An outline of the family Cucurbitariaceae

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The Cucurbitariaceae is a relatively poorly known family of Dothideomycetes. *Cucurbitaria berberidis*, the type species of *Cucurbitaria*, was collected on dead twigs of *Berberis vulgaris* in Austria and this new collection is used to epitypify the taxon and described and illustrated in detail with regard to its sexual and asexual state. Sequence data from 18S nrDNA (SSU) and 28S nrDNA (LSU) gene regions of several isolates of the epitype or isoepitype specimens were compared with representative isolates of *C. berberidis* and closely related sequences in GenBank. The phylogenetic results show that Cucurbitariaceae is a well-resolved family within *Pleosporineae*. A *Pyrenochaeta*-like coelomycetous asexual state formed in the *Cucurbitaria* culture, and is illustrated here. The types of *Curreya, Rhytidiella* and *Syncarpella* are also studied and illustrated. Taxonomic placements of each genus with their asexual states are discussed. We provisionally accept six genera in Cucurbitariaceae i.e. *Cucurbitaria* with *Pyrenochaeta*-like asexual states, *Curreya* (including *Cucurbidothis*) with *Coniothyrium*-like asexual states, *Rhytidiella* with *Phaeoseptoria*-like asexual states, *Syncarpella* and the asexual genera *Pyrenochaeta* and *Pyrenochaetopsis*. Placement of *Syntholus* in Cucurbitariaceae is uncertain as the generic type has not been linked and further studies are needed.

Keywords: asexual morphs, *Cucurbidothis*, *Cucurbitaria berberidis, Curreya, Pyrenochaeta berberidis,* phylogeny, *Rhytidiella, Syncarpella,* taxonomy, types.

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The family Cucurbitariaceae was introduced by Winter (1885) and is typified by *Cucurbitaria berberidis* (Pers.) Gray. This family is characterized by ascomata, which develop in erumpent clusters beneath the host periderm, or are superficial on decorticated wood (Barr 1990). The ascomata occur on a common basal pseudostroma or subiculum, have a rounded ostiole, and the peridium is relatively thick, reddish brown and comprises brown, thin-walled, angular cells. The hamathecium is made up of relatively wide filamentous pseudoparaphyses, and the asci are cylindrical to cylindric-clavate, while the ascospores are brown and muriform or tranversely septate (Barr 1990, Cannon & Kirk 2007). *Cucurbitaria* has 466 associated names (Index Fungorum 2013), most of which have not been studied since their introduction in the late 19th or early 20th century. *Curreya* (23 names), *Rhytidiella* (4 names), *Syncarpella* (10 names) and *Pyrenochaetopsis* (5 names) are also included in the family (Lumbsch & Huhndorf 2010, de Gruyter *et al.* 2010, Index Fungorum 2013).

Recent phylogenetic studies showed that *Phoma*-like species, *Pyrenochaetopsis* and *Pyrenochaeta* also belong in Cucurbitariaceae (Aveskamp *et al.* 2010; de Gruyter *et al.* 2010, 2013). Other putative asexual states listed by Wijayawardene *et al.* (2012) are *Camarosporium, Coniothyrium*-like, *Diplodia*-like, *Pleurophoma* and *Syntholus*.

Fresh material of *Cucurbitaria berberidis* was collected in Austria, and cultures were prepared from ascospores. We use this material to epitypify *C. berberidis* in order to stabilize the taxonomy of the species. We also describe the *Pyrenochaeta*-like asexual morph from the natural host and from artificial culture. *Curreya*, *Rhytidiella* and *Syncarpella* of Cucurbitariaceae are also illustrated from their holotypes and their familial placement is considered. We also discuss the asexual states related to Cucurbitariaceae. We hope to initiate recollection and further study of genera of Cucurbitariaceae at the molecular level by providing high quality descriptions and illustrations of the type species of the included genera.

Materials and methods

Collection and culture preparation

Material of *Cucurbitaria berberidis* was collected on dead twigs of *Berberis vulgaris* in Austria. Isolations were made from single ascospores as described by Jaklitsch (2009) except that 2 % MEA was used as the isolation medium, or alternatively, following the method described in Chomnunti *et al.* (2011): after surface sterilization (3 min in 70 % ethanol) ascomata were cut horizontally and the contents transferred to a drop of sterile water on a glass container. The spore suspension was dropped on squares on a plate of malt extract agar (MEA). After incubating the unsealed plate at 25 °C for 24 hours, single germinating ascospores were transferred to fresh potato dextrose agar (PDA) plates and checked by microscopy to ensure that a single spore had been transferred. Representative isolates are deposited at the Mae Fah Lu-

ang Culture Collection (MFLUCC) and Centraalbureau voor Schimmelcultures (CBS).

Morphology

Type materials of *Curreya* (*Homostegia conorum*), *Rhytidiella moriformis* and *Syncarpella tumefaciens* were borrowed from S, DAOM and NY (Thiers 2013). Sections of ascomata were made by free-hand under a stereomicroscope. Miscoscopic mounts were prepared in water from herbarium specimens rehydrated in 5 % KOH for 5–10 min and preserved in lactoglycerol. Ascomata on the natural host were photographed with a Canon digital IXUS 110 IS fitted Olympus SZ40 microscope, conidiomata on the natural host by using a Nikon Coolpix 4500 attached to a Nikon SMZ 1500 stereomicroscope. Colony characteristics are recorded from cultures grown on MEA. Pycnidia were variably produced in MEA cultures after growth at 25 °C and subsequent storage at 15 °C for 1–10 months. Alternatively, formation of pycnidia was induced by transferring isolates to water agar (WA) overlaid with sterilized pine needles as a substrate and incubated at 22 °C for 4 months. Mycelia were mounted in lactoglycerol, pycnidia in 3 % KOH. Morphological observations and photomicrographs were made using either a Nikon Eclipse E600 DIC microscope and a Nikon DS-U2 camera or a Nikon Eclipse 80i compound microscope fitted with a Canon 450D digital camera. Measurements were made with the NIS-Elements D version 3.0 software or Tarosoft (R) Image Frame Work in water (Liu *et al.* 2011).

DNA extraction, PCR amplification and sequencing

Fungal isolates were grown on PDA for 1 week. Total genomic DNA was extracted from mycelia using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer's protocol (Hangzhou, P. R. China). DNA amplification was performed by polymerase chain reaction (PCR). Two different regions of the rDNA gene (characterised by different rates of evolution) were amplified. The primer pair SR7R (5′-AGTTAAAAAGCTCGTAGTTG-3′) (Vilgalys 1990) and NS24 (5′-AAACCTTGTTACGACTTTTA-3′) (Gargas & Taylor 1992) was used for the small 18S subunit (SSU; White *et al.* 1990). The LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′) primer pair was used to amplify a segment of the large 28S subunit (LSU; Vilgalys & Hester 1990).

The amplifications were performed in a 50 µl reaction volume containing 1× PCR buffer, 0.2 mM dNTP, 0.3 µM of each primer; 1.5 mM $MgCl_2$, 0.8 units Taq Polymerase and 5–10 ng DNA (Cai *et al.* 2009). The amplification conditions consisted of initial denaturation of 5 min at 95 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 48 °C and 90 s at 72 °C, and a final extension period of 10 min at 72 °C (Phillips *et al.* 2008). The PCR products were checked on 1 % agarose electrophoresis gels stained with ethidium bromide.

PCR products were then purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). The sequencing were carried out by Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P. R. China). For each fungal strain, sequences obtained for the respective primers (LR0R and LR5, SR7R and NS24) were manually aligned to obtain an assembled sequence using BioEdit (Hall 1999). The reference nucleotide sequences of LSU and SSU regions of various taxa were obtained from GenBank (Tab. 1).

Tab. 1. Isolates and sequence data used in this study.

Taxon	Culture accession no.1	GenBank accession no.2	
		LSU	SSU
Aigialus grandis	BCC 18419	GU479774	GU479738
Aigialus grandis	BCC 20000	GU479775	GU479739
Amniculicola lignicola	CBS 123094	EF493861	EF493863
Ascocratera manglicola	HHUF 30032	GU479783	GU479748
Ascocratera manglicola	BCC 09270	GU479782	GU479747
Botryosphaeria dothidea	CMW 8000	AY928047	EU673173
Chaetosphaeronema hispidulum	CBS 216.75	EU754144	EU754045
Cochliobolus heterostrophus	CBS 134.39	AY544645	AY544727
Cochliobolus sativus	DAOM 226212	DQ678045	DQ677995
Cucurbitaria berberidis	MFLUCC 11-0384	**KC506793**	**KC506797**
Cucurbitaria berberidis	MFLUCC 11-0385	**KC506794**	**KC506798**
Cucurbitaria berberidis	MFLUCC 11-0386	**KC506795**	**KC506799**
Cucurbitaria berberidis	MFLUCC 11-0387	**KC506796**	**KC506800**
Cucurbitaria berberidis	CBS 394.84	GQ387605	GQ387544
Cucurbitaria berberidis	CBS 363.93	GQ387606	GQ387545
Didymella cucurbitacearum	IMI 373225	AY293792	AY293779
Didymella exigua	CBS 183.55	EU754155	EU754056
Dothidotthia aspera	CPC 12933	EU673276	EU673228
Dothidotthia symphoricarpi	CBS119687	EU673273	EU673224
Entodesmium rude	CBS 650.86	GU301812	–
Fissuroma maculans	MFLUCC 10-0886	JN846724	JN846734
Fissuroma maculans	MFLUCC 10-0887	JN846725	JN846736
Herpotrichia juniperi	CBS 468.64	DQ384093	DQ384077
Leptosphaeria doliolum	CBS 505.75	GU301827	GU296159
Leptosphaeria dryadis	CBS 643.86	GU301828	–
Leptosphaeria maculans	DAOM 229267	DQ470946	–
Leptosphaerulina australis	CBS 939.69	EU754167	EU754068
Lindgomyces breviappendiculatus	HHUF 28193	AB521748	AB521733
Lindgomyces ingoldianus	ATCC 200398	AB521736	AB521719
Lindgomyces rotundatus	HHUF 27999	AB521740	AB521723
Lophiostoma arundinis	CBS 621.86	DQ782384	DQ782383
Lophiostoma crenatum	CBS 629.86	DQ678069	DQ678017
Lophiostoma fuckelii	CBS 113432	EU552139	–
Lophiostoma fuckelii	CBS 101952	DQ399531	–
Lophiostoma macrostomum	HHUF 27293	AB433274	AB521732
Lophiostoma macrostomum	HHUF 27290	AB433273	AB521731
Melanomma pulvis-pyrius	IFRD 002	FJ201984	FJ201985
Monascostroma innumerosum	CBS 345.50	GU301850	GU296179

Taxon	Culture accession no.1	GenBank accession no.2	
		LSU	SSU
Murispora rubicunda	IFRD 2017	FJ795507	GU456308
Neoastrosphaeriella krabiensis	MFLUCC 11-0022	JN846727	JN846735
Neoastrosphaeriella krabiensis	MFLUCC 11-0023	JN846729	JN846739
Neomassariosphaeria typhicola	CBS 123126	FJ795504	GU296174
Neophaeosphaeria filamentosa	CBS 102202	GQ387577	GQ387516
Ophiosphaerella herpotricha	CBS 620.86	DQ678062	DQ678010
Phaeosphaeria avenaria	DAOM 226215	AY544684	AY544725
Phaeosphaeria eustoma	CBS 573.86	DQ678063	DQ678011
Phaeosphaeriopsis musae	CBS 120026	GU301862	GU296186
Phoma herbarum	CBS 615.75	EU754186	EU754087
Platystomum scabridisporum	BCC 22835	GQ925844	GQ925831
Platystomum scabridisporum	BCC 22836	GQ925845	GQ925832
Pleospora herbarum	CBS 191.86	DQ247804	DQ247812
Preussia minima	CBS 524.50	DQ678056	DQ678003
Preussia terricola	DAOM 230091	AY544686	AY544726
Pyrenochaeta acicola	CBS 101634	GQ387603	GQ387542
Pyrenochaeta acicola	CBS 812.95	GQ387602	GQ387541
Pyrenochaeta acicola	CBS 124142	GQ387604	GQ387543
Pyrenochaeta cava	CBS 257.68	EU754199	EU754100
Pyrenochaeta cava	CBS 115953	GQ387607	GQ387546
Pyrenochaeta corni	CBS 248.79	GQ387608	GQ387547
Pyrenochaeta corni	CBS 102828	GQ387609	GQ387548
Pyrenochaeta nobilis	CBS 566.75	GQ387616	GQ387555
Pyrenochaeta nobilis	CBS 407.76	EU754206	EU754107
Pyrenochaeta unguis-hominis	CBS 378.92	GQ387621	GQ387560
Pyrenochaeta unguis-hominis	CBS 111112	GQ387623	GQ387562
Pyrenochaetopsis leptospora	CBS 101635	GQ387627	GQ387566
Pyrenochaetopsis leptospora	CBS 122789	EU754204	EU754105
Pyrenochaetopsis leptospora	CBS 536.66	GQ387628	GQ387567
Pyrenochaetopsis leptospora	CBS 131.69	GQ387629	GQ387568
Pyrenophora phaeocomes	DAOM 222769	DQ499596	DQ499595
Pyrenophora tritici-repentis	OSC 100066	AY544672	AY544716
Rimora mangrovei	JK 5246A	GU301868	GU296193
Rimora mangrovei	JK 5437B	GU479798	–
Setomelanomma holmii	CBS 110217	GU301871	GU296196
Setosphaeria monoceras	CBS 154.26	AY016368	AY016352
Sporormia lignicola	CBS 363.69	DQ384098	DQ384087

BCC: BIOTEC Culture Collection, Bangkok, Thailand; **CBS:** Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CMW:** Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; **DAOM:** Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; **HHUF:** Herbarium of Hirosaki University, Japan; **IFRD:** Culture Collection, International Fungal Research & Development Centre, Chinese Academy of Forestry, Kunming, China; **IMI:** International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K; **MFLUCC:** Mae Fah Luang University Culture Collection, ChiangRai, Thailand; **OSC:** Oregon State University Herbarium, U.S.A. **JK:** J. Kohlmeyer.

Phylogenetic analysis

The aligned SSU and LSU datasets were first analysed separately and then the individual datasets were concatenated into a combined dataset. A BLAST search was performed to reveal the closest matches with fungal members from different families of the order Pleosporales, which comprises the families Amniculicolaceae, Aigialaceae, Cucurbitariaceae, Didymellaceae, Dothidotthiaceae, Leptosphaeriaceae, Lindgomycetaceae, Lophiostomataceae, Melanommataceae, Phaeosphaeriaceae, Pleosporaceae and Sporormiaceae. DNA sequences were aligned using BioEdit (Hall 1999) and Clustal X 2.0.11 (Thompson *et al.* 1997) with other sequences obtained from GenBank. The alignments were checked visually and improved manually where necessary. Phylogenetic analyses were performed using PAUP* version 4.0b10 (Swofford 2002) for Maximum parsimony (MP) and Mr. Bayes v. 3.0b4 (Ronquist & Huelsenbeck 2003) for Bayesian analyses. MP analysis was performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 100 replicates of random stepwise addition of taxa (Hillis & Bull 1993). Maximum Likelihood analysis was performed in RAxML (Stamatakis 2006) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak 2012). The search strategy was set to rapid bootstrapping and the analysis carried out using the GTRGAMMAI model of nucleotide substitution. The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1000000 generations and trees were sampled every 100th generation (resulting in 10000 total trees). Phylogenetic trees were drawn using Treeview (Page 1996). Sequences derived in this study are deposited in GenBank.

Results

The combined LSU and SSU data set consists of 75 taxa, with *Botryosphaeria dothidea* as the outgroup taxon. The dataset consisted of 2292 characters after alignment, of which 2190 sites were included in the ML and MP analyses. Of the included bases 373 (17 %) are parsimony informative. A heuristic search with random addition of taxa (1000 replicates) and treating gaps as missing characters generated six equally parsimonious trees. All trees were similar in topology and not significantly different (data not shown). A best scoring RAxML tree is shown in Fig. 60. Bootstrap support (BS) values of ML and MP (equal to or above 50 % based on 1000 replicates) are shown on the upper branches. Values of the Bayesian posterior probabil-

ities (PP) (equal to or above 0.90 based on 1000 replicates) from MCMC analyses are shown under the branches.

The phylogenetic trees obtained from maximum likelihood, Bayesian and maximum parsimony analyses gave similar results relating to family relationships and were similar to previous studies based on maximum likelihood (Schoch *et al.* 2009, Suetrong *et al.* 2009, Liu *et al.* 2011, Zhang *et al.* 2012). The families Cucurbitariaceae, Didymellaceae, Dothidotthiaceae, Leptosphaeriaceae, Phaeosphaeriaceae and Pleosporaceae clustered into the suborder Pleosporineae, however, the strains in Leptosphaeriaceae did not form a well-supported clade in all three phylogenetic analyses (ML, MP and Bayesian). The four new strains of *Cucurbitaria berberidis* clustered together with two other strains (CBS 363.93 and CBS 394.84) and formed a strongly supported (100%, 100% and 1.00) clade within Cucurbitariaceae.

Taxonomy

Cucurbitariaceae G. Winter [as 'Cucurbitarieae'], Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 308 (1885), MycoBank MB 80667

Necrotrophic or saprobic on woody plants. Ascomata immersed in host tissue, erumpent, solitary or mostly gregarious, scattered or clustered on a subiculum or hypostroma, globose to subglobose, brown to black, ostiolate; surface smooth, rough or hairy. Ostiole inconspicuous or papillate, ostiolar canal periphysate. Peridium thick, comprising two layers, outer layer blackened and often amorphous; inner layer composed of light brown to reddish brown cells of *textura angularis*; peridium often thickened at the base, sometimes merging with the hypostroma. Hamathecium of numerous, filiform, hyaline, septate pseudoparaphyses embedded in a gelatinous matrix. Asci cylindrical to cylindric-clavate, 8-spored, bitunicate, fissitunicate, with short pedicel and minute ocular chamber, arising from the ascoma base. Ascospores overlapping uniseriate to biseriate, ellipsoid, light brown, golden brown to dark brown, muriform or transversely septate, typically constricted at the first-formed septum. Asexual states coelomycetous, *Camarosporium*-, *Coniothyrium*-, *Phaeoseptoria*- or *Pyrenochaeta*-like.

Type genus. – ***Cucurbitaria*** Gray, Nat. Arr. Brit. Pl. (London) 1: 508, 519 (1821), MycoBank MB 1348

Probable synonyms (from www.indexfungorum.org 2013):
= *Crotonocarpia* Fuckel, Jb. nassau. Ver. Naturk. 23–24: 163 (1870) [1869–70]
= *Cucurbitariopsis* Vassilkov, Botanicheskiĭ Zhurnal 45: 1369 (1960)
= *Cyathisphaera* Dumort., Comment. bot. (Tournay): 87 (1822)
= *Gemmamyces* Casagr., Phytopath. Z. 66: 119 (1969)
= *Gibberidea* (Fr.) Kuntze, Revis. gen. pl. (Leipzig) 3(2): 481 (1898)
= *Leucothyridium* Speg., Anal. Mus. nac. B. Aires, Ser. 3 12: 388 (1909)
= *Megalospora* Naumov, Mater. Mikol. Fitopat. Ross. 6(1): 9, 10 (1927)
= *Phialospora* Raf., Gard. Mag. & Reg. Rural Domest. Improv. 8: 248 (1832)

= *Sphaeria* sect. *Gibberidea* Fr., Summa veg. Scand., Section Post. (Stockholm): 395 (1849)

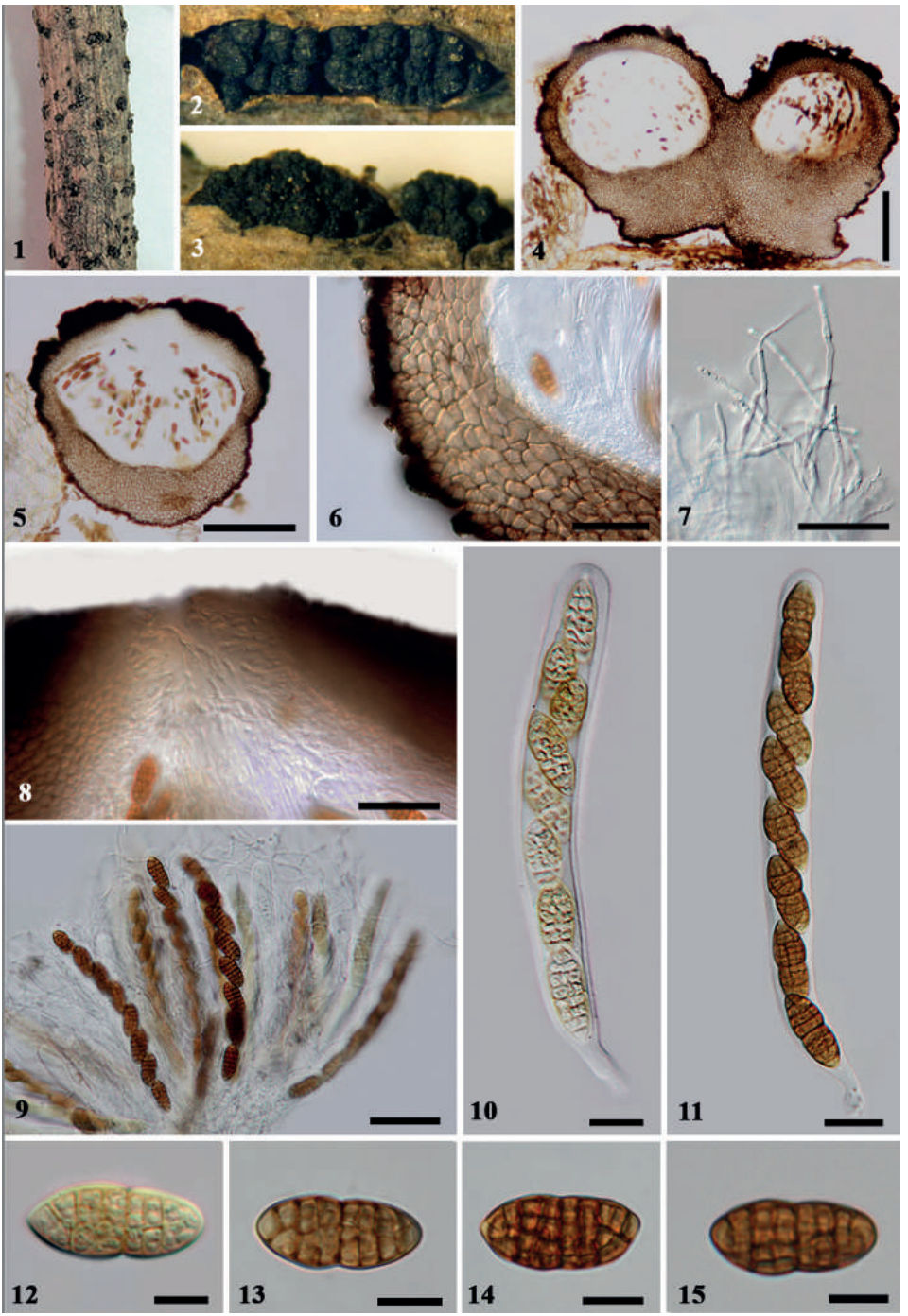
Saprobic or necrotrophic on branches. Ascomata scattered or clustered in small to large groups on a subiculum or hypostroma, initially immersed beneath the host periderm, becoming erumpent to superficial, globose to turbinate, brown to black; apex obtuse or papillate. Ostiole central, short, sometimes visible as a minute, light-coloured area. Peridium firm, comprising two layers, outer layer variable, of black sclerotial cells, inner part comprising several layers of pale to reddish brown cells, arranged as *textura angularis*, outwardly smooth to rough or hairy, sometimes thickened basally or apically. Hamathecium of numerous, dense, filiform, hyaline pseudoparaphyses embedded in a gelatinous matrix. Asci cylindrical, 8-spored, bitunicate, fissitunicate, short-pedicellate, apically thick-walled, rounded, with a small ocular chamber, arising from the base of the ascoma. Ascospores uniseriate, ellipsoid, initially hyaline, become golden brown to dark brown, muriform, constricted at the primary septum, with obtuse or acute ends. Asexual state *Pyrenochaeta*-like in the type species. Pycnidia superficial on wood in bark fissures, scattered or aggregated, (sub)globose or obpyriform, unilocular, dark brown to black, with thick pseudoparenchymatous wall, setose. Setae dark brown, thick-walled, sometimes light brown and thin-walled, ends narrowly rounded and broad at the base, septate, numerous. Conidiogenous cells enteroblastic, phialidic, hyaline, smooth, Conidia agglutinated in masses, oblong to ellipsoid, hyaline, aseptate, guttulate, smooth (Sutton 1980). *Camarosporium*-like asexual morphs described for other species: pycnidial, forming brown, muriform conidia.

Notes. – *Cucurbitaria* was introduced by Gray (1821) with *C. berberidis* as the type of the genus. *Cucurbitaria* is one of the oldest pyrenomycete genera separated from *Sphaeria* Haller in the sense of Tode (1790) and Persoon (1801), while Fries (1823) considered it under Pyrenomycetes along with the genus *Sphaeria*. The original generic diagnosis given by Gray (1821) is as "irregular spreading thallus, thecae in tufts, placed on the thallus" but the scope of the genus was unlimited. Thus, Greville (1824–1826), Tulasne & Tulasne (1863), Saccardo (1883), Winter (1885), Ellis & Everhart (1892), and Berlese (1900), described the genus in more detail, but the identity remained inconclusive. In the arrangement of Winter (1885) the genus *Cucurbitaria* was placed under the family Cucurbitariaceae with other taxa having aggregated fruit bodies. A subsequent monograph was provided by Welch (1926). He studied 77 taxa and accepted only five species (*C. arizonica* Ellis & Everh., *C. berberidis*, *C. caraganae* P. Karst., *C. elongata* (Fr.) Grev. *C. laburni* (Pers.) De Not.), excluding, synonymising, transferring or considering others as doubtful. Mirza (1968) later studied the genus and recognized 28 taxa. Barr (1990) gave an account of North American species and accepted 11 species providing keys, illustrations and brief descriptions. No species have been introduced since Barr (1990).

Type species – ***Cucurbitaria berberidis*** (Pers.) Gray, Nat. Arr. Brit. Pl. (London) 1: 508, 519 (1821), MycoBank MB 239072 (Figs. 1–24, 56–59)

≡ *Sphaeria berberidis* Pers., Neues Mag. Bot. 1: 83 (1794)
≡ *Gibberidea berberidis* (Pers.) Rabenh. ex Kuntze
≡ *Hypoxylon berberidis* (Pers.) J. Kickx f., Rech. Serv. Fl. Crypt. Fland. 1: 18 (1841)
= *Crotonocarpia moriformis* Fuckel, Jb. nassau. Ver. Naturk. 23–24: 163 (1870) [1869–1870]
= *Cucurbitaria moriformis* (Fuckel) M. E. Barr, Mycotaxon 29: 503 (1987)
= *Pyrenochaeta berberidis* (Sacc.) Brunaud, Act. Soc. linn. Bordeaux, Trois. sér. 40: 83 (1886)
≡ *Phoma berberidis* Sacc., Michelia 1 (no. 2): 259 (1878)

Saprobic on stems of *Berberis* spp. Ascomata (435)500–650(870) µm high × 380–595 µm diam. (mean = 619 × 502 µm, n = 20), immersed-erumpent through bark fissures, gregarious to densely crowded, less commonly solitary, subglobose to turbinate, seated on a basal pseudostroma and often surrounded by dark brown hyphae. Ostiole central, short, slightly sunken, minute and inconspicuous at the surface, hyaline or pale yellowish or reddish. Peridium (47)66–80 µm thick at the sides, elongated up to 420 µm at the base, comprising two layers, outer layer black, carbonaceous and coarsely warted or cracked at the surface, inner part laterally comprising 5–6 layers of light brown to reddish brown, compressed, angular cells. Hamathecium comprising numerous, 2–3 µm wide, filiform, hyaline, septate, branched pseudoparaphyses, embedded in a gelatinous matrix. Asci (130)180–200(220) × 15–20 µm (mean = 183 × 17 µm, n = 20), cylindrical, 8-spored, fissitunicate, thick-walled; pedicel rather short and rounded, apex rounded with an ocular chamber; arising from the ascoma base. Ascospores 23–32 × 9–14 µm (mean = 27 × 11 µm, n = 30), overlapping uniseriate, ellipsoid to widely fusiform, muriform, with (4)7–9(11) transverse septa and (1)3–4 longitudinal septa, constricted at the central septum, initially hyaline, later golden brown to dark brown, with obtuse or acute, slightly paler ends. Asexual state *Pyrenochaeta*-like: Pycnidia superficial on wood in bark fissures, scattered, aggregated, also directly associated with ascomata, (sub)globose or obpyriform, soft, black, (80)105–240 µm diam., 65–225 µm high (without setae), with a central ostiolar papilla. In MEA culture pycnidia formed superficially among aerial hyphae and immersed in the agar. Pycnidial wall pseudoparenchymatous, covered by dark brown, thick-walled, subacute, (7)15–70(140) µm long, 3–5 µm wide setae, with bases sometimes swollen up to 8.5 µm, on the entire surface, but concentrated in the central apical ostiolar region; pycnidial surface also with many subglobose projecting cells 6–10 µm diam., similar to setae in walls and pigmentation. Conidiogenous cells enteroblastic, of small solitary, terminal, lageniform, hyaline phialides. Conidia (2.7)3.0–4.0(5.3) × (1.0)1.2–1.5(1.8) µm (mean = 3.5 × 1.3 µm, n = 50), mostly agglutinated in masses, oblong, straight or slightly curved, hyaline, smooth, with 1–2 minute guttules or mostly eguttulate. Cultural characteristics: Ascospores germinating on 2 % MEA within 24–37 hours. Germ tubes produced from multiple cells of the as-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cospores. Colonies growing to ca. 5–6 cm at 25 °C under 12 h light/12 h darkness after 1 month upon first isolation, additional isolates reaching only 2.5 cm per month at 25–28 °C; evenly effuse, first white, soon turning pale to dark brown or grey-olivaceous, covered by a loose mat of aerial hyphae.

Typification. – Three specimens of Persoon, L0112523, L0112525, and L0112524 (no collection data given), which were confirmed to represent *Cucurbitaria berberidis*, are extant in L. We here select and designate L0112523 as *lectotype* of *Sphaeria berberidis*, because it is well preserved and contains typical asci and ascospores. *Epitype* of *Sphaeria berberidis* (and thus of *Cucurbitaria berberidis*), here designated in order to firmly link the name with the fungal material and molecular data: Austria, Kärnten, St. Margareten im Rosental, Wograda, grid square 9452/3 on twigs of *Berberis vulgaris*, 30 April 2011, *leg.* W. Jaklitsch (WU 31405; part deposited as iso-epitype MFLU 12-0111; ex-epitype culture CB1 = CBS 130007 = MFLUCC11-0384). Additional cultures from different ascomata of the iso-epitype specimen: MFLUCC 11-0385, MFLUCC 11-0386, MFLUCC 11-0387.

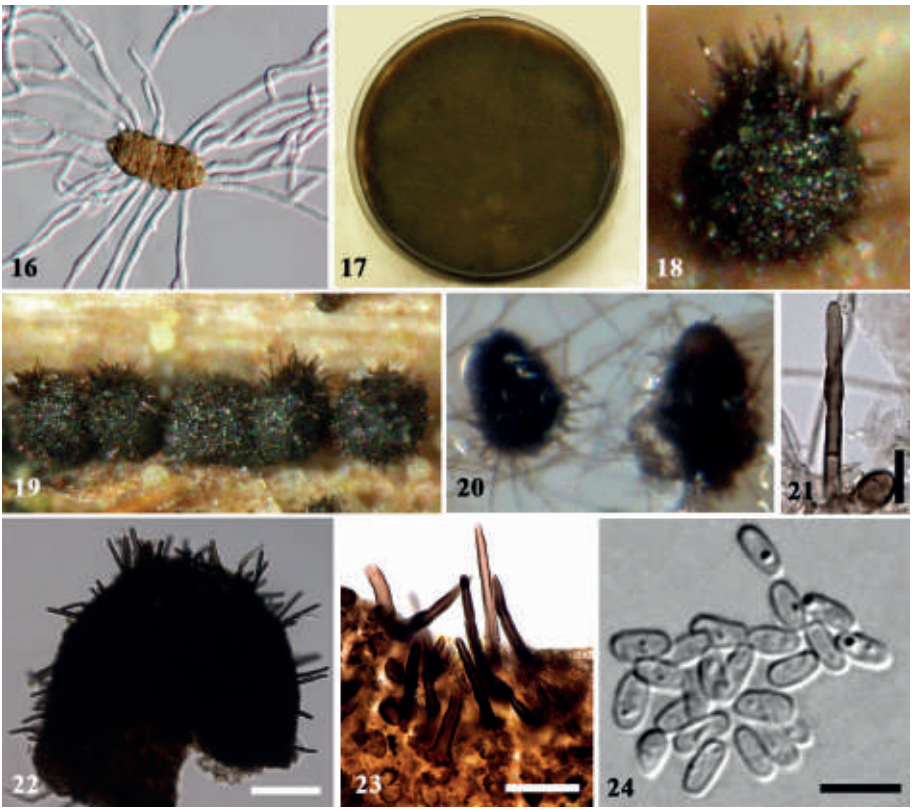
Additional material examined. – AUSTRIA, Niederösterreich, Klosterneuburg, Donau-Auen, on twigs of *Berberis vulgaris*, May 1939, *leg.* F. Petrak (BPI, 1111780).

Notes. – Images of the lectotype were produced in the very last moment after having finished the main illustrations, therefore the lectotype is illustrated further below along with images of the epitype (Figs. 56–59).

The description of the asexual state above is combined from both the natural host and from MEA cultures. Only few differences were noted: In culture pycnidia were surrounded by numerous subhyaline to brown hyphae and the number of setae was much reduced and they were lighter in colour when formed within the agar; setae were sometimes up to 140 µm long.

The epitypification of the type species of the genus will allow comparison with other species in the genus using molecular data. The designation thus allows us to work with living material and subject *Cucurbitaria berberidis* to phylogenetic analysis. In this way we can move forward and stabilize the taxonomy of the species, genus and family this taxon represents. Many of the numerous epithets in *Cucurbitaria* may be synonyms, doubtful or belong in other genera, but many additional species may be established. The epitypification allows us to compare fresh collections with the epitype, which is fully described and well illustrated.

Figs. 1–15. *Cucurbitaria berberidis* (MFLU12-0111, iso-epitype). **1–3.** Ascomata on the natural substrate. **4–5.** Section through ascomata. **6.** Peridium. **7.** Pseudoparaphyses. **8.** Ostiolar canal with periphyses. **9.** Asci with pseudoparaphyses. **10–11.** Asci with eight overlapping uniseriate spores. **12–15.** Muriform ascospores. Scale bars: **4, 5** 200 µm. **6, 9** 50 µm. **8** 30 µm. **7, 11** 20 µm. **10, 12–15** 10 µm.



Figs. 16–24. *Asexual state of Cucurbitaria berberidis* (epitype WU 31405 and ex-epitype culture CBS 130007). **16.** Germinated ascospore. **17.** Culture on MEA. **18.** Close-up of a pycnidium. **19, 20.** Pycnidia. **21.** Seta and projecting subglobose cell from the pycnidial surface. **22.** Pycnidium with setae. **23.** Close-up of setae. **24.** Hyaline unicellular conidia. Scale bars: **21** 10 µm. **22** 50 µm. **23** 20 µm. **24** 5 µm.. 20, 21, 24 from MEA culture; 18, 19, 22, 23 from the natural substrate.

Curreya Sacc., Syll. fung. (Abellini) 2: 651 (1883), MycoBank MB 1356

Possible synonym:
= *Cucurbidothis* Petr., Annales mycol. 19(3/4): 201 (1921)

Saprobic on cone scales and dead wood. Ascomata black, immersed, with the upper part becoming erumpent, scattered or gregarious, sometimes laterally fused-stromatic, sphaeroid, apex rounded, papilla short. Peridium comprising pseudoparenchymatous cells, outer layer thickened and dark, inner layer thinner and lighter in colour, dark brown to black. Hamathecium of filiform, hyaline, septate, branched pseudoparaphyses. Asci cylindric-clavate to broadly clavate, 8-spored, bitunicate, fissitunicate, with short pedicel, thickened and rounded at the apex, with a wide, flattened, inconspicuous, ocular chamber. Ascospores biseriate or partially uniseri-

ate, fusiform-ellipsoid to broadly ellipsoid, brown to reddish brown, muriform, smooth. Asexual state *Coniothyrium*-like (Wijayawardene *et al*. 2012).

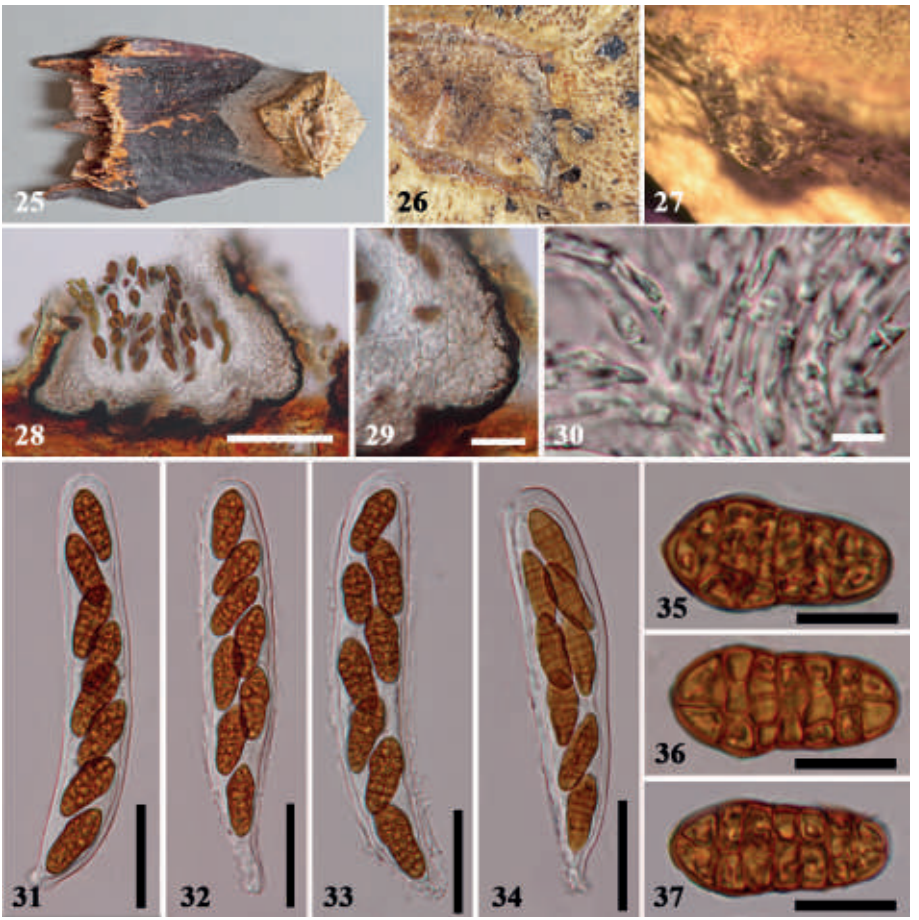
Notes. – The genus *Curreya* was described by Saccardo (1883) based on *Homostegia conorum* Fuckel. Theissen & Sydow (1915) classified the genus in the *Dothideales*. Petrak (1940) classified *Curreya* under *Cucurbitaria*. Later Barr (1981) synonymised *Curreya* under *Pleospora*, not considering the *Coniothyrium*-like, pycnidial asexual morph. Arx & van der Aa (1983) maintained *Curreya* as a distinct genus, based on its *Coniothyrium* asexual state and considered *Curreya* to be closely related to *Didymosphaeria, Melanomma, Paraphaeosphaeria* or *Massarina*. Based on the characters of the ascomatal wall composed of small pseudoparenchymatous cells and the narrower, thinner-walled asci along with the *Coniothyrium*-like asexual state, Barr (1990) assigned *Curreya* to Leptosphaeriaceae while Zhang *et al*. (2012) referred *Curreya* to Cucurbitariaceae. Currently 23 species are listed for *Curreya* in Index Fungorum (2013).

There have been few molecular investigations of *Curreya* as compared to morphological studies. Currently there are 27 hits for *Curreya* in GenBank. Previous phylogenetic studies indicate that type strains of *Curreya austroafricana* (EU552116), *C. proteae* (EU552117), *C. grandicipis* (CBS 114272) and *C. grandicipis* (CBS 111702) clustered within Massarineae (Kruys et al. 2006, Crous *et al*. 2011). The type species of *Curreya* has not been sequenced and thus the familial placement of *Curreya* is still debatable. Recollection of the type specimens and molecular data from type strains are essential to resolve the placement of this genus.

Type species. – ***Curreya conorum*** (Fuckel) Sacc., Syll. fung. (Abellini) 2: 651 (1883), MycoBank MB 217136 (Figs. 25–37)

≡ *Homostegia conorum* Fuckel, Jb. nassau. Ver. Naturk. 29–30: 38 (1875) [1877]
≡ *Pleospora conorum* (Fuckel) M.E. Barr, Mycologia 73(4): 601 (1981)

Saprobic on cone scales of *Pinus silvestris* (Pinaceae). Ascomata 210–235 × 300–315 µm (mean = 225 × 310 µm, n = 10), immersed, becoming erumpent with the upper part, scattered or gregarious, occasionally united by stromatic tissues, sphaeriod, coriaceous, black. Ostiolar apex rounded, papilla short. Peridium 20–28 µm thick (mean = 23 µm, n = 10), dark brown to black, consisting of pseudoparenchymatous cells, outer layer thickened and dark, inner layer thinner and lighter. Hamathecium composed of 1–3 µm wide, filiform, hyaline, septate, apically branched pseudoparaphyses. Asci (50)68–100 × 20–32 µm (mean = 77 × 28 µm, n = 20), cylindric-clavate to broadly clavate, 8-spored, bitunicate, fissitunicate, with a short pedicel, thickened and rounded at the apex with a minute ocular chamber; arising from the base of the ascoma. Ascospores 20–30 × 10–14 µm (mean = 29 × 11 µm, n = 30), biseriate or partially uniseriate, fusiform-ellipsoid to broadly ellipsoid, brown to reddish brown, with 5–7 transverse and 1–3 longitudinal septa, constricted often at A2 septa, wall smooth. Asexual state *Conio-*



Figs. 25–37. *Curreya conorum* (S F8461, holotype). **25–27.** Habit of erumpent ascomata on the natural substrate. **28.** Section of ascoma. **29.** Close up of the peridium. **30.** Pseudoparaphyses. **31–34.** Asci with reddish brown ascospores. **35–37.** Muriform ascospores. Scale bars: **28** 100 µm. **29** 10 µm. **30** 5 µm. **31–34** 25 µm. **35–37** 10 µm.

thyrium-like. Conidiomata pycnidial, unilocular, globose, brown or dark brown, separate, pycnidial wall comprising thick-walled cells of *textura angularis* or *globulosa*. Conidiophores absent. Conidiogenous cells holoblastic, annellidic, hyaline or pale brown, smooth. Conidia cylindrical, pale brown, thick-walled, aseptate (Crous *et al.* 2004).

Notes. – The type species of *Curreya*, *C. conorum*, which is based on *Homostegia conorum*, occurs on cone scales of *Pinus sylvestris* in Germany (Barr 1981). Barr (1981) transferred *Curreya conorum* to *Pleospora*, while retaining the related *Cucurbidothis* in Cucurbitariaceae, but Arx & van der

Aa (1983) raised arguments against this. The latter authors also reported on an associated presumed *Coniothyrium*-like asexual morph and suggested that *Curreya conorum* may be a cone-inhabiting relative of *Cucurbidothis pityophila* (J. C. Schmidt & Kunze) Petr., with larger ascospores and smaller ascomata. *Cucurbidothis pityophila* also differs by superficial growth on conifer wood and strictly cylindrical asci from *Curreya conorum* (Barr 1990, Checa 2004). No strains of *Curreya conorum* or *Cucurbidothis pityophila* were available to us for sequencing and we could not locate type material of *Cucurbidothis pityophila*. Therefore, establishing the correct placement of *Curreya* and its relationship with *Cucurbidothis* and *Cucurbitaria* depends on recollecting type specimens and analysis of molecular data derived from authentic cultures.

Material examined. – GERMANY, Hessen, Oestrich, on cone scales of *Pinus silvestris*, *leg*. L. Fuckel (S F8461, holotype).

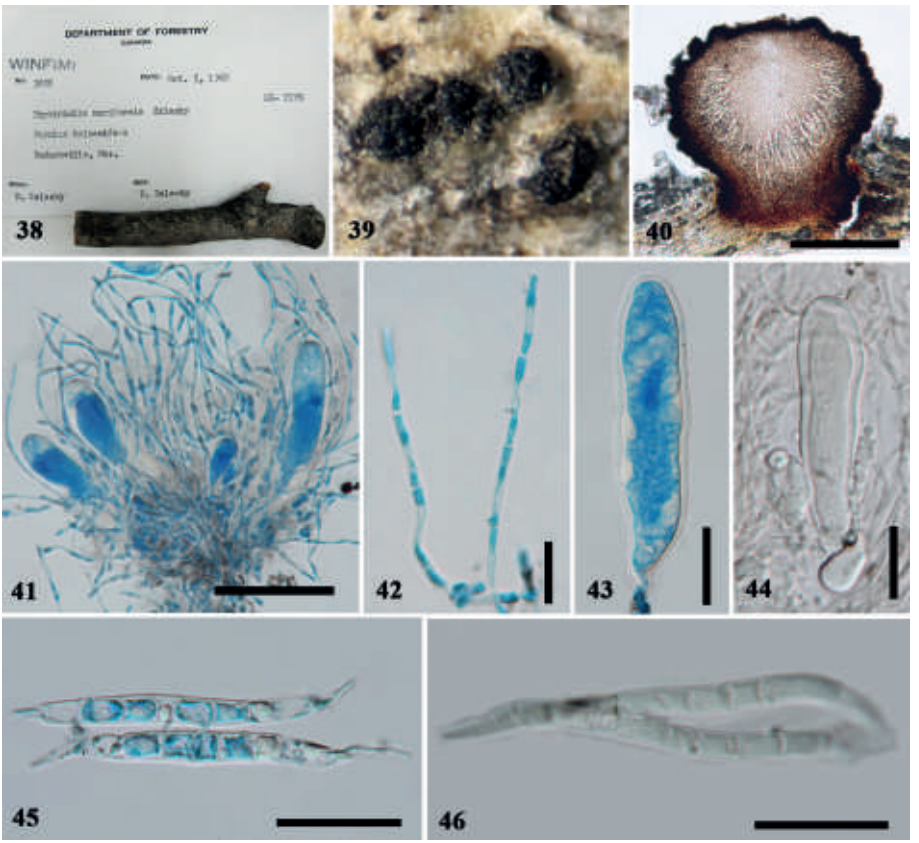
Rhytidiella Zalasky, Can. J. Bot. 46(11): 1383 (1968), MycoBank MB 4741

Pathogenic on woody plants. Ascomata initially immersed in the bark, erumpent, visible as blackened structures with coarsely rugose walls, solitary or gregarious, semi-immersed, globose or subglobose, seated on basal pseudostroma, truncate at the base, with a minute apical, non-papillate ostiole. Peridium comprising two layers, outer layer darkly pigmented and amorphous, inner layer of small pale brown cells forming a *textura angularis*, usually on an erumpent wide stalk. Hamathecium comprising numerous wide, septate, filiform pseudoparaphyses embedded in a gelatinous matrix. Asci oblong to cylindric-clavate, 8-spored, bitunicate, short pedicellate, arising from the base and sides of the ascoma. Ascospores, fusoid, sigmoid to curved, septate, with several transverse septa, hyaline to yellow brown. Asexual state *Phaeoseptoria*-like: Conidiomata pycnidial, scattered, erumpent, globose, with ostiolate, slightly papilliform pore, truncate at the base. Conidiophores hyaline, branched, phialidic, fasciculate, 0–1-septate, short subuliform, obclavate, or cylindrical. Macroconidia hyaline to olivaceous or yellowish, filiform, trans-septate. Microconidia 1-celled, hyaline to light olivaceous, ovoid, guttulate (Zalasky 1968).

Notes. – *Rhytidiella* was described by Zalasky (1968) with *R. moriformis* as the type species. Barr (1987) placed *Rhytidiella* in the Cucurbitariaceae, although Eriksson (2006) suggested that this placement should be confirmed with molecular data. No strain of *Rhytidiella* appears to be available for molecular work and no sequence data is available for the type or other species in GenBank.

Type species. – ***Rhytidiella moriformis*** Zalasky, Can. J. Bot. 46(11): 1383 (1968), MycoBank MB 338606 (Figs. 38–46)

Pathogenic causing perennial rough-bark of *Populus balsamifera* L. Ascomata (365)385–400(420) µm high × 360–405 µm diam. (mean = 397×



Figs. 38–46. *Rhytidiella moriformis* (WINF(M) No. 3825, holotype). **38.** Details of the holotype specimen. **39.** Ascomata erumpent from the host bark. **40.** Section through ascoma. **41.** Immature asci and pseudoparaphyses. **42.** Pseudoparaphyses. **43–44.** Immature asci. **45–46.** Fusoid ascospores. 41–43, 45 in lacto-phenol cotton blue. Scale bars: **40** 200 µm. **41**.100 µm. **42–46** 20 µm.

383 µm n = 15), initially immersed in the bark, erumpent, visible as semi-immersed, blackened structures with coarsely rugose walls, solitary or gregarious, globose or subglobose, seated on a basal pseudostroma, truncate at the base, with a minute apical, non-papillate ostiole. Peridium 52–85 µm thick at sides, comprising two layers, outer layer darkly pigmented and amorphous, inner layer of small pale brown cells in a *textura angularis*, usually on an erumpent, ca. 200 µm wide stalk. Hamathecium comprising numerous, more than 2 µm wide, septate, filiform pseudoparaphyses embedded in a gelatinous matrix. Asci (75)85–110(120) × 15–19 µm (mean = 89 × 17 µm), 8-spored, bitunicate, oblong to cylindric-clavate, short pedicellate, arising from the base and sides of the ascomata. Ascospores 61–84 × 5–6 µm

(mean = 74 × 6 µm, n = 10), fusoid, sigmoid to curved, septate, with several transverse septa, hyaline to yellow brown. Asexual state *Phaeoseptoria*-like. Conidiomata pycnidial, scattered, erumpent, globose, with ostiolate, slightly papilliform pore, truncate at the base. Conidiophores hyaline, branched, apically phialidic, fasciculate, 0–1-septate, short subuliform, obclavate, or cylindrical. Macroconidia filiform, 0–10 septate, wider at the lower end and tapering gradually upwards, hyaline to olivaceous or yellowish. Microconidia 1-celled, hyaline to light olivaceous, ovoid, guttulate (Zalasky 1968).

Notes. – *Rhytidiella moriformis* produces sexual and asexual states in nature. The asexual state belongs to *Phaeoseptoria* according to Zalasky (1968). *Rhytidiella moriformis* is parasitic, causing perennial rough-bark of *Populus balsamifera*. However, there are two similar diseases of bark of *P. balsamifera* (balsam poplar) in Manitoba and Saskatchewan. Rough-bark is caused by *R. moriformis*, by woody galls and by *Diplodia tumefaciens* (Shear) Zalasky (1964). Both fungi affect the periderm, cortex, and phloem tissues. There are obvious differences in their symptoms, host reactions, and fungal succession (Zalasky 1968).

There are four species in *Rhytidiella* (*R. baranyayi* A. Funk & Zalasky, *R. beloniza* (Stirt.) M. B. Aguirre, *R. hebes* P. R. Johnst. and *R. moriformis* Zalasky) listed in Index Fungorum (2013). *Rhytidiella beloniza* has ascomata macroscopically similar to *R. moriformis* with similarly sized and shaped asci and ascospores, *R. beloniza* differs from *R. moriformis* based on biology, being a putative saprobe on *Cordyline australis* bark (Aguirre-Hudson 1991), its geographic distribution and in that its hamathecium does not react in iodine (Galloway 1985, Aguirre-Hudson 1991), while *R. moriformis* stains bright red in iodine (Zalasky 1968). *Rhytidiella baranyayi* differs from *R. moriformis* in lacking a *Phaeoseptoria* conidial state, in ascospore size, in the ascomata being smooth-walled and the hamathecium not reacting to iodine (Funk & Zalasky 1975). *Rhytidiella hebes* is distinguished from the three other species in *Rhytidiella* by biology and in having much wider ascospores (Johnston 2007).

Material examined. – CANADA, Hadashville, Man, on brown bark of living *Populus balsamifera*, 5 October 1965, *leg*. H. Zalasky (WINF(M) No. 3825, holotype).

Syncarpella Theiss. & Syd., Annales mycol. 13(5/6): 631 (1915), MycoBank MB 5331

Saprobic on woody stems. Ascomata erumpent, gregarious or scattered, subglobose, with a papillate ostiole, black, seated on a hypostroma. Peridium comprising two layers, outer layer pigmented, thick-walled, inner layer comprising 3–6 layers of thin-walled, flattened, pale brown cells forming a *textura angularis*. Hamathecium comprising hyaline, filiform, branched pseudoparaphyses embedded in a gelatinous matrix. Asci subcylindrical, 8-spored, bitunicate, short pedicellate. Ascospores biseriate,

cylindric-fusiform, with 3 transverse septate, slightly constricted at the septa, yellow to brown. Asexual state *Syntholus*-like.

Notes. – *Syncarpella* was introduced by Theissen & Sydow (1915) as a genus of Montagnellaceae within *Dothideales*, with *Syncarpella tumefaciens* (Ellis & Harkn.) Theiss. & Syd. as the type of the genus. *Syncarpella* was considered to be closely related to *Leptosphaeria* (Clements & Shear 1931). Barr & Boise (1989) transferred *Syncarpella* to Cucurbitariaceae based on abundant globose, ovoid to turbinate ascomata with minute papillae which are seated on a common base. There are ten species in the genus (Index Fungorum 2013). Currently there is no sequence data available for the type species of *Syncarpella* in GenBank. Ramaley & Barr (1997) described *Syntholus ribis* A. W. Ramaley & M. E. Barr to accommodate the asexual state of *Syncarpella ribis* A. W. Ramaley & M. E. Barr.: Conidiomata of *Syntholus ribis* eustromatic, at first immersed but strongly erumpent, multilocular or rarely unilocular, the locules more or less distinct in upper parts, separated below by columns of hyaline to pale brown pseudoparenchyma, each with one or more non- or slightly papillate ostioles. Conidiophores absent. Conidiogenous cells enteroblastic, phialidic, determinate, discrete. Conidia aseptate, thick-walled. Microconidia formed on the upper wall near the ostiole and near the ostiolar canal (Ramaley & Barr 1997).

We cannot be certain that *Syncarpella ribis* is closely related to the type species of *Syncarpella* and therefore cannot synonymize *Syntholus* with *Syncarpella*.

Type species. – ***Syncarpella tumefaciens*** (Ellis & Harkn.) Theiss. & Syd., Annales mycol. 13(5/6): 633 (1915), MycoBank MB 229427 (Figs. 47–55)

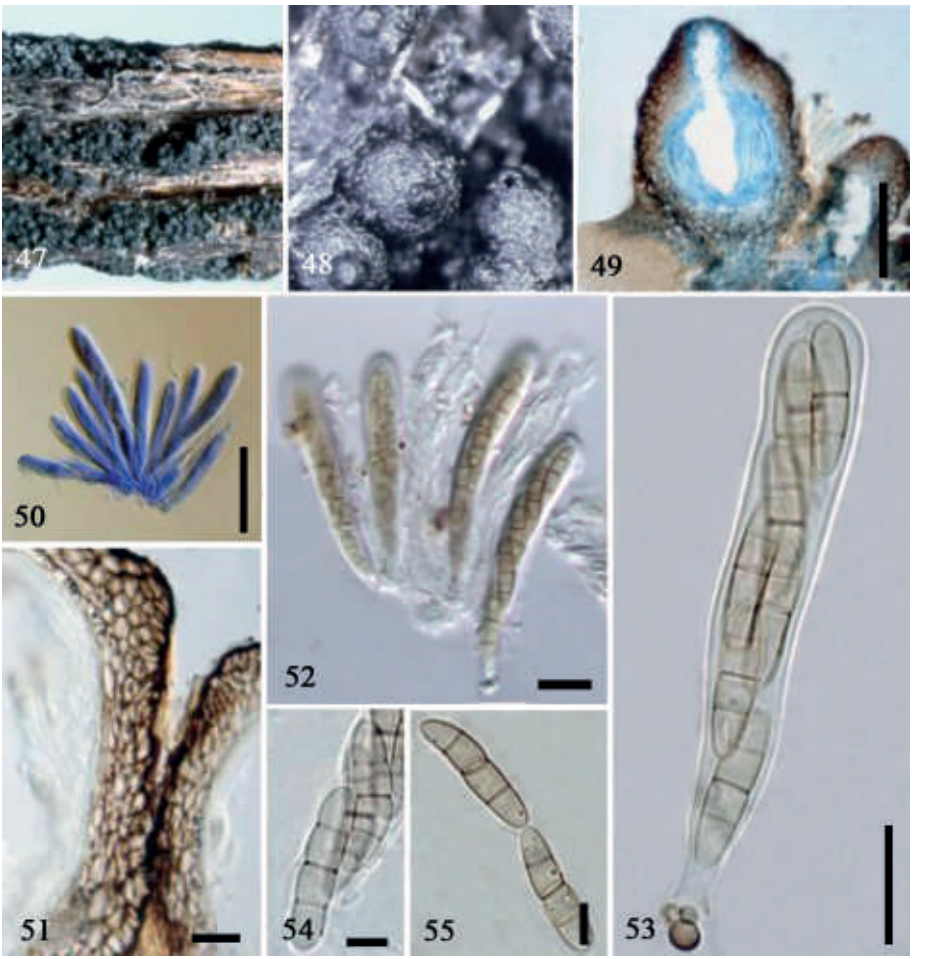
≡ *Sphaeria tumefaciens* Ellis & Harkn., J. Mycol. 2(4): 41 (1886)

≡ *Leptosphaeria tumefaciens* (Ellis & Harkn.) Petr., Annales mycol. 32(5/6): 361 (1934)

≡ *Montagnella tumefaciens* (Ellis & Harkn.) Berl. & Voglino, in Saccardo, Syll. fung. (Abellini) 4: 244 (1886).

Ascomata (305)470–650 µm high × (315)410–525 µm diam. (mean = 479 × 416 µm, n = 10), united on a crustose hypostroma, subglobose to amygdaliform, erumpent through longitudinal fissures in the bark and forming densely compacted clusters, black, rough-walled, cupulate on drying, with a papillate ostiole. Peridium 52–105 µm wide, comprising two layers, outer layer darkly pigmented, small, thick-walled, inner layer comprising 3–6 layers of brown-walled cells forming a *textura angularis*. Hamathecium of dense, septate, filiform, hyaline, branched pseudoparaphyses, embedded in a gelatinous matrix. Asci (150)170–220 × 23–27 µm (mean = 174 × 26 µm, n = 10), subcylindrical, 8-spored, bitunicate, pedicel short. Ascospores (38)50–64 × 11–13 µm (mean = 54 × 12 µm, n = 10), biseriate, cylindric-fusiform, slightly curved, with three transverse septa, yellowish brown.

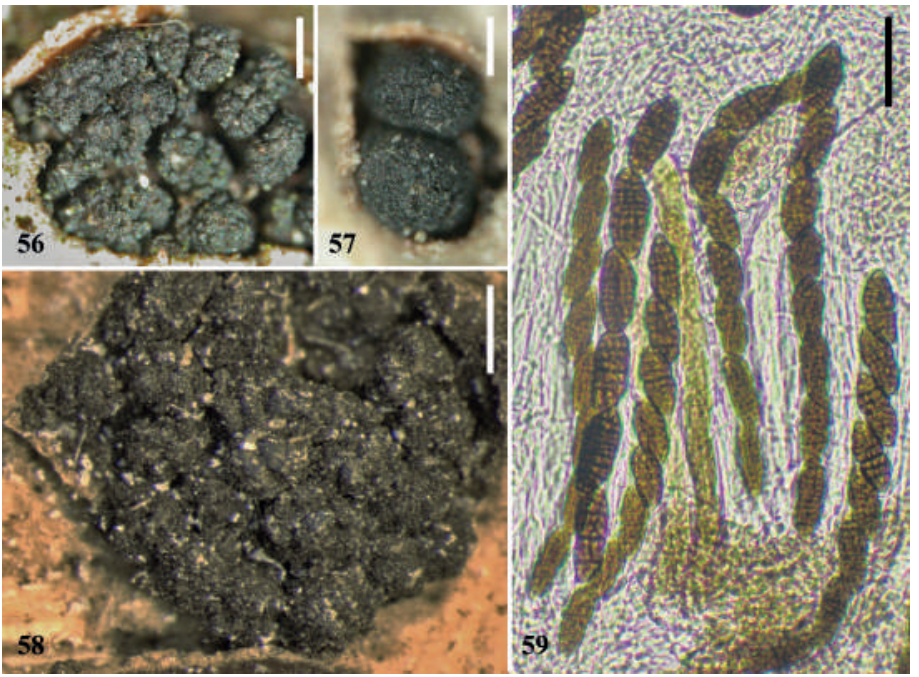
Material examined. – USA, New York, on dead limbs of *Artemisia californica,* 1886, *leg*. H. K. Harkness (NY, 1677, holotype).



Figs. 47–55. *Syncarpella tumefaciens* (NY, holotype). **47.** Crowded ascomata on the host stem. **48.** Close-up of ascomata on the natural host. **49.** Section through ascoma. **50.** Squash mount showing asci. **51.** Peridium. **52.** Asci and pseudoparaphyses. **53.** Ascus with ascospores. **54–55.** Ascospores. 49, 50 in cotton blue. Scale bars: **48** 50 µm. **49** 200 µm. **50** 100 µm **51–52** 25 µm. **53–55** 5 µm.

Discussion

In this paper we used four isolates of *Cucurbitaria berberidis* (MFLUCC 11-0384, MFLUCC 11-0385, MFLUCC 11-0386 and MFLUCC 11-0387) from different ascomata collected in the same area on twigs of several individual plants that had been united into one specimen. The phylogenetic analysis showed that all new isolates of *C. berberidis* sequenced in this study are phylogenetically identical (99 % bootstrap support). The newly sequenced strains



Figs. 56–59. *Cucurbitaria berberidis*. **56–57.** Ascomata of the epitype WU 31405. **58.** Ascomata of the lectotype L0112523. **59.** Asci and ascospores of the lectotype. Scale bars: **56, 58** 500 µm. **57** 200 µm. **59** 30 µm.

also clustered together with two other strains (CBS 363.93 and CBS 394.84) from GenBank and formed a strongly supported (100 % ML, 100 % MP and 1.00 Bayesian) clade within Cucurbitariaceae. Therefore the new collection of *C. berberidis* (WU 31405; part deposited as MFLU12-0111), which comes from the same host as given in the protologue, is here designated as epitype.

There are no sequence data available for the generic type of *Curreya*, *Rhytidiella* or *Syncarpella* species in GenBank and thus we could not include them in our analysis. These genera are, however, placed in Cucurbitariaceae (Tab. 2) based on abundant ascomata which are seated on a basal pseudostroma. In these genera the peridium is obviously two layered, with an outer blackened, amorphous layer and an inner layer composed of pale brown cells of *textura angularis*. The ascomata are on a thick base or stalk and form on a basal pseudostroma.

Sivanesan (1984) reported that *Cucurbitaria* has *Pyrenochaeta* asexual states. This was confirmed by Farr *et al.* (1989). De Gruyter (2010, 2013) showed that *Pyrenochaeta nobilis* De Not. and *Pyrenochaetopsis leptospora* (Sacc. & Briard) Gruyter, Aveskamp & Verkley, the generic types of *Pyrenochaeta* and *Pyrenochaetopsis*, can be accommodated in Cucurbitariaceae. *Pyrenochaetopsis* was introduced to accommodate several *Phoma* spp. and

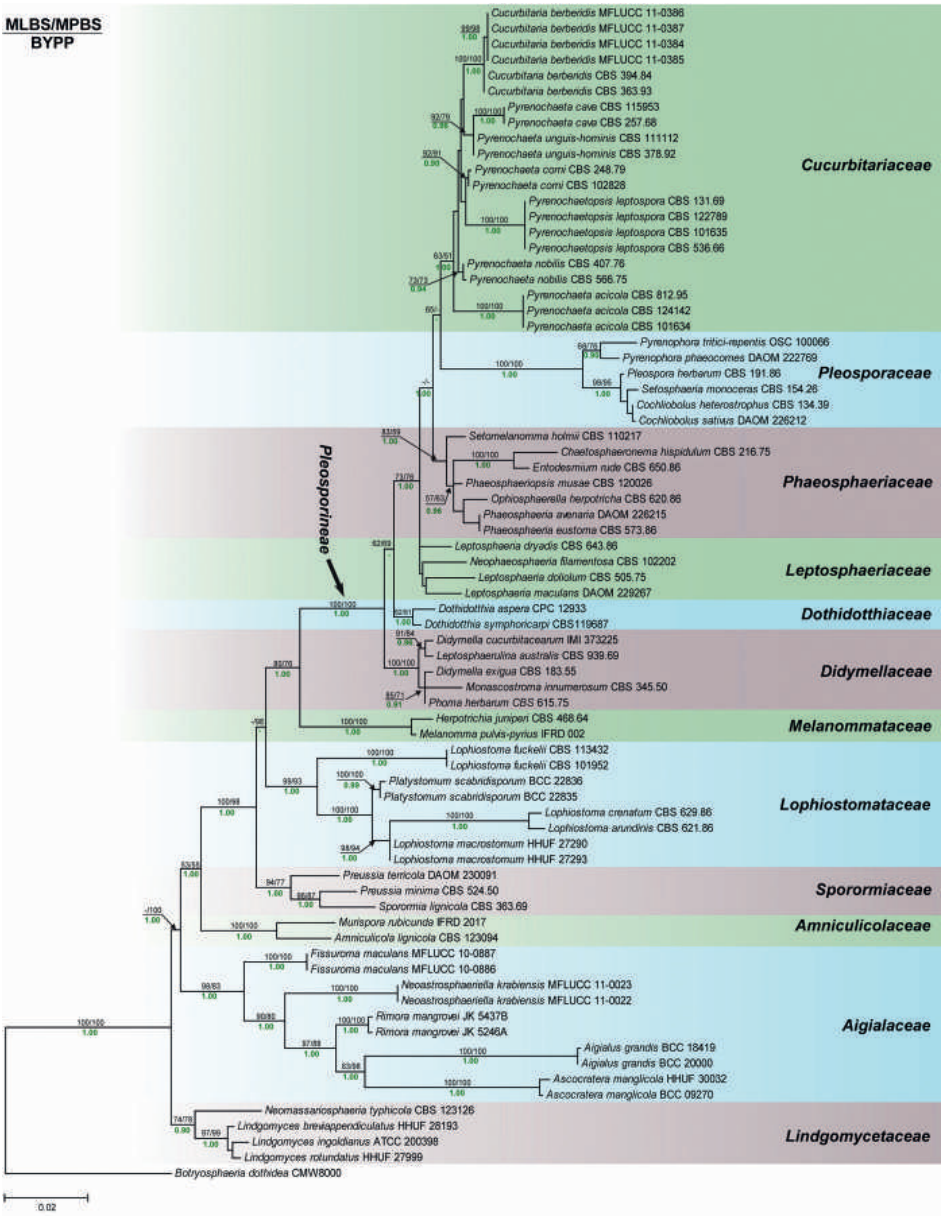


Fig. 60. RAxML tree based on a combined dataset of SSU (1010 bp) and LSU (1279 bp) nrDNA sequences. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 50 % are given above the nodes; Bayesian posterior probabilities above 0.90 are given below the nodes. Hyphen (–) indicates a value lower than 50 % (BS) or 0.90 (PP). The original isolate numbers are given after the species names. The tree was rooted with *Botryosphaeria dothidea*.

Phoma cava Schulzer was transferred to *Pyrenochaeta* by de Gruyter *et al.* (2010). Furthermore, de Gruyter *et al.* (2010) showed phylogenetically that *Pyrenochaeta nobilis* De Not., the generic type of *Pyrenochaeta* grouped with *C. berberidis* (= *P. berberidis*) in the same clade. However, *Pyrenochaeta* spp. are not placed as one group in our study. The type species, *P. nobilis* grouped as distinct from *P. berberidis*. Hence, we currently do not reduce *Pyrenochaeta* as a synonym under *Cucurbitaria* although some *Pyrenochaeta* spp. are linked with *Cucurbitaria* spp.

Tab. 2. Classifications of genera in Cucurbitariaceae.

Kirk *et al.* (2008)	Lumbsch & Huhndorf (2010)	This paper
Cucurbitaria	*Cucurbitaria*	*Cucurbitaria*
Curreya	*Curreya*	*Curreya*
Gibberidea	*?Rhytidiella*	*Pyrenochaeta*
?Rhytidiella	*Syncarpella*	*Pyrenochaetopsis*
Syncarpella		*?Rhytidiella*
		?Syncarpella

Mirza (1968) and Sivanesan (1984) obtained from *Cucurbitaria* species asexual states that are morphologically assignable to *Camarosporium* Schulzer, but *Camarosporium quaternatum* Sacc., the type species of *Camarosporium*, was shown to cluster with leptosphaeriaceous taxa by Schoch *et al.* (2009). Recently, Liu *et al.* (2012) accepted *Camarosporium* as a genus in Botryosphaeriales *incertae sedis*. Hence we do not accept *Camarosporium sensu stricto* in Cucurbitariaceae, but *Camarosporium*-like asexual states can be produced by some species and this may be an indication that *Cucurbitaria* in its current scope is not monophyletic. Wijayawardene *et al.* (2012) listed *Pleurophoma* in Cucurbitariaceae, but the generic type of *Pleurophoma*, *P. pleurospora* (Sacc.) Höhn. was shown to cluster far from Cucurbitariaceae by de Gruyter *et al.* (2010; 2013). Hence, we do not accept *Pleurophoma* in Cucurbitariaceae.

Curreya conorum (Fuckel) Sacc., the generic type of *Curreya* was shown to be linked with *Coniothyrium glomerulatum* Sacc. (Arx & van der Aa 1983), while Marincowitz *et al.* (2008) also reported that *Curreya* has *Coniothyrium*-like asexual morphs. The generic type of *Coniothyrium*, *C. palmarum* Corda, however, clusters in a separate clade in *Dothideomycetes* and de Gruyter *et al.* (2013) and Hyde *et al.* (unpubl.) accept *Coniothyrium sensu stricto* in Coniothyriaceae as a distinct family in *Dothideomycetes*. *Curreya* probably has *Coniothyrium*-like asexual morphs, but they may not belong in *Coniothyrium sensu stricto*. This was interpreted similarly by Crous *et al.* (2011) who showed that *Coniothyrium grandicipis* Joanne E. Taylor & Crous grouped with *Curreya austroafricana* Marinc., M. J. Wingf. & Crous and *Curreya proteae* Marinc., M. J. Wingf. & Crous. As it is not appropriate to keep

Coniothyrium grandicipis in *Coniothyrium,* it was transferred to *Curreya* (Crous *et al*. 2011). However, the generic type of *Curreya* requires sequencing in order to confirm these generic assignments.

Zalasky (1968) and Sivanesan (1984) reported that the generic type of *Rhytidiella, R. moriformis* Zalasky has *Phaeoseptoria* asexual morphs. However, *Phaeoseptoria* is a genus of Phaeosphaeriaceae (Camara *et al*. 2002). Thus, asexual morphs of *Rhytidiella* may only be called *Phaeoseptoria*-like.

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