... ***Laboulbenia dubia***
– Insertion cell attached to the posterior margin of the perithecium (not free); outer appendage not branched **105**
105 Outer appendage with dark septa between basal cells; insertion cell near the base of the perithecium ..... ***Laboulbenia stilicicola***
– Outer appendage without dark septa at the basal cells; insertion cell near the middle of the perithecium ..... ***Laboulbenia atlantica***
106 Outer appendage with at least one dark septum at the basal and suprabasal cells **108**
– Outer appendage without dark septa at the basal cells **107**
107 Outer appendage forming a tuft of branches, posterior margins of both its basal and suprabasal cell entirely darkened ..... ***Laboulbenia barbara***
– Outer appendage simple or forked once; posterior margin of suprabasal cell of outer appendage with black remains of primary appendage .... ***Laboulbenia cristata***

108 Cell II hyaline; one black septum above the basal cell of the outer appendage ..... ***Laboulbenia littoralis***

– Anterior part of cell II pigmented black; black septa between all basal cells of inner and outer appendage..... ***Laboulbenia philonthi***

109 Perithecium with conspicuous outgrowths (spikes, thorns) **110**

– Perithecium without outgrowths; receptacle with outgrowth..... **111**

110 Perithecial outgrowth, arising from the apical-most wall cell ..... ***Chitonomyces paradoxus***

– Perithecial outgrowth lateral, arising from sub-apical wall cell..... ***Chitonomyces aculeifer***

111 Suprabasal cell of the receptacle (Ia) flattened ..... ***Chitonomyces bidessarius***

– Suprabasal cell of the receptacle (Ia) isodiametric **112**

112 Receptacular outgrowth hyaline, straight or arcuate.... ***Chitonomyces italicus***

– Receptacular outgrowth black, straight, with conspicuously hooked apex ..... ***Chitonomyces melanurus***

113 Primary appendage with 1 antheridium, always situated in the lowest cell .... ***Troglomyces manfrediae***

– Primary appendage with 3 antheridia situated in the third, fourth and fifth cell..... ***Troglomyces triandrus***

114 Appendage branched **115**

– Appendage an unbranched axis **116**

115 Basal cell of appendage small, pigmented; appendage cells normal..... ***Fanniomyces burdigalensis***

– Basal cell of appendage elongate, not pigmented; appendage cells elongated. ***Fanniomyces ceratophorus***

116 Cell VI shorter than cell III; appendage consisting isodiametric to elongated cells..... **117**

– Cells III and VI equally long; appendage with dark basal cell, consisting of flattened cells; on Sphaeroceridae (Diptera) **120**

117 Venter of perithecium without protuberances **118**

– Venter of perithecium with protuberances; on Ephydridae (Diptera) ..... ***Stigmatomyces hydrelliae***

118 Appendage arcuated or sigmoid; perithecial neck shorter than the venter; on *Musca* (Diptera, Muscidae) ..... ***Stigmatomyces baeri***

– Appendage not arcuated; perithecial neck longer than venter; on *Drosophila* (Diptera, Drosophilidae)..... **119**

119 Perithecial neck as long as venter; appendage hyaline, its axis composed of 4 cells; On *Drosophila* subg. *Sophophora* (Diptera, Drosophilidae) ..... ***Stigmatomyces majewskii***

– Perithecial neck 2× as long as venter; appendage brown, its axis composed of 6 cells; On *Drosophila* subg. *Drosophila* (Diptera, Drosophilidae) ..... ***Stigmatomyces entomophilus***

120 Venter of perithecium without protuberances **121**
– Venter of perithecium with protuberances **122**
121 Perithecial basal cells elongated, longer than the appendage ..... ***Stigmatomyces limosinae***
– Perithecial basal cells not elongated, never longer than the appendage ..... ***Stigmatomyces crassicollis***
122 Perithecial apex abruptly becoming conical; appendage not proliferating ..... ***Stigmatomyces platensis***
– Perithecial apex gradually tapering; appendage proliferating distally..... **123**
123 Perithecial venter with numerous knobs, below the neck and also downwards ..... ***Stigmatomyces minilimosinae***
– Perithecial venter with 4 knobs, only below the neck ..... ***Stigmatomyces divergatus***
124 Basal cells of the primary appendage dark brown on the outer side ..... ***Cryptandromyces euplecti***
– Primary appendage entirely hyaline..... **125**
125 Cell I supporting cell II; thallus > 125 µm long .... ***Cryptandromyces elegans***
– Cell I supporting cells II and III; thallus < 100 µm long ..... ***Cryptandromyces bibloplecti***
126 Basal cell of the receptacle with a black upgrowth; on *Rugilus* (Staphylinidae)..... ***Corethromyces stilici***
– Basal cell of the receptacle normal, without a black upgrowth; on *Choleva* (Leiodidae) ..... ***Corethromyces henrotii***
127 Upper and lower lobes of perithecium of equal length, not exceeding perithecial tip..... ***Hesperomyces coccinelloides***
– Upper lobes long, exceeding the perithecial tip; lower lobes short **128**
128 On *Halyzia* (Coleoptera, Coccinellidae)..... ***Hesperomyces halyziae***
– On *Harmonia*, *Tytthaspis* (Coleoptera, Coccinellidae) ..... ***Hesperomyces virescens* sensu lato**
129 Cell II of receptacle blackened ..... ***Cantharomyces denigratus***
– Cell II never black, at most brownish or with a black spot **130**
130 Basal cell of primary appendage supporting a 300–415 µm long unbranched series of 7–11 elongate cells..... ***Cantharomyces elongatus***
– Primary appendage not so long..... **131**
131 Primary appendage not ramified ..... ***Cantharomyces italicus***
– Primary appendage with ramifications **132**
132 Primary appendage ramified above the suprabasal cell; basal cell not spherical..... **133**
– Primary appendage ramified above the basal cell, the latter spherical..... ***Cantharomyces robustus***
133 Antheridium small, not reaching the upper and lower septa of the basal cell of the appendage..... ***Cantharomyces platystethi***
– Antheridium larger, reaching the lower (and upper) septum of the basal cell of the appendage..... ***Cantharomyces orientalis***

134 Sterile appendages blackish brown, without conspicuous black septa ..... ***Peyritschiella heinemanniana***

– Sterile appendages hardly pigmented, with black septa..... **135**

135 Lower horizontal tier of cells pigmented black, septa between cells obscured... **136**

– Lower horizontal tier of cells not as pigmented, septa between cells visible.... **138**

136 Distal part of receptacle broad; upper tier composed of > 20 cells ..... ***Peyritschiella biformis***

– Upper tier of the receptacle with < 15 cells; lateral sides of median tier each produce one pigmented outgrowths (some reach the upper tier) **137**

137 Thallus with 2 perithecia..... ***Peyritschiella furcifera***

– Thallus with (2–)3–12 perithecia ..... ***Peyritschiella vulgata***

138 Median cell of both lowest horizontal tiers as long as neighboring cells..... ***Peyritschiella protea***

– Median cell of both lowest horizontal tiers much larger than neighboring cells, the latter becoming smaller outwards. **139**

139 Perithecium usually with auricula; Third and second horizontal tier of similar length ..... ***Peyritschiella dubia***

– Perithecium without auricula; third horizontal tier longer than second..... ***Peyritschiella princeps***

140 Secondary receptacula unicellular..... **141**

– Secondary receptacula multicellular **146**

141 Secondary receptacula pigmented **145**

– Secondary receptacula hyaline..... **142**

142 Cell VI much longer than perithecium ..... ***Monoicomyces myllaenae***

– Cell VI shorter than perithecium **143**

143 Perithecium asymmetrical and bent; antheridium usually without apical branchlets ..... ***Monoicomyces matthiatis***

– Perithecium symmetrical, straight; antheridium with several apical branchlets..... **144**

144 Basal cell of primary appendage at least partially pigmented, not narrowing towards the apex ..... ***Monoicomyces homalotae***

– Basal cell of primary appendage entirely hyaline, narrowing towards the apex ..... ***Monoicomyces britannicus***

145 Antheridial and primary appendages very long, reaching beyond the perithecial apex..... ***Monoicomyces fragilis***

– Antheridial and primary appendages shorter, not reaching beyond the perithecial apex..... ***Monoicomyces nigrescens***

146 Secondary receptaculum pigmented..... ***Monoicomyces bolitocharae***

– Secondary receptaculum hyaline **147**

147 Secondary and antheridial appendages blackish brown ..... ***Monoicomyces californicus***

– Secondary and antheridial appendages hyaline or hardly pigmented, never blackish brown..... ***Monoicomyces invisibilis***

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Three new *Scheffersomyces* species associated with insects and rotting wood in China

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Abstract

Three species of *Scheffersomyces* were identified during a diversity study of yeasts. All three are associated with insects and rotting wood in China. Phylogenetic analyses of a genomic dataset combining ITS and nrLSU revealed that these new collections are distinct from known species, thus three new species are introduced i.e. *S. jinghongensis*, *S. paraergatensis*, and *S. anoplophorae*. In our phylogenetic analyses, *Scheffersomyces jinghongensis* possesses a strong independent lineage and is closely related to *S. titanus*. *S. paraergatensis* is closely related to *S. ergatensis*, while *S. anoplophorae* is related to *S. stambukii*. Several differences in physiological traits and molecular data indicate that *S. jinghongensis*, *S. paraergatensis*, and *S. anoplophorae* are three newly identified species.

Keywords

Debaryomycetaceae; *Saccharomycetales*; taxonomy; D-xylose-fermenting yeast

Introduction

Kurtzman and Suzuki (2010) introduced the genus *Scheffersomyces* Kurtzman. ex M. Suzuki. (2010) to include D-xylose-fermenting species in the *Pichia stipitis* clade, viz. *P. segobiensis*, *P. spartinae*, and *P. stipitis*. The genus *Scheffersomyces* was later expanded by including seven related *Candida* species as new combinations and by three novel species, *S. illinoinensis*, *S. quercinus*, and *S. virginianus*, which were discovered in rotting wood (Urbina and Blackwell 2012). More recently, several new species and combinations from the genus *Scheffersomyces*, (*S. cryptocerci*, *S. parashehatae*, *S. titanus*, and

S. xylosifermentans) have been identified in wood-ingesting insects (Suh et al. 2013; Urbina et al. 2013; Liu et al. 2016b), and in rotting wood (*S. amazonensis*, *S. ergatensis*, *S. henanensis*, and *S. stambukii*) (Ren et al. 2013; Urbina and Blackwell 2013; Lopes et al. 2018). This genus comprises 21 species, though the status of *S. gosingicus* and *S. lignicola* is currently unclear due to nomenclatural issues regarding the Melbourne Code (Index Fungorum, accessed 17.07.2020). Of the 19 valid species, five of them are known to form ascospores (Kurtzman 2011; Ren et al. 2013, Liu et al. 2016b) and 14 of them display asexual morphs (Urbina and Blackwell 2012; Suh et al. 2013; Urbina and Blackwell 2013; Urbina et al. 2013; Lopes et al. 2018).

Species in the genus *Scheffersomyces* are characterized by pseudohyphae formation, an inability to utilize nitrates, and the possession of the co-enzyme Q-9 (Kurtzman and Suzuki 2010; Kurtzman 2011). Asexual reproduction occurs via multilateral budding on a narrow base; sexual reproduction occurs via the formation of 1–2 hat-shaped ascospores released soon after formation (Kurtzman 2011). Several *Scheffersomyces* species, such as *S. henanensis*, *S. shehatae*, and *S. stipitis*, strongly ferment D-xylose, which is important for producing bioethanol from the residue of plant waste (du Preez and van der Walt 1983; Agbogbo and Wenger 2006; Ren et al. 2013; Suh et al. 2013; Lopes et al. 2018). Despite the existence of these microbes, obtaining high ethanol yields from pentose sugars on a large scale remains a challenge (Chandel et al. 2011) because scientists have yet to identify microbes that convert pentose sugars into ethanol at high yields, while withstanding fermentation inhibitors (Agbogbo and Wenger 2006; Long et al. 2012). Therefore, there is a need to identify new yeasts capable of efficient xylose fermentation in order to produce bioethanol.

Several D-xylose-fermenting yeasts were collected from different regions in China during a study on fungal diversity in insects and rotting wood. Two *Scheffersomyces* species, *S. henanensis* and *S. titanus*, have already been described in published studies (Ren et al. 2013; Liu et al. 2016b). In this study, the other three new species are described and characterized based on their morphology and phylogenetics, increasing the species diversity of *Scheffersomyces* in China.

Materials and methods

Sample collection, morphological studies, and isolation

Samples of insects and rotting wood were collected from Henan Province and Yunnan Province from 2015–2017. Strains of yeast were isolated from the insect guts according to the methods described by Liu et al. (2016a, b). Prior to dissection, the insects were placed in Petri dishes for 1–3 days without food, which eliminates some of the contaminating organisms found in the gut. Surface disinfection was performed by submersion in 95% ethanol for 1–2 min, followed by rinsing in a 0.7% saline solution. The insect gut was removed aseptically under a dissecting microscope. The gut segments were streaked on acidified yeast extract–malt extract (YM) agar plates (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% plain agar; pH adjusted to 3.5 with

HCl) and incubated at 25 °C for 3–4 days. Strains were isolated from the samples of rotting wood via the enrichment technique with YM broth, supplemented by 0.025% sodium propionate and 0.01% chloramphenicol (Ren et al. 2013). All yeast isolates were purified by repeated streak-inoculation on YM agar plates and preserved in 15% glycerol at -80 °C.

The morphological, physiological, and biochemical properties were determined according to those used by Kurtzman et al. (2011). The beginning of the sexual stage was determined by incubating single or mixed cultures of each of the two strains on cornmeal (CM) agar, YM agar, or 5% malt extract (ME) agar at 25 °C for 6 weeks (Ren et al. 2013, Liu et al. 2016b). The assimilation of carbon and nitrogen compounds and related growth requirements were tested at 25 °C. The effects of temperature from 25–40 °C were examined in a liquid culture and on agar plates.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from the yeast using an Ezup Column Yeast Genomic DNA Purification Kit, according to the manufacturer's instructions (Sangon Biotech, Shanghai, China). The nuc rDNA ITS1-5.8S-ITS2 (ITS) region was amplified using primer pairs ITS1/ITS4 (White et al. 1990). The D1/D2 domain of nrLSU rDNA (nrLSU) was amplified using the primer pair NL1/NL4 (Kurtzman and Robnett 1998). The PCR protocols used for the ITS and nrLSU were those outlined by Liu et al. (2016a). PCR products were directly purified and sequenced by Sangon Biotech Inc. (Shanghai, China). We confirmed the identity and accuracy of the resulting sequences by assembling them using BioEdit and comparing them to sequences in GenBank (Hall 1999). The sequences were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Table 1).

Phylogenetic analysis

The sequences obtained from this study and the reference sequences obtained from GenBank (Table 1) were aligned using MAFFT v. 6 (Katoh and Toh 2010) and manually edited using MEGA v. 7 (Kumar et al. 2016). The best-fit nucleotide substitution models for each gene were selected using jModelTest v2.1.7 (Darriba et al. 2012) according to the Akaike information criterion. Phylogenetic analyses of combined gene regions (ITS and nrLSU) were performed using MEGA v7 for maximum parsimony (MP) analysis (Kumar et al. 2016) and PhyML v3.0 for Maximum Likelihood (ML) analysis (Guindon et al. 2010). *Saccharomyces cerevisiae* CBS 1171^{T} was chosen as the outgroup after consulting Liu et al. (2016b) and Suh et al. (2013).

MP analysis was performed using a heuristic search option with tree-bisection reconnection (TBR) branch swapping (Nei and Kumar 2000) and 1,000 random sequence additions. ML analysis was performed using GTR+I+G models for each partition (Nei and Kumar 2000) and a proportion of invariant sites with 1000 rapid

Table 1. Sequences used in molecular phylogenetic analysis. Entries in bold are newly generated for this study.

Species	Strain	ITS	D1/D2
Candida broadrunensis	CBS 11838^T	HQ263349	NG_064320
Candida thasaenensis	CBS 12529^T	NR_111028	NG_055174
Scheffersomyces anoplophorae	**NYNU 15730^T**	**KU128714**	**KU128724**
Scheffersomyces anoplophorae	**NYNU 15733**	**MT133542**	**MT133540**
Scheffersomyces coipomoensis	NRRL Y-17651^T	HQ652070	HQ651966
Scheffersomyces cryptocerci	CBS 12658^T	NR_120091	NG_055704
Scheffersomyces ergatensis	NRRL Y-17652^T	EU343826	U45746
Scheffersomyces gosingicus	CBS 11433^T	HQ999978	HQ999955
Scheffersomyces henanensis	CBS 12475^T	HQ127627	HQ127626
Scheffersomyces illinoinensis	NRRL Y-48827^T	JN943261	JN703959
Scheffersomyces insectosa	NRRL Y-12854^T	NR_111587	NG_055695
Scheffersomyces jinghongensis	**NYNU 17926^T**	**MG255722**	**MG255714**
Scheffersomyces jinghongensis	**NYNU 17977**	**MT133547**	**MT133543**
Scheffersomyces lignicola	CBS 10610^T	HQ652074	AY845350
Scheffersomyces lignosus	NRRL Y-12856^T	R_120020	NG_055694
Scheffersomyces paraergatensis	**NYNU 16782^T**	**KY213803**	**KY213826**
Scheffersomyces paraergatensis	**NYNU 16969**	**MT133541**	**MT133546**
Scheffersomyces parashehatae.	CBS 12535^T	NR_138230	NG_055697
Scheffersomyces queiroziae	NRRL Y-48722^T	HM566445	HM566445
Scheffersomyces quercinus	NRRL Y-48825^T	JN943260	JN703957
Scheffersomyces segobiensis	NRRL Y-11571^T	NR_111217	NG_055696
Scheffersomyces shehatae	NRRL Y-12858^T	JN943264	JQ025409
Scheffersomyces spartinae	NRRL Y-7322^T	HQ876044	U45764
Scheffersomyces stambukii	CBS 14217^T	KT033721	KT033720
Scheffersomyces stipitis	NRRL Y-7124^T	JN943257	U45741
Scheffersomyces titanus	CBS 13926^T	KP054263	KP054262
Scheffersomyces virginianus	NRRL Y-48822^T	NR_120018	NG_055702
Scheffersomyces xylosifermentans	CBS 12540^T	KY105362	KY109586
Saccharomyces cerevisiae	CBS 1171^T	NR_111007	NG_042623

Abbreviations: **CBS**: CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **NRRL**: the Agricultural Research Service Culture Collection, Peoria, IL, USA; **NYNU**: Microbiology Lab, Nanyang Normal University, Henan, China; **T**: type strain.

bootstrap replicates. The phylogenies from MP and ML analyses were displayed using Mega 7 and FigTree v1.4.3 (Rambaut 2016), respectively. Bootstrap support values ≥ 50% are shown at the nodes.

Results

The alignment was based on the combined sequence dataset (ITS and nrLSU) and included 26 in-group taxa and one out-group taxon (*Saccharomyces cerevisiae* CBS 1171^T), comprised of 1085 characters in the aligned matrix. Of these, 541 characters were constant, 356 variable characters were parsimony-uninformative, and 188 characters were parsimony-informative. The MP analysis resulted in three equally parsimonious trees; the first tree (TL = 589, CI = 0.640, RI = 0.833, RC = 0.533) is shown in Fig. 1. The

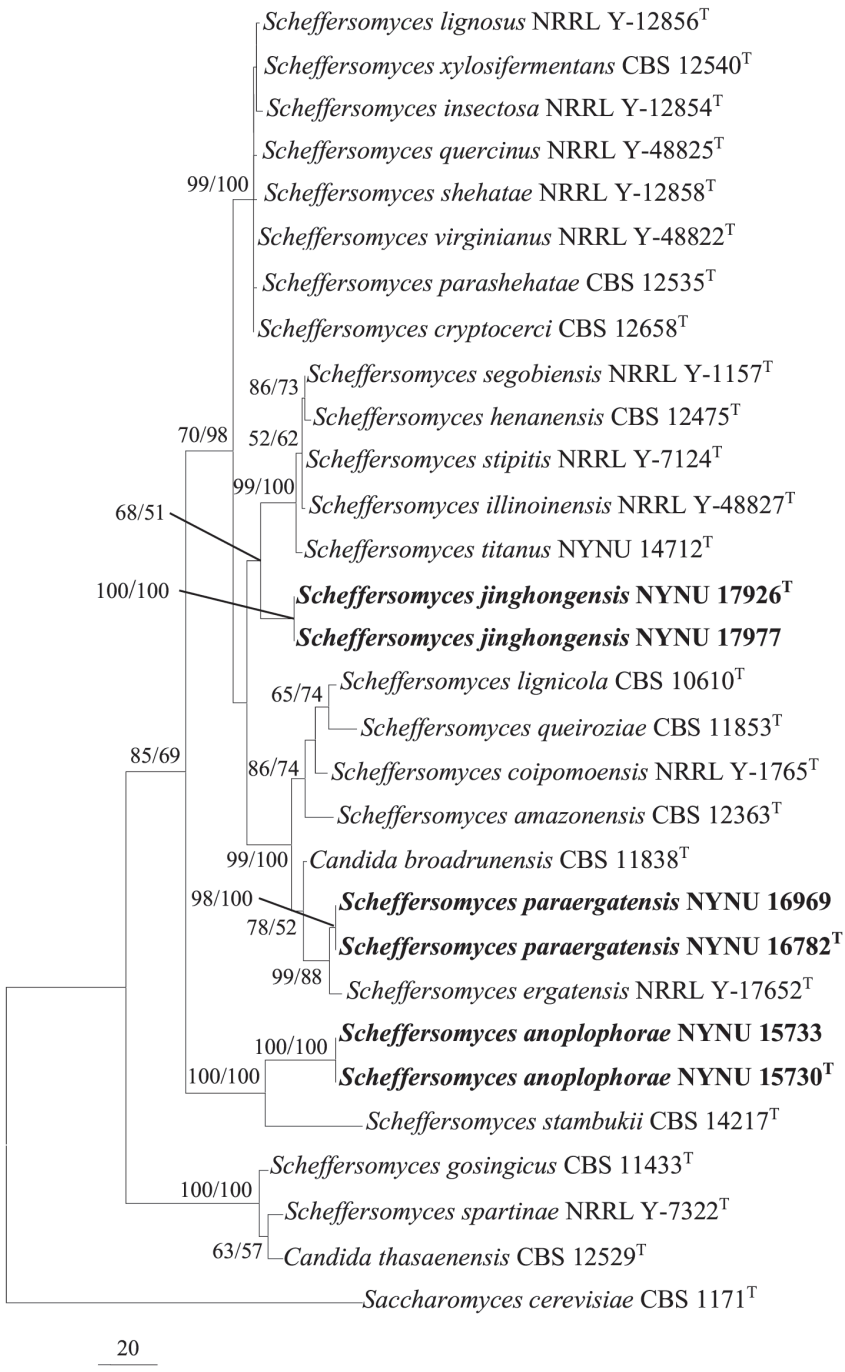

Figure 1. Maximum parsimony phylogenetic tree generated from analysis of combined ITS and nrLSU sequences dataset for 24 taxa of *Scheffersomyces* and related *Candida* species. *Saccharomyces cerevisiae* CBS 1171T is the out-group taxon. Bootstrap values ≥ 50% for MP/ML analyses are presented at the branches. Scale bar = 20 nucleotide substitutions. The species from this study are indicated in bold letters.

three MP trees were identical, except for the species order within different clades. ML analyses revealed that tree topologies of the best tree were identical to those of the MP tree (not shown). The sequences of each three new species formed a well-supported monophyletic group (MP = 98–100%, ML = 100%). *S. paraergatensis* and *S. anoplophorae* were related to *S. ergatensis* and *S. stambukii*, respectively, while *S. jinghongensis* possessed a strongly independent lineage that is distinct from other species (Fig. 1).

Taxonomy

***Scheffersomyces jinghongensis* C.Y. Chai & F.L. Hui, sp. nov.**
MycoBank No: 835004
Figure 2

Etymology. The species name *jinghongensis* (N.L. fem. adj.) refers to the geographical origin of the type strain of this species.

Holotype. NYNU 17926^{T}.

Isolation data. China, Yunnan Province, Jinghong, in rotting wood, under a tropical rainforest, July 2017, K.F. Liu & Z.W. Xi (ex-holotype: CICC 33270; CBS 15230).

Description. The cells are ovoid to elongate (3–4 ×3–7.5 μm) and occur singly or in pairs after being placed in YM broth for 3 days at 25 °C (Fig. 2A). Budding is multilateral. After 3 days of growth on YM agar at 25 °C, the colonies are white to cream-colored, buttery, and smooth, with entire margins. After 7 days at 25 °C on a Dalmau plate culture with CM agar, pseudohyphae were observed but true hyphae were not (Fig. 2B). Asci or signs of conjugation were not observed on sporulation media. Glucose, galactose, trehalose, cellobiose, and D-xylose (weak) are fermented, but maltose, sucrose, melibiose, lactose, melezitose, raffinose, and inulin are not. Glucose, galactose, L-sorbose, D-glucosamine, D-ribose, D-xylose, sucrose, maltose, trehalose, cellobiose, arbutin, melezitose, inulin, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, D-glucono-1, 5-lactone, 2-keto-D-gluconate, D-glucuronate, succinate, and ethanol are assimilated. No growth was observed in L-arabinose, D-arabinose, L-rhamnose, methyl α-D-glucoside, salicin, melibiose, lactose, raffinose, L-arabinitol, galactitol, *myo*-inositol, 5-keto-D-gluconate, D-gluconate, DL-lactate, citrate, or methanol. For the assimilation of nitrogen compounds, growth on L-lysine, glucosamine, or D-tryptophan is positive, while growth on nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine, or imidazole is negative. Growth is observed at 37 °C but not at 40 °C. Growth in the presence of 0.1% cycloheximide is positive, but growth in the presence of 10% NaCl with 5% glucose and 1% acetic acid is negative. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are also negative.

Additional isolate examined. China, Yunnan Province, Jinghong, in rotting wood, under a tropical rainforest, July 2017, K.F. Liu & Z.W. Xi, NYNU 17977.

GenBank accession numbers. holotype NYNU 17926^{T} (ITS:MG255722; nrLSU D1/D2: MG255714); additional isolate NYNU 17977 (ITS: MT133547; nrLSU D1/D2: MT133543).

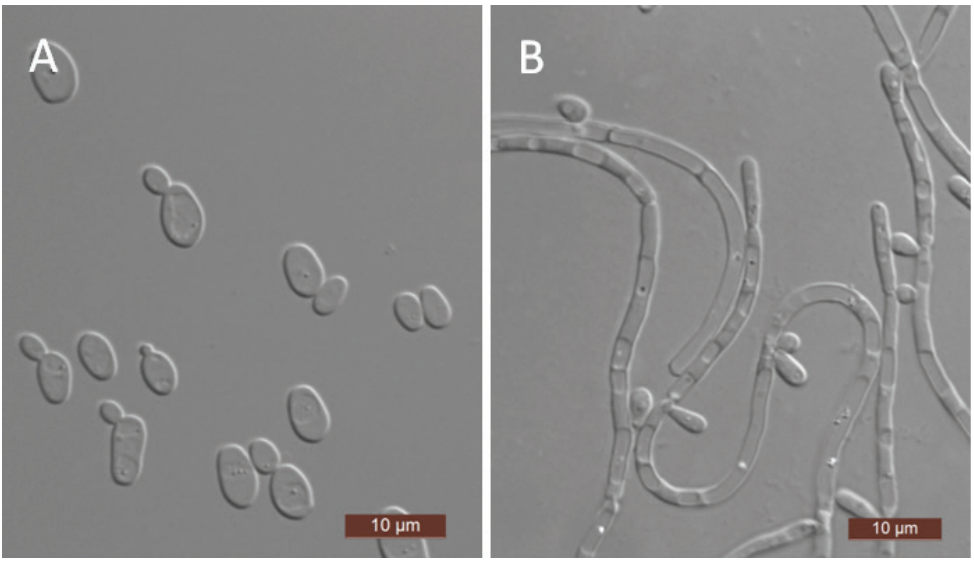

Figure 2. Morphology of *S. jinghongensis*. **A** budding cells **B** pseudohyphae. Scale bars: 10 μm.

Notes. Two strains representing *S. jinghongensis* were grouped in an independent lineage and are related to *S. titanus* and other *Scheffersomyces* species. The nucleotide differences between the new species and the close relative *S. titanus* (Liu et al. 2016b) are 1.6% substitutions in the D1/D2 domain and 4.9% substitutions in the ITS region, respectively. Physiologically, *S. jinghongensis* can be differentiated from *S. titanus* based on growth in L-arabinose, L-rhamnose, methyl α-D-glucoside, salicin, lactose, *myo*-inositol, and 5-keto-D-gluconate, all of which were positive for *S. titanus* and negative for the new species. Additionally, *S. jinghongensis* ferments cellobiose and grows at 37 °C, but not for *S. titanus*.

***Scheffersomyces paraergatensis* C.Y. Chai & F.L. Hui, sp. nov.**
MycoBank No: 835005
Figure 3

Etymology. The species name *paraergatensis* (Gr. prep.) refers to its phylogenetic similarity to *S. ergatensis*.

Holotype. NYNU 16782^{T}.

Isolation data. China, Henan Province, Nanyang, in rotting wood, under a mixed forest, July 2016, K.F. Liu & Z.W. Xi (ex-holotype: CICC 33165; CBS 14694).

Description. The cells are ovoid to elongate (2.5–5×3.5–6 μm) and occur singly or in pairs after grown in a YM broth for 3 days at 25 °C (Fig. 3A). Budding is multilateral. After 3 days of growth on YM agar at 25 °C, the colonies are white to cream-colored, buttery, and smooth with entire margins. After 7 days at 25 °C, on a Dalmau plate culture with CM agar, pseudohyphae were observed but true hyphae were not.

Conjugated asci formed after 6 days at 25 °C on CM agar and 5% ME agar, with each ascus containing one or two hat-shaped ascospores (Fig. 3B). Glucose, galactose, and D-xylose are weakly fermented, but maltose, sucrose, trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, and inulin are not. Glucose, galactose, D-ribose, D-xylose, L-arabinose, D-arabinose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, lactose, raffinose, inulin, glycerol, ribitol, xylitol, D-glucitol, D-mannitol, D-glucono-1, 5-lactone, D-gluconate, succinate, citrate, and ethanol are assimilated. No growth was observed in L-sorbose, D-glucosamine, L-rhamnose, melibiose, melezitose, erythritol, galactitol, *myo*-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucuronate, DL-lactate, or methanol. For the assimilation of nitrogen compounds, growth on L-lysine, glucosamine, and D-tryptophan is positive, while growth on nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine, and imidazole is negative. Growth was observed at 30 °C, but not at 35 °C. Growth in the presence of 0.1% cycloheximide is positive, but growth in the presence of 10% NaCl with 5% glucose and 1% acetic acid is negative. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are also negative.

Additional isolate examined. China, Henan Province, Nanyang, in rotting wood, under a oak forest, August 2016, K.F. Liu & Z.W. Xi, NYNU 16969.

GenBank accession numbers. holotype NYNU 16782^{T} (ITS: KY213803; nrLSU D1/D2: KY213826); additional isolate NYNU 16969 (ITS: MT133541; nrLSU D1/D2: MT133546).

Notes. Two strains formed a group related to *S. ergatensis* and *Candida broadrunensis*, which represent a new species, *S. paraergatensis*. The nucleotide differences between the new species and its closest relative, *S. ergatensis*, were 1.1% substitutions in the D1/D2 domain and 0.8% substitutions in ITS region, respectively. Similarly,

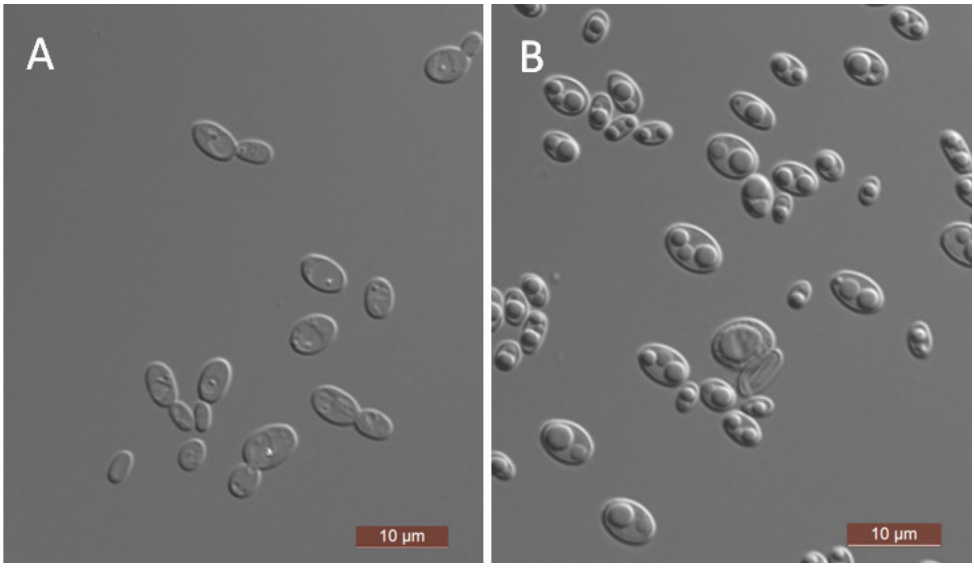

Figure 3. Morphology of *S. paraergatensis*. **A** budding cells **B** ascus and ascospores. Scale bars: 10 μm.

S. paraergatensis and *C. broadrunensis* displayed 0.9% substitutions in the D1/D2 domain and 2.4% substitutions in the ITS region, respectively. Physiologically, *S. paraergatensis* can be differentiated from its closest relative, *S. ergatensis* (Lachance et al. 2011), by its ability to ferment D-xylose and assimilate L-arabinose, raffinose, inulin, and D-gluconate and its inability to assimilate L-sorbose. Additionally, *S. paraergatensis* can grow in 0.1% cycloheximide and at 30 °C, but not for *S. ergatensis*.

Scheffersomyces anoplophorae **C.Y. Chai & F.L. Hui, sp. nov.**
MycoBank No: 835006
Figure 4

Etymology. The species name *anoplophorae* (N.L. fem. Gen. n.) refers to the genus of the host beetle, *Anoplophora leechi*.

Holotype. NYNU 15730^{T}.

Isolation data. China, Henan Province, Nanyang, in the gut of *Anoplophora leechi*, in the People's Park, July 2015, R.C. Ren & K.F. Liu (ex-holotype: CICC 33086; CBS 14170).

Description. The cells are spherical or ovoid (2.5–6 × 2.5–7.5 μm) and occur singly or in pairs (Fig. 4A) when placed in YM broth after 3 days at 25 °C. Budding is multilateral. After 3 days of growth on YM agar at 25 °C, the colonies are white to cream-colored, buttery, and smooth with entire margins. After 12 days at 25 °C on a Dalmau plate culture with CM agar, pseudohyphae were observed but true hyphae were not (Fig. 4B). Asci or signs of conjugation were not observed on sporulation media. Glucose, galactose, trehalose, cellobiose (weak), and D-xylose (weak) are fermented, but maltose, sucrose, melibiose, lactose, melezitose, raffinose, and inulin are not. Glucose, galactose, D-glucosamine, D-xylose, maltose, trehalose, cellobiose, salicin, glycerol, ribitol, D-glucitol, D-mannitol, D-glucono-1, 5-lactone, 2-keto-D-gluconate, 5-keto-D-gluconate, succinate, citrate, and ethanol are all assimilated. No growth was observed in L-sorbose, D-ribose, L-arabinose, D-arabinose, L-rhamnose, sucrose, methyl α-D-glucoside, arbutin, melibiose, lactose, raffinose, melezitose, inulin, erythritol, xylitol, L-arabinitol, galactitol, *myo*-inositol, D-gluconate, D-glucuronate, DL-lactate, or methanol. For the assimilation of nitrogen compounds, growth on L-lysine, glucosamine, or D-tryptophan is positive, while growth on nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine, and imidazole is negative. Growth is observed at 37 °C, but not at 40 °C. Growth in the presence of 0.01% cycloheximide is positive, but growth in the presence of 0.1% cycloheximide, 10% NaCl with 5% glucose, and 1% acetic acid is negative. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are also negative.

Additional isolate examined. China, Henan Province, Nanyang, in the gut of *Anoplophora leechi*, in the People's Park, July 2015, R.C. Ren & K.F. Liu, NYNU 15733.

GenBank accession numbers. holotype NYNU 15730^{T} (ITS: KU128714; nrLSU D1/D2: KU128724); additional isolate NYNU 15733 (ITS: MT133542; nrLSU D1/D2: MT133540).

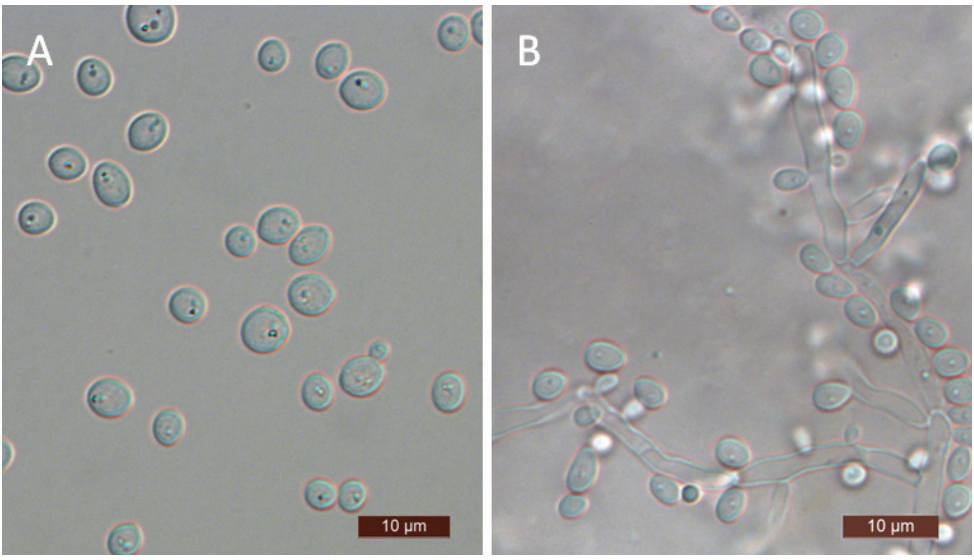

Figure 4. Morphology of *S. anoplophorae*. **A** budding cells **B** pseudohyphae. Scale bars: 10 μm.

Notes. Two strains, representing *S. anoplophorae*, were clustered in a well-supported clade and were phylogenetically related to *S. stambukii* (Lopes et al. 2018). The nucleotide differences between the new species and its closest relative, *S. stambukii*, were 2.3% substitutions in the D1/D2 domain and 6.6% substitutions in the ITS region, respectively. Physiologically, the ability to assimilate D-glucosamine and the inability to assimilate D-gluconate are the primary differences between *S. anoplophorae* and its closest relative, *S. stambukii*. Additionally, *S. stambukii* can grow in 5% glucose medium with 10% NaCl, while *S. anoplophorae* cannot.

Discussion

This study introduced and characterized three new D-xylose-fermenting species based on their morphology and phylogeny: *S. jinghongensis*, *S. paraergatensis*, and *S. anoplophorae*. While these new species share high morphological similarities with their closest relatives, *S. titanus*, *S. ergatensis*, and *S. stambukii*, they are different species due to their physiological traits and nucleotide differences in the D1/D2 domain and ITS region.

The genus *Scheffersomyces* accommodates a monophyletic group based on phylogenetic analyses of two sequence datasets (nrSSU and nrLSU) (Kurtzman and Suzuki 2010). Since it was first proposed, 18 new species and combinations have been categorized in this genus. Kurtzman and Robnett (2013) compared the species type of 70 recognized genera by analyzing sequence divergence in five-genes (nrSSU, nrLSU, *EF-1a*, *RPB1*, and *RPB2*), and determined that the genus *Scheffersomyces* is polyphyletic. *S. spartinae* was placed in a clade with *Spathaspora passalidarum*, distinct from the type species *S. stipitis*, although this placement was weakly supported by statistical

analyses. However, Urbina and Blackwell (2012) suggested that the genus can be divided into three groups based on phylogenetic analysis of a multilocus dataset (nrLSU, ITS, *COXII*, and *MtSm*) with 14 described species. These results were later supported by Ren et al. (2013) and Suh et al. (2013). The results of our phylogenetic analyses of combined gene sequences (ITS and nr LSU) with all currently known species indicated that the genus is not monophyletic, but that it consists of three phylogenetically distinct groups (Fig. 1): (i) *S. stipitis* (the type species), *S. coipomoensis*, *S. shehatae*, and their related species, (ii) *S. stambukii* and *S. anoplophorae* (described in this paper) and (iii) *S. gosingicus*, *S. spartinae*, and *C. thasaenensis*. These results suggest that the genus *Scheffersomyces* should be limited to species of the group comprising the type species *S. stipitis*. The remaining two groups, which have previously been considered members of *Scheffersomyces*, could become two novel genera, although their phylogenetic relationships with other genera were not fully examined by this study (Fig. 1). As such, a careful phylogenetic analysis of *Scheffersomyces* species is required to clarify the possible heterogeneity of the genus.

Yeasts of the genus *Scheffersomyces* have been found to occupy habitats rich in xylose, including rotting wood (Ren et al. 2013; Lopes et al. 2018), wood-feeding insects (Suh et al. 2013; Urbina et al. 2013; Liu et al. 2016b), and the resulting frass (Lachance et al. 2011). We have reported the isolation and identification of several D-xylose-fermenting yeasts from wood-feeding insects and rotting wood (Ren et al. 2013; Liu et al. 2016b), including those detailed in this study. Although the samples of insects and rotting wood were collected in a relatively small geographical area in China, the D-xylose-fermenting yeasts are diverse and several of them were identified as new species (Ren et al. 2013; Liu et al. 2016b). These yeasts demonstrated several physiological traits related to the full utilization of lignocellulosic biomass, such as assimilating and fermenting xylose and/or cellobiose (Ren et al. 2013; Suh et al. 2013). Surveying D-xylose-fermenting yeasts in insects and rotting wood from various regions in different climates will help identify valuable biological and genetic resources to aid in the production of ethanol from biomass.

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RESEARCH ARTICLE

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Multi-gene phylogenetic evidence suggests *Dictyoarthrinium* belongs in Didymosphaeriaceae (Pleosporales, Dothideomycetes) and *Dictyoarthrinium musae* sp. nov. on *Musa* from Thailand

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Abstract

Dead leaves of *Musa* sp. (banana) were collected in northern Thailand during an investigation of saprobic fungi. Preliminary morphological observations revealed that three specimens belong to *Dictyoarthrinium*. Phylogenetic analyses of combined SSU, LSU, ITS and *tef*1-α sequence data revealed that *Dictyoarthrinium* forms a clade in Didymosphaeriaceae (Massarineae, Pleosporales, Dothideomycetes) sister to *Spegazzinia.* Based on contrasting morphological features with the extant taxa of *Dictyoarthrinium*, coupled with the multigene analyses, *Dictyoarthrinium musae* sp. nov. is introduced herein. Our study

provides the first detailed molecular investigation for *Dictyoarthrinium* and supports its placement in Didymosphaeriaceae (Massarineae, Pleosporales, Dothideomycetes). Previously, *Dictyoarthrinium* was classified in Apiosporaceae (Xylariales, Sordariomycetes).

Keywords
Banana, *Dictyoarthrinium sacchari*, DNA sequences, Musaceae, one new species, saprobes, taxonomy

Introduction

Hughes (1953) documented seven hyphomycete genera (*Arthrinium*, *Catenospegazzinia*, *Cordella*, *Dictyoarthrinium*, *Endocalyx*, *Pteroconium* and *Spegazzinia*) that had unique basauxic conidiogenous cell development. Hyde et al. (1998) accommodated *Dictyoarthrinium*, *Endocalyx*, *Scyphospora* (= *Arthrinium*) and *Spegazzinia* in Apiosporaceae (Xylariales, Sordariomycetes), based on morphological characteristics. Based on molecular phylogenetic data (LSU and ITS), *Cordella* and *Pteroconium* were synonymised under *Arthrinium* by Crous and Groenewald (2013) and *Arthrinium* was confirmed as the asexual morph of *Apiospora*. With the availability of molecular data (SSU, LSU, ITS and *tef*1-α), Tanaka et al. (2015) transferred *Spegazzinia* to Didymosphaeriaceae. Wijayawardene et al. (2018) and Hyde et al. (2020) accommodated *Arthrinium*, *Dictyoarthrinium* and *Endocalyx*, all with basauxic conidiogenous cell development, in Apiosporaceae.

Dictyoarthrinium was introduced by Hughes (1952) with *D. quadratum* as the type species. *Dictyoarthrinium africanum* was simultaneously introduced. Damon (1953) re-examined the type material, descriptions and illustrations of *Tetracoccosporium sacchari* (Johnston and Stevenson 1917) and mentioned that *T. sacchari* was congeneric with *Dictyoarthrinium quadratum*. Therefore, Damon (1953) combined *T. sacchari* as *Dictyoarthrinium sacchari*. Damon (1953) also named *D. quadratum* as the heterotypic synonym of *D. sacchari*. Rao and Rao (1964) introduced *D. lilliputeum* and *D. microsporum*, while Kobayasi et al. (1971) introduced *D. rabaulense* as novel taxa to the genus. Somrithipol (2007) introduced *D. synnematicum* and currently seven epithets of *Dictyoarthrinium* are listed in Index Fungorum (2020). All *Dictyoarthrinium* species were introduced, based only on morphological data. Vu et al. (2019) sequenced *D. sacchari* (CBS 529.73) and submitted LSU data to GenBank as the only valid molecular record for the genus.

Dictyoarthrinium is characterised by basauxic conidiogenous cell development (Hughes 1952; Damon 1953; Matsushima 1971). Basauxic development is demonstrated by conidiogenous cells in which elongation occurs at a basal growing point after formation of a single, terminal blastic conidium at its apex (Cole 1976). Conidiophores of *Dictyoarthrinium* are minutely verruculose, subhyaline and transversely septate (Ellis 1971). Usually, the septa are dark brown and appear as thick stripes on the conidiophore. Conidiophore mother cells are often hyaline or pale brown and cup-shaped (Hughes 1952) or subspherical (Ellis 1971). The length of conidiophores

varies within the genus, but in some species, the dimensions are more or less similar. Conidia of *Dictyoarthrinium* arise from the conidiophore at terminal or lateral parts. Conidiogenesis is monoblastic or polyblastic and integrated (Ellis 1971). Conidia are simple, solitary, dematiaceous and often four-celled. Some taxa (e.g. *D. africanum*) have 16-celled conidia (Hughes 1952). The surface of conidia is verruculose and most species have warts on the surface. However, the conidia of *D. rabaulense* are densely echinulate with long spines (Kobayasi 1971). The conidia vary in shape from square to spherical, subspherical or oblong. Most conidia appear flattened on one side. As a specific feature, only *D. synnematicum* possesses synnemata with filaments (Somrithipol 2007). Stroma, setae and hyphopodia have not been observed in *Dictyoarthrinium*.

Many *Dictyoarthrinium* species are saprobes that colonise dead plant materials, although *D. rabaulense* was recorded even from soil and air (Kobayasi et al. 1971; Ellis 1976). Most *Dictyoarthrinium* species occur on monocotyledonous plants. The genus is widely distributed across the tropics, mainly in terrestrial environments (Ellis 1971; 1976). The sexual morph of *Dictyoarthrinium* is unknown. Hosts, substrates and geographical distributions of extant *Dictyoarthrinium* species are listed in Table 1.

A study was undertaken to determine the saprobic fungi associated with *Musa* sp. (banana) in Thailand, during the dry season. Three hyphomycetous taxa that morpho-

Table 1. Hosts, substrates and geographical distribution of *Dictyoarthrinium* species.

Species	Hosts/substrates	Geographical distribution	References
Dictyoarthrinium africanum S. Hughes	*Miscanthus*, *Panicum*, *Paspalum virgatum*, *Saccharum*, leaf litter of *Typha latifolia*	Argentina, Ghana, Solomon Islands, Venezuela	Hughes (1952); Ellis (1971); McKenzie and Jackson (1986); Urtiaga (1986); Tarda et al. (2019)
D. lilliputeum P. Rag. Rao and D. Rao	Leaf litter of *Bambusa*	India	Rao and Rao (1964); Sushma et al. (2020)
D. microsporum P. Rag. Rao and D. Rao	Dead leaves of *Borassus flabellifer*	India	Rao and Rao (1964)
D. rabaulense Matsush.	*Brassica campestris*, *Dendrocalamus strictus*, *Gossypium*, *Xylia xylocarpa*, air and soil	Bismarck Archipelago, Britain, Congo, India, New Caledonia, Nigeria, Tanzania.	Kobayasi et al. (1971); Ellis (1976); Bhat (2010)
D. sacchari (J.A. Stev.) Damon = *D. quadratum* S. Hughes	Dead stems and leaves of *Ananas*, *Bambusa*, *Borassus*, *Cassia*, *Cosmos bipinnatus*, *Cymbopogon*, *Delonix elata*, *Dracaena*, *Erythrina*, *Lithachne pauciflora*, *Musa acuminata*, *M. paradisiaca*, *Neolitsea scrobiculata*, *Pandanus*, *Persea mechrantha*, *Phragmites*, *Prunus amygdalus*, *Saccharum* sp., *S. officinarum*, *S. spontanium*, *Zinnia*, leaf litter of *Typha latifolia*, decaying plant materials of dicots	Brazil, Cuba, Federated Ghana, India, Malaysia, Pakistan, Puerto Rico, Solomon Islands, Spain, States of Micronesia, Thailand, Venezuela, Zambia	Hughes (1952); Subramanian (1952); Nair and Tyagi (1961); Srivastava et al. (1964); Dennis (1970); Ellis (1971); Matsushima (1971); Stevenson (1975); Srivastava and Gupta (1981); Arnold (1986); McKenzie and Jackson (1986); Paul and Singh (1986); Gene et al. (1990); McKenzie and Jackson (1990); Ahmad et al. (1997); Pande and Rao (1998); Lumyong et al. (2003); Saravanan and Vittal (2007); Leão-Ferreira et al. (2010); Tarda et al. (2019)
D. synnematicum Somrith.	Decaying leaves of *Musa* sp.	India, Thailand	Somrithipol (2007)

logically resembled *Dictyoarthrinium* were examined. According to our phylogenetic analyses of combined SSU, LSU, ITS and *tef*1-α sequence data, *Dictyoarthrinium* clustered in Didymosphaeriaceae (Pleosporales, Dothideomycetes) with strong statistical support, sister to *Spegazzinia.* Hence, we propose to transfer *Dictyoarthrinium* from Apiosporaceae (Xylariales, Sordariomycetes) to Didymosphaeriaceae (Pleosporales, Dothideomycetes) and introduce *Dictyoarthrinium musae* sp. nov. as a saprobe recorded from *Musa* sp. We also provide detailed morphological illustrations, descriptions and DNA sequence data for *D. sacchari*, recorded on *Musa* sp. from Thailand, which further validates the novel taxonomic placement of *Dictyoarthrinium* in Didymosphaeriaceae.

Materials and methods

Sample collection, morphological studies and isolation

Dead leaves of *Musa* sp. were collected from Thailand during the dry season (December to August) of 2018 and 2019. Specimens were transferred to the laboratory in cardboard boxes. Samples were examined with a Motic SMZ 168 Series microscope. Powder-like masses of fungal conidia were mounted in water for microscopic studies and photomicrography. The specimens were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 550D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work programme and images used for figures were processed with Adobe Photoshop CS3 Extended v. 10.0 software (Adobe Systems, USA).

Single spore isolation was carried out following the method described in Chomnunti et al. (2014). Germinated spores were individually transferred to potato dextrose agar (PDA) plates and incubated at 25 °C in daylight. Colony characteristics were observed and measured after 3 weeks at 25 °C. Herbarium specimens were deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures were deposited in the Culture Collection of Mae Fah Luang University (MFLUCC). Faces of fungi numbers (Jayasiri et al. 2015) and MycoBank numbers (http://www.MycoBank.org) were obtained for the respective taxa.

DNA extraction, PCR amplification and sequencing

Fungal isolates grown on potato dextrose agar (PDA) for 4 weeks at 25 °C were used to extract total genomic DNA. DNA was extracted from 50 to 100 mg of axenic mycelium of the 4-weeks-old growing cultures. The mycelium was ground to a fine powder in liquid nitrogen and fungal DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) according to the manufacturer's instructions. Four gene regions, the internal transcribed spacer (ITS), partial 18S small sub unit (SSU), partial 28S large sub unit (LSU) and partial translation elongation fac-

tor 1-alpha gene (*tef*1-α) were amplified using ITS5/ITS4 (White et al. 1990), NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990) and EF1-983F /EF1-2218R (Rehner 2001) primers, respectively.

Polymerase chain reactions (PCR) were conducted according to the following protocol. The total volume of the PCR reaction was 25 µl and consisted of 12.5 µl of 2 × Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µlTaq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl pH 8.3, 100 mMKCl, 3 mM $MgCl_2$, stabiliser and enhancer), 1 µl of each primer (10 pM), 2 µl genomic DNA extract and 8.5 µl double distilled water (ddH_2O). The reaction was conducted by running for 40 cycles. The annealing temperature was 56 °C for ITS and LSU, 57.2 °C for *tef*1-α and 55 °C for SSU and initially 95 °C for 3 min, denaturation at 95 °C for 30 seconds, annealing for 1 min, elongation at 72 °C for 30 seconds and final extension at 72 °C for 10 min for all gene regions. PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR fragments were sent to a commercial sequencing provider (TsingKe Biological Technology Co., Beijing, China). The nucleotide sequence data acquired were deposited in GenBank.

Sequence alignment

Sequences obtained in this study were subjected to BLAST search in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST search results and initial morphological studies supported that our isolates belong to Didymosphaeriaceae. Other sequences used in the analyses were obtained from GenBank based on recently published papers (Tanaka et al. 2015; Jayasiri et al. 2019) (Table 2) and BLAST search results. The single gene alignments were done by MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/large.html; Katoh et al. 2019) using the default settings and later refined, where necessary, using BioEdit v. 7.0.5.2 (Hall 1999).

Table 2. Selected taxa with their corresponding GenBank accession numbers in the family Didymosphaeriaceae that are used in the phylogenetic analyses. Type strains are indicated as superscript T and newly-generated strains are indicated in bold.

Taxa	Culture collection	ITS	LSU	SSU	*tef*1-α
Alloconiothyrium aptrootii	CBS 980.95^T	JX496121	JX496234	NA	NA
A. aptrootii	CBS 981.95^T	JX496122	JX496235	NA	NA
Austropleospora archidendri	CBS 168.77^T	JX496049	JX496162	NA	NA
A. keteleeriae	MFLUCC 18-1551^T	NR_163349	MK348021	MK347910	MK360045
Bambusistroma didymosporum	MFLU 15-0057^T	KP761733	KP761730	KP761737	KP761727
B. didymosporum	MFLU 15-0058	KP761734	KP761731	KP761738	KP761728
Bimuria novae zelandiae	CBS 107.79^T	MH861181	AY016356	AY016338	DQ471087
Chromolaenicola lampangensis	MFLUCC 17-1462^T	MN325016	MN325004	MN325010	MN335649
C. thailandensis	MFLUCC 17-1510^T	MN325018	MN325006	MN325012	MN335651
Cylindroaseptospora leucaenae	MFLUCC 17-2424^T	NR_163333	NG_066310	MK347856	MK360047

Taxa	Culture collection	ITS	LSU	SSU	*tef*1-α
Deniquelata barringtoniae	MFLUCC 11-0422[T]	NR_111779	NG_042696	JX254656	NA
D. vittalii	NFCCI4249[T]	MF406218	MF182395	MF622059	MF182398
Dictyoarthrinium musae	**MFLUCC 20-0105[T]**	**MT482323**	**MT482320**	**MT482326**	**MT495602**
D. musae	**MFLUCC 20-0106[T]**	**MT482324**	**MT482321**	**MT482327**	**MT495603**
D. sacchari	**MFLUCC 20-0107**	**MT482325**	**MT482322**	**MT482328**	NA
D. sacchari	CBS 529.73	NA	MH872479	NA	NA
Didymocrea sadasivanii	CBS 438.65[T]	MH858658	DQ384103	NA	NA
Didymosphaeria rubi-ulmifolii	MFLUCC 14-0023[T]	NA	KJ436586	NG_063557	NA
D. rubi-ulmifolii	MFLUCC 14-0024	NA	KJ436585	KJ436587	NA
Kalmusia italica	MFLUCC 14-0560[T]	KP325440	KP325441	KP325442	NA
K. variisporum	CBS 121.517[T]	NR_145165	JX496143	NA	NA
Kalmusibambusa triseptata	MFLUCC 13-0232[T]	KY682697	KY682695	KY682696	NA
Karstenula rhodostoma	CBS 690.94	NA	GU301821	GU296154	GU349067
K. rhodostoma	CBS 691.94	LC014559	AB807531	AB797241	AB808506
Laburnicola hawksworthii	MFLUCC 13-0602[T]	KU743194	KU743195	KU743196	NA
L. muriformis	MFLUCC 14-0921[T]	KU743200	KU743201	KU743202	NA
Letendraea cordylinicola	MFLUCC 11-0150	KM213996	KM213999	KM214002	NA
L. cordylinicola	MFLUCC 11-0148[T]	NR_154118	NG_059530	KM214001	NA
Montagnula bellevaliae	MFLUCC 14-0924[T]	KT443906	KT443902	KT443904	KX949743
M. cirsii	MFLUCC 13-0680	KX274242	KX274249	KX274255	KX284707
M. scabiosae	MFLUCC 14-0954[T]	KT443907	KT443903	KT443905	NA
Neokalmusia brevispora	KT 1466[T]	LC014573	AB524600	AB524459	AB539112
N. scabrispora	KT 1023	LC014575	AB524593	AB524452	AB539106
Neptunomyces aureus	CMG12[T]	MK912121	NA	NA	MK948000
N. aureus	CMG13	MK912122	NA	NA	MK948001
Paracamarosporium fagi	CPC 24890	KR611886	KR611904	NA	NA
P. fagi	CPC 24892[T]	KR611887	KR611905	NA	NA
Paraconiothyrium cyclothyrioides	CBS 972.95[T]	JX496119	JX496232	AY642524	NA
Paramassariosphaeria anthostomoides	CBS 615.86	MH862005	GU205223	GU205246	NA
P. anthostomoides	MFLU 16-0172[T]	KU743206	KU743207	KU743208	NA
Paraphaeosphaeria rosae	MFLUCC 17-2549[T]	MG828937	MG829046	MG829152	MG829223
P. rosicola	MFLUCC 15-0042[T]	NR_157528	MG829047	MG829153	NA
Phaeodothis winteri	CBS 182.58	NA	GU301857	GU296183	NA
Pseudocamarosporium propinquum	MFLUCC 13-0544	KJ747049	KJ813280	KJ819949	NA
P. pteleae	MFLUCC 17-0724[T]	NR_157536	MG829061	MG829166	MG829233
Pseudopithomyces entadae	MFLUCC 17-0917[T]	NA	NG_066305	MK347835	MK360083
P. rosae	MFLUCC 15-0035[T]	MG828953	MG829064	MG829168	NA
Spegazzinia bromeliacearum	URM 8084[T]	MK804501	MK809513	NA	NA
S. deightonii	MFLUCC 20-0002	MN956768	MN956772	MN956770	NA
S. intermedia	CBS 249.89[T]	MH862171	MH873861	NA	NA
S. lobulata	CBS 361.58[T]	MH857812	MH869344	NA	NA
S. musae	MFLUCC 20-0001[T]	MN930512	MN930514	MN930513	NA
S. neosundara	MFLUCC 15-0456[T]	KX965728	KX954397	KX986341	NA
S. radermacherae	MFLUCC 17-2285[T]	MK347740	MK347957	MK347848	MK360088
S. tessarthra	SH 287	JQ673429	AB807584	AB797294	AB808560
Trematei arundicola	MFLU 16-1275[T]	KX274241	KX274248	KX274254	KX284706
T. guiyangensis	GZAAS01[T]	KX274240	KX274247	KX274253	KX284705
T. murispora	GZCC 18-2787[T]	NR_165916	MK972751	MK972750	MK986482
Verrucoconiothyrium nitidae	CBS:119209	EU552112	NA	NA	NA
Xenocamarosporium acaciae	CBS:139895	NR_137982	NG_058163	NA	NA
X. acaciae	MFLUCC 17-2432	MK347766	MK347983	MK347873	MK360093

*Abbreviations of culture collections: **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, **CPC**: Working collection of Pedro Crous housed at CBS, **GZAAS**: Guizhou Academy of Agricultural Sciences Herbarium, China, **KT**: K. Tanaka, **MFLU**: Mae Fah Luang University, Chiang Rai, Thailand, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, **SH**: Academia Sinica People's Republic of China. Shanghai, **URM**: Universidade Federal de Pernambuco.

Phylogenetic analyses

Maximum Likelihood (ML) trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008; Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) using GTR+I+G model of evolution. Bootstrap supports were obtained by running 1000 pseudo-replicates. Maximum Likelihood bootstrap values (ML) ≥ 60% are given above each node of the phylogenetic tree in blue (Fig. 1).

Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (BMCMC). Two parallel runs were conducted, using the default settings, but with the following adjustments: four simultaneous Markov chains were run for 2,000,000 generations, trees were sampled every 100th generation and 20,001 trees were obtained. The first 4,000 trees, representing the burn-in phase of the analyses, were discarded. The remaining 16,001 trees were used for calculating PP in the majority rule consensus tree. Branches with Bayesian posterior probabilities (BYPP) ≥ 0.95 are indicated above each node of the phylogenetic tree (Fig. 1). Phylogenetic trees were visualised with the FigTree v1.4.0 programme (Rambaut 2011).

Results

Phylogenetic analyses

The combined SSU, LSU, ITS and *tef*1-α matrix comprised 61 sequences that represents the genera in Didymosphaeriaceae. The best scoring RAxML tree is shown (Fig. 1) with a final ML optimisation likelihood value of -19278.64. The matrix had 1091 distinct alignment patterns, with 39.08% of undetermined characters or gaps. Estimated base frequencies were: A = 0.234095, C = 0.252628, G = 0.278053, T = 0.235224; substitution rates AC = 1.252730, AG = 2.198875, AT = 1.318760, CG = 0.953798, CT = 5.276095, GT = 1.000000; proportion of invariable sites I = 0.491333; gamma distribution shape parameter α = 0.446418. All trees (ML and BYPP) were similar in topology and did not differ at the generic relationships, which are in agreement with multi-gene phylogeny of Tanaka et al. (2015) and Jayasiri et al. (2019). All *Dictyoarthrinium* strains analysed herein clustered as a highly-supported monophyletic clade (ML = 100%, BYPP = 1.00) in Didymosphaeriaceae (Fig. 1) sister to *Spegazzinia* (ML = 75%, BYPP = 0.98). We have included LSU sequence data of *D. sacchari* (CBS 529.73) of Vu et al. (2019) in our phylogenetic analyses. According to GenBank, CBS 529.73 was classified in Apiosporaceae (Sordariomycetes). In our analyses, *D. sacchari* (CBS 529.73) clustered with MFLUCC 20-0105, MFLUCC 20-0106 and MFLUCC 20-0107 strains in Didymosphaeriaceae with a strong statistical support (ML = 100%, BYPP = 1.00). Our strain MFLUCC 20-0107 grouped with *D. sacchari* (CBS 529.73). The novel isolates of *D. musae* (MFLUCC 20-0105 and MFLUCC 20-0106) were sister to *D. sacchari* (CBS 529.73 and MFLUCC 20-0107) with strong statistical support (ML = 100%, BYPP = 1.00).

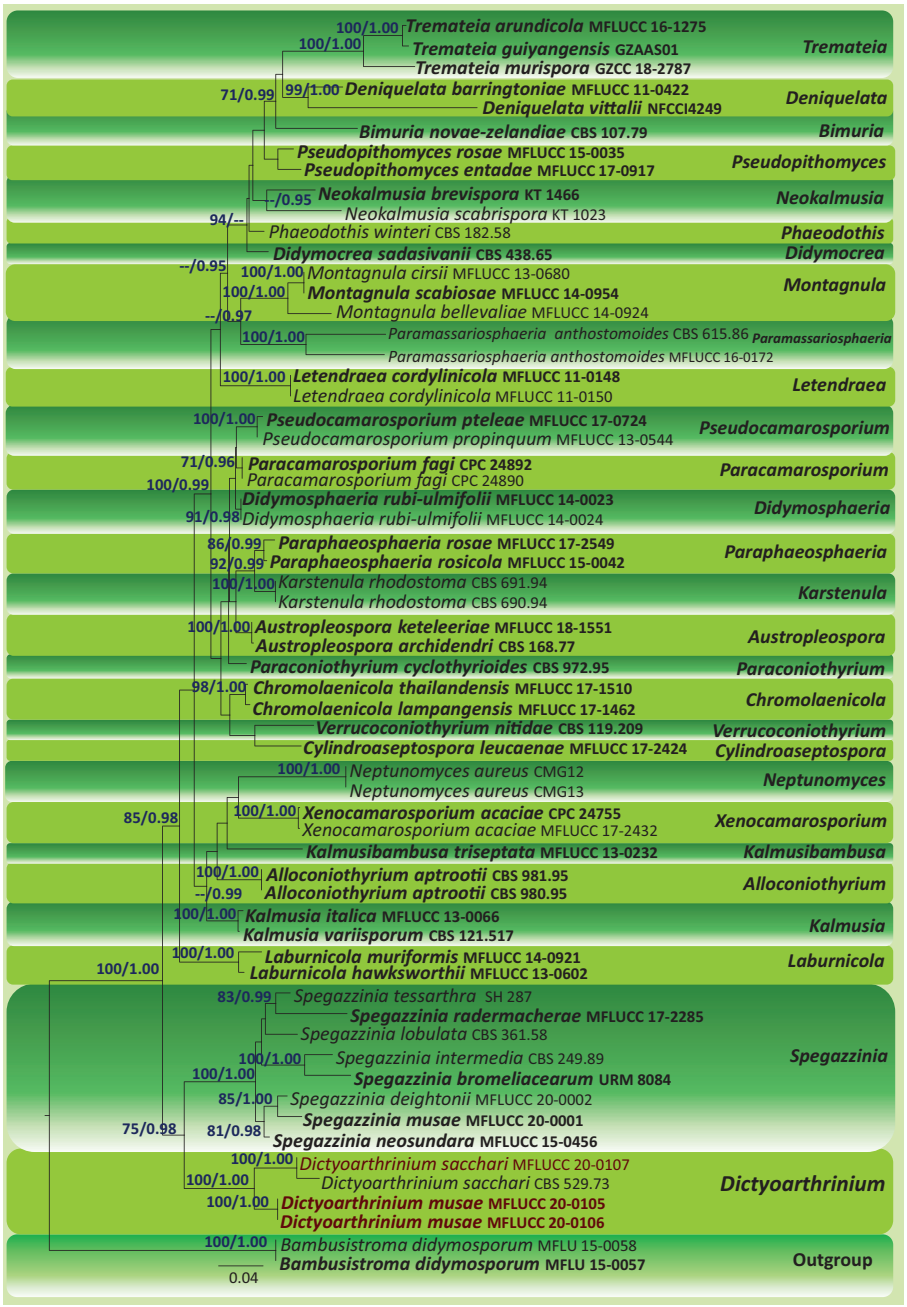

Figure 1. Maximum Likelihood tree revealed by RAxML from an analysis of SSU, LSU and ITS and *tef*1-α sequence data of the genera of Didymosphaeriaceae, showing the phylogenetic position of *Dictyoarthrinium musae* (MFLUCC 20-0105, MFLUCC 20-0106) and *D. sacchari* (MFLUCC 20-0107). ML bootstrap supports (≥ 60%) and Bayesian posterior probabilities (≥ 0.95 BYPP) are given above the branches, respectively. The tree is rooted with *Bambusistroma didymosporum* (MFLU 15-0057 and MFLU 15-0058). Strains generated in this study are indicated in brown bold type. Ex-type strains are indicated in black bold. The scale bar represents the expected number of nucleotide substitutions per site.

Taxonomy

Dictyoarthrinium musae **Samarakoon, Chomnunti & K.D. Hyde, sp. nov.**
MycoBank No: 835764
Facesoffungi Number: FoF08467
Figure 2

Etymology. Name reflects the host genus, *Musa* (Musaceae).

Holotype. MFLU 20-0437

Description. *Saprobic* on dead leaves of *Musa* sp. **Sexual morph:** Undetermined. **Asexual morph:** *Colonies* compact or effuse, black, often pulvinate. *Mycelium* superficial, a close network of branched and anastomosing hyphae. *Stromata* none. *Setae* and *hyphopodia* absent. *Conidiophores* 30–140 × 1–2 µm ($\bar{x}$ 81.5 × 1.6 µm, n = 25), basauxic, arising usually singly from subspherical, subhyaline to light brown conidiophore mother cells, 4.5–4.8 × 4.3–4.5 µm ($\bar{x}$ = 4.6 × 4.4 µm, n = 10), macronematous, mononematous, straight or flexuous, narrow, cylindrical, rough, subhyaline to pale brown, with thick brown or dark brown transverse septa that appear as stripes with distances of 6.3–5.8 µm at apex and 2.3–3 µm at base of the conidiophore. *Conidiogenous cells* 4.1–4.5 × 4.3–4.7 µm ($\bar{x}$ = 4.4 × 4.5 µm, n = 10), blastic, integrated, terminal and intercalary, cylindrical, smooth, denticles absent, hyaline. *Conidia* 7–11.5 × 6.5–9 µm ($\bar{x}$ = 8.7 × 7.9 µm, n = 40), solitary, dry, acropleurogenous, simple, square, rounded at the corners, 4-celled, spherical or subspherical, often flattened in one plane, pale to dark brown at maturity, verrucose, with light brown to dark brown warts, immature conidia often 1-celled and subhyaline. Terminal conidium with four cells, sometimes absent or fallen before lateral conidia, mature conidia split along one line of the septa, most conidia arranged obliquely downwards on the conidiophore, conidial formation observed as a bunch starting after conidiophore 1–3 septate.

Culture characteristics. Conidia germinating on PDA within 18 hrs. Colonies on PDA reaching a diameter of 50 mm after 14 days at 25 °C, slightly raised, hairy, filamentous, moderately dense, middle light grey, periphery white; reverse white to greyish-white.

Material examined. THAILAND. Chiang Rai. On dead leaves of *Musa* sp. (Musaceae), 7 December 2018, M. C. Samarakoon, BNS265 (MFLU 20-0437, ***holotype***), ex-type living culture (MFLUCC 20-0105); *ibid*. 20 February 2019, B. C. Samarakoon BNS2239 (MFLU 20-0438, ***paratype***), ex-paratype living culture (MFLUCC 20-0106).

Notes. Based on BLAST search results of SSU, LSU, ITS and *tef*1-α sequence data, *Dictyoarthrinium musae* (MFLUCC 20-0105 and MFLUCC 20-0106) showed high similarity as follows: SSU = 99.15% to *Paraconiothyrium hawaiiense* (CBS 120025), LSU = 95.57% to *Cylindroaseptospora siamensis* (MFLUCC 17-2527), ITS = 98.24% to *Kalmusia italica* (isolate 5), *tef*1-α = 97.75% to *Spegazzinia neosundara* (MFLUCC 13-0211) with 100%, 100%, 87% and 99% query covers, respectively. In the multigene phylogeny, the *Dictyoarthrinium* clade was sister to *Spegazzinia* (ML = 75%, BYPP = 0.98). Within the *Dictyoarthrinium* clade, *D. musae* (MFLUCC 20-0105 and MFLUCC 20-0106) separated from the sister taxon, *D. sacchari* with strong statisti-

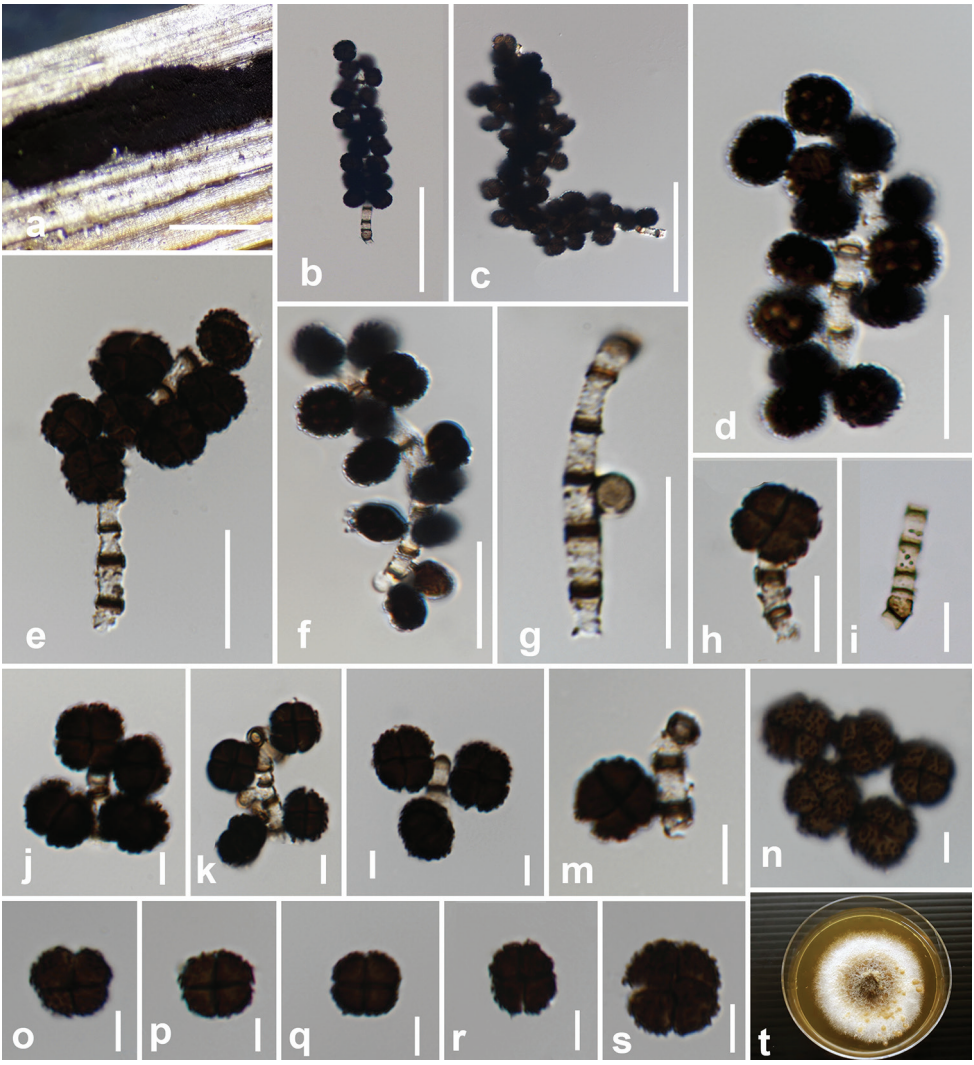

Figure 2. *Dictyoarthrinium musae* (MFLU 20-0437, holotype) **a** conidia on the host **b** conidiophore and conidia with conidiophore mother cell **c–f** conidia with conidiophores on stalk **g** developmental stage of an immature lateral conidium **h** four-celled terminal conidium **i** conidiophore **j** conidiophores and conidia with terminal conidium **k, l** conidiophores without terminal conidium **m** attachment of a mature lateral conidium **n–q** warted four-celled mature conidia **r, s** mature conidia that split at septa **t** colony on PDA after 21 days. Scale bars: 500 μm (**a**); 50 μm (**b, c**); 20 μm (**d–g, i**); 10 μm (**h**); 5 μm (**j–s**).

cal support (ML = 100%, BYPP = 1.00). ITS sequence comparison revealed 7.84% base pair differences between *D. musae* and *D. sacchari* (MFLUCC 20-0107), which is in agreement with the new species concept outlined by Jeewon and Hyde (2016). *Dictyoarthrinium musae* differs from *D. sacchari* by its unique conidial development in the apex. The terminal conidia of *D. musae* are always 4-celled and similar in colour

to mature lateral conidia. In addition, the terminal conidia of *D. musae* are sometimes absent or fallen before the lateral conidia. In contrast, the terminal conidia of *D. sacchari* can be 2-celled or 4-celled, pale brown with respect to lateral mature conidia and always persist on the conidiophore. In addition, the mature conidia of *D. musae* split along one line of the septa and this specific feature is absent in *D. sacchari*. *Dictyoarthrinium musae* has a subhyaline, spherical conidiophore mother cell while *D. sacchari* has a distinct cup-shaped, brown conidiophore mother cell. Therefore, based on contrasting morphological differences to *D. sacchari* and strong statistical support from our molecular phylogeny, *D. musae* is herein introduced as a new species.

***Dictyoarthrinium sacchari* (J.A. Stev.) Damon, Bull. Torrey bot. Club 80: 164 (1953)**
Facesoffungi Number: FoF08468
Figure 3

Description. *Saprobic* on dead leaves of *Musa* sp. **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* compact or effuse, black, often pulvinate. *Mycelium* superficial, a close network of branched and anastomosing hyphae. *Stromata* none. *Setae* and *hyphopodia* absent. *Conidiophores* 50–110 × 1–2 µm ($\bar{x}$ = 72.0 × 1.6 µm, n = 15), basauxic, arising from cup-shaped, brown, distinct conidiophore mother cells, 3.4–4.4 × 2.9–4.7 µm ($\bar{x}$ = 4 × 3.7 µm, n = 10), macronematous, mononematous, usually straight or flexuous, narrow, cylindrical, rough-walled, subhyaline to pale brown, with dark brown transverse septa as stripes with distances of 6.3–5.8 µm at apex and 2.3–3 µm at base of the conidiophore. *Conidiogenous cells* 4–4.5 × 4.3–4.7 µm ($\bar{x}$ = 4.4 × 4.5 µm, n = 10), blastic, integrated, terminal and intercalary, cylindrical, smooth, hyaline. *Conidia* at maturity 8.5–11.5 × 8.5–10 µm ($\bar{x}$ = 9.9 × 9.3 µm, n = 40), solitary, dry, acropleurogenous, simple, square, rounded at the corners, 4-celled, but difficult to distinguish the cells due to their blackish-brown nature, spherical or subspherical, often flattened in one plane, blackish-brown at maturity, with brown warts on surface of the cells, terminal conidium always 4-celled or 2-celled, light brown when compared with lateral conidia, most conidia arranged perpendicular to the conidiophore, some directed obliquely upwards.

Culture characteristics. Conidia germinating on PDA within 18 hrs. Colonies on PDA reaching a diameter of 55 mm after 14 days at 25 °C, raised, moderately dense, entire margined, brownish-grey at maturity; reverse white to greyish-white.

Material examined. Thailand, Chiang Mai. On mid-rib of a dead leaf of *Musa* sp. (Musaceae), S. Phongeun, 18 July 2018, BNS2287, (MFLU 20-0439), living culture MFLUCC 20-0107.

Notes. Based on BLAST search results of SSU, LSU, ITS and *tef*1-α sequence data, our strain (MFLUCC 20-0107) showed high similarity to the taxa in GenBank as follows (SSU = 99.26% to *Paraconiothyrium brasiliense* (isolate GF1), LSU = 96.14% to *Alloconiothyrium aptrooti* (CBS 981.95), ITS = 93.00% to *Kalmusia italica* (MFLUCC 13-0066). In the multigene phylogeny, MFLUCC 20-0107 groups with *Dictyoarthrinium sacchari*, sister to *D. musae* with strong statistical support (ML = 100%, BYPP =

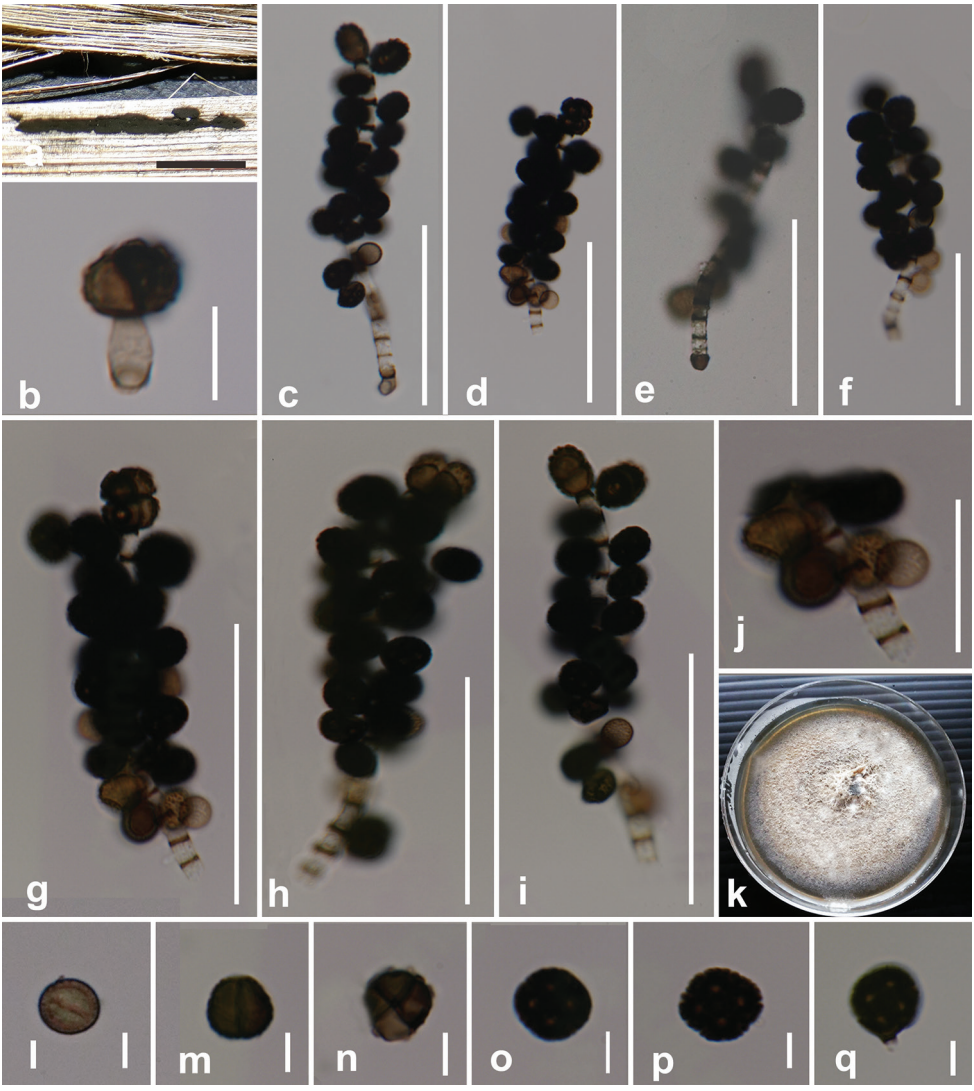

Figure 3. *Dictyoarthrinium sacchari* (MFLU 20-0439) **a** conidia on the host **b** developmental stage of terminal conidium attached to the conidiophore **c–f** Conidiophores and conidia (**e**, with distinct mother cell) **g, h** mature conidiophores with four-celled terminal conidium **i** conidiophore with two celled terminal conidium **j** developmental stages of conidia on conidiophore **k** colony on PDA after 21 days **l–q** conidia. Scale bars: a = 1000 μm (**a**); 20 μm (**b, j**); 50 μm (**c–i**); 5 μm (**l–q**).

1.00). Our strain shares similar morphological features with *D. sacchari* (Subramanium 1952; Ellis 1971) and did not differ significantly. There are slight differences in conidial dimensions and the length of conidiophores of our collection and other *D. sacchari* collections by previous studies. Conidial dimensions and the length of conidiophores may differ due to diverse environmental effects and host associations. LSU sequence data of *D. sacchari* (CBS 529.73) are identical with our strain (MFLUCC 20-0107). Unfortunately, ITS, SSU and *tef*1-α sequence data of CBS 529.73 are not

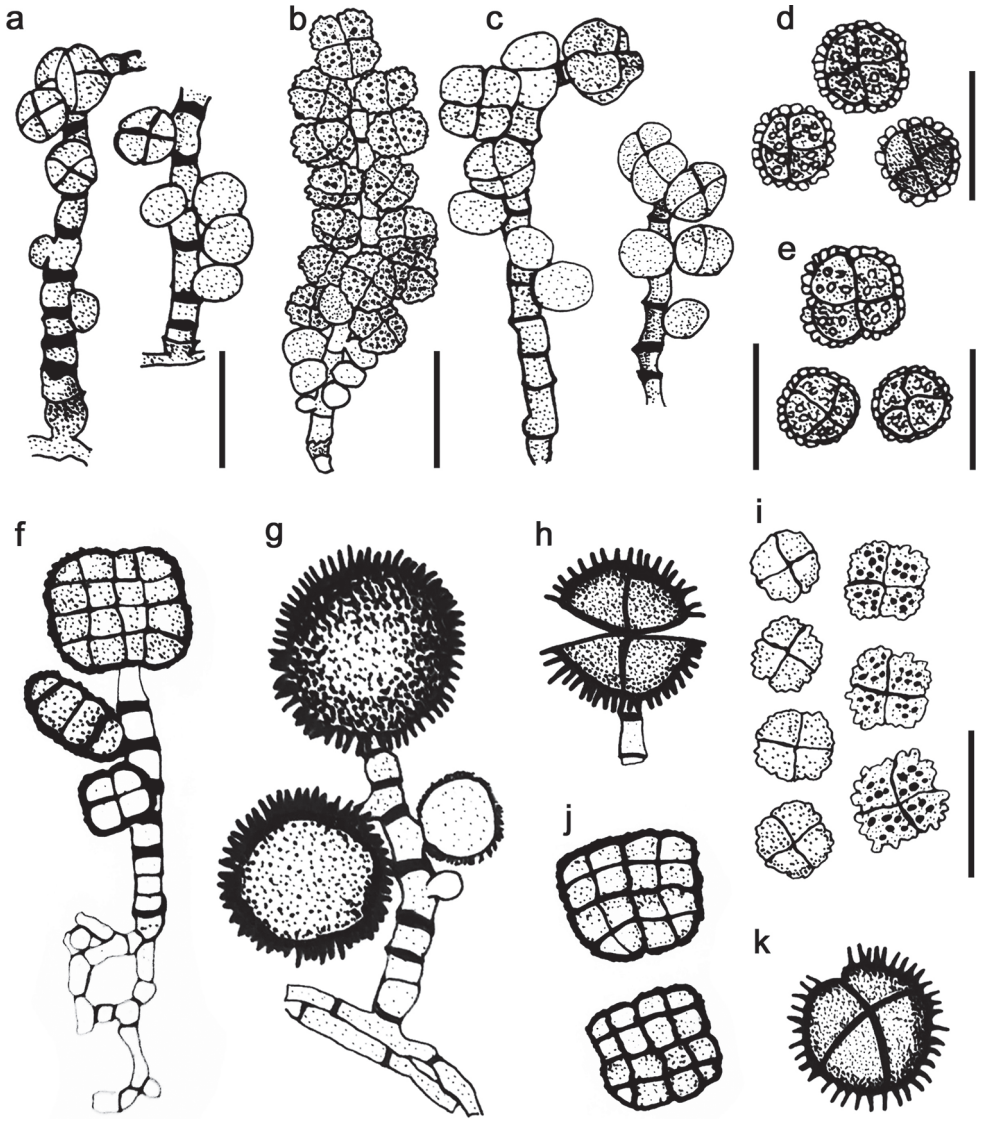

Figure 4. Morphology of conidia and conidiophores of previously described *Dictyoarthrinium* species **a, d** *D. microsporum* **b, i** *D. synnematicum* **c, e** *D. lilliputeum* **f, j** *D. africanum* **g, h, k** *D. rabaulense.* Scale bars: 20 μm (**a, c, d, e**); 10 μm (**b, i**). Magnification × 650 (**f, g, h, j, k**). Redrawn from Rao and Rao (1964), Ellis (1971), Kobayasi et al. (1971) and Somrithipol (2007).

available in GenBank to compare with our strain. LSU data of *Dictyoarthrinium musae* have 2.24% of base pair difference with *D. sacchari* (CBS 529.73 and MFLUCC 20-0107). *Dictyoarthrinium sacchari* was reported on *Musa* sp. from Thailand in Lumyong et al. (2003) without morpho-molecular justifications. In this study, we document *D. sacchari* with detailed morphological illustrations, description, herbarium material and a living culture coupled with DNA sequence data (SSU, LSU, ITS) for a better taxonomic resolution.

Discussion

Both *Dictyoarthrinium* and *Spegazzinia* are characterised by basauxic conidiophores (Hughes 1952; Ellis 1971; Tanaka et al. 2015). *Spegazzinia* often has stellate (α) and disc-shaped (β) conidia (Ellis 1971; Tanaka et al. 2015). The conidia of *Dictyoarthrinium* (except *D. africanum*) share some similar characteristics with disc-shaped, β conidia of *Spegazzinia*. Both conidia are brown, 4-celled and constricted at the septa. Conidia of *Dictyoarthrinium* have characteristic hyaline or brown warts. Rarely, some taxa of *Spegazzinia*, for example, *S. deightonii*, also bear blunt ended spines. Most disc-shaped conidia of *Spegazzinia* are not warted. In addition, stellate conidia of *Spegazzinia* are always 4–5-celled and spinulose (Ellis 1971; Tanaka et al. 2015). There are contrasting morphological features of the basauxic conidiophores of both genera. The conidiophores of *Dictyoarthrinium* are hyaline to subhyaline with septa that appear as dark brown or light brown stripes throughout the conidiophore. The conidiophores (in stellate conidia) of *Spegazzinia* are more elongated, narrow, aseptate and dematiaceous.

Dictyoarthrinium quadratum (type of *Dictyoarthrinium*) is the heterotypic synonym of *D. sacchari. Dictyoarthrinium quadratum* has a terminal mature conidium with one to two cells. As described in Hughes (1952), these 2-celled conidia remain on the conidiophore, even when other conidia fall off. This feature is absent in *D. musae*. The terminal conidium of *D. musae* always ends up with four cells. The conidia of *D. quadratum* are obliquely upwardly directed, whereas the conidia of *D. musae* are obliquely downwardly directed (Fig. 2). The conidiophores of *D. quadratum* are erect and straight while *D. musae* has more curved conidiophores.

Dictyoarthrinium africanum differs significantly from *D. musae* by having 16-celled conidia. The conidia of *D. rabaulense* are completely black and densely echinulate with spines sometimes up to 4 μm long (Ellis 1976). However, *D. musae* has brown warts on the surface of conidia, while *D. lilliputeum* has hyaline warts. *Dictyoarthrinium microsporum* has longer conidiophores (250 μm) than *D. musae*. Morphological features of *Dictyoarthrinium* species are illustrated in Fig. 4. A key to the species of *Dictyoarthrinium* is provided below.

Key to the species of *Dictyoarthrinium*

1 Synnemata present ..... ***D. synnematicum***
– Synnemata absent **2**
2 Conidia 2- or 4-celled **3**
– Conidia 16-celled ..... ***D. africanum***
3 Conidia with brown warts **4**
– Conidia with hyaline warts ..... ***D. lilliputeum***
4 Conidiophores up to 130 μm long **5**
– Conidiophores up to 250 μm long ..... ***D. microsporum***

5 Terminal conidium always 4-celled, mature conidia split along one line of the septa..... ***D. musae***

– Terminal conidium 2- or 4-celled, mature conidia do not split along septa ... .***D. sacchari***

To date, the taxonomy and phylogeny of most genera that have basauxic conidiogenesis (Hughes 1952) have been resolved with their correct taxonomic placements. *Dictyoarthrinium* and *Endocalyx* represented the sole unresolved genera. We transferred *Dictyoarthrinium* to Didymosphaeriaceae based on morphological and molecular evidence. This study uses multigene sequence data of SSU, LSU, ITS and *tef*1-α for the first time to confirm the taxonomic placement of *Dictyoarthrinium* in Didymosphaeriaceae.

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RESEARCH ARTICLE

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Ascomycetes from the Qilian Mountains, China – Hypocreales

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Abstract

To investigate fungi from the Qilian Mountains in Gansu Province, ascomycetous specimens were collected and hypocrealean fungi were examined. Eighteen species belonging to six genera in the families Hypocreaceae and Nectriaceae were identified, including 11 species of *Hypomyces* and *Trichoderma* in Hypocreaceae and seven species of *Nectria*, *Stylonectria*, *Thelonectria*, and *Thyronectria* in Nectriaceae. Among them, *Stylonectria qilianshanensis* and *Trichoderma gansuanum* are new to science. DNA sequence analyses of combined ACL1, ITS, RPB2, and TEF1 regions confirmed their taxonomic placements. Morphological distinctions between the new species and their close relatives are discussed. *Hypomyces tremellicola* is reported for the first time in China.

Keywords

Biodiversity, Hypocreaceae, Nectriaceae, sequence analysis, taxonomy

Introduction

The Qilian Mountains is located across the northeastern Qinghai and western Gansu provinces (35°50'–39°19'N, 94°10'–103°04'E) at elevations ranging from 4000 to 6000 m. The area has a temperate climate with mean annual precipitation around 400 mm. Forests are composed mainly of mixed broad-leaf and coniferous trees. The Qilian Mountain National Nature Reserve, where ascomycetes were surveyed, is ex-

tremely diverse in climate, vegetation, and geographic structure. Previous investigations of fungal resources have mainly focused on Basidiomycota (Gui et al. 2010; Xi et al. 2011). Our understanding of ascomycetes of the region needs to be broadened.

The order Hypocreales includes about 2700 species in 240 genera, which are divided into 12 families (Rossman et al. 1999; Lumbsch and Huhndorf 2007; Kirk et al. 2008; Lombard et al. 2015; Maharachchikumbura et al. 2016; Sun et al. 2017; Zhuang and Zeng 2017). Hypocrealean collections from the Qilian Mountains belong to the families Hypocreaceae and Nectriaceae. These families are ubiquitous in nature and exhibit very high species diversity in temperate and tropical regions (Rossman 1996; Rossman et al. 1999). They are economically important in fields of industry, environment protection, and agriculture. For instance, some species of *Trichoderma* Pers. play vital roles in production of industrial enzymes and antibiotics (Jangiret et al. 2017), while several species of *Hypomyces* (Fr.) Tul. & C. Tul. are pathogens of cultivated mushrooms (Tamm and Põldmaa 2013). Some members of *Nectria* (Fr.) Fr., *Thelonectria* P. Chaverri & C. Salgado, and *Thyronectria* Sacc. cause *Abies* and *Rubus* cankers (Hirooka et al. 2011, 2012; Salgado-Salazar et al. 2015). Therefore, discovery of fungi in Hypocreales is of theoretical and practical importance. Improvement and updating our knowledge of the group will provide useful information about sustainable utilization and conservation of natural resources.

Studies on fungi of this group in China dates back to 1895 when *Trichoderma cornu-damae* (Pat.) Z.X. Zhu & W.Y. Zhuang (as *Hypocrea cornu-damae* Pat.) was reported on rotten wood in Sichuan Province (Patouillard 1895). Early studies were initiated by Teng (1934, 1935, 1936, 1963), and recent studies are summarized by Liang (2007), Zhuang (2013), and Zhuang and Zeng (2017). A survey of ascomycetes in the Qilian Mountains was carried out in 2018. A total of 67 specimens were examined in this study. Eighteen taxa belonging to seven genera were identified, including 11 species of *Hypomyces* and *Trichoderma* in Hypocreaceae, and seven of *Nectria*, *Thelonectria*, *Thyronectria*, and *Stylonectria* Höhn. in Nectriaceae. *Stylonectria qilianshanensis* and *Trichoderma gansuanum* are described and illustrated as new species. *Hypomyces tremellicola* is reported for the first time from China.

Materials and methods

Sampling and morphological studies

Specimens were collected from the Qilian Mountains in Gansu Province, and they are deposited in the Herbarium Mycologicum Academia Sinica (HMAS). Cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. The methods of Luo and Zhuang (2010) and Põldmaa et al. (2019) were followed for morphological observations. The ascomatal wall reactions to 3% potassium hydroxide (KOH) and 100% lactic acid (LA) were tested. To observe microscopic characteristics of perithecial walls, sections were made with a freezing microtome (YD-1508-III, Jinhua, China) at a thickness of 6–8 μm.

Cotton blue lactophenol solution and lactic acid solution were used as mounting media for examinations of anatomical structures and measurements of perithecia, asci, and ascospores. Photographs were taken with a Leica DFC450 digital camera (Wetzlar, Germany) attached to a Leica M125 stereomicroscope (Milton Keynes, UK) for gross morphology, and a Zeiss AxioCamMRc 5 digital camera (Jena, Germany) attached to a Zeiss Axio Imager A2 microscope (Göttingen, Germany) for anatomy. Measurements of individual structures were based on n = 30, except as otherwise noted. The culture of *Hypomyces tremellicola* was isolated from conidia found on the surface of the host. To determine colony features and growth rates, strains were grown on malt extract agar [MEA, 2% (w/v) malt extract+ 2% (w/v) agar] and potato dextrose agar [PDA, 20% (w/v) potato + 2% (w/v) dextrose + 2% (w/v) agar] in 90 mm plastic Petri dishes at 25 °C for 7 d. For observation of conidiophores and conidia, cultures were grown on PDA at 25 °C with alternating periods of light and darkness (12 h/12 h).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dry specimens or fresh mycelia following the method of Wang and Zhuang (2004). Four primer pairs, acl1-230up/acl1-1220low (Nowrousian et al. 2000), ITS5/ITS4 (White et al. 1990), RPB2-5f/RPB2-7cR (Liu et al. 1999), EF1-728F/TEF1LLErev (Carbone and Kohn 1999; Jaklitsch et al. 2005) were used to amplify the ACL1, ITS, RPB2, and TEF1 gene regions, respectively. PCR reactions were performed using an ABI 2720 Thermal Cycler (Applied Biosciences, Foster City, USA) with a 25 µl reaction system consisting of 12.5 µl Taq MasterMix, 1 µl each primer (10 µM), 1 µl template DNA, and 9.5 µl ddH_2O. DNA sequencing was carried out in both directions on an ABI 3730XL DNA Sequencer (Applied Biosciences, Foster City, USA) based on the procedures detailed in Gräfenhan et al. (2011), Jaklitsch et al. (2005), and Chaverri et al. (2011).

Sequence alignment and phylogenetic analyses

Newly obtained sequences and those retrieved from GenBank are listed in Tables 1 and 2, respectively. The sequences were assembled, aligned and the primer sequences were trimmed using BioEdit 7.0.5 (Hall 1999), and converted to NEXUS files by ClustalX 1.83 (Thompson et al. 1997). The aligned sequences were combined in BioEdit and analyzed with Bayesian inference (BI) and maximum parsimony (MP) methods to determine the phylogenetic positions of the new species. The MP analysis was performed with PAUP 4.0b10 (Swofford 2002) using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR (tree bisection and reconnection) branch swapping. Topological confidence of the resulting trees was tested by maximum parsimony bootstrap proportion (BP) with 1000 replications, each with 10 replicates of random addition of taxa. The Bayesian inference (BI) analysis was conducted by

Table 1. List of *Stylonectria* species and the relatives, herbarium/strain numbers and GenBank accession numbers of materials used in this study.

Species	Herbarium/strain numbers	GenBank accession numbers		
		ACL1	ITS	RPB2
Albonectri rigidiuscula (Berk. & Broome) Rossman & Samuels	CBS 122570	HQ897896	HQ897815	HQ897760
Clonostachys rosea (Preuss) Mussat	CML817/CBS 114056	KX184866	KC806254	DQ522415
Cyanonectria cyanostoma (Sacc. & Flageolet) Samuels & P. Chaverri	CBS 101734	HQ897895	FJ474076	HQ897759
Dialonectria episphaeria (Tode) Cooke	CBS 125494	HQ897892	HQ897811	HQ897756
Fusarium sambucinum Fuckel	CBS 14695	KM231015	KM231813	KM232381
Fusicolla matuoi (Hosoya & Tubaki) Gräfenhan & Seifert	CBS 58178	HQ897858	KM231822	HQ897720
Geejayessia cicatricum (Berk.) Schroers	CBS 125552	HQ728171	HQ728145	HQ728153
Macroconia leptosphaeriae (Niessl) Gräfenhan & Schroers	CBS 100001	HQ897891	HQ897810	HQ897755
Microcera coccophila Desm.	CBS 31034	HQ897843	HQ897794	HQ897705
Neocosmospora vasinfecta E.F. Sm.	CBS 32554	KM231005	KM231803	KM232370
Stylonectria applanata Höhn.	CBS 125489	HQ897875	HQ897805	HQ897739
Stylonectria carpini Gräfenhan	DAOM 235819	HQ897909	HQ897823	HQ897773
Stylonectria norvegica Lechat, J. Fourn. & Nordén	CBS 139239	–	NR154415	–
Stylonectria purtonii (Grev.) Gräfenhan	DAOM 235818	HQ897919	HQ897831	HQ897783
Stylonectria qilianshanensis Z.Q. Zeng & W.Y. Zhuang	HMAS 255803	**MT087289**[a]	**MT084413**	**MT087288**
Stylonectria wegeliniana (Rehm) Gräfenhan, Voglmayr & Jaklitsch	CBS 125490	HQ897890	KM231817	HQ897754
Trichoderma parareesei Atan., Jaklitsch, Komoń-Zel., C.P. Kubicek & Druzhin.	CBS 125925	KJ665112	MH863773	HM182963

[a] Numbers in bold indicate the newly provided sequences.

MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using a Markov chain Monte Carlo algorithm. MrModeltest v. 2.3 was used to determine the nucleotide substitution models (Nylander 2004). Four Markov chains were run simultaneously for 1000000 generations with the trees sampled every 100 generations. A 50% majority rule consensus tree was computed after excluding the first 2500 trees as 'burn-in'. Bayesian inference posterior probability (PP) was determined from the remaining trees. Branch support measures were calculated with 1000 bootstrap replicates. Trees were examined by TreeView 1.6.6 (Page 1996). The BIPP greater than 90% and MPBP greater than 70% were shown at the nodes.

Results

To determine taxonomic positions of the *Hypomyces* collections, sequences of ITS and 28S rDNA were searched against the NCBI GenBank database using BLASTN. Sequence comparisons showed that HMAS 247843 shares 99% sequence similarity with *H. tremellicola*, which confirmed its taxonomic position in the genus.

To place the *Stylonectria* specime n within a phylogenetic context, sequences of ACL1, ITS, and RPB2 regions from 15 species of the genus and relatives were analyzed

Table 2. List of *Trichoderma* species, herbarium/strain numbers, and GenBank accession numbers of specimens used in this study.

Species	Herbarium/ strain numbers	GenBank accession numbers	
		RPB2	TEF1
Hypocrella discoidea (Berk. & Broome) Sacc.	BCC 8237	DQ452461	–
Hypocrella nectrioides Thaxt.	GJS 8910	DQ522448	–
Trichoderma alutaceum Jaklitsch	CBS 120535	FJ179600	FJ179567
	CBS 33269	FJ179601	FJ179568
Trichoderma gansuanum Z.Q. Zeng & W.Y. Zhuang	HMAS 279687	**MT087287** [a]	**MT095060**
Trichoderma gelatinosum P. Chaverri & Samuels	CPK 1618	FJ179604	FJ179569
Trichoderma leucopus Jaklitsch	CBS 122495	FJ179606	FJ179570
	CBS 122499	FJ179605	FJ179571
Trichoderma lixii (Pat.) P. Chaverri	CPK 1934	FJ179608	FJ179573
Trichoderma minutisporum Bissett	CBS 121276	FJ179610	FJ179574
Trichoderma nybergianum (T. Ulvinen & H.L. Chamb.) Jaklitsch & Voglmayr	CBS 122496	FJ179612	FJ179576
	CBS 122500	FJ179611	FJ179575
Trichoderma parapiluliferum (B.S. Lu, Druzhin. & Samuels) Jaklitsch & Voglmayr	CBS 20921	FJ179614	FJ179578
Trichoderma pezizoides (Berk. & Broome) Samuels, Jaklitsch & Voglmayr	GJS 01257	EU248608	AY937438
Trichoderma piluliferum J. Webster & Rifai	CBS 120927	KJ842159	FJ860674
Trichoderma placentula Jaklitsch	CBS 120924	FJ179616	FJ179580
Trichoderma polysporum (Link) Rifai	CPK 3131	FJ860558	FJ860661
Trichoderma poronioideum (Möller) Samuels	GJS 01203	–	KP109823
Trichoderma seppoi Jaklitsch	CBS 122497	FJ179618	FJ179582
	CBS 122498	FJ179617	FJ179581
Trichoderma strictipile Bissett	CPK 1601	FJ860594	FJ860704

[a] Numbers in bold indicate the newly provided sequences.

using BI and MP methods. *Clonostachys rosea* (Preuss) Mussat and *Trichoderma parareesei* Atan., Jaklitsch, Komoń-Zel., C.P. Kubicek & Druzhin. were used as outgroup taxa. The partition homogeneity test (PHT) (P = 0.01) indicated that the individual partitions were not highly incongruent (Cunningham 1997), the three loci were thus combined for phylogenetic analyses. The combined datasets include 2197 characters, of which 964 were constant, 310 were variable and parsimony-uninformative and 923 were parsimony-informative. The MP analysis resulted in two most parsimonious trees (tree length = 4081, CI = 0.5285, HI = 0.4715, RI = 0.4459, RCI = 0.2357) with similar topology. The final matrix was deposited in TreeBASE with accession no. S25189. The BI tree is shown in Figure 1. The MP tree is similar to that of the BI tree in topology. HMAS 255803 was associated with other *Stylonectria* species forming a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed its taxonomic position in the genus.

To place the *Trichoderma* collections with clavate fruit bodies within a phylogenetic context, the sequences of RPB2 and TEF1 from 15 species of the genus were analyzed using BI and MP. *Hypocrella discoidea* (Berk. & Broome) Sacc. and *Hypocrella nectrioides* Thaxt. were used as outgroup taxa. The PHT (P = 0.01) indicated that the individual parti-

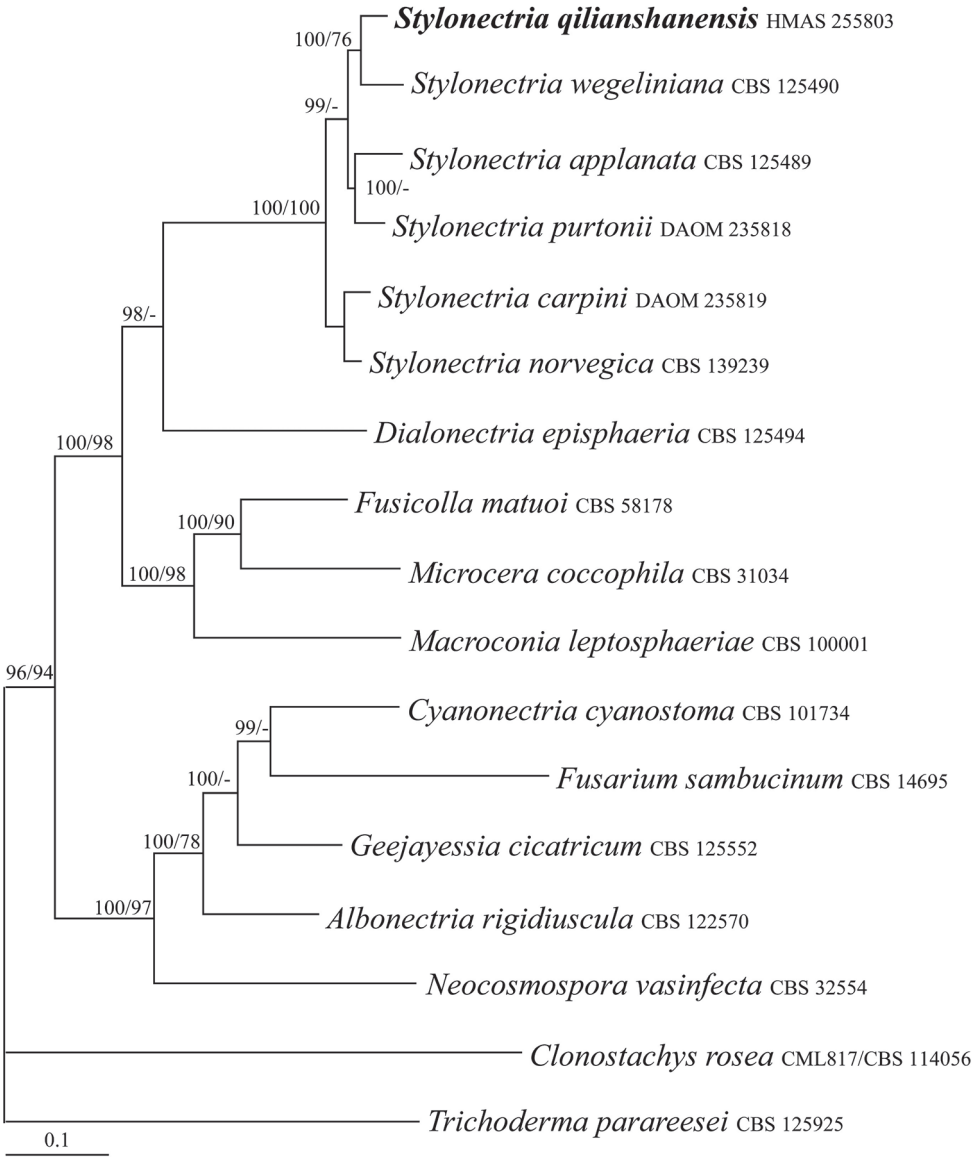

Figure 1. BI tree generated based on the combined datasets of ACL1, ITS and RPB2 sequences of *Stylonectria* and its relatives. Supporting values showing at branches: BIPP (left) and MPBP (right). BIPP greater than 90% and MPBP greater than 70% are shown at the nodes.

tions were not highly incongruent (Cunningham 1997), the two loci were thus combined for phylogenetic analyses. The combined datasets include 1436 characters, of which 688 were constant, 167 were variable and parsimony-uninformative and 581 were parsimony-informative. The MP analysis resulted in one most parsimonious tree (tree length =1828, CI = 0.6510, HI = 0.3490, RI = 0.6706, RCI = 0.4366). The final matrix was deposited in TreeBASE with accession no. S25188. The BI tree is shown in Figure 2, which is similar

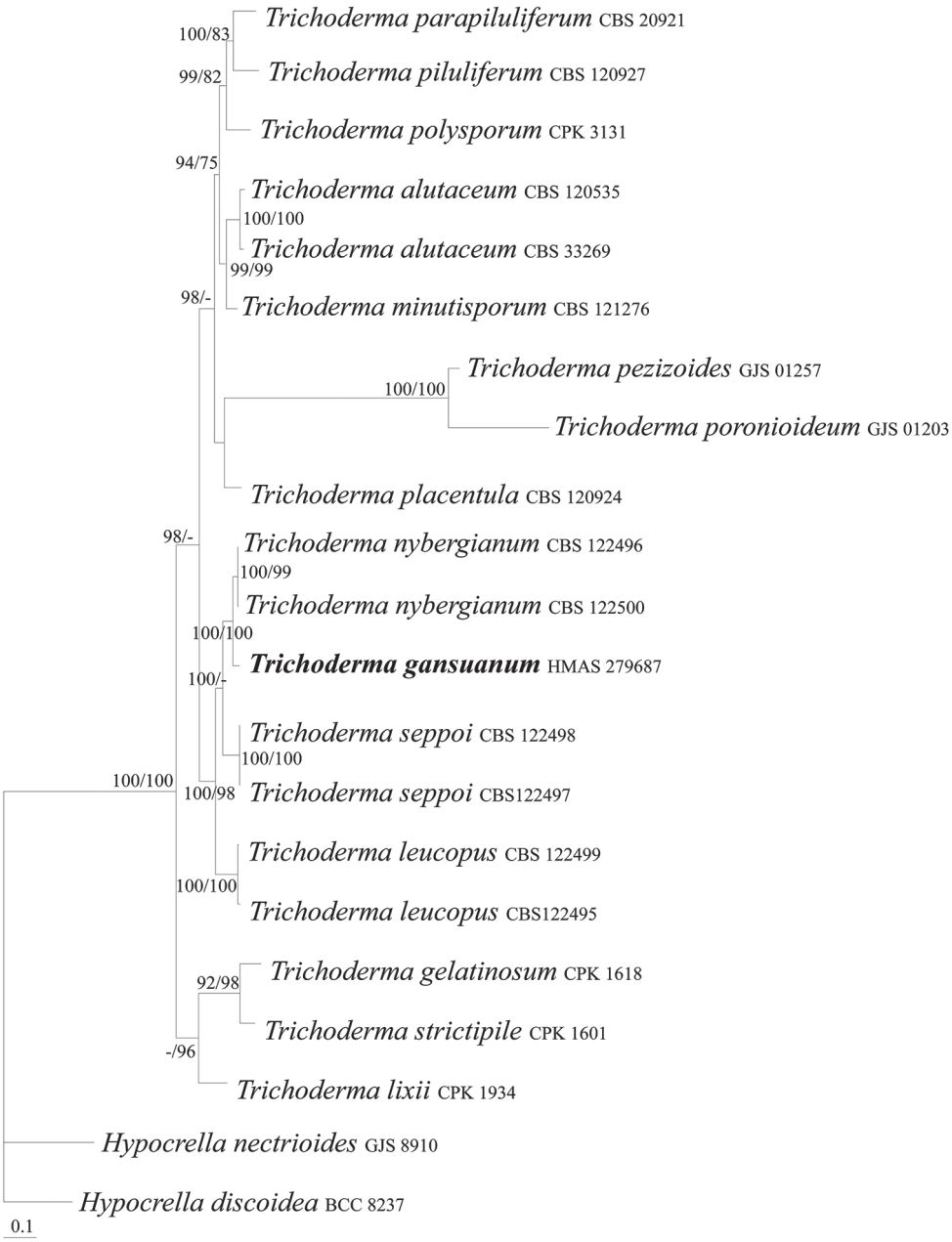

Figure 2. BI tree generated based on the combined datasets of RPB2 and TEF1 sequences of *Trichoderma* species. Supporting values showing at branches: BIPP (left) and MPBP (right). BIPP greater than 90% and MPBP greater than 70% are shown at the nodes.

to the MP tree in topology. Among the investigated species, HMAS 279687 was distinct from but associated with other *Trichoderma* species forming a highly supported monophyletic group (BIPP/MPBP = 100%/100%), which confirmed its taxonomic position.

Taxonomy

***Stylonectria qilianshanensis* Z.Q. Zeng & W.Y. Zhuang, sp. nov.**
Fungal Names: FN 570729
Figure 3

Holotype. China. Gansu Province, Wuwei, Chashugou, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 26 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12155 (HMAS 255803).

Paratypes. China. Zhangye, Longchanghe, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 24 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12016, 12017 (HMAS 255804, 255805); Kangle, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 24 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12035, 12036, 12037, 12038 (HMAS 255806, 255807, 255808, 255809); Shandan, Yanzhishan, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 25 August 2018, H.D. Zheng, X.C. Wang & Z.Q. Zeng 12082 (HMAS 255810); Yanzhishan, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 25 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12086, 12087, 12088, 12089, 12090 (HMAS 255811, 255812, 255813, 255814, 255815); Wuwei, Chashugou, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 26 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12148, 12153, 12156, 12158 (HMAS 255816, 255817, 255818, 279708); Tianzhu, Kelacun, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 27 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12229 (HMAS 279709); Haxi, on decayed fruitbodies of an ascomycete on bark of *Picea asperata*, 28 August 2018, Z.Q. Zeng, X.C. Wang & H.D. Zheng 12276, 12278 (HMAS 255819, 255820).

Etymology. The specific epithet refers to the type locality.

Description. Perithecia gregarious, up to 30 in a group, parasitic on decayed fruitbodies of an ascomycete, deep red to dark red, turning black red in 3% KOH and light yellow in 100% LA, subglobose to globose, not becoming cupulate upon drying, (216–)255–344 × (186–)206–304 µm (n = 12), apex broadly discoid, flattened, 50–70 µm high, 160–220 µm in diameter, slightly constricted below, with a tiny papilla. Perithecial wall of two layers, 25–38 µm thick, outer layer 20–31 µm, of textura angularis, cells 5–8 × 2–4 µm, walls 0.8–1.0 µm thick; inner layer 5–7 µm, of textura prismatica, cells 8–12.5 × 3–5 µm, walls 0.5–0.8 µm thick. Asci clavate, with an apical ring, 8-spored, 55–88 × 5–8(–10) µm. Ascospores ellipsoidal, ends rounded, 1-septate, light brown, smooth, uniseriate, 10–13 × 5–5.5 µm. Asexual state unknown.

Distribution. China.

Notes. *Stylonectria qilianshanensis* is morphologically similar to *S. wegeliniana* in having the perithecia with a broad, discoid apex, clavate asci with an apical ring, and ellipsoidal ascospores with rounded ends (Petch 1938). However, *S. qilianshanensis* differs in having smaller asci [55–88 × 5–8(–10) µm vs 90–100 × 9–10 µm] and ascospores (10–13 × 5–5.5 µm vs 10–18 × 6–9 µm) (Petch 1938). Sequence comparisons indicate that ITS of *S. qilianshanensis* differs from that of *S. wegeliniana* by 20 bp in a total length of 576 bp; ACL1 and RPB2 of the former differ from those of the

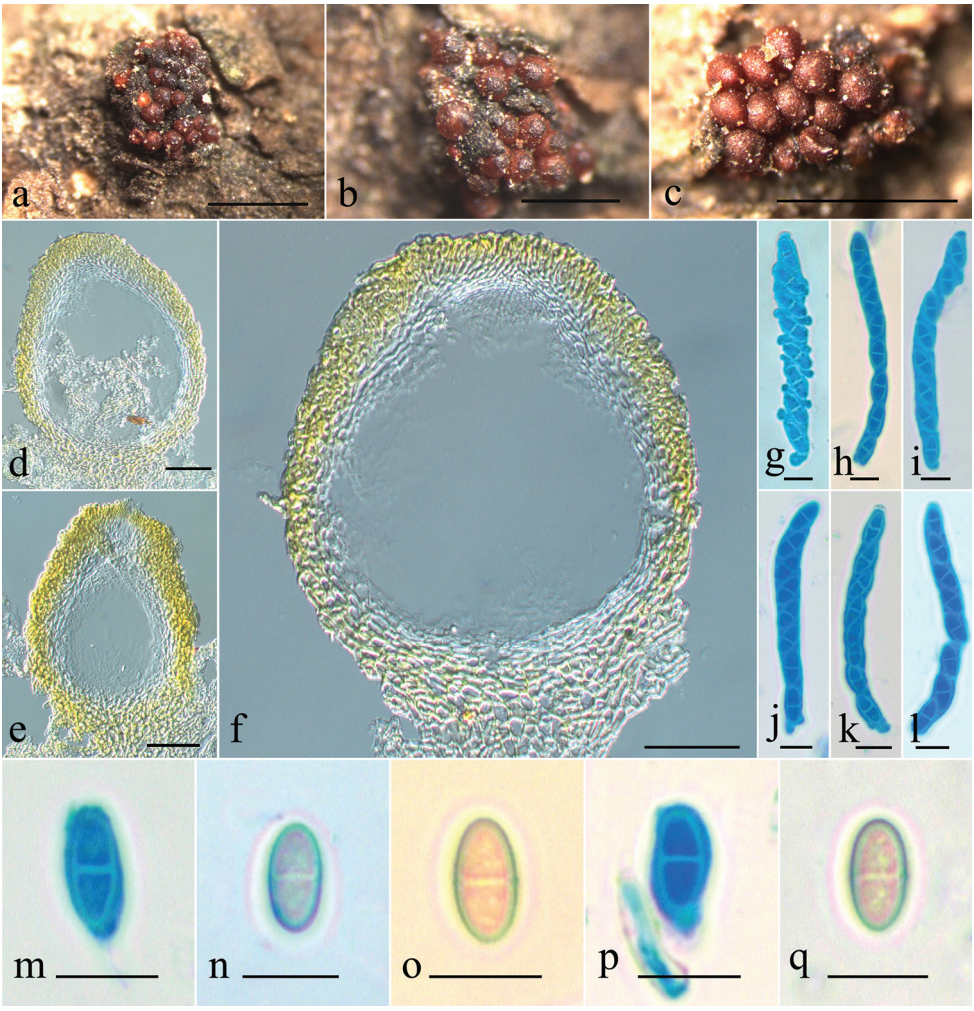

Figure 3. *Stylonectria qilianshanensis* **a–c** ascomata on natural substrate **d–f** median section through perithecium **g–l** ascus with ascospores **m–q** ascospores. From HMAS 255803. Scale bars: 1 mm (**a–c**); 50 μm (**d–f**); 10 μm (**g–q**).

latter by 84 bp and 38 bp, respectively among 722 bp and of 869 bp in length. The asexual state of the fungus remains unknown until culture is available.

***Trichoderma gansuanum* Z.Q. Zeng & W.Y. Zhuang, sp. nov.**
Fungal Names: FN 570730
Figure 4

Holotype. China. Gansu Province, Shandan, Yanzhishan, on mossy humus, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12100 (HMAS 279687).

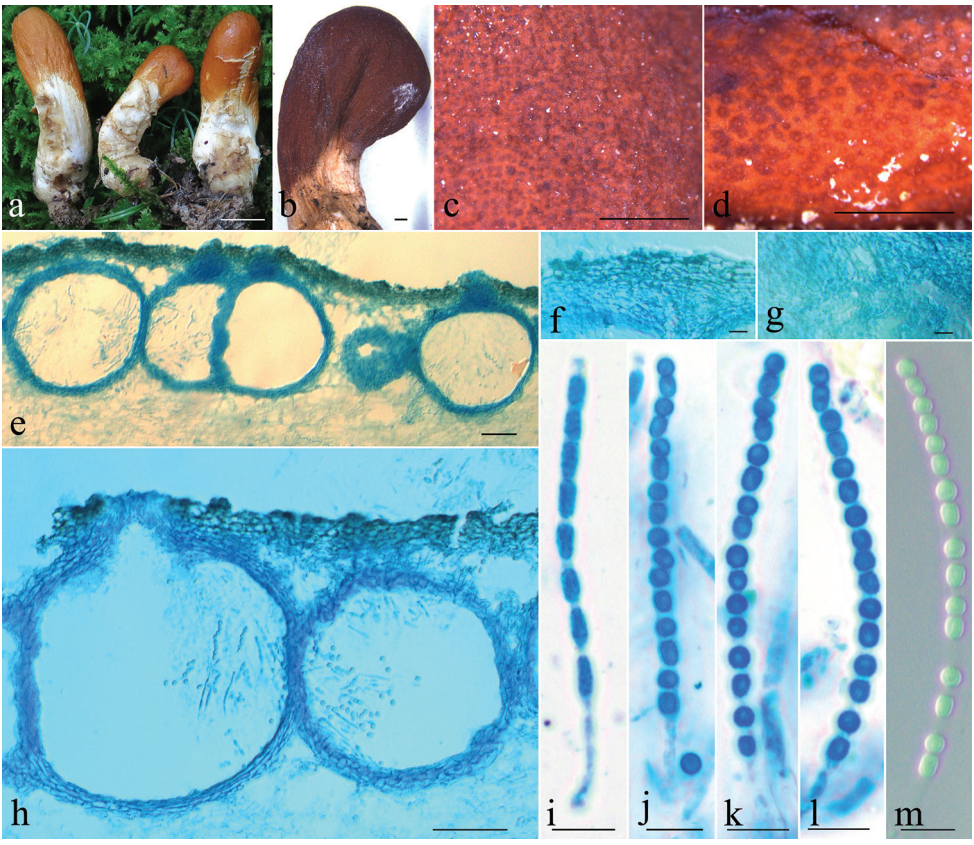

Figure 4. *Trichoderma gansuanum* **a** fresh stroma **b** dry stroma **c** stromatal surface **d** color of stroma after rehydration **e** median section through stromata **f** cortical tissue in section **g** subperithecial tissues in section **h** perithecia in section **i** ascus with ascospores **j–m** ascus with part-ascospores **a** from HMAS 279684, **b–m** from HMAS 279687. Scale bars: 1 cm (**a**); 1 mm (**b–d**); 50 μm (**e–f**); 10 μm (**g–m**).

Paratypes. China. Gansu Province, Shandan, Yanzhishan, on mossy humus, 25 August 2018, Z.Q. Zeng, H.D. Zheng 12043, 12044 (HMAS 279684, 279685), on mossy humus, 25 August 2018, X.Z. Liu & Z.Q. Zeng 12045 (HMAS 279686); Wuwei, Chashugou, on mossy humus, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12104, 12105 (HMAS 279688, 279689); Wuwei, Xiama, on mossy humus, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12162, 12163 (HMAS 279690, 279691).

Etymology. The specific epithet refers to the type locality.

Description. Stromata simple, rare dichotomously branched, clavate, 20–54 mm long. Fertile part clavate, 5–18 mm long, 1.2–4 mm wide at apex, only slightly broader than stipe, distinctly laterally compressed or longitudinally furrowed, gradually tapered downwards, reddish brown to brownish orange, KOH+; sterile part 15–36 mm long, 1–3 mm wide, beige to cream KOH+. Stromatal surface slight tuberculate from papillate perithecial elevations. Ostiolar openings visible, 30–58 μm high. In section, cortical tissue of textura angularis,15–35 μm thick, cells hyaline to light yellow, 5–15 × 2–3 μm;

subcortical tissue of textura angularis, 8–28 μm thick, cell hyaline to light yellow, 8–15 × 3–5 μm; subperithecial tissue of textura epidermoidea, rare textura angularis, cells hyaline to light yellow. Perithecia globose to subglobose, 196–206 × 167–235 μm; peridium 8–18 μm thick at flanks, 9–20 μm thick at the base. Papilla prominent, blunt or truncate, brown, 15–35 μm high, 18–43 μm wide at the base. Asci subcylindrical, 50–80 × 3–4 μm. Part-ascospores green, spinulose, dimorphic, distal cells globose, rarely ellipsoidal, 2.5–4 × 2.5–4 μm; proximal cells ellipsoidal to oblong, 3–5.5 × 2.5–3 μm. Asexual state unknown.

Distribution. China.

Notes. Among the known stipitate species of *Trichoderma*, *T. gansuanum* resembles *T. nybergianum* in habitat and having simple, clavate, erect stromata, cylindrical asci and disarticulating ascospores (Chamberlain et al. 2004). *Trichoderma gansuanum* differs from the latter in shorter ascomatal stipe (20–54 mm vs 22–220 mm long), smaller perithecia (196–206 × 167–235 μm vs 180–450 × 65–315 μm), asci (50–80 × 3–4 μm vs 63–130 × 3.2–7.5 μm), and part-ascospores (distal: 2.5–4 × 2.5–4 μm vs 3–6 × 3–4.5 μm; proximal: 3–5.5 × 2.5–3 μm vs 3–6 × 2.5–4.5 μm) (Chamberlain et al. 2004). Sequence comparisons reveal that there are 25 bp and 16 bp divergences between the two species in the regions of RPB2 and TEF1. Both morphological and molecular data support distinguish them at the species level. The asexual state of *T. gansuanum* remains unknown until a culture is available.

Hypomyces cervinigenus Rogerson & Simms, Mycologia 63: 418, 1971.

Specimens examined. China, Gansu Province, Wuwei, Tianzhu, on *Helvella* sp., 26 August 2018, X.C. Wang, Z.Q. Zeng & H.D. Zheng 12103 (HMAS 279612); Wuwei, Tianzhu, on *Helvella* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12210, 12221, 12225 (HMAS 279667, 279668, 279669).

Distribution. Canada, China, United States.

Hypomyces chrysospermus Tul. & C. Tul., Annls Sci. Nat., Bot., Sér. 4, 13: 16, 1860.

Specimens examined. China, Gansu Province, Tianzhu, Zhuchacun, on *Boletus* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12206 (HMAS 279670); Tianzhu, Kelacun, on *Boletus* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12220 (HMAS 279671).

Distribution. Australia, Belgium, Canada, China, French, Japan, New Zealand, United Kingdom, United States.

Hypomyces lateritius (Fr.) Tul. & C. Tul., Annls Sci. Nat., Bot., Sér. 4, 13: 11, 1860.

Specimens examined. China, Gansu Province, Shandan, Yanzhishan, on *Lactarius* sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12060, 12061 (HMAS 254608,

254609); Tianzhu, Kelacun, on *Lactarius* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12216, 12217, 12218, 12219 (HMAS 279672, 279673, 279674, 279675).

Distribution. Austria, Belgium, Canada, China, Czech, Denmark, Finland, France, Germany, Italy, Mexico, New Zealand, Russia, Sweden, United Kingdom, United States.

***Hypomyces perniciosus* Magnus, Bot. Ztg. 46: 394, 1888.**

Specimen examined. China, Gansu Province, Tianzhu, Kelacun, on *Agaricus* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12222 (HMAS 279705).

Sequences. ITS (MT676396) and LSU (MT669266).

Distribution. China, France, Germany, United Kingdom.

***Hypomyces rosellus* (Alb. & Schwein.) Tul. & C. Tul., Annls Sci. Nat., Bot., Sér. 4 13: 12, 1860.**

Specimen examined. China, Gansu Province, Shandan, Yanzhishan, on *Lactarius* sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12056 (HMAS 279706).

Sequences. ITS (MT676395).

Distribution. China, Estonia, Poland, Ukraine, United States.

***Hypomyces stephanomatis* Rogerson & Samuels, Mycologia 77: 775, 1985.**

Specimens examined. China, Gansu Province, Shandan, Yanzhishan, on *Humaria* sp., 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12063 (HMAS 279676); Tianzhu, Zhuchacun, on *Humaria* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12205, 12207, 12208, 12209, 12211 (HMAS 279677, 279678, 279679, 279680, 279681); Tianzhu, Kelacun, on *Humaria* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12223, 12226 (HMAS 279682, 279683).

Distribution. Canada, China, Germany, United States.

***Hypomyces tremellicola* (Ellis & Everh.) Rogerson, Mem. N. Y. Bot. Gdn. 26(3): 20, 1976.**

Specimen examined. China, Gansu Province, Zhangye, Minyue, on (?) *Agaricus* sp., August 2018, C.H. Dong & S.J. Li 12287 (HMAS 247843).

Sequences. ITS (MT084414) and LSU (MT078664).

Description. On MEA, colony radius 33 mm after 7d at 25 °C, velvet, surface white, reverse light brown; aerial hyphae white. On PDA, colony radius 20 mm after 7d at 25 °C, floccose, surface grey white, reverse light sienna; aerial hyphae white. Simple branches of aerial hyphae terminating in 1–2-verticillate conidio-

phores with terminal whorl of 2–4 phialides. Phialides subulate, tapering towards apex, smooth, 8–25 × 1.5–2 μm. Conidia ellipsoidal to rod-shaped, aseptate, hyaline, smooth, 2.5–8 × 1–3 μm. Chlamydospores globose, hyaline, smooth, 5–8 μm in diameter, rare ellipsoidal, 6–12 × 5–10 μm, formed singly or in chains in intercalary position.

Distribution. Canada, China, Germany, New Zealand, The Netherlands, United States, Venezuela.

Notes. *Hypomyces tremellicola* is a new record for China. This species was originally described as *Hypocrea tremellicola* Ellis & Everh. (Ellis and Everhart 1892), and later transferred to *Hypocreopsis* P. Karst. (Seaver 1910) and *Nectriopsis* Maire (Gams and Zaayen 1982). Samuels (1976) redescribed the species and assigned it to *Hypomyces*. It usually grows on *Crepidotus* spp., and less frequently on *Polyporus* spp. and *Pleurotus* spp. The shape and size of conidia and chlamydospores of the Chinese material match well with the description provided by Zare and Gams (2019). Sequence comparisons showed that 4 bp and 1 bp divergences existed in ITS and 28S rDNA between the Chinese material (HMAS 247843) and a collection from Germany (CBS 441.65). We treat them as infraspecific variations.

Nectria asiatica **Hirooka, Rossman & P. Chaverri, Stud. Mycol. 68: 44, 2011.**

Specimen examined. China, Gansu Province, Tianzhu, Zhuchacun, on rotten twigs, 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12214 (HMAS 254610).

Distribution. China, Japan.

Nectria berberidicola **Hirooka, Lechat, Rossman & P. Chaverri, in Hirooka, Rossman, Samuels, Lechat & Chaverri, Stud. Mycol. 71: 48, 2012.**

Specimens examined. China, Gansu Province, Shandan, Yanzhishan, on rotten twigs, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12084 (HMAS 279707); Tianzhu, Zhuchacun, on rotten twigs of *Berberis* sp., 27 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12212 (HMAS 254611); Tianzhu, Haxia, on rotten twigs of *Berberis* sp., 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12279, 12280, 12281 (HMAS 254612, 254613, 255801).

Sequences. ITS from HMAS 254613 (MT676394).

Distribution. China, France.

Nectria nigrescens **Cooke, Grevillea 7(no. 42): 50, 1878.**

Specimen examined. China, Gansu Province, Shandan, Yanzhishan, on rotten twigs of broadleaf tree, 25 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12085 (HMAS 255802).

Sequences. ITS (MT676393) and LSU (MT669265).
Distribution. Canada, China, France, Germany, United Kingdom, United States.

***Thelonectria discophora* (Mont.) P. Chaverri & C. Salgado sensu lato, in Chaverri, Salgado, Hirooka, Rossman & Samuels, Stud. Mycol. 68: 76, 2011.**

Specimen examined. China, Gansu Province, Wuwei, Chashugou, on rotten twigs, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12149 (HMAS 279710).
Distribution. Chile, China, United Kingdom.

***Thyronectria rosellinii* (Carestia) Jaklitsch & Voglmayr, Persoonia 33: 204, 2014.**

Specimens examined. China, Gansu Province, Wuwei, Chashugou, on rotten twigs, 26 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12150, 12151, 12154 (HMAS 279711, 255821, 255822).
Distribution. Canada, China, France, Germany, Italy, Japan, United States.

***Thyronectria zangii* (Z.Q. Zeng & W.Y. Zhuang) Voglmayr & Jaklitsch, in Voglmayr, Akulov & Jaklitsch, Mycol. Progr. 15: 934, 2016.**

Specimens examined. China, Gansu Province, Wuwei, Chengshanqizu, on rotten twigs of *Populus* sp., 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12285, 12286 (HMAS 255823, 255824).
Sequences. ITS from HMAS 255824 (MT676392).
Distribution. China.

***Trichoderma alutaceum* Jaklitsch, Fungal Diversity 48: 69, 2011.**

Specimen examined. China, Gansu Province, Zhangye, Dayekou, on mossy ground under *Picea asperata*, 3 September 1958, Q.M. Ma 890 (HMAS 23955).
Distribution. Austria, China, Denmark, Finland, Germany, Japan, Sweden, United Kingdom, United States.

***Trichoderma paraviridescens* Jaklitsch, Samuels & Voglmayr, Persoonia 31: 128, 2013.**

Specimen examined. China, Gansu Province, Tianzhu, Haxi, on rotten twigs, 28 August 2018, Z.Q. Zeng, H.D. Zheng & X.C. Wang 12282 (HMAS 255825).

Sequences. ITS (MT676391) and TEF1 (MT674562).

Distribution. Austria, China, Colombia, France, Germany, Greece, Iran, Italy, Japan, Mexico, Spain, Switzerland, United States.

***Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx, Beih. Nova Hedwigia 87: 288, 1987.**

Specimen examined. China, Gansu Province, Yongchang, in soil, April 2016, K. Chen TC896.

Distribution. China, United States.

Discussion

The genus *Stylonectria*, typified by *S. applanata*, was established as a monotypic genus by Höhnel (1915). It was included in *Nectria* by Booth (1959) and then treated as a synonym of *Cosmospora* Rabenh. by Rossman et al. (1999). Gräfenhan et al. (2011) resurrected the generic name *Stylonectria* and accepted four species. Lechat et al. (2015) described *S. norvegica* Lechat, J. Fourn. & Nordén from Norway. The morphological features of *S. qilianshanensis*, such as red perithecia growing on other ascomycetes, with flattened discoid apices, and two-layered wall bearing thick-walled outer cells, fit well the generic concept defined by Gräfenhan et al. (2011), which was confirmed by sequence analyses of the ACL1, ITS, and RPB2 regions. *Stylonectria qilianshanensis* is associated with *S. wegeliniana* (BIPP/MPBP = 100%/76%). *Stylonectria purtonii* [as *Cosmospora purtonii* (Grev.) Rossman & Samuels] on carbonaceous pyrenomycetes was the only species of the genus reported from China (Zhang and Zhuang 2003).

For some nectriaceous fungi, substrate type was considered of taxonomic importance (Zeng and Zhuang 2016). Species of *Stylonectria* are fungicolous and sometimes host-specific. For example, *S. applanata* is known only from *Melogramma bulliardii* Tul. & C. Tul. on *Corylus avellana*, *S. carpini* is restricted to a pyrenomycete on *Carpinus*, and *S. wegeliniana* colonizes solely on *Hapalocystis bicaudata* Fuckel on *Ulmus glabra* (Gräfenhan et al. 2011; Lechat et al. 2015). Similarly, *S. qilianshanensis* occurs on decayed fruit bodies of an ascomycete on the bark of *Picea asperata*. However, *S. norvegica* and *S. purtonii* have slightly wider host ranges. The former occurs on pyrenomycetes on Betulaceae and Fagaceae, while the latter is found on pyrenomycetes on coniferous trees. *Stylonectria* is currently a poorly known genus. Further investigations may provide useful information about its selection of hosts or substrates.

Gross morphology like stroma, ascospore features and asexual states were stressed for generic delimitations of the hypocrealean fungi. Species of Hypocreaceae possessing clavate to cylindrical, fleshy, bright-colored stromata were previously accommodated in *Podostroma* P. Karst. (Karsten 1892; Rossman et al. 1999). The accumulated

molecular evidence argued that shape of fruit body is not phylogenetically distinctive within genus. Chamberlain et al. (2004) then synonymized *Podostroma* with *Hypocrea* Fr. (= *Trichoderma*). The diagnostic characteristics for *Trichoderma* include substrate, fruit body gross morphology, anatomy, extent of fertile region, surface pigmentation of stromata, and ascospore shape, size and ornamentation (Chamberlain et al. 2004). The genera *Aphysiostroma* Barrasa, A.T. Martinez & G. Moreno, *Pseudohypocrea* Yoshim. Doi, and *Sarawakus* Lloyd, which possess gliocladium-, trichoderma- and verticillim-like asexual states, were also synonymized with *Trichoderma* (Jaklitsch et al. 2014; Jaklitsch and Voglmayr 2015; Zeng and Zhuang 2017). The taxonomic position of *T. gansuanum* is confirmed by the combined sequence analyses of RPB2 and TEF1 regions and morphological characteristics, such as the stipitate, clavate, upright stromata, cylindrical asci, and disarticulating ascospores. A few stipitate species of *Trichoderma* have not been cultured or linked to asexual states (Chamberlain et al. 2004). Knowing the whole fungus is surely our future goal.

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