Studies in Mycology

Stemphylium revisited

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Abstract: In 2007 a new *Stemphylium* leaf spot disease of *Beta vulgaris* (sugar beet) spread through the Netherlands. Attempts to identify this destructive *Stemphylium* sp. in sugar beet led to a phylogenetic revision of the genus. The name *Stemphylium* has been recommended for use over that of its sexual morph, *Pleospora*, which is polyphyletic. *Stemphylium* forms a well-defined monophyletic genus in the *Pleosporaceae, Pleosporales* (*Dothideomycetes*), but lacks an up-to-date phylogeny. To address this issue, the internal transcribed spacer 1 and 2 and intervening 5.8S nr DNA (ITS) of all available *Stemphylium* and *Pleospora* isolates from the CBS culture collection of the Westerdijk Institute (N = 418), and from 23 freshly collected isolates obtained from sugar beet and related hosts, were sequenced to construct an overview phylogeny (N = 350). Based on their phylogenetic informativeness, parts of the protein-coding genes calmodulin and glyceraldehyde-3-phosphate dehydrogenase were also sequenced for a subset of isolates (N = 149). This resulted in a multi-gene phylogeny of the genus *Stemphylium* containing 28 species-clades, of which five were found to represent new species. The majority of the sugar beet isolates, including isolates from the Netherlands, Germany and the UK, clustered together in a species clade for which the name *S. beticola* was recently proposed. Morphological studies were performed to describe the new species. Twenty-two names were reduced to synonymy, and two new combinations proposed. Three epitypes, one lectotype and two neotypes were also designated in order to create a uniform taxonomy for *Stemphylium*.

Key words: Morphology, Multi-gene phylogeny, *Pleospora*.

Taxonomic novelties: New combinations: *Stemphylium armeriae* (Corda) Woudenb. & Crous, *S. halophilum* (J. Webster) Woudenb. & Crous; **New species:** *S. canadense* Woudenb. & Crous, *S. chrysanthemicola* Woudenb. & Crous, *S. lucomagnoense* Woudenb. & Crous, *S. novae-zelandiae* Woudenb. & Crous, *S. simmonsii* Woudenb. & Crous; **Typification (Basionyms): Epitypifications:** *Alternaria lancipes* Ellis & Everh., *Stemphylium solani* G.F. Weber, *Thyrospora astragali* Yoshii; **Lectotypification:** *Thyrospora astragali* Yoshii; **Neotypifications:** *Pleospora pomorum* A.S. Horne, *Thyrospora lycopersici* Enjoji.

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INTRODUCTION

In 2007 a new leaf spot disease associated with a *Stemphylium* sp. was first discovered on sugar beet (*Beta vulgaris*) in the Netherlands, which subsequently spread rapidly throughout the country in the following years (Hanse 2013). The causal agent was recently formally named as *Stemphylium beticola* (Crous *et al.* 2016), but the genus itself was not treated in that study.

Stemphylium is a dematiaceous hyphomycete, which can be distinguished from other hyphomycetes forming phaeodictyospores based on the percurrent rejuvenation of its conidiophores, and apically swollen conidiogenous cells. Other closely related genera mostly display a geniculate, sympodial proliferation, e.g. *Alternaria* (Simmons 2007). *Stemphylium*, with *S. botryosum* as type species, forms a well-defined monophyletic genus in the family *Pleosporaceae, Pleosporales* (Câmara *et al.* 2002; Inderbitzin *et al.* 2009). However, the sexual morph to which *Stemphylium* is linked, *Pleospora*, is known to be polyphyletic. The type species of *Pleospora*, *Pleospora herbarum*, has *Stemphylium herbarum* as asexual morph (Simmons 1985), but several *Pleospora* spp. have been linked to a range of different asexual genera (e.g. Inderbitzin *et al.* 2006; De Gruyter *et al.* 2013; Ariyawansa *et al.* 2015; Crous & Groenewald 2017). The latest comprehensive phylogenetic study on *Pleospora* species with *Stemphylium* asexual morphs was published in 2009 (Inderbitzin *et al.* 2009), which left many unnamed and potentially new *Stemphylium* species. The *Pleospora herbarum* clade *sensu* Inderbitzin *et al.* (2009) illustrated the problems with identification in the genus. Based on a multi-gene phylogeny five species should be synonymised, but RAPD fingerprints (Chaisrisook *et al.* 1995), morphology and ecology studies supported them to be separate species. Some researchers therefore chose to retain all the species names (e.g. Inderbitzin *et al.* 2009), while others again chose to synonymise them (e.g. Köhl *et al.* 2009). With the uptake of the one fungus-one name initiative in the International Code of Nomenclature for algae, fungi and plants (ICN, McNeill et al., 2012), name changes in these genera became necessary. The use of *Stemphylium* over *Pleospora* has subsequently been recommended by the Working Group on *Dothideomycetes* of the International Committee on the Taxonomy of Fungi (Rossman *et al.* 2015).

The aim of the present study was to construct a phylogenetic overview of the genus *Stemphylium*. All available *Stemphylium* and *Pleospora* isolates from the CBS collection, together with *Stemphylium* isolates collected from sugar beet from different parts of the Netherlands as well as from the UK and Germany, were included in the study. The internal transcribed spacer 1 and 2 and intervening 5.8S nr DNA (ITS) were sequenced to construct a draft overview phylogeny. Using a reduced set of isolates, the phylogenetic informativeness of six commonly used protein-coding genes, namely partial actin (*actA*), beta-tubulin (*tub2*), calmodulin (*cmdA*), translation elongation factor 1-alpha

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(*tef1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and DNA-directed RNA polymerase second largest subunit (*rpb2*) were also evaluated. Based on these results, the two most promising genes were additionally sequenced for the genus *Stemphylium*, and used to construct a multi-gene phylogeny.

MATERIALS AND METHODS

Isolates

Four-hundred-and-forty-one isolates were included in this study, comprising of 418 *Pleospora* and *Stemphylium* isolates from the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands (Supplementary Table 1) and 23 isolates received from the IRS (the research and knowledge centre for sugar beet cultivation in The Netherlands), Bergen op Zoom, the Netherlands (Supplementary Table 2). The dataset includes 48 (ex-)type strains. Freeze-dried strains from the CBS culture collection were revived in 2 mL malt/peptone (50 %/50 %) and subsequently transferred to oatmeal agar (OA) (Crous *et al.* 2009). Strains stored in liquid nitrogen were transferred to OA directly from the −185 °C storage. For the isolation methods of the IRS isolates see Hanse *et al.* (2015).

Morphology

Isolates were grown on potato carrot agar (PCA, Crous et al., 2009) and synthetic nutrient-poor agar (SNA, Nirenberg, 1976) at moderate temperatures under CoolWhite fluorescent light with an 8 h photoperiod. After 7 and 14 d the growth rates were measured and the colony characters noted. Colony colours were rated according to Rayner (1970). Morphological descriptions of asexual structures were made for isolates grown on SNA for 7 d. Slides were prepared with the cellotape technique (Schubert *et al.* 2007) using Titan Ultra Clear Tape (Conglom Inc., Toronto, Canada) and Shear's medium as mounting fluid. Morphological descriptions of sexual structures were made for isolates grown on PCA for 14 d, with 85 % lactic acid as mounting fluid. The mean plus/minus standard deviation values were derived from measurements of 30 structures, with extremes given in parentheses. Photographs of characteristic structures were made with a Zeiss Axio Imager A2 microscope equipped with a Nikon DS-Ri2 high-definition colour camera using differential interference contrast (DIC) optics and the Nikon software NIS-elements D v. 4.50. Adobe Bridge CS5.1 and Adobe Photoshop CS5 Extended, v. 12.1, were used for the final editing and photographic preparation. Nomenclatural data were deposited in MycoBank (Crous *et al.* 2004).

DNA isolation, PCR and sequencing

DNA extraction was performed using the Wizard® Genomic DNA purification kit (Promega, Madison, USA) according to the manufacturer's instructions. The ITS region, *gapdh*, *tef1* and *rpb2* gene regions were amplified and sequenced with respectively the primers V9G (De Hoog and Gerrits van den Ende, 1998)/ITS4 (White *et al.* 1990), gpd1/gpd2 (Berbee *et al.* 1999), EF1-728F/EF1-986R (Carbone & Kohn 1999), and RPB2-5F2 (Sung *et al.* 2007)/fRPB2-7cR (Liu *et al.* 1999) as described in Woudenberg *et al.* (2013). The *actA* gene region was amplified and sequenced with ACT-512F/ACT-783R (Carbone & Kohn 1999) as described in De Gruyter *et al.* (2009). For the *tub2* gene region several primer combinations and PCR programs were tested, but no PCR product could be obtained. The *cmdA* gene region was amplified and sequenced with the primers CALDF1/CALDR2 (Lawrence *et al.* 2013). The PCR mixture consisted of 1 µl 50× diluted genomic DNA, 1× NH4+ reaction buffer (Bioline, Luckenwalde, Germany), 2 mM $MgCl_2$, 20 µM of each dNTP, 0.2 µM of each primer and 0.25 U *Taq* DNA polymerase (Bioline). The PCR conditions consisted of an initial denaturation step of 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 59 °C and 1 min at 72 °C, and a final elongation step of 7 min at 72 °C. The PCR products were sequenced in both directions using a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Bleiswijk, the Netherlands) and analysed with an ABI Prism 3730xl DNA Analyser (Thermo Fisher Scientific) according to the manufacturer's instructions. Consensus sequences were computed from forward and reverse sequences using the Bionumerics v. 4.61 software package (Applied Maths, St-Marthens-Latem, Belgium). Generated sequences were deposited in GenBank (Table 1, Supplementary Table 1).

Identification of best loci

Based on the ITS sequence results and former sequence data (Inderbitzin *et al.* 2009), seven isolates representing clade 10 (Fig. 1), namely CBS 378.54, CBS 116598, CBS 116599, CBS 134496, CBS 136590, GV11-196-a1-3 and IFZ2013-024, were selected to determine which gene would be the most informative in distinguishing species within this clade. In addition to ITS, the *actA*, *cmdA*, *gapdh*, *rpb2* and *tef1* gene regions were amplified and sequenced as described above. Unfortunately the beta-tubulin PCRs did not give any results, even when following previously published PCR primers and methods (Bt2a/Bt2b, Glass & Donaldson 1995) which are supposed to work on *Stemphylium* species (Lawrence *et al.* 2013). A sequence comparison from the five additional gene regions of the seven selected isolates was made in Bionumerics v. 4.61 (Applied Maths) and by eye (Table 2).

Phylogenetic analyses

In Bionumerics v. 4.61 (Applied Maths), a quick UPGMA phylogeny was constructed from the ITS sequences of the 441 included isolates to assign them to clusters of closely related or identical isolates. For those isolates belonging to the *Stemphylium* clade, a multiple sequence alignment of the ITS sequences was generated with MAFFT v. 7.271 (http://mafft.cbrc.jp/alignment/server/index.html) using the FFT-NS-i method. With Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) the best nucleotide substitution model was determined. Bayesian analyses were performed with MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The sample frequency was set at 1 000 and the temperature value of the heated chain was set at 0.1. The run stopped when the average standard deviation of split frequencies reached below 0.01. Burn-in was set to 25 % after which the likelihood values were

Table 1. Collection details and GenBank accession numbers of the *Stemphylium* cultures included in the multi-gene phylogeny.

Name	Old name[1]	Strain number[2]	Other collection number[2]	Host/Substrate	Country	GenBank accession numbers		
						ITS	*gapdh*	*cmdA*
Alternaria alternata		GV14-634a1		*Chenopodium album*	Netherlands	KU850502	KU850649	KU850790
Stemphylium amaranthi	*S. phaseolina*^{T}	CBS 124650	HSAUP VI1538	*Phaseolus vulgaris*	China	KU850503	KU850650	KU850791
		CBS 124651	HSAUP VI1682	*Phaseolus vulgaris*	China	KU850504	KU850651	KU850792
		CBS 124746^{T}	HSAUPpyf1835	*Amaranthus retroflexus*	China	KU850505	KU850652	KU850793
		CBS 124750	HSAUPpyf1902	*Malus sieversii*	Chile	KU850506	KU850653	KU850794
	S. microsporum^{T}	CBS 124753	HSAUPpyf1904	*Malus sieversii*	China	KU850507	KU850654	KU850795
		CBS 124984	HSAUPpyf2018	*Raphanus sativus*	China	KU850508	KU850655	KU850796
	S. luffae^{T}	CBS 124985	HSAUPpyf2016	*Luffa cylindrica*	China	KU850509	KU850656	KU850797
		CBS 136589	E.G.S. 48.098	*Lotus pendunculatus*	New Zealand	KU850510	KU850657	KU850798
Stemphylium armeriae comb. nov.	*P. armeriae*	CBS 338.73		*Armeria maritima*	UK	KU850511	KU850658	KU850799
Stemphylium astragali		CBS 116583^{ET}	E.G.S. 08.174	*Astragalus* sp.	Japan	KU850512	KU850659	KU850800
Stemphylium beticola	*P. armeriae*	CBS 378.54		*Lychnis* sp.	Canada	KU850513	KU850660	KU850801
		CBS 116599	UAMH 10489	Herbaceous dicot	Canada	KU850514	KU850661	KU850802
		CBS 133512	E.G.S. 30.152	*Pisum sativum*	Canada	KU850515	KU850662	KU850803
		CBS 133892	E.G.S. 38.090	*Lens culinaris*	USA	KU850516	KU850663	KU850804
		CBS 136590	E.G.S. 48.097	*Passiflora edulis*	New Zealand	KU850517	KU850664	KU850805
		CBS 136699	E.G.S. 48.126	*Panax* sp.	USA	KU850518	KU850665	KU850806
		CBS 137492	E.G.S. 50.095	*Spinacia oleracea*	USA	KU850519	KU850666	KU850807
		CBS 141024^{T}	GV11-265a	*Beta vulgaris*	Netherlands	KU850520	KU850667	KU850808
		CBS 141025	GV12-288-2	*Beta vulgaris*	Netherlands	KU850521	KU850668	KU850809
		CBS 141026	GV12-474-a1	*Beta vulgaris*	Netherlands	KU850522	KU850669	KU850810
		GV11-196a1-3		*Beta vulgaris*	Netherlands	KU850523	KU850670	KU850811
		GV12-275a1		*Beta vulgaris*	Netherlands	KU850524	KU850671	KU850812
		GV12-276a1		*Beta vulgaris*	Netherlands	KU850525	KU850672	KU850813
		GV12-287a1		*Beta vulgaris*	Netherlands	KU850526	KU850673	KU850814
		GV12-336a1		*Beta vulgaris*	Netherlands	KU850527	KU850674	KU850815
		GV12-356a1		*Beta vulgaris*	Netherlands	KU850528	KU850675	KU850816
		GV12-367a1		*Beta vulgaris*	Netherlands	KU850529	KU850676	KU850817
		GV12-368a1		*Beta vulgaris*	Netherlands	KU850530	KU850677	KU850818
		GV12-403a1		*Beta vulgaris*	Netherlands	KU850531	KU850678	KU850819

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Table 1. (Continued).

Name	Old name[1]	Strain number[2]	Other collection number[2]	Host/Substrate	Country	GenBank accession numbers		
						ITS	*gapdh*	*cmdA*
		GV13-425a1		*Beta vulgaris*	Netherlands	KU850532	KU850679	KU850820
		GV13-436c2		*Beta vulgaris*	Netherlands	KU850533	KU850680	KU850821
		GV14-693a1		*Beta vulgaris*	UK	KU850534	KU850681	KU850822
		IFZ2013-024		*Beta vulgaris*	Germany	KU850535	KU850682	KU850823
		IFZ2013-035		*Beta vulgaris*	Germany	KU850536	KU850683	KU850824
		IFZ2014-020		*Beta vulgaris*	Germany	KU850537	KU850684	KU850825
Stemphylium botryosum		CBS 714.68^{T}	E.G.S. 04.118c; IMI 135456; MUCL 11717; QM 1379	*Medicago sativa*	Canada	**KC584238**	**AF443881**	KU850826
		CBS 116596	E.G.S. 08.069; QM 7066	*Medicago sativa*	USA	KU850538	KU850685	KU850827
Stemphylium callistephi		CBS 527.50^{T}		*Callistephus chinensis*	USA	KU850539	KU850686	KU850828
Stemphylium canadense sp. nov.		CBS 116602^{T}	UAMH 10491	*Salicornia* sp.	Canada	KU850641	KU850782	KU850932
		CBS 118081	UAMH 10491	*Salicornia* sp.	Canada	KU850642	KU850783	KU850933
Stemphylium chrysanthemicola sp. nov.		CBS 117255^{T}	E.G.S. 31.008	*Chrysanthemum* sp.	New Zealand	KU850640	KU850781	KU850931
Stemphylium drummondii		CBS 346.83^{T}		*Phlox drummondii*	Germany	**GQ395365**	KU850687	KU850829
Stemphylium eturmiunum	*S. vesicarium*	CBS 668.80		*Solanum lycopersicum*	Greece	KU850540	KU850688	KU850830
		CBS 109845^{T}	E.G.S. 29.099; IMI 386969	*Solanum lycopersicum*	New Zealand	KU850541	KU850689	KU850831
		CBS 122124		*Asphodelus aestivus*	Greece	KU850542	KU850690	KU850832
	S. variabilis^{T}	CBS 122641	HSAUPIVI1508	*Allium sativum*	France	KU850543	KU850691	KU850833
		CBS 124652	HSAUP1559	*Solanum lycopersicum*	China	KU850544	KU850692	KU850834
	S. vesicarium	CBS 133528	E.G.S. 30.002	*Allium sativum*	India	KU850545	KU850693	KU850835
	S. capsici^{T}	CBS 138495	E.G.S. 53.123	*Capsicum annuum*	China	KU850546	KU850694	KU850836
Stemphylium gracilariae	*P. lycopersici*	CBS 308.36	ATCC 10737	*Solanum lycopersicum*	USA	KU850547	KU850695	KU850837
	P. herbarum f. *lactucum*^{T}	CBS 273.55		*Lactuca* sp.	Unknown	KU850548	KU850696	KU850838
		CBS 482.90^{T}	E.G.S. 37.073; ATCC 669721	*Gracilaria* sp.	Israel	KU850549	**AF443883**	KU850839
		CBS 115179	STE-U 5216; CPC 5216	*Leucospermum* sp.	Spain	KU850550	KU850697	KU850840
		CBS 115180	STE-U 5217; CPC 5217	*Leucospermum* sp.	Spain	KU850551	KU850698	KU850841
	S. cucumis^{T}	CBS 125060	HSAUPpyf2377	*Cucumis melo*	China	KU850552	KU850699	KU850842
Stemphylium halophilum comb. nov.		CBS 337.73^{T}		*Limonium vulgare*	UK	KU850553	KU850700	KU850843
		CBS 410.73		*Armeria maritima*	UK	KU850554	KU850701	KU850844

Stemphylium ixeridis		CBS 124748^{T}		*Ixeris denticulata*	China	KU850590	KU850737	KU850881
Stemphylium lancipes		CBS 101217		*Aquilegia* sp.	New Zealand	KU850594	KU850741	KU850885
		CBS 116584	E.G.S. 46.182	*Aquilegia* sp.	New Zealand	KU850595	**AF443886**	KU850886
		CBS 133314^{ET}	E.G.S. 10.022	*Aquilegia canadensis*	USA	KU850596	KU850742	KU850887
Stemphylium loti		CBS 407.54^{T}	ATCC 11718	*Lotus corniculatus*	USA	KU850597	KU850743	KU850888
Stemphylium lucomagnoense sp. nov.	*P. gigaspora*	CBS 116601^{T}	E.G.S. 37.017	*Minuartia hybrida*	Switzerland	KU850629	KU850770	KU850920
Stemphylium lycii		CBS 115192	STE-U 5223; CPC 5223	*Protea cynaroides*	Portugal	KU850598	KU850744	KU850889
		CBS 116582	E.G.S. 48.089	*Pistacia vera*	USA	KU850599	KU850745	KU850890
		CBS 124982	HSAUPpyf1828	*Apium graveolens*	China	KU850600	KU850746	KU850891
		CBS 125240	HSAUP1826	*Cucurbita moschata*	China	KU850601	KU850747	KU850892
		CBS 125241^{T}	HSAUP 1833	*Lycium chinense*	China	KU850602	KU850748	KU850893
Stemphylium lycopersici	*S. lancipes*	CBS 333.73	PD 72/1118	*Platycodon* sp.	Netherlands	KU850603	KU850749	KU850894
	S. vesicarium	CBS 436.76		Unknown	Indonesia	KU850604	KU850750	KU850895
	S. lancipes	CBS 463.78		*Solanum tuberosum*	Peru	KU850605	KU850751	KU850896
		CBS 321.87		*Solanum lycopersicum*	Senegal	KU850606	KU850752	KU850897
	S. xanthosomatis^{T}	CBS 116585	E.G.S. 17.137	*Xanthosoma sagittifolium*	New Caledonia	KU850607	**AY317010**	KU850898
		CBS 116587	E.G.S. 46.001	*Solanum lycopersicum*	Dominican Republic	KU850608	KU850753	KU850899
	S. sophorae^{T}	CBS 120325		*Sophora microphylla*	China	KU850609	KU850754	KU850900
	S. oblongum^{T}	CBS 120326		*Gossypium hirsutum*	China	KU850610	KU850755	KU850901
		CBS 122639^{NT}	HSAUPV0893	*Solanum lycopersicum*	China	KU850611	KU850756	KU850902
	S. pyrina^{T}	CBS 122803	HSAUP wy0006	*Pyrus sinkiangensis*	China	KU850612	KU850757	KU850903
		CBS 123008	HSAUP0475	*Brassica pekinensis*	China	KU850613	KU850758	KU850904
	S. pruni^{T}	CBS 124980	HSAUPIII00159; E.G.S. 53.121	*Prunus persica*	China	KU850614	KU850759	KU850905
	S. plantaginis^{T}	CBS 124981	HSAUPIII00532	*Plantago major*	China	KU850615	KU850760	KU850906
		CBS 124983	HSAUPpyf1842(2)	*Clinopodium polycephalum*	China	KU850616	KU850761	KU850907
		CBS 135778	E.G.S. 46.183	*Salvia officinalis*	New Zealand	KU850617	**AY317026**	KU850908
Stemphylium majusculum		CBS 717.68^{T}	E.G.S. 29.094; ATCC 18520; IMI 135459; MUCL 11720; MUCL 18568; NRRL 5269; QM 8382	*Lathyrus maritimus*	USA	KU850618	**AF443891**	KU850909
		CBS 133424	E.G.S. 16.068; IMI 135459; QM 8382	*Lathyrus maritimus*	USA	KU850619	**AF443891**	KU850910

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Table 1. (Continued).

Name	Old name[1]	Strain number[2]	Other collection number[2]	Host/Substrate	Country	GenBank accession numbers		
						ITS	*gapdh*	*cmdA*
Stemphylium novae-zelandiae sp. nov.		CBS 138157	E.G.S. 52.147	*Avicennia resinifera*	New Zealand	KU850630	KU850771	KU850921
		CBS 138295^{T}	E.G.S. 52.148	*Avicennia resinifera*	New Zealand	KU850631	KU850772	KU850922
Stemphylium paludiscirpi		CBS 109842^{T}	E.G.S. 31.016; IMI 386966	*Scirpus* sp.	USA	KU850620	KU850762	KU850911
Stemphylium sarciniforme		CBS 335.33		*Trifolium pratense*	USA	KU850621	KU850763	KU850912
		CBS 364.49	ATCC 10828	*Trifolium pratense*	USA	KU850622	KU850764	KU850913
	"*S. kaiseri*"	CBS 110049	E.G.S. 31.011	*Cicer arietinum*	Iran	KU850591	KU850738	KU850882
		CBS 116579	E.G.S. 38.121	*Trifolium pratense*	USA	KU850623	**AF443892**	KU850914
		CBS 116581	E.G.S. 29.188	*Cicer arietinum*	Iran	KU850592	KU850739	KU850883
		CBS 133723	E.G.S. 36.006	*Trifolium pratense*	USA	KU850624	KU850765	KU850915
		CBS 136810	E.G.S. 49.033	*Cicer arietinum*	Iran	KU850593	KU850740	KU850884
		CBS 138345	E.G.S. 53.018	*Trifolium pratense*	New Zealand	KU850625	KU850766	KU850916
Stemphylium simmonsii sp. nov.	*S. globuliferum*	CBS 716.68	ATCC 18518; IMI 135458; MUCL 11718; QM 8729	*Commelina* sp.	USA	KU850632	KU850773	KU850923
		CBS 116598	UAMH 10487	*Phragmitis* sp.	Canada	KU850633	KU850774	KU850924
		CBS 116603	UAMH 10493	*Lactuca muralis*	Canada	KU850634	KU850775	KU850925
		CBS 116604	UAMH 10494	*Guem macrophyllum*	Canada	KU850635	KU850776	KU850926
		CBS 133515	E.G.S. 30.153	*Solanum lycopersicum*	Canada	KU850636	KU850777	KU850927
		CBS 133518^{T}	E.G.S. 30.154	*Fragaria* sp.	Canada	KU850637	KU850778	KU850928
	S. globuliferum	CBS 133894	E.G.S. 38.115	*Trifolium pratense*	USA	KU850638	KU850779	KU850929
	S. globuliferum	CBS 134496	E.G.S. 42.138	*Malus sylvestris*	Australia	KU850639	KU850780	KU850930
Stemphylium solani		CBS 408.54	ATCC 11128	*Solanum lycopersicum*	USA	KU850626	KU850767	KU850917
		CBS 116586^{ET}	E.G.S. 41.135	*Solanum lycopersicum*	USA	KU850627	KU850768	KU850918
		CBS 118082	E.G.S. 42.055; CBS 134293	*Euphorbia marginata*	USA	KU850628	KU850769	KU850919
Stemphylium symphyti		CBS 115268^{T}		*Symphytum uplandicum*	USA	KU850643	KU850784	KU850934
		CBS 118796		*Symphytum uplandicum*	New Zealand	KU850644	KU850785	KU850935
		CBS 138069	E.G.S. 52.041	*Borago officinalis*	New Zealand	KU850645	KU850786	KU850936
		CBS 138070	E.G.S. 52.042	*Borago officinalis*	New Zealand	KU850646	KU850787	KU850937
Stemphylium trifolii		CBS 116580^{T}	E.G.S. 12.142	*Trifolium repens*	USA	KU850647	KU850788	KU850938
Stemphylium triglochinicola		CBS 718.68^{T}	ATCC 18516; IMI 122774ii; IMI 135460; MUCL 11716; MUCL 18569; NRRL 5270; QM 8752	*Triglochin maritima*	UK	KU850648	KU850789	KU850939

Stemphylium vesicarium		CBS 155.24		*Allium* sp.	Unknown	KU850555	KU850702	KU850845
		CBS 157.24		*Abies* sp.	Unknown	KU850556	KU850703	KU850846
	P. pomorum[NT]	CBS 184.25		*Malus domestica*	UK	KU850557	KU850704	KU850847
		CBS 273.31		Unknown	Unknown	KU850558	KU850705	KU850848
		CBS 274.31		*Phaseolus vulgaris*	Unknown	KU850559	KU850706	KU850849
		CBS 307.36		*Citrus* sp.	Tunisia	KU850560	KU850707	KU850850
		CBS 156.45		*Dianthus caryophyllus*	Netherlands	KU850561	KU850708	KU850851
		CBS 322.49		*Lathyrus odoratus*	Netherlands	KU850562	KU850709	KU850852
		CBS 370.51		*Trigonella foenum-graecum*	Netherlands	KU850563	KU850710	KU850853
		CBS 368.59		*Linum usitatissimum*	Denmark	KU850564	KU850711	KU850854
	S. vesicarium	CBS 715.68	E.G.S. 12.171; ATCC 18521; DAOM 48576a; IMI 135457; MUCL 11719; NRRL 5	*Pisum sativum*	Canada	KU850565	KU850712	KU850855
		CBS 406.76		*Solanum lycopersicum*	Germany	KU850566	KU850713	KU850856
		CBS 205.82		*Lunaria annua*	Netherlands	KU850567	KU850714	KU850857
	S. herbarum[T]	CBS 191.86	E.G.S. 36.138; IMI 276975	*Medicago sativa*	India	**KC584239**	**AF443884**	KU850858
	S. alfalfae[T]	CBS 192.86	E.G.S. 36.088; IMI 269683	*Medicago sativa*	Australia	KU850568	KU850715	KU850859
	S. vesicarium	CBS 311.92		*Allium cepa*	Netherlands	KU850569	KU850716	KU850860
	S. vesicarium	CBS 486.92		*Allium cepa*	Netherlands	KU850570	KU850717	KU850861
	P. sedicola[T]	CBS 109843	E.G.S. 48.095; IMI 386967	*Sedum spectabile*	New Zealand	KU850571	KU850718	KU850862
	P. tomatonis[T]	CBS 109844	E.G.S. 29.089; IMI 386968	*Solanum lycopersicum*	USA	KU850572	KU850719	KU850863
		CBS 115182	STE-U 5229; CPC 5229	*Leucadendron* sp.	South Africa	KU850573	KU850720	KU850864
		CBS 115204	STE-U 5224; CPC 5224	*Leucadendron* sp.	Portugal	KU850574	KU850721	KU850865
	S. mali[T]	CBS 122640	HSAUP Vlwy1542	*Malus sieversii*	China	KU850575	KU850722	KU850866
		CBS 123005	HSAUPV 0366	*Fabaceae*	China	KU850576	KU850723	KU850867
	S. alfalfae	CBS 123803	HSAUP 0366	*Allium sativum*	China	KU850577	KU850724	KU850868
	S. eturmiunum	CBS 124279		*Malus domestica*	Denmark	KU850578	KU850725	KU850869
	S. cremanthodii[T]	CBS 124747	HSAUPpyf1830(1)	*Cremanthodium discoideum*	China	KU850579	KU850726	KU850870
	S. brassicicola[T]	CBS 124749	HSAUPpyf1858(2)	*Brassica pekinensis*	China	KU850580	KU850727	KU850871
		CBS 124751	HSAUPpyf2328	*Pyrus sinkiangensis*	China	KU850581	KU850728	KU850872
		CBS 124752	HSAUPpyf2371	*Populus tomentosa*	China	KU850582	KU850729	KU850873
		CBS 125242	HSAUP 1914	*Dahlia pinnata*	China	KU850583	KU850730	KU850874
	S. tomatonis	CBS 133474	E.G.S. 29.089	*Solanum lycopersicum*	USA	KU850584	KU850731	KU850875
	S. alfalfae	CBS 133737	E.G.S. 36.088; IMI 269683	*Medicago sativa*	Australia	KU850585	KU850732	KU850876

(*continued on next page*)

Table 1. (Continued).

Name	Old name[1]	Strain number[2]	Other collection number[2]	Host/Substrate	Country	GenBank accession numbers		
						ITS	*gapdh*	*cmdA*
	S. alfalfae	CBS 133905	E.G.S. 39.127	*Medicago sativa*	USA	KU850586	KU850733	KU850877
	S. alfalfae	CBS 133914	E.G.S. 40.038	*Medicago sativa*	USA	KU850587	KU850734	KU850878
		CBS 138138	E.G.S. 52.113	*Lonicera* sp.	Netherlands	KU850588	KU850735	KU850879
		GV11-355-a1-2		*Beta vulgaris*	Netherlands	KU850589	KU850736	KU850880

[1] The [T] indicates the ex-type isolate of the synonymised species.

[2] ATCC: American Type Culture Collection, Manassas, VA, USA; CBS: Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CPC: Personal collection of P.W. Crous, Utrecht, the Netherlands; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; E.G.S.: Personal collection of Dr. E.G. Simmons; HSAUP: Department of Plant Pathology, Shandong Agricultural University, China; IMI: Culture Collection of CABI Europe-UK, Egham, UK; MUCL: (Agro) Industrial Fungi and Yeast Collection of the Belgian Co-ordinated Collections of Micro-organisms (BCCM), Louvain-la Neuve, Belgium; NRRL: ARS Culture Collection, U.S. Department of Agriculture, Peoria, IL, USA; PD: Plant Protection Service, Wageningen, the Netherlands; QM: Quarter Master Culture Collection, Amherst, MA, USA; STE-U: Department of Plant Pathology, University of Stellenbosch, South Africa; UAMH: University of Toronto, UAMH Centre for Global Microfungal Biodiversity, Toronto, Canada; Ex-epitype, -type, and -neotype isolates are indicated with [ET], [T], and [NT], respectively.

stationary. Tracer v. 1.5.0 (Rambaut & Drummond 2009) was used to confirm the convergence of chains. A maximum-likelihood analysis including 500 bootstrap replicates using RAxML v. 7.2.6 (Stamatakis & Alachiotis 2010) was also run. Sequences of *A. alternata* (GV14-634-a1) were used as outgroup. The same steps were applied to generate the multi-gene phylogeny, on both the single gene alignments and the multi-gene alignment, with the only difference being that the L-INS-I method was used in MAFFT v. 7.271 for generating the multiple sequence alignment. The resulting trees were printed with TreeView v. 1.6.6 (Page 1996) and, together with the alignments, deposited into TreeBASE (http://www.treebase.org).

RESULTS

Identification of best loci

The ITS, *rpb2* and *actA* gene regions were the least informative, since only two sequence alleles were observed, all splitting the seven isolates in the same two allele groups (Table 2). For the ITS sequences the sequence difference between the two allele groups is in two T-repeats, which are not considered informative by standard phylogeny software. Differences in repeat regions are normally regarded as sequence errors, and are not included in calculations for phylogenetic trees. However, when these differences are compared with the results from the other gene information, the difference in number of T-repeats does seem to be relevant in this case. The *tef1* gene region showed three different sequence alleles, additionally splitting CBS 134496 from the second allele group (Table 2). The *cmdA* and *gapdh* gene regions seem to have the highest potential of being most informative as respectively four and five different sequence alleles were observed (Table 2). Based on these results the *cmdA* and *gapdh* gene regions were sequenced for a selection of 150 isolates (including the outgroup isolate GV14-634-a1), representing all possible species in *Stemphylium* based on ITS sequence data and ecological data (Table 1).

ITS phylogeny

The initial UPGMA phylogeny constructed in Bionumerics v. 4.61 placed 356 isolates in the *Stemphylium* clade (data not shown). Together with the outgroup-isolate GV14-634-a1, an *Alternaria alternata* isolated from sugar beet, these 357 isolates form the dataset of the *Stemphylium* ITS phylogeny. The aligned sequences contained 545 nucleotides with 101 unique site patterns. The TrN model with a gamma-distributed rate variation was suggested as model for the Bayesian analysis. The average standard deviation of split frequencies never reached below 0.01 while running MrBayes at different temperature values. Therefore, the temperature value was lowered to 0.05, and the run was stopped after 5 M generations for which the convergence of chains was confirmed in Tracer. After discarding the burn-in phase trees, the runs resulted in 7 502 trees from which the majority rule consensus tree and posterior probabilities were calculated.

The phylogeny based on the ITS sequences divides the 356 *Stemphylium* isolates into 22 clades (Fig. 1). In clade 10, 33 isolates were found, 18 sugar beet isolates and 15 isolates from the CBS collection. The three sugar beet isolates from Germany

Table 2. Gene test on selected isolates from clade 10 (see Fig. 1). The numbers in the body of the table represent the number of the sequence allele for the given locus.

Isolate number	Original name	Host	Location	ITS[1]	*actA*	*rpb2*	*tef1*	*cmdA*	*gapdh*	*tub2*[2,3]
CBS 116599	*Pleospora* sp.	Herbaceous dicot	Canada	1	1	1	1	1	1	np
GV11-196-a1-3	*Stemphylium* sp.	*Beta vulgaris*	Netherlands	1	1	1	1	1	1	np
CBS 378.54	*P. armeriae*	*Lychnis* sp.	Canada	1	1	1	1	1	2	np
IFZ2013-024	*Stemphylium* sp.	*Beta vulgaris*	Germany	1	1	1	1	2	1	np
CBS 136590	*Pleospora* sp.	*Passiflora edulis*	New Zealand	1	1	1	1	2	3	np
CBS 116598	*Pleospora* sp.	*Phragmitis* sp.	Canada	2	2	2	2	3	4	np
CBS 134496	*S. globuliferum*	*Malus sylvestris*	Australia	2	2	np[3]	3	4	5	np

[1] ITS difference is only in two T-repeat regions.
[2] *tub2* gave no PCR products, despite of testing different primer combinations and PCR conditions.
[3] np: no PCR product.

and the one from the UK cluster here amidst the Dutch sugar beet isolates. The phylogenetic tree shows a straight vertical line for this clade, implying that the sequences are phylogenetically identical. However, by eye two different sequences are observed with a T repeat of 7 nt starting on position 139 in the ITS alignment (deposited in TreeBASE) in combination with a T repeat of 6 nt starting on position 491, versus a T repeat of 6 nt starting on position 139 in combination with a T repeat of 7 nt starting on position 491 in the alignment. Although not phylogenetically recognised, this difference splits the CBS isolates in two subgroups, with seven isolates, CBS 378.54, CBS 116599, CBS 133512, CBS 133892, CBS 136590, CBS 136699 and CBS 137492, having an ITS sequence identical to the sugar beet isolates.

Multi-gene phylogeny

From the ITS phylogeny 149 isolates were selected to represent the genus *Stemphylium* and the partial *gapdh* and *cmdA* gene sequences were added to the existing ITS sequence data (Table 1). The selection included all ex-type isolates, all isolates from potential new species, and at least one representative per clade of the ITS phylogeny. The aligned sequences of the ITS (545 characters), *gapdh* (595 characters) and *cmdA* (860 characters) gene regions of the 150 isolates (including the outgroup isolate) had a total length of 2 000 characters, with respectively 95, 199, and 317 unique site patterns. The HKY model with a gamma-distributed rate variation was suggested as model for the ITS and *gapdh* alignments and the GTR model with a gamma-distributed rate variation for the *cmdA* alignment. After discarding the burn-in phase trees, the multi-gene Bayesian analysis resulted in 6 978 trees from which the majority rule consensus tree and posterior probabilities were calculated.

The multi-gene phylogeny divided the isolates in 28 species clades (Fig. 2). New species descriptions are provided in the taxonomy section below.

Taxonomy

As a result of the multi-gene phylogenetic analysis, 22 species names are synonymised, and two new combinations and five new species proposed. Synonyms and descriptions of the new species and new combinations are provided below.

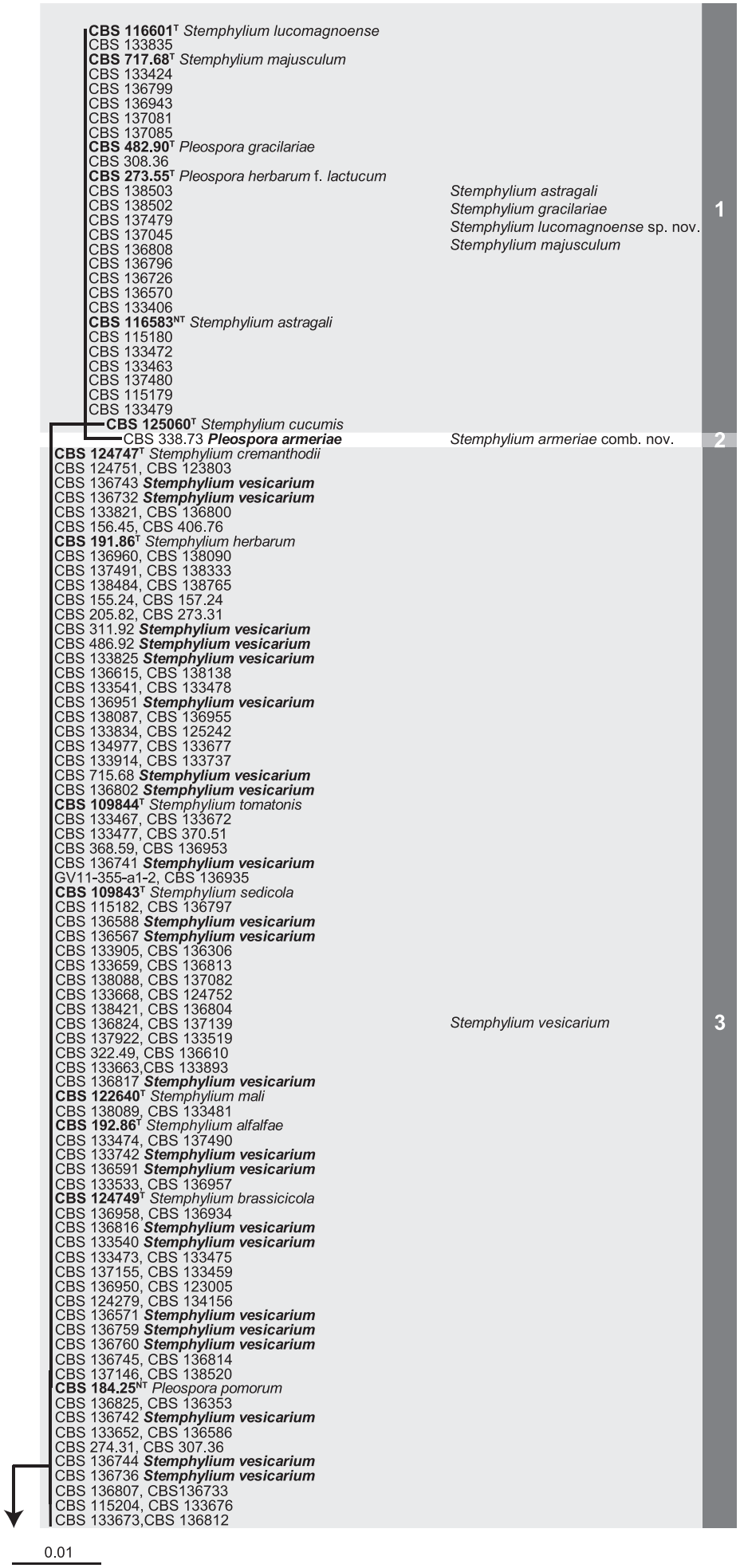
Stemphylium amaranthi Y.F. Pei & X.G. Zhang, Mycotaxon 109: 495. 2009.

Synonyms: *Stemphylium microsporum* Y.F. Pei & X.G. Zhang, Mycotaxon 111: 171. 2010.
Stemphylium phaseolina Yong Wang bis & X.G. Zhang, Mycologia 102: 709. 2010.
Stemphylium luffae Y.F. Pei & X.G. Zhang, Mycol. Progr. 10: 166. 2011.

Specimens examined: **China**, Hebei Province, Zhaoxian, from *Phaseolus vulgaris* leaves, Sep. 2006, Y. Wang (culture **ex-type** of *S. phaseolina* CBS 124650); Shandong Province, Tai'an, from *Phaseolus vulgaris* leaves, Oct. 2006, Y. Wang, CBS 124651; Sinkiang Province, Korla, from *Amaranthus retroflexus* leaves, 17 Oct. 2008, Y.F. Pei (culture **ex-type** CBS 124746); Sinkiang Province, Yili, from *Luffa cylindrica* leaves, collection date unknown, Y.F. Pei (culture **ex-type** of *S. luffae* CBS 124985); Sinkiang Province, Yili, from *Malus sieverii* leaves, 10 Aug. 2009, Y.F. Pei, (culture **ex-type** of *S. microsporum* CBS 124753).

Notes: The species *S. amaranthi* and *S. microsporum* were described based on morphological data only (Pei *et al.* 2009, 2010), and no sequence data were available on GenBank. Their morphological descriptions differ, especially their spore sizes (22–35 × 10–19 for *S. amaranthi* versus 15–24 × 9–15 for *S. microsporum*). However, our measurements of the ex-type isolate of *S. microsporum* (CBS 124753) resulted in a spore size of (24.5–)27–35 (–42) × (12–)13.5–16(–18), which would fit the description of *S. amaranthi*. Both *S. luffae* and *S. phaseolina* are described based on morphological and molecular data, although in the later description of *S. luffae*, the sequences of *S. phaseolina* are not incorporated in the phylogenetic tree. The published ITS sequences of the ex-type isolate of *S. luffae* and *S. phaseolina* (GU182943 and GQ395369 respectively) are 100 % identical, but their *gapdh* sequences (GU182938 and GQ395374 respectively) are only 98 % identical. However, the *gapdh* sequence we obtained from the ex-type strain of *S. luffae* (KU850656) is only 99 % identical to the originally published sequence (GU182938), and also the *gapdh* sequence we obtained from the ex-type strain of *S. phaseolina* (KU850650) is only 99 % identical to the originally published sequence (GQ395374). This led to a 100 % identity of the *gapdh* sequences of the ex-type isolates of *S. luffae* and *S. phaseolina*. When looking at the described morphological characters, *S. luffae* and *S. phaseolina* also fit in the morphological species description *of S. amaranthi*. The only remark is that *S. luffae* and *S. phaseolina* are described with a conspicuously punctate conidial wall, although *S. amaranthi* was originally described with an inconspicuously micromaculate conidial wall.

Stemphylium armeriae (Corda) Woudenb. & Crous, **comb. nov.** MycoBank MB820657.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CBS 137081
CBS 137085
CBS 482.90[T] *Pleospora gracilariae*
CBS 308.36
CBS 273.55[T] *Pleospora herbarum* f. *lactucum*
CBS 138503
CBS 138502
CBS 137479
CBS 137045
CBS 136808
CBS 136796
CBS 136726
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CBS 133406
CBS 116583[NT] *Stemphylium astragali*
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CBS 133472
CBS 133463
CBS 137480
CBS 115179
CBS 133479
Stemphylium astragali
Stemphylium gracilariae
Stemphylium lucomagnoense sp. nov.
Stemphylium majusculum
1
CBS 125060[T] *Stemphylium cucumis*
CBS 338.73 ***Pleospora armeriae***
Stemphylium armeriae comb. nov.
2
CBS 124747[T] *Stemphylium cremanthodii*
CBS 124751, CBS 123803
CBS 136743 ***Stemphylium vesicarium***
CBS 136732 ***Stemphylium vesicarium***
CBS 133821, CBS 136800
CBS 156.45, CBS 406.76
CBS 191.86[T] *Stemphylium herbarum*
CBS 136960, CBS 138090
CBS 137491, CBS 138333
CBS 138484, CBS 138765
CBS 155.24, CBS 157.24
CBS 205.82, CBS 273.31
CBS 311.92 ***Stemphylium vesicarium***
CBS 486.92 ***Stemphylium vesicarium***
CBS 133825 ***Stemphylium vesicarium***
CBS 136615, CBS 138138
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CBS 133834, CBS 125242
CBS 134977, CBS 133677
CBS 133914, CBS 133737
CBS 715.68 ***Stemphylium vesicarium***
CBS 136802 ***Stemphylium vesicarium***
CBS 109844[T] *Stemphylium tomatonis*
CBS 133467, CBS 133672
CBS 133477, CBS 370.51
CBS 368.59, CBS 136953
CBS 136741 ***Stemphylium vesicarium***
GV11-355-a1-2, CBS 136935
CBS 109843[T] *Stemphylium sedicola*
CBS 115182, CBS 136797
CBS 136588 ***Stemphylium vesicarium***
CBS 136567 ***Stemphylium vesicarium***
CBS 133905, CBS 136306
CBS 133659, CBS 136813
CBS 138088, CBS 137082
CBS 133668, CBS 124752
CBS 138421, CBS 136804
CBS 136824, CBS 137139
CBS 137922, CBS 133519
CBS 322.49, CBS 136610
CBS 133663,CBS 133893
CBS 136817 ***Stemphylium vesicarium***
CBS 122640[T] *Stemphylium mali*
CBS 138089, CBS 133481
CBS 192.86[T] *Stemphylium alfalfae*
CBS 133474, CBS 137490
CBS 133742 ***Stemphylium vesicarium***
CBS 136591 ***Stemphylium vesicarium***
CBS 133533, CBS 136957
CBS 124749[T] *Stemphylium brassicicola*
CBS 136958, CBS 136934
CBS 136816 ***Stemphylium vesicarium***
CBS 133540 ***Stemphylium vesicarium***
CBS 133473, CBS 133475
CBS 137155, CBS 133459
CBS 136950, CBS 123005
CBS 124279, CBS 134156
CBS 136571 ***Stemphylium vesicarium***
CBS 136759 ***Stemphylium vesicarium***
CBS 136760 ***Stemphylium vesicarium***
CBS 136745, CBS 136814
CBS 137146, CBS 138520
CBS 184.25[NT] *Pleospora pomorum*
CBS 136825, CBS 136353
CBS 136742 ***Stemphylium vesicarium***
CBS 133652, CBS 136586
CBS 274.31, CBS 307.36
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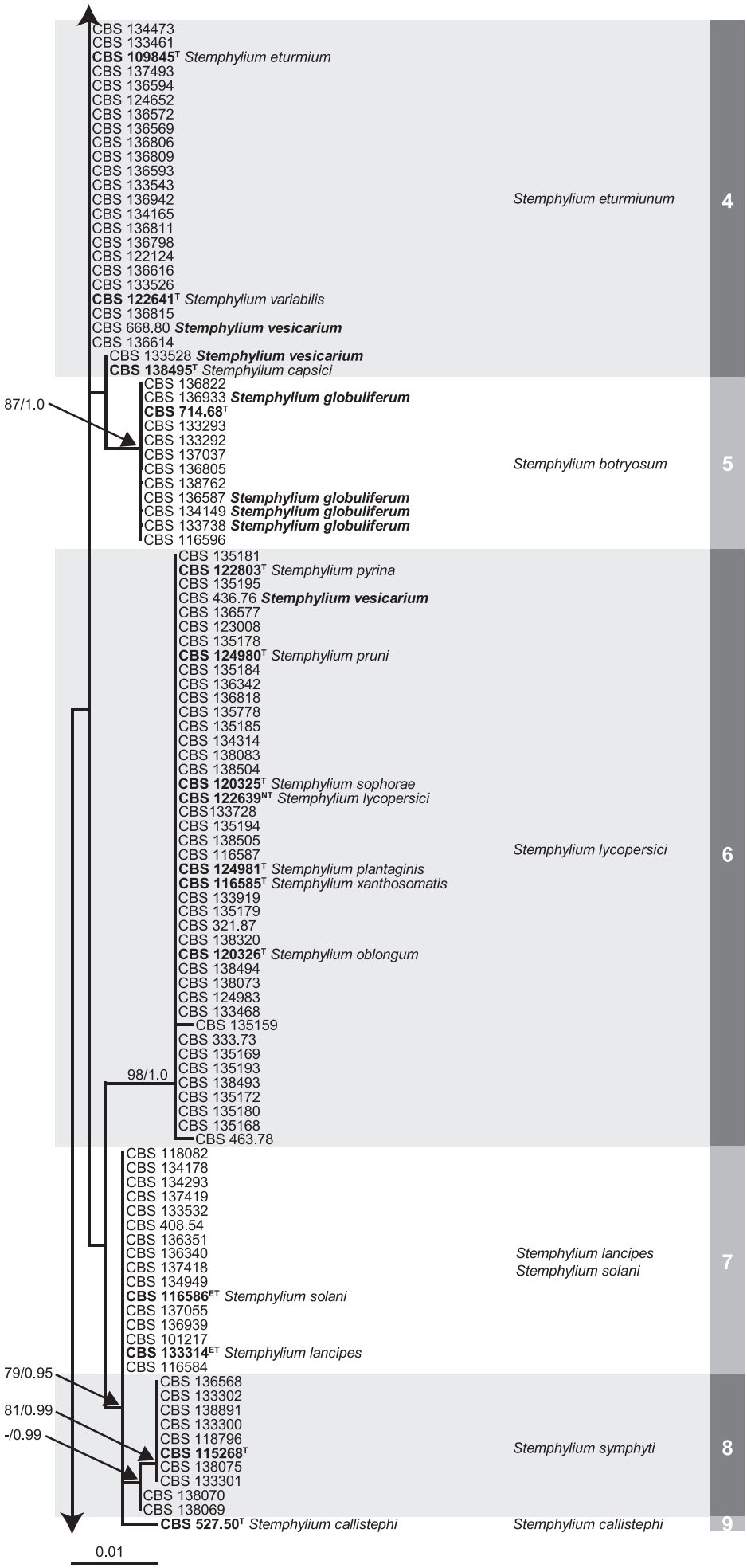

Fig. 1. (Continued).

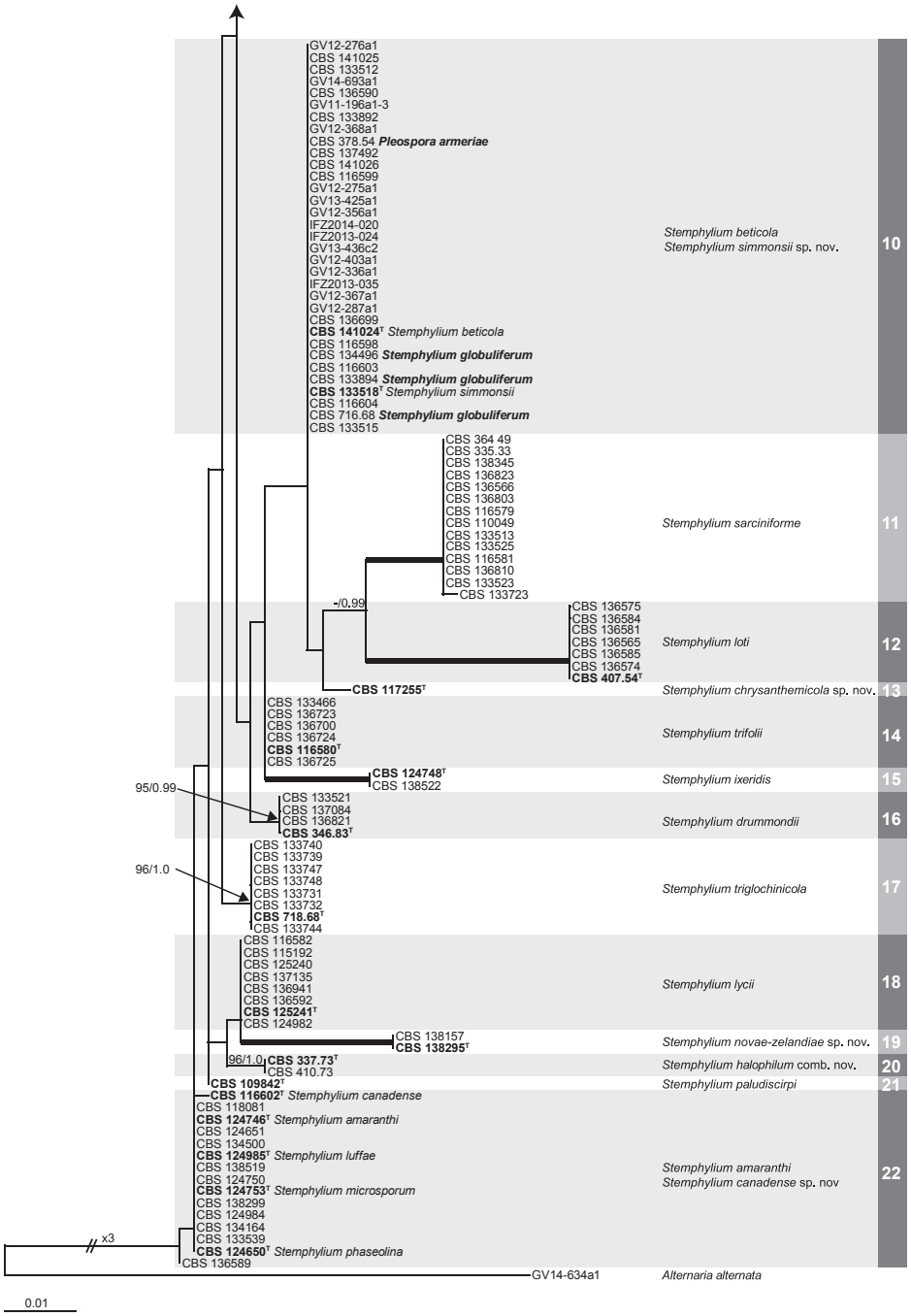

Fig. 1. (Continued).

Basionym: *Sphaeria armeriae* Corda, Icon. Fungorum hucusque Cogn. 4: 41, t. 8:119. 1840.

Synonyms: *Pleospora armeriae* (Corda) Ces. & De Not., Comment. Soc. Crittog. Ital. 1: 218. 1863.

Pleospora herbarum f. *armeriae* (Corda) Sacc., Syll. Fungorum (Abellini) 2: 247. 1883.

Pyrenophora armeriae (Corda) Berl., Nuovo Giorn. Bot. Ital. 20: 242. 1888.

Pleospora herbarum var. *armeriae* (Corda) J. Webster, Trans. Brit. Mycol. Soc. 44: 418. 1961.

Specimen examined: **UK**, England, Devon, Budleigh Salterton Salt Marsh, from *Armeria maritima*, 12 Aug. 1972, J. Webster, CBS 338.73.

Notes: *Sphaeria armeriae* was described from flower stalks of *Armeria vulgaris* (= *A. maritima*) in Germany (Corda 1840). Later it was transferred to the genus *Pleospora* (Cesati & De Notaris 1863). Saccardo (1883) treated it as a form of *P. herbarum*, while others treated it as synonym of *P. herbarum* (Winter 1887; Müller 1951). Wehmeyer (1952) and Webster & Lucas (1961) both studied the holotype specimen (Herb A.C.I. Corda no. 155637), and concluded that it was immature; no fully mature ascospores could be found. A study comparing *P. herbarum* var. *armeriae* isolates from *Armeria* with cultures of *P. herbarum* from other hosts in culture, showed conidia similar to the *Stemphylium* type (Webster & Lucas 1961). However, they did observe a

Fig. 1. Maximum likelihood tree based on the ITS sequences of 357 isolates. The RAxML bootstrap support values > 75 % (BS) and Bayesian posterior probabilities > 0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100 % and a PP of 1.0. Ex-type strain numbers are in **bold** face and indicated with ^{T} (or ^{NT} or ^{ET} when respectively neo- or epi-typified in this study). Species names in **bold** face represent unconfirmed species names. The tree was rooted to *A. alternata* GV14-634a1.

difference in the ascus width between the two species, with var. *armeriae* having wider asci. Isolate CBS 338.73 was deposited in the CBS collection as *S. herbarum* var. *armeriae* by J. Webster, the author of this variety. We therefore propose the new combination for *Sphaeria armeriae* as *Stemphylium armeriae*.

Stemphylium astragali (Yoshii) W. Yamam., Trans. Mycol. Soc. Japan 2: 92. 1960.
Basionym: *Thyrospora astragali* Yoshii, J. Pl. Protect. 16: 536. 1929.

Specimen and material examined: **Japan**, (**lectotype designated here** of *T. astragali*, Journal of Plant Protection, Tokyo 16: illustration on page 534, 1929, Yoshii H, MBT375877); Fukuoka, from *Astragalus* sp., collection date unknown, H. Yoshii, (**epitype designated here** of *T. astragali* CBS H-23050, MBT375505, culture ex-epitype CBS 116583 = E.G.S. 08.174).

Notes: *Stemphylium astragali*, with *Thyrospora astragali* as basionym, does not refer to a holotype specimen in its original description (Yoshii 1929), nor could we locate one. However, in 1956 Yoshii sent an isolate (CBS 116583) named *Thyrospora astragali* to Emory G. Simmons, who recognised this as authentic isolate. Since no holotype specimen is known, we designated the original illustration on page 534 as lectotype, and propose CBS 116583 as ex-epitype culture of *Thyrospora astragali* here.

Stemphylium beticola Woudenb. & Hanse, Persoonia 36: 403. 2016. Fig. 3.

Conidiophores solitary, straight to flexuous, occasionally branched, septate, smooth, pale brown, (41–)45–72(–88) × 4–5 µm, bearing 1–3 darkened percurrent rejuvenation sites. *Conidiogenous cells* swollen at the apex, darkened, 5–6 µm wide. *Conidia* solitary, conidium body pale olivebrown, verrucose, ellipsoid to cylindrical, (21–)22–26(–30) × (13–)14–16(–18) µm, L/W = 1.6, with 2–4 *transverse septa* and 1–3 *longitudinal* and 0–2 *oblique septa* per transverse sector. Constricted at 1–2 darkened transverse septa. Occasionally with an apical *secondary conidiophore*. Immature ascomata of *sexual morph* observed on agar, pseudothecia globose, ellipsoid or irregular, single or aggregated, ranging from 100 to 300 µm tall (from Crous *et al.* 2016).

Culture characteristics: After 7 d cultures on SNA flat, fimbriate, colourless with abundant black ascomatal initials in the agar, aerial mycelium is sparse, white, colonies reaching 45–55 mm diam; cultures on PCA flat, entire to undulate, colourless with abundant black ascomata in the agar, aerial mycelium is sparse floccose, (greenish) olivaceous; colonies reaching 50–60 mm diam.

Specimens examined: **Netherlands**, Noord-Brabant, Langenboom, on leaves of *Beta vulgaris*, 17 Aug. 2011, P. Wilting, (**holotype** CBS H-22486, culture ex-type CBS 141024 = GV11-265a); Groningen, Nieuwe Pekela, on leaves of *Beta vulgaris,* 17 July 2012, J. Lingbeek, GV12-288-2 = CBS 141025; Drenthe, Eerste Exloërmond, on leaves of *Beta vulgaris,* 11 Sept. 2012, B. Hanse, CBS 141026 = GV12-474a1.

Notes: *Stemphylium beticola* causes a leaf spot disease on sugar beet (*Beta vulgaris*) (Hanse *et al.* 2015), which has been detected in multiple European countries (Crous *et al.* 2016). Host range tests demonstrated that the species was not restricted to *Beta vulgaris* (Hanse *et al.* 2015), which is confirmed in this study by the clustering of multiple isolates from different hosts in the *S. beticola* clade. This study further shows that *S. beticola* also occurs in the USA, Canada and New Zealand. Molecularly it is closely related to *S. simmonsii*, another species with a broad host range, but which does not include isolates from Europe. They can be separated morphologically by their ascomata, which have dark hyphal outgrows in *S. simmonsii*.

Stemphylium canadense Woudenb. & Crous, **sp. nov.** MycoBank MB820658. Fig. 4.

Etymology: Named after the country from which it was collected, Canada.

Conidiophores solitary, straight to flexuous, occasionally branched, septate, smooth, light olive brown, (46–)62.5–107(–137.5) × (3–)4–5.5(–7) µm, bearing 1–2 thickened, darkened, percurrent rejuvenation sites. *Conidiogenous cells* swollen at the apex, darkened, (5.5–)6.5–8.5(–10.5) µm wide. *Conidia* solitary, conidium body pale olive brown, verrucose, ovoid with pointed apex, (37.5–)43.5–59(–71.5) × (13.5–)15–18(–20) µm, L/W = 3.1, with 5–8 *transverse septa* and 1–2(–3) *longitudinal* or *oblique septa* per transverse sector. Constricted at multiple darkened transverse septa. *Sexual morph* not observed.

Culture characteristics: After 7 d cultures on SNA flat, entire, aerial mycelium is scarce, woolly, white, colonies colourless, with pale olivaceous grey centre, colonies 20–29 mm diam; cultures after 7 d on PCA effuse, entire, aerial mycelium scarce, fine felty to woolly, olivaceous grey, colonies colourless with greenish olivaceous zones, colonies reaching 20–31 mm diam.

Specimens examined: **Canada**, British Colombia, near Roberts Bank Port, from *Salicornia* sp., 24 May 2001, A. & R. Bandoni (**holotype** F 14991, culture ex-type CBS 116602 = UAMH 10491); British Colombia, Hornby Island, beach of Cape Gurney, from *Salicornia* sp., collection date unknown, A. & R. Bandoni, CBS 118081 = UAMH 10492.

Notes: *Stemphylium canadense* includes two cultures (CBS 116602 and CBS 118081) isolated from *Salicornia* spp. in Canada. In fig. 2 of Inderbitzin *et al.* (2009) these two isolates were already mentioned as an unnamed species in Clade E1. A *Pleospora* sp. has already been described from *Salicornia* sp. in France, namely *Pleospora salicorniae* (Dangeard 1888). However no sexual morph was observed in our isolates of *Stemphylium canadense*, and therefore we could not confirm conspecificity.

Stemphylium chrysanthemicola Woudenb. & Crous, **sp. nov.** MycoBank MB820659. Fig. 5.

Etymology: Named after the host genus from which it was collected, *Chrysanthemum.*

Conidiophores solitary, straight to curved, occasionally branched, septate, smooth, sub-hyaline, (71–)106–186(–282) × (3–)4–5 µm, bearing multiple darkened percurrent rejuvenation sites. *Conidiogenous cells* swollen at the apex, sub-hyaline, (5–)5.5–7(–7.5) µm wide. *Conidia* solitary, conidium body brown, verrucose, ellipsoid to cylindrical, (24.5–)26–29(–30.5) × (11–)


CBS 368.59 Linum usitatissimum, Denmark
CBS 115204 Leucadendron sp., Portugal
GV11-355a1-2 Beta vulgaris, Netherlands
CBS 155.24 Allium sp., unknown
CBS 486.92 Allium cepa, Netherlands
CBS 273.31 unknown, unknown
CBS 156.45 Dianthus caryophyllus, Netherlands
CBS 406.76 Solanum lycopersicum, Germany
CBS 133474 Solanum lycopersicum, USA
CBS 184.25 Malus domestica, UK (P. pomorum NT)
CBS 109843 Sedum spectabile, New Zealand (S. sedicola T)
CBS 115182 Leucadendron sp., South Africa
CBS 124747 Cremanthodium discoideum, China (S. cremanthodii T)
CBS 109844 Solanum lycopersicum, USA (S. tomatonis T)
CBS 124279 Malus domestica, Denmark
CBS 311.92 Allium cepa, Netherlands
CBS 274.31 Phaseolus vulgaris, unknown
CBS 307.36 Citrus sp., Tunisia
CBS 205.82 Lunaria annua, Netherlands
CBS 124751 Pyrus sinkiangensis, China
CBS 192.86 Medicago sativa, Australia (S. alfalfae T)
CBS 138138 Lonicera sp., Netherlands
CBS 124752 Populus tomentosa, China
CBS 370.51 Trigonella foenum-graecum, Netherlands
CBS 133914 Medicago sativa, USA
CBS 715.68 Pisum sativum, Canada
CBS 157.24 Abies sp., unknown
CBS 122640 Malus sieversii, China (S. mali T)
CBS 133737 Medicago sativa, Australia
CBS 123005 Fabaceae, China
CBS 123803 Allium sativum, China
CBS 125242 Dahlia pinnata, China
CBS 191.86 Medicago sativa, India (S. herbarum T)
CBS 322.49 Lathyrus odoratus, Netherlands
CBS 133905 Medicago sativa, USA
CBS 124749 Brassica pekinensis, China (S. brassicicola T)
Stemphylium vesicarium
1
CBS 338.73 Armeria maritima, UK
Stemphylium armeriae comb. nov.
2
CBS 273.55 Lactuca sp., USA (P. herbarum f. lactucum T)
CBS 308.36 Solanum lycopersicum, USA
CBS 115179 Leucospermum sp., Spain
CBS 115180 Leucospermum sp., Spain
CBS 125060 Cucumis melo, China (S. cucumis T)
CBS 482.90T Gracilaria sp., Israel
Stemphylium gracilariae
3
CBS 133424 Lathyrus maritimus, USA
CBS 717.68T Lathyrus maritimus, USA
Stemphylium majusculum
4
CBS 116601T Minuartia hybrida, Switzerland
Stemphylium lucomagnoense sp. nov.
5
CBS 116583NT Astragalus sp., Japan
Stemphylium astragali
6
CBS 124652 Solanum lycopersicum, China
CBS 122641 Allium sativum, France (S. variabilis T)
CBS 668.80 Solanum lycopersicum, Greece
CBS 109845T Solanum lycopersicum, New Zealand
CBS 138495 Capsicum annuum, China (S. capsici T)
CBS 133528 Allium sativum, India
CBS 122124 Asphodelus aestivus, Greece
Stemphylium eturmium
7
CBS 116596 Medicago sativa, USA
CBS 714.68T Medicago sativa, Canada
Stemphylium botryosum
8
CBS 124980 Prunus persica, China (S. pruni T)
CBS 436.76 unknown, Indonesia
CBS 122803 Pyrus sinkiangensis, China (S. pyrina T)
CBS 116587 Solanum lycopersicum, Dominican Republic
CBS 122639NT Solanum lycopersicum, China
CBS 321.87 Solanum lycopersicum, Senegal
CBS 463.78 Solanum tuberosum, Peru
CBS 124981 Plantago major, China (S. plantaginis T)
CBS 116585 Xanthosoma sagittifolium, New Caledonia (S. xanthosomatis T)
CBS 124983 Clinopodium polycephalum, China
CBS 135778 Salvia officinalis, New Zealand
CBS 333.73 Platycodon sp., Netherlands
CBS 120325 Sophora microphylla, China (S. sophorae T)
CBS 123008 Brassica pekinensis, China
CBS 120326 Gossypium hirsutum, China (S.oblongum T)
Stemphylium lycopersici
9
CBS 138070 Borago officinalis, New Zealand
CBS 138069 Borago officinalis, New Zealand
CBS 115268T Symphytum uplandicum, USA
CBS 118796 Symphytum uplandicum, New Zealand
Stemphylium symphyti
10
CBS 124748T Ixeris denticulata, China
Stemphylium ixeridis
11
CBS 118082 Euphorbia marginata, USA
CBS 408.54 Solanum lycopersicum, USA
CBS 116586ET Solanum lycopersicum, USA
Stemphylium solani
12
CBS 527.50T Callistephus chinensis, USA
Stemphylium callistephi
13
CBS 116584 Aquilegia sp., New Zealand
CBS 101217 Aquilegia sp., New Zealand
CBS 133314ET Aquilegia canadensis, USA
Stemphylium lancipes
14
-/0.99
-/0.98
81/-
78/-
92/1.0
84/-
99/1.0
88/1.0
98/1.0
92/1.0
99/1.0
97/1.0
78/95
-/0.98
89/1.0
90/0.97
90/-
-/0.99
88/1.0
99/1.0
92/1.0
0.1

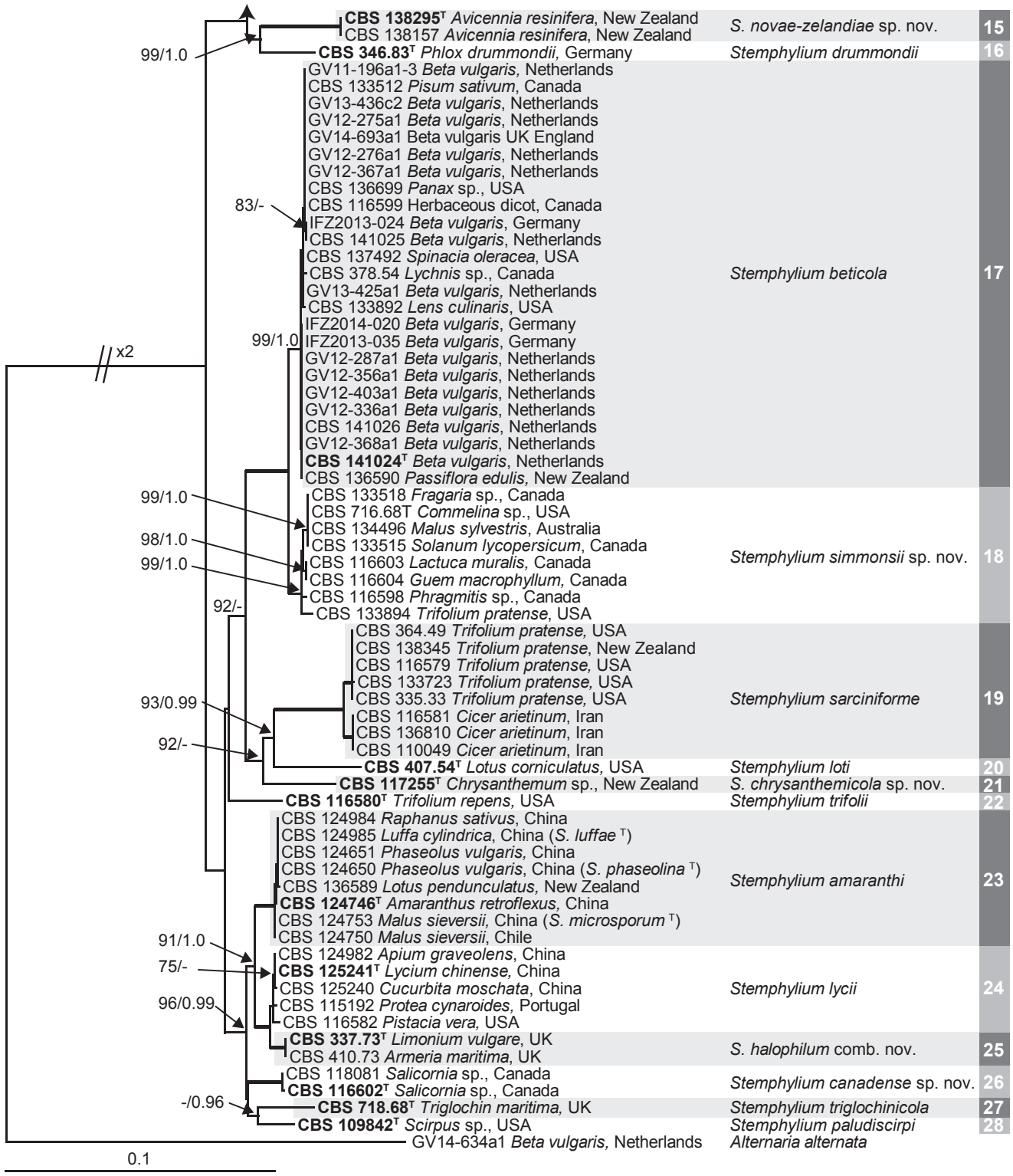

Fig. 2. (Continued).

13.5–15.5(–16.5) µm, L/W = 1.9, with 2–3 *transverse septa* and 1(–2) *longitudinal* or *oblique septa* per transverse sector. Constricted at 1–2 darkened transverse septa. Forms hyphal plaques at the bottom of PCA plates. *Sexual morph* not observed.

Culture characteristics: After 7 d cultures on SNA flat, rhizoid, aerial mycelium is fine felty, grey olivaceous, colonies colourless, pale olivaceous grey coloured by aerial conidia, black hyphal plaques at the bottom of the plate, colonies reaching 42 mm

Fig. 2. Maximum likelihood tree based on the combined ITS, *gapdh* and *cmdA* sequence alignment of 150 isolates. The RAxML bootstrap support values > 75 % (BS) and Bayesian posterior probabilities > 0.95 (PP) are given at the nodes. Thickened lines indicate a BS of 100 % and a PP of 1.0. Species names between parentheses represent synonymised species names. Ex-type strains are in **bold** face and indicated with T (or NT or ET when respectively neo- or epi-typified in this study). The tree was rooted to *A. alternata* GV14-634a1.

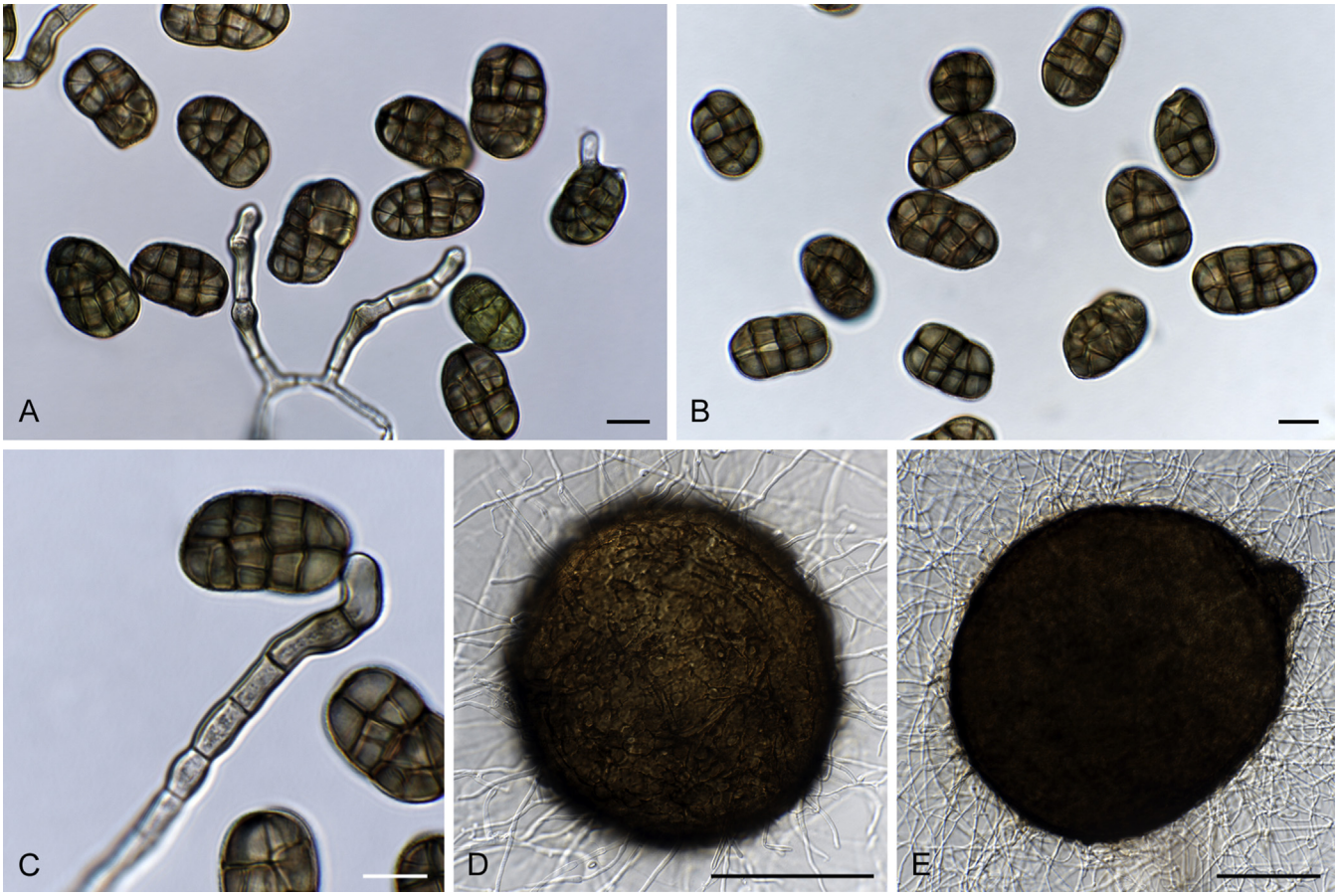

Fig. 3. *Stemphylium beticola* (CBS 141024). **A, C.** Conidiophores and conidia. **B.** Conidia. **D–E.** Ascoma. Scale bars: A–C = 10 µm; D–E = 100 µm.

diam; cultures on PCA flat, entire, aerial mycelium is floccose, pale olivaceous grey, colonies colourless with grey olivaceous rings, centre olivaceous, black hyphal plaques at the bottom of the plate; colonies reaching 46 mm diam.

Specimen examined: **New Zealand**, from *Chrysanthemum* sp., before May 1973, K.S. Milne (**holotype** CBS H-23045, culture ex-type CBS 117255 = E.G.S. 31.008).

Notes: Characteristic for the new species *S. chrysanthemicola* are the hyphal plaques which are formed at the bottom of the agar plates. These hyphal plaques are also observed in *S. novae-zelandiae* but after 14 d on PCA only.

Stemphylium drummondii Nirenberg & Plate, Phytopathol. Z. 107: 365. 1983.
Synonyms: *Pleospora drummondii* Nirenberg & Plate, Phytopathol. Z. 107: 365. 1983.
Stemphylium spinaciae B.J. Li, Yan F. Zhou & Y.L. Guo, Mycosystema 30: 380. 2011.

Notes: Comparison of the ITS (HQ622100) and *gapdh* (JF489118) sequence of the type of *S. spinaciae* (Zhou *et al.* 2011) with the type of *S. drummondii* showed identical ITS sequences and nearly identical *gapdh* sequences (1 nt difference in 374 nt). Together with the matching spore size (*S. spinaciae* 20–40 × 17.5–25 µm, *S. drummondii* 33.8 × 22.6 µm), we propose to synonymise these species. The description of a smooth conidial wall in *S. spinaciae*, which is incongruent with the verrucose conidia in *S. drummondii*, is questioned, since in fig. 1D of the original description (Zhou *et al.* 2011) verrucose conidia can be seen.

Stemphylium eturmiunum E.G. Simmons, Harvard Pap. Bot. 6: 204. 2001.
Synonyms: *Pleospora eturmiuna* E.G. Simmons, Harvard Pap. Bot. 6: 206. 2001.
Stemphylium variabilis Yong Wang bis & X.G. Zhang, Mycologia 102: 711. 2010.
Stemphylium capsici Yong Wang bis & X.G. Zhang, Mycotaxon 96: 80. 2006.

Specimens examined: **China**, Yunnan Province, Dali, from *Capsicum annuum* leaves, 5 Aug. 2002, X.G. Zhang (culture ex-type of *S. capsici* CBS 138495 = E.G.S. 53.123). **France**, Angres, from *Allium sativum* leaves, Aug. 2006, X.G. Zhang (culture ex-type of *S. variabilis* CBS 122641). **New Zealand**, Levin, from *Solanum lycopersicum* fruit, 1969, G.F. Laundon (culture ex-type of *P. eturmiuna* CBS 109845 = E.G.S. 29.099).

Notes: Morphological examination supports the synonymy of *S. capsici* and *S. variabilis* under *S. eturmiunum* (Fig. 6). *Stemphylium capsici* was described based on morphology only (Wang & Zhang 2006). Although the description of *S. capsici* describes smooth-walled conidia, our morphological examination of the ex-type isolate (CBS 138495) clearly shows densely verrucose conidia (Fig. 6B). *Stemphylium variabilis* was described based on morphological characters and molecular phylogenetic analyses (Wang *et al.* 2010). However, some sequence differences between the published sequences of *S. variabilis* (ITS GQ395366, *gapdh* GQ395373) and our sequences (ITS KU850543, *gapdh* KU850691, 3 and 4 nt

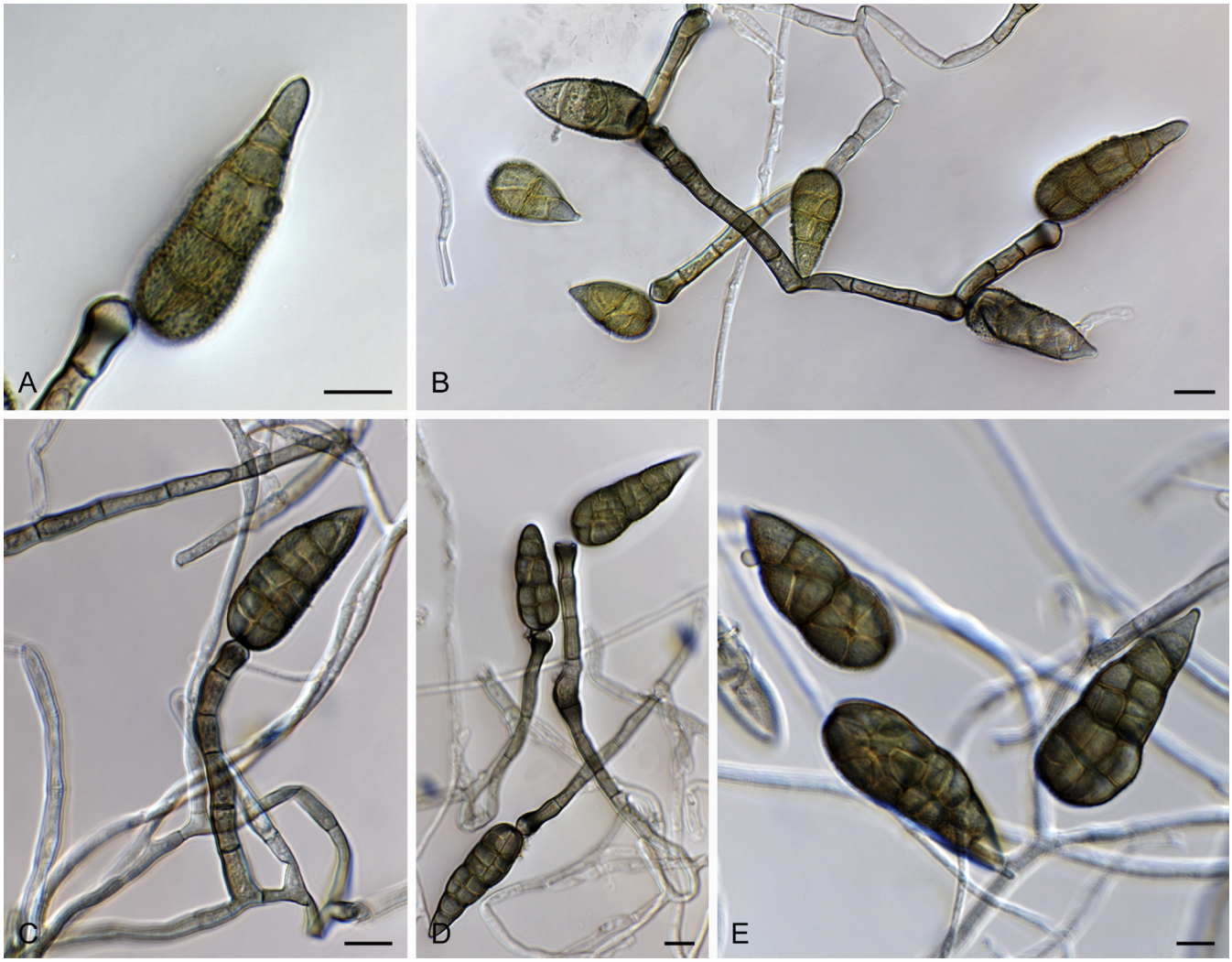

Fig. 4. *Stemphylium canadense* sp. nov. (CBS 116602). **A–D.** Conidiophores and conidia. **E.** Conidia. Scale bars = 10 µm.

difference respectively) placed *S. variabilis* in synonymy with *S. eturmiunum* instead of the close phylogenetic relationship published originally. Morphologically the variable shape of conidia and abundant secondary conidiophores were mentioned as being unique for *S. variabilis*, and different from the broadly ovoid or ellipsoid conidia of *S. eturmiunum* (Wang *et al.* 2010). However, our morphological examination did not show extensive secondary conidiophore formation or highly variable shaped conidia in the type isolate of *S. variabilis* (CBS 122641, Fig. 6C).

Stemphylium gracilariae E.G. Simmons, Mem. New York Bot. Gard. 49: 305. 1989.
Synonyms: *Pleospora herbarum* f. *lactucum* Padhi & Snyder, Phytopath. 44: 179. 1954. (nom. inval.)
Pleospora gracilariae E.G. Simmons & S. Schatz, Mem. New York Bot. Gard. 49: 305. 1989.
Stemphylium cucumis Y.F. Pei & X.G. Zhang, Mycol. Progr. 10: 167. 2011.

Specimens examined: **China**, Sinkiang province, Korla, from *Cucumis melo* leaves, collection date unknown, Y.F. Pei (culture ex-type of *S. cucumis* CBS 125060). **Israel**, from *Gracilaria* sp., collection date unknown, S. Schatz (culture ex-type of *S. gracilariae* CBS 482.90 = E.G.S. 37.073). **Spain**, Tenerife, from *Leucospermum* sp. (Rigoletto), 1 Apr. 2000, S. Denman, CBS 115179; Tenerife, from *Leucospermum* sp. (Succession), 1 Apr. 2000, S. Denman, CBS 115180. **USA**, California, from *Solanum lycopersicum* fruit, collection date unknown, G.B. Ramsey, CBS 308.36 = ATCC 10737. **Unknown**, from leaf of *Lactuca* sp., collection date unknown, W.C. Snyder (culture ex-type *P. herbarum* f. *lactucum* CBS 273.55).

Notes: In this study CBS 273.55 is recognised as ex-type culture of *Pleospora herbarum* f. *lactucum* based on the study of the original data deposited in the CBS culture collection archive. This correspondence showed that the isolate was deposited in the collection by the original author of the species (W.C. Snyder), after a request from the curator of the CBS collection to deposit the new species. Therefore *P. herbarum* f. *lactucum* will be synonymised with *S. gracilariae* instead of *P. herbarum* under which name it is currently synonymised. The description of *S. cucumis* was based on morphology and molecular phylogenetic analyses (Pei *et al.* 2011). Although their phylogenetic tree places *S. cucumis* distant from *S. gracilariae*, their sequences published for *S. gracilariae* and *S. cucumis* are identical (*S. cucumis* GU182942, GU182939, *S. gracilariae* AF442784, AF443883, for ITS and *gapdh* respectively). In the tree, *S. cucumis* was probably exchanged with *S. luffae* which is placed in close phylogenetic relation with *S. gracilariae* in the tree. However, sequence comparisons between the ex-type isolate of *S. luffae* and *S. gracilariae* show multiple nucleotide differences. The morphological description of *S. cucumis* also fits the description of *S. gracilariae* and is therefore synonymised here. Culture CBS 308.36, isolated from tomato in California, USA, was stored as *Pleospora lycopersici* in the CBS collection.

Fig. 5. *Stemphylium chrysanthemicola* sp. nov. (CBS 117255). **A–B.** Conidiophores and conidia. **C–D.** Conidia. **E.** Hyphal plaques. Scale bars = 10 µm.

However, the original description of *P. lycopersici* was from *Solanum lycopersicum* in Belgium (Marchal & Marchal 1921). Therefore, based on this single strain, we choose not to synonymise *P. lycopersici* with *S. gracilariae* at this point pending the collection of more isolates.

Stemphylium halophilum (J. Webster) Woudenb. & Crous, **comb. nov.** MycoBank MB820660.
Basionym: *Pleospora halophila* J. Webster, in Subramanian, Taxonomy of Fungi, (Proc. Int. Symp. Madras 1973) Part 2 (Madras): 349. 1984.

Specimens examined: **UK**, England, Devon, Exeter, Dawlish Warren, from *Limonium vulgare*, coll. date unknown, J. Webster (**holotype** HME 3143, culture ex-type CBS 337.73); England, Devon, near Exeter, from *Armeria maritima*, 10 Aug. 1972, J. Webster, CBS 410.73.

Note: The transfer of *P. halophila* to the genus *Stemphylium* is in congruence with an earlier study based on the large subunit 28S nr DNA (Kodsueb *et al.* 2006).

Stemphylium lancipes (Ellis & Everh.) E.G. Simmons, Mycologia 61: 21. 1969.
Basionym: *Alternaria lancipes* Ellis & Everh., J. Mycol. 4: 45. 1888.

Specimens examined: **New Zealand**, from *Aquilegia* sp., collection date and collector unknown, CBS 116584 = E.G.S. 46.182; from *Aquilegia* sp., Jul. 1998, HM Dance, CBS 101217. **USA**, Kansas, from *Aquilegia canadensis*, collection date and collector unknown (**epitype designated here** CBS H-23043, MBT375502, culture ex-epitype CBS 133314 = E.G.S. 10.022).

Notes: The type material from *Alternaria lancipes*, basionym of *Stemphylium lancipes,* was originally described from *Argemone* sp. collected in Manhattan, Kansas, USA (Ellis & Everhart 1888). The holotype material, stored at the NY herbarium (ID 00830044), was studied by Emory G. Simmons, who subsequently transferred the species to the genus *Stemphylium* (Simmons 1969). However, two other collections from the same locality are on *Aquilegia* sp., which yielded the isolate Emory G. Simmons studied (Simmons 1969). Here we propose this isolate (CBS 133314), isolated from *Aquilegia canadensis* in Kansas, USA, as epitype of *A. lancipes*.

Stemphylium lucomagnoense Woudenb. & Crous, **sp. nov.** MycoBank MB820661. Fig. 7.

Etymology: Named after the place of isolation, Lucomagno, the Lukmanier Pass in Switzerland.

On PCA after 14 d: *Conidiophores* solitary, straight to flexuous, occasionally branched, septate, smooth, sub-hyaline, (34–)46–95(–119) × (2.5–)3–4(–4.5) µm, bearing multiple darkened percurrent rejuvenation sites. *Conidiogenous cells* swollen at the apex, darkened, (4–)5–6.5(–7.5) µm wide. *Conidia* solitary or in

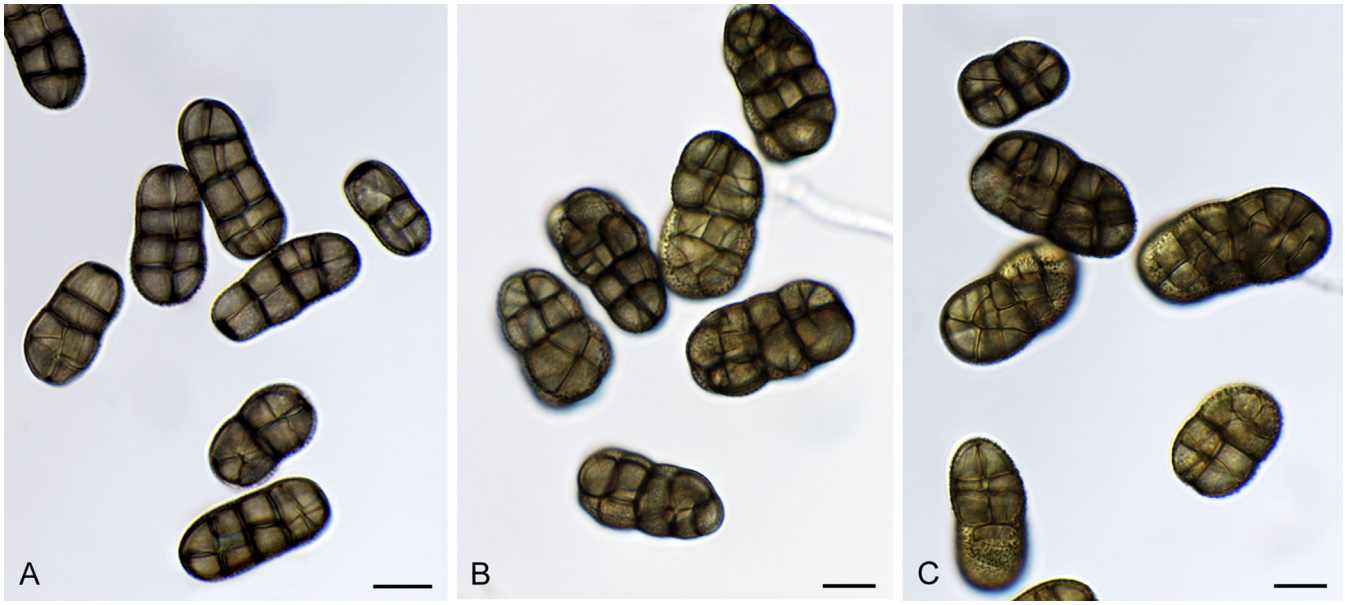

Fig. 6. *Stemphylium eturmiunum* conidiophores and conidia. **A.** CBS 109845. **B.** CBS 138495. **C.** CBS 122641. Scale bars = 10 µm.

short chains of 2 conidia, conidium body is dark brown, inconspicuously verrucose, ellipsoid to broad ovoid, (18.5–)20–27(–31) × (9.5–)11–16(–18) µm, L/W = 1.8, with (2–)3 *transverse septa* and 1–2 *longitudinal* or *oblique septa* per transverse sector. Constricted at 1–3 darkened transverse septa. Immature ascomata of *sexual morph* observed in agar, pseudothecia globose or broad ovoid, single, covered with dark hyphal outgrows, ranging in size to 485 µm tall.

Culture characteristics: After 7 d cultures on SNA flat, rhizoid, aerial mycelium is scarce, colonies colourless, no sporulation, colonies 5 mm diam; cultures after 7 d on PCA flat, entire, aerial mycelium woolly, pale olivaceous grey, colonies greenish olivaceous with two olivaceous rings, young colourless ascomata in agar which become black after 14 d, colonies reaching 28–30 mm diam.

Specimen examined: **Switzerland**, Ticino, Lucomagno, from *Minuartia hybrida*, 19 Jun. 1981, P.G. Crivelli (**holotype** CBS H-23046, culture ex-type CBS 116601 = E.G.S. 37.017).

Notes: Culture CBS 116601 was deposited as *Pleospora gigaspora* in the CBS collection, as diagnosed by Crivelli (Inderbitzin *et al.* 2009). *Pleospora gigaspora* was originally described from dead shoots of "herbarum majorum" from the inlands of "Maris glacialis, Kildin", Russia (Karsten 1884), with smooth ascomata of 300–400 µm and no description of the asexual morph. Since our species has dark hyphal outgrows on its ascomata and is obviously different, we provided this species with a new name. *Pleospora minuartiae* is described from dry leaves of *Minuartia taurica* from Mt. Babugan-Yayla, Tauria, Crimea, Ukraine (Gucevicz 1972). This species is described with small ascomata measuring 140–180 µm, which significantly differs from our species for which ascomata of up to 485 µm tall are observed. Since there is also a morphologically different *Pleospora* species named after the country of isolation, *P. helvetica* with small ascomata measuring 180–200 µm (Niessl 1867), we named our isolate after the place of isolation, Lucomagno, the Lukmanier Pass.

Stemphylium lycopersici (Enjoji) W. Yamam., Trans. Mycol. Soc. Japan 2: 93. 1960. Fig. 8.

Basionym: *Thyrospora lycopersici* Enjoji, J. Pl. Protect. 18: 52. 1931.

Synonyms: *Stemphylium xanthosomatis* B. Huguenin, as "*xanthosomae*", Bull. Soc. Mycol. France 81: 697. 1966.

Stemphylium plantaginis Yong Wang bis & X.G. Zhang, Mycotaxon 96: 79. 2006.

Stemphylium pruni Yong Wang bis & X.G. Zhang, Mycotaxon 96: 78. 2006.

Stemphylium oblongum Yong Wang bis & X.G. Zhang, Nova Hedwigia 88: 201. 2009.

Stemphylium pyrina Yong Wang bis & X.G. Zhang, Mycol. Progr. 8: 303. 2009.

Stemphylium sophorae Yong Wang bis & X.G. Zhang, Nova Hedwigia 88: 200. 2009.

Stemphylium platycodontis J.X. Deng & S.H. Yu, Mycol. Progr. 13: 479. 2014.

Specimens examined: **China**, Guizhou Province, Guiyang, from *Solanum lycopersicum* leaves, collection date unknown, Y. Wang (**neotype designated here** of *T. lycopersici* CBS H-23051, MBT375506, culture ex-neotype CBS 122639); Guizhou Province, Guiyang, from *Prunus persica* leaves, 16 Aug. 2003, Y. Wang (culture ex-type of *S. pruni* CBS 124980); Shandong Province, Taian, from *Gossypium hirsutum* leaves, 3 Oct. 2004, X.G. Zhang (culture ex-type of *S. oblongum* CBS 120326); Shandong Province, Mountain Tai, from *Plantago major* leaves, 5 Oct. 2003, Y. Wang (culture ex-type of *S. plantaginis* CBS 124981); Shandong Province, Mountain Tai, from *Sophora microphylla* leaves, 3 Oct. 2004, Y. Wang (culture ex-type of *S. sophorae* CBS 120325); Sinkiang Province, Korla, from *Pyrus sinkiangensis* leaves, 9 Aug. 2006, Y. Wang (culture ex-type of *S. pyrina* CBS 122803). **New Caledonia**, Nouméa, from *Xanthosoma sagittifolium*, 1962, B. Huguenin (culture ex-type of *S. xanthosomatis* CBS 116585 = E.G.S. 17.137 = IMI 98083).

Notes: *Stemphylium lycopersici*, with *Thyrospora lycopersici* as basionym, was originally described from *Solanum lycopersicum* in Japan, but lacks a holotype specimen (Enjoji 1931). The culture CBS 116587, isolated from *Solanum lycopersicum* in the Dominican Republic, was considered by Emory G. Simmons to fit the concept of this species (Inderbitzin *et al.* 2009). Here we propose CBS 122639, isolated from *Solanum lycopersicum* in

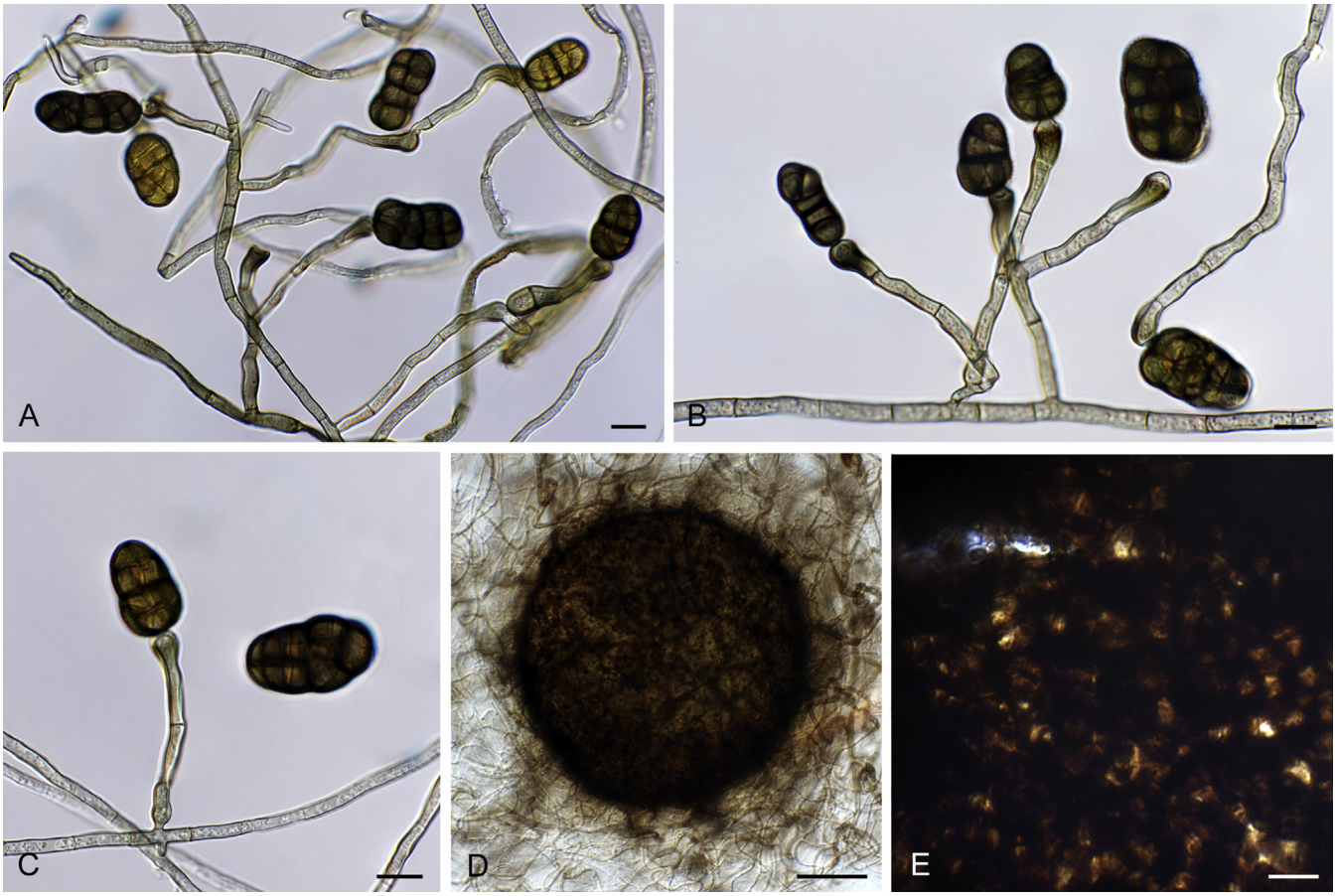

Fig. 7. *Stemphylium lucomagnoense* sp. nov. (CBS 116601). **A–C.** Conidiophores and conidia. **D.** Ascoma. **E.** Ascomatal wall. Scale bars: A–C, E = 10 µm; D = 100 µm.

China, as ex-neotype of *T. lycopersici*, since this isolate is from a geographically closer location, and also clusters in the same phylogenetic species clade. The type-isolate of *S. platycodontis* (CNU 111092) is not included in this study, but another one is included, namely CBS 333.73, also isolated from *Platycodon* sp. and regarded as *S. platycodontis* (Deng *et al.* 2014). *Stemphylium platycodontis* was described based on phylogenetic study of the ITS, *gapdh* and *tef1* partial gene sequences in combination with morphology studies. When comparing the ITS, *gapdh* and *cmdA* sequence of isolate CBS 333.73, only the *gapdh* sequence is unique for the two isolates from *Platycodon*, with only 1 nt difference. Together with the minor morphological differences described, slightly larger spore size (*S. platycodontis* 33–80 × 12–22, *S. lycopersici* 21–60 × 12–24 µm) and no production of brown pigment in PDA of *S. platycodontis*, we propose to synonymise *S. platycodontis* under *S. lycopersici*. Five synonymised species under *S. lycopersici* were described based on morphology alone. *Stemphylium oblongum, S. plantaginis, S. pruni, S. pyrina* and *S. sophorae* were described as new species from China (Wang & Zhang 2006; Wang *et al.* 2009; Wang & Zhang 2009), with some even appearing in the same manuscript. However, the broad conidial size range (21–60 × 12–24 µm) and described shape of conidia (ellipsoidal, ovoid, short cylindrical or shortly obclavate) of *S. lycopersici* by Yamamoto (1960), results in the fact that all described species fit the concept of *S. lycopersici*. The only difference in the descriptions is the structure of the conidial wall. This ranges from smooth (*S. plantaginis* and *S. pruni*) to densely tuberculate (*S. pyrina*) including descriptions with both smooth and finely postulate/micromaculate conidia (*S. oblongum* and *S. sophorae*). The description of *Stemphylium lycopersici* mentions echinulate (with sharply pointed spines) conidia. Morphological examination showed that all studied isolates have roughened conidia (Fig. 8), including the ex-type isolates of *S. plantaginis* (CBS 124981, Fig. 8E–F) and *S. pruni* (CBS 124980, Fig. 8G).

Stemphylium subglobuliferum was described based on a phylogenetic study of the ITS and *gapdh* partial gene sequences in combination with morphological studies (Xue *et al.* 2005). The ITS sequence of *S. subglobuliferum* (AY751454) is 100 % identical with *S. lycopersici*, and the *gapdh* sequence (AY751459) only has 1 unique nt compared to our *S. lycopersici gapdh* sequences. However, *S. subglobuliferum* was described as a new species based on the smaller spore size (9–20 × 5–13) and smooth conidial wall. A re-examination of the type-isolate is needed to clarify if this is indeed another synonym of *S. lycopersici*.

Based on our specimens examined, *Stemphylium lycopersici* has a broad host range infecting plant leaves from at least six different families (*Araceae*, *Fabaceae*, *Malvaceae*, *Plantaginaceae*, *Rosaceae* and *Solanaceae*).

Stemphylium novae-zelandiae Woudenb. & Crous, **sp. nov.** MycoBank MB820662. Fig. 9.

Etymology: Named after the country where it was isolated, New Zealand.

Conidiophores solitary, straight to flexuous, unbranched, septate, smooth, sub-hyaline, (46.5–)64.5–111(–144.5) × (2.5–)3–4.5(–5.5) µm, bearing 1–2 thickened percurrent rejuvenation

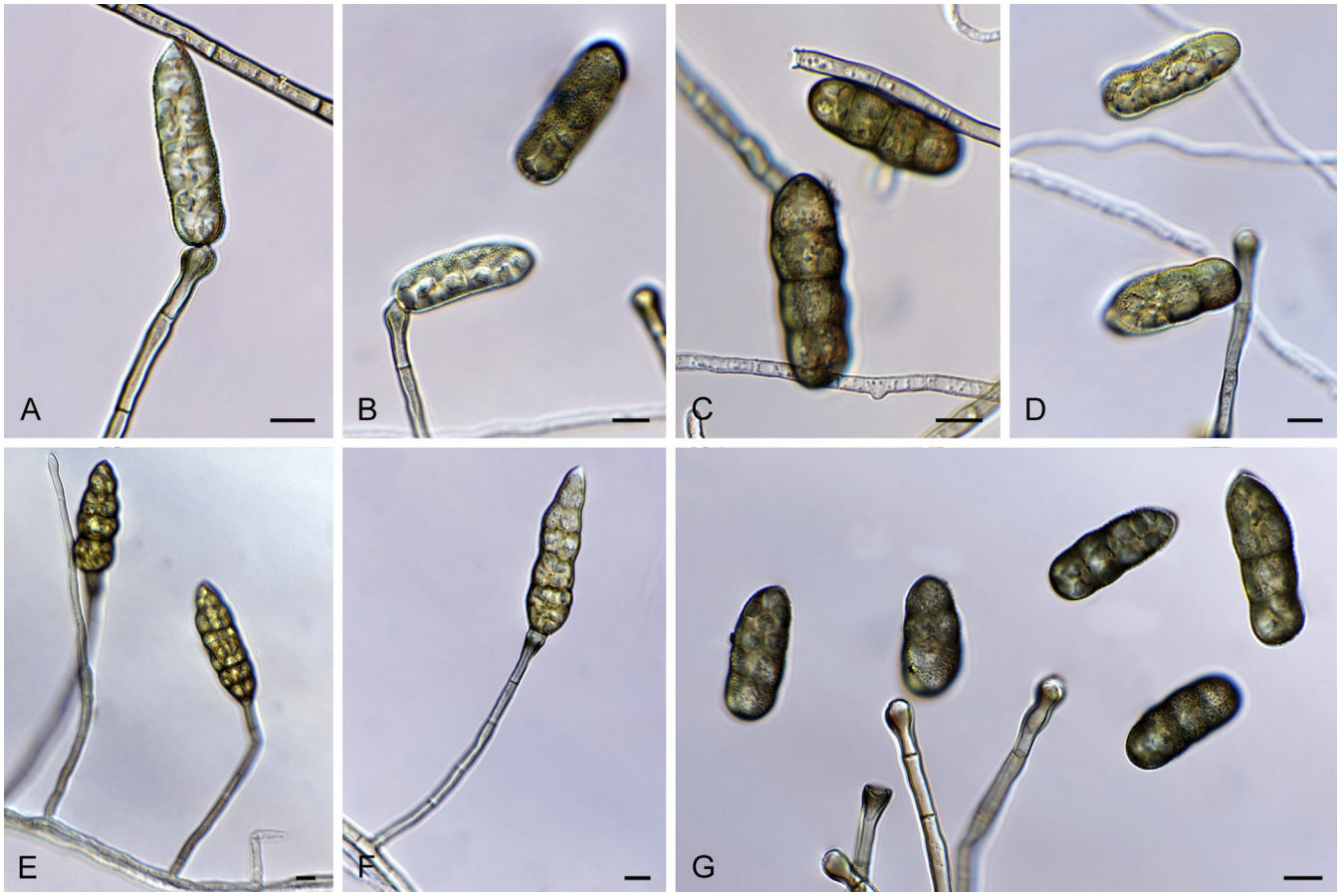

Fig. 8. *Stemphylium lycopersici* conidiophores and conidia after 7 d on PCA. **A–B.** CBS 122639. **C.** CBS 120325. **D.** CBS 122803. **E–F.** CBS 124981. **G.** CBS 124980. Scale bars = 10 µm.

sites. *Conidiogenous cells* swollen at the apex, darkened, (5–)6–7.5(–8.5) µm wide. *Conidia* solitary, conidium body is light olive brown, verrucose, cylindrical, (31–)34–40.5(–45.5) × (9–)11–13(–14.5) µm, L/W = 3.1, with 3–5(–7) *transverse septa* and 1–2 *longitudinal* or *oblique septa* per transverse sector. Constricted at 2–3 darkened transverse septa. Forms hyphal plaques at the bottom of PCA plates after 14 d. *Sexual morph* not observed.

Culture characteristics: After 7 d cultures on SNA flat, entire, aerial mycelium is scarce, wooly, white, colonies colourless, with three pale olivaceous grey rings and centre, colonies 20–24 mm diam; cultures after 7 d on PCA flat, entire, aerial mycelium fine felty, pale olivaceous grey, colonies white to olivaceous buff with two grey olivaceous rings and a greenish olivaceous outer ring, colonies reaching 35 mm diam.

Specimens examined: **New Zealand**, Waitakaruru, from dead leaf of *Avicennia resinifera*, 10 Sep. 2006, C.F. Hill (**holotype** CBS H-23047, culture ex-type CBS 138295 = E.G.S. 52.148 (06/5200B)); additional strain from the same source CBS 138157 = E.G.S. 52.147 (06/5200A).

Notes: To avoid confusion with the species *Pleospora avicenniae* (Borse 1987), we named the species after the country where it was isolated, New Zealand, instead of the host of isolation. Recently *Pleospora avicenniae* was placed in the new genus *Halojulella* based on a morphological and molecular examination (Ariyawansa *et al.* 2013). As in *S. chrysanthemicola*, *S. novae-zelandiae* forms hyphal plaques at the bottom of the PCA plate but these are only observed after 14 d.

Stemphylium simmonsii Woudenb. & Crous, **sp. nov.** MycoBank MB820663. Fig. 10.

Etymology: Named after Emory G. Simmons, who extensively studied *Pleospora* and *Stemphylium* species.

Conidiophores solitary, straight to flexuous, occasionally branched, septate, smooth, sub-hyaline, (18–)30–93(–159) × (2–)3–4(–5) µm, bearing multiple darkened percurrent rejuvenation sites. *Conidiogenous cells* swollen at the apex, darkened, (4.5–)5–6.5(–7.5) µm wide. *Conidia* solitary, conidium body is pale olive brown, verrucose, ellipsoid to broad ovoid, (18–)20.5–24.5(–28) × (11–)13–16(–18.5) µm, L/W = 1.6, with (2–)3 *transverse septa* and (1–)2(–3) *longitudinal* or *oblique septa* per transverse sector. Often constricted at the middle, darkened transverse septum. Immature ascomata of *sexual morph* observed in and on agar, pseudothecia subglobose or broad ovoid, single, covered with dark hyphal outgrows, ranging from 175 to 365 µm tall.

Culture characteristics: After 7 d cultures on SNA flat, rhizoid, aerial mycelium is fine felty, pale olivaceous grey, colonies colourless, pale olivaceous grey coloured by aerial conidia in rhizoid shape, colonies 45–55 mm diam; cultures on PCA flat, entire, aerial mycelium scarce, woolly, pale olivaceous grey, colonies colourless with three grey olivaceous rings, and centre olivaceous to iron-grey with ascomata in and on agar, colonies reaching 60 mm diam.

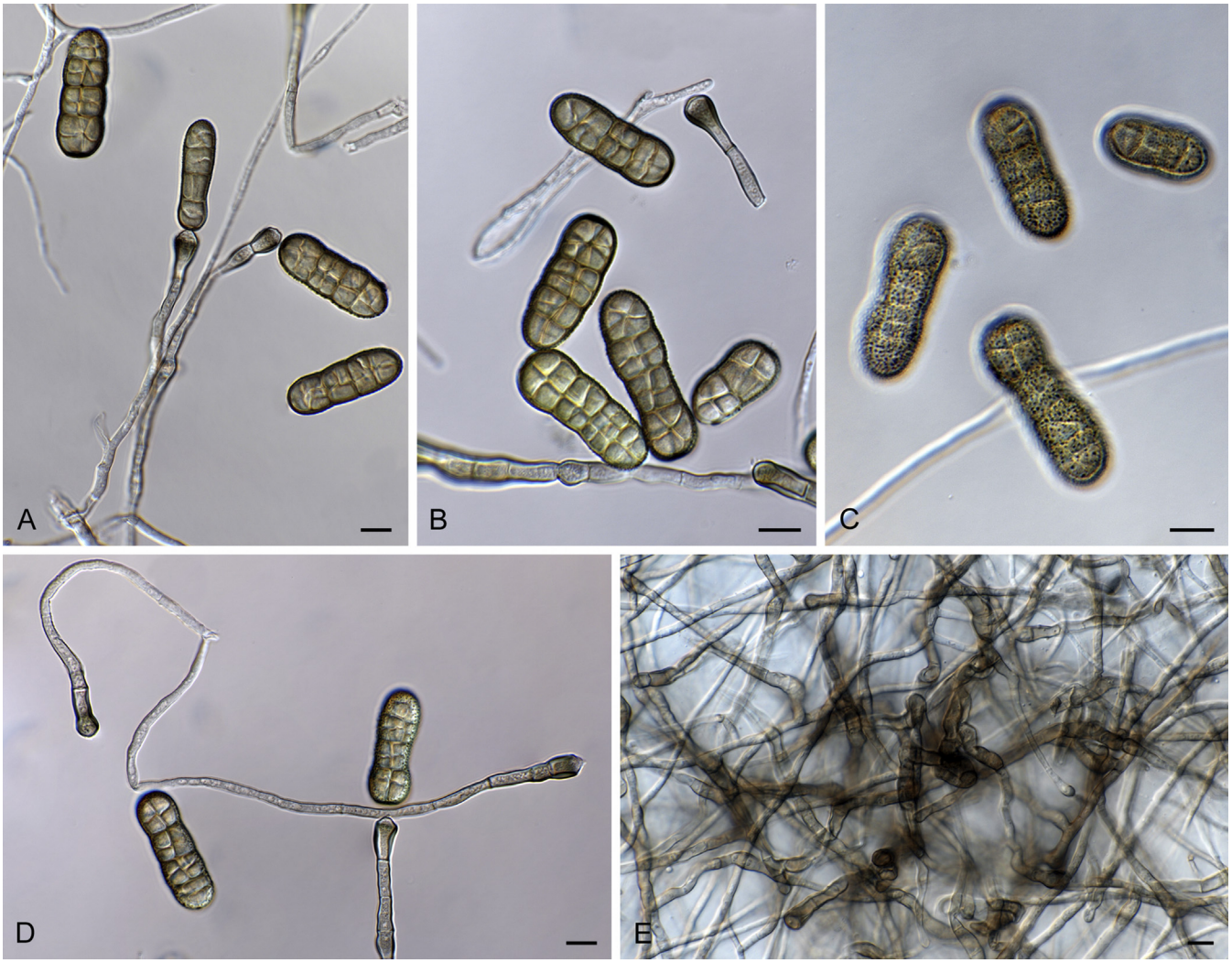

Fig. 9. *Stemphylium novae-zelandiae* sp. nov. (CBS 138295). **A, D.** Conidiophores and conidia. **B–C.** Conidia. **E.** Hyphal plaques. Scale bars = 10 µm.

Specimens examined: **Australia**, from *Malus sylvestris* fruit, 1 Apr. 1976, C. Robertson, CBS 134496 = E.G.S. 42.138. **Canada**, from *Fragaria* sp., before 1971, C.O. Gourlay (**holotype** CBS H-23048, culture ex-type CBS 133518 = E.G.S. 30.154); from *Solanum lycopersicum* leaf, before 1971, C.O. Gourlay, CBS 133515 = E.G.S. 30.153; British Colombia, Ladner, from *Phragmites* sp. leaves, 7 Feb. 1999, A. & R. Bandoni & S. landvik & P. Inderbitzin, CBS 116598 = UAMH 104876; British Colombia, Sidney, from *Lactuca muralis*, 22 May 2001, M.E. Barr, CBS 116603; British Colombia, Sidney, from *Geum macrophyllum*, 22 May 2001, M.E. Barr, CBS 116604. **USA**, Maryland, Laurel, from *Commelina* sp. leaf, 14 Aug. 1966, E.G. Simmons, CBS 716.68. = E.G.S. 17.151 = ATCC 18518 = IMI 135458 = MUCL 11718; Massachusetts, Hadley, from *Trifolium pratense* leaf, 20 Jun. 1985, E.G. Simmons, CBS 133894 = E.G.S. 38.115.

Notes: Three examined isolates were named *S. globuliferum* by E.G. Simmons (CBS 716.68, CBS 133894, CBS 134496). Since the original description of *M. globuliferum* was from *Lotus corniculatus* (*Fabaceae*) from Gotland, Sweden (Vestergren 1896), we did not follow this identification but introduced the new name *S. simmonsii*. Morphologically *S. simmonsii* resembles *S. botryosum*, which is phylogenetically only distantly related. Phylogenetically it is closely related to *S. beticola*, which can easily be distinguished from *S. simmonsii* by its glabrous ascomata (Fig. 3D–E; *S. simmonsii* has ascomata with dark hyphal outgrows, Fig. 10E). See the general discussion below for additional information.

Stemphylium solani G.F. Weber, Phytopathol. 20: 516. 1930.

Synonym: *Thyrospora solani* (G.F. Weber) Sawada, Rep. Dept. Agric. Gov. Res. Inst. Formosa 51: 115. 1931.

Specimens examined: **USA**, Indiana, Darlington, from *Solanum lycopersicum*, Sep. 1993, E.G. Simmons (**epitype designated here** CBS H-23049, MBT375504, culture ex-epitype CBS 116586 = E.G.S. 41.135); Kansas, Riley County, from *Euphorbia marginata* leaf, 6 Nov. 1994, D. Stuterille, CBS 118082 = E.G.S. 42.055; South Carolina, Charleston, from *Solanum lycopersicum*, 1952, C.F. Andrus, CBS 408.54 = ATCC 11128.

Notes: *Stemphylium solani* was originally described from diseased tomato plants collected in Florida, USA (Weber 1930). The holotype material is stored in the Florida Agricultural Experiment Station Herbarium, now named University of Florida Herbarium, under the specimen number FLAS-F-13571. According to Emory G. Simmons, CBS 116586, isolated from *Solanum lycopersicum* from Indiana, USA, was a good representative of the species after examination of the type material (Inderbitzin *et al.* 2009). We follow his suggestion and designate CBS 116586 as ex-epitype culture of *S. solani*.

Stemphylium vesicarium (Wallr.) E.G. Simmons, Mycologia 61: 9. 1969. Fig. 11.
Basionym: *Helminthosporium vesicarium* Wallr. [as 'Helmisporium'], Fl. Cryptog. German. 2: 166. 1833.
Synonyms: *Macrosporium vesicarium* (Wallr.) Sacc., Syll. Fungorum 4: 537. 1886.

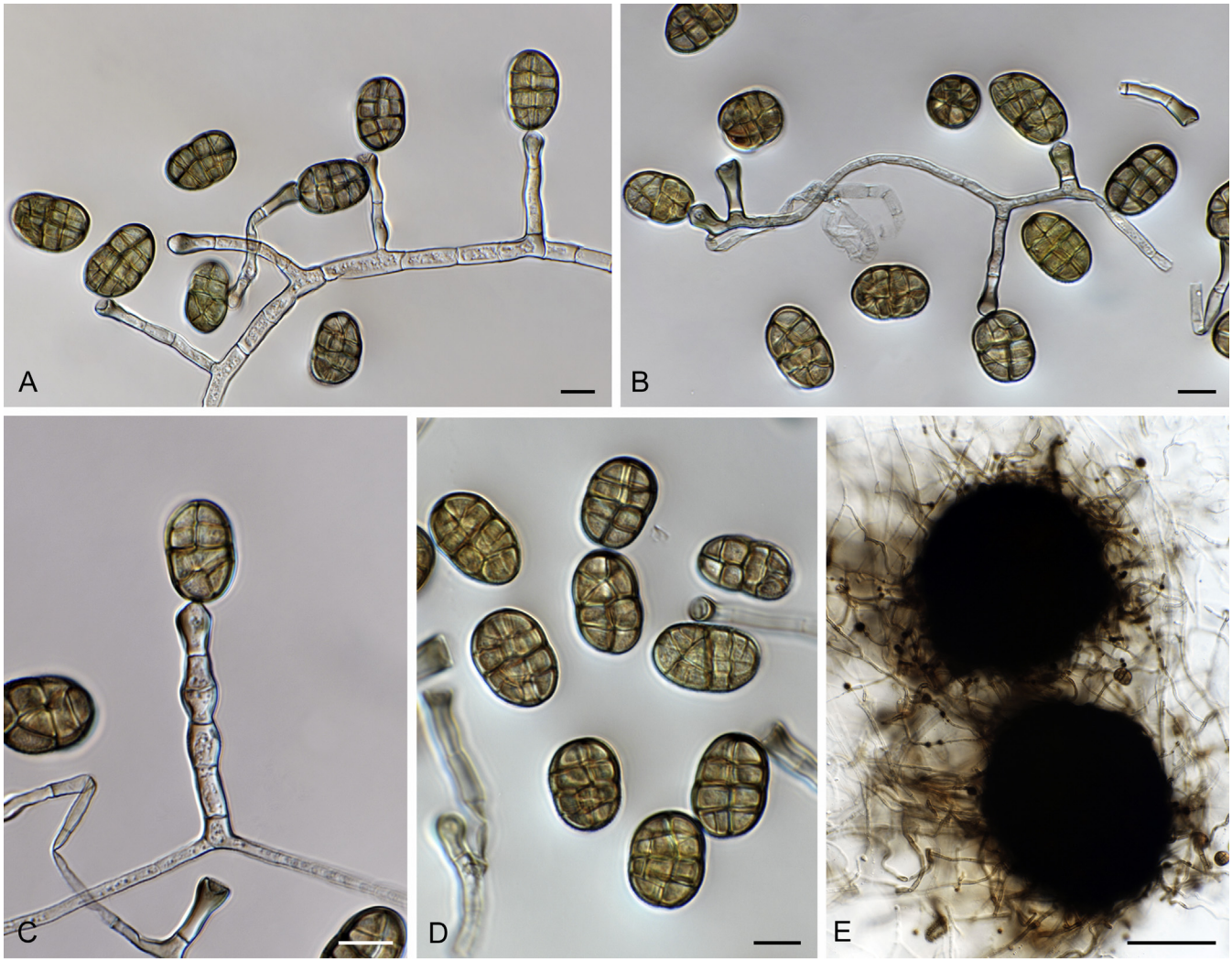

Fig. 10. *Stemphylium simmonsii* sp. nov. (CBS 133518). **A–C.** Conidiophores and conidia. **D.** Conidia. **E.** Ascomata. Scale bars: A–D = 10 µm; E = 100 µm.

Sphaeria herbarum Pers.: Fr, Syn. Meth. Fungorum 1: 78. 1801.
Pleospora herbarum (Pers.: Fr) Rabenh. ex Ces. & De Not.: Fr Comment. Soc. Crittog. Ital. 1:217. 1863.
Pleospora pomorum A.S. Horne, J. Bot. 58: 239. 1920.
Stemphylium herbarum E.G. Simmons, Sydowia 38: 291. 1986.
Pleospora alfalfae E.G. Simmons, Sydowia 38: 292. 1986.
Stemphylium alfalfae E.G. Simmons, Sydowia 38: 292. 1986.
Pleospora sedicola E.G. Simmons, Harvard Pap. Bot. 6: 202. 2001.
Stemphylium sedicola E.G. Simmons, Harvard Pap. Bot. 6: 202. 2001.
Pleospora tomatonis E.G. Simmons, Harvard Pap. Bot. 6: 204. 2001.
Stemphylium tomatonis E.G. Simmons, Harvard Pap. Bot. 6: 204. 2001.
Stemphylium cremanthodii Y.F. Pei & X.G. Zhang, Mycotaxon 109: 494. 2009.
Stemphylium mali Yong Wang bis & X.G. Zhang, Mycol. Progr. 8: 303. 2009.
Stemphylium brassicicola Y.F. Pei & X.G. Zhang, Mycotaxon 111: 169. 2010.
See Index Fungorum for additional synonyms.

Specimens examined: **Australia**, Western Australia, Harvey, from *Medicago sativa*, 30 Jul. 1982, collector unknown (culture ex-type of *P. alfalfae* CBS 192.86 = E.G.S. 36.088 = IMI 269683). **China**, Sinkiang province, Korla, from *Cremanthodium discoideum* leaves, 16 Oct. 2008, Y.F. Pei (culture ex-type of *S. cremanthodii* CBS 124747); Sinkiang province, Korla, from *Brassica pekinensis* leaves, 7 Aug. 2009, Y.F. Pei (culture ex-type *of S. brassicicola* CBS 124749); Sinkiang Province, Yili, from *Malus sieversii* leaves, 19 Jul. 2005, Y. Wang (culture ex-type of *S. mali* CBS 122640). **India**, Uttar Pradesh, Jhansi, from *Medicago sativa*, 1983, H.K. Joshi (culture ex-type of *S. herbarum* CBS 191.86 = E.G.S. 36.138 = IMI 276975). **New Zealand**, Auckland, from *Sedum spectabile* leaf lesion, Mar. 2000, E.G. Simmons (culture ex-type of *P. sedicola* CBS 109843 = E.G.S. 48.095 = IMI 386967). **UK**, England, from *Malus domestica* fruit, collection date unknown, M.N. Kidd (**neotype** of *P. pomorum* **designated here** CBS H-23044, MBT375503, culture ex-neotype CBS 184.25). **USA**, California, Central Valley, from *Solanum lycopersicum* fruit, Oct. 1968, E.G. Simmons (culture ex-type of *P. tomatonis* CBS 109844 = E.G.S. 29.089 = IMI 386968).

Notes: *Pleospora pomorum* was originally described from spotted apples in Britain, without the designation of a holotype specimen (Horne 1920). A second publication on the species was done by Kidd & Beaumont (1924), who deposited isolate CBS 184.25, from apple fruit in England in the CBS collection. Since no holotype specimen is known, we propose CBS 184.25 as ex-neotype culture of *Pleospora pomorum*. Therefore, *P. pomorum* will be synonymised with *S. vesicarium*. The first molecular study of *Stemphylium* species showed that *S. alfalfae, S. herbarum*, and *S. vesicarium* were identical based on their ITS and *gapdh* sequences (Câmara *et al.* 2002). A more extensive phylogenetic analysis on DNA sequences from four loci ITS, *gapdh*, *tef1* and the intergenic spacer between *vmaA* and *vpsA* (Inderbitzin *et al.* 2009) showed the same clustering, and added the species

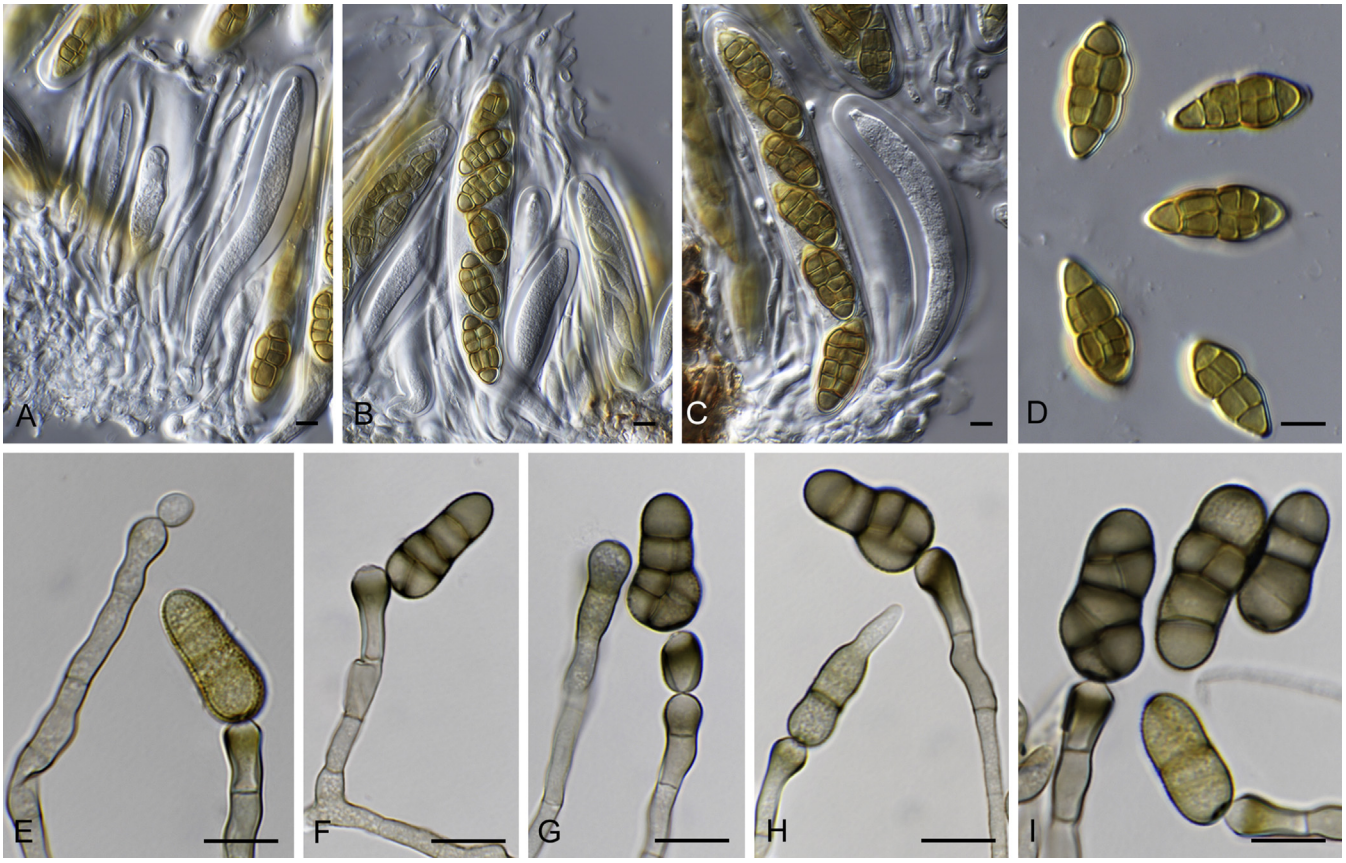

Fig. 11. *Stemphylium vesicarium*. **A–D.** (CPC 29939). Asci, pseudoparaphyses, and ascospores. **E–I.** (CBS 123005). Conidiophores and conidia. Scale bars = 10 µm.

P. sedicola and *P. tomatonis* to the species clade. However, small morphological differences have been used to distinguish among the species now synonymised under *S. vesicarium* (summarised in Table 3). Although the described differences seem sometimes considerable, they are not always obvious when performing morphological studies (illustrated by pictures 1–5 in fig. 4 of Inderbitzin *et al.* 2009). See the general discussion below for additional information on the synonymies proposed here for *S. vesicarium.* Only the synonyms of *S. vesicarium* proposed in this research are listed, for the full list of synonyms see Index Fungorum (http://www.indexfungorum.org).

DISCUSSION

This manuscript presents a molecular phylogenetic overview of species in the genus *Stemphylium* known from culture, initiated due to our inability to unequivocally identify a *Stemphylium* sp. causing yellow leaf spot in sugar beet. To be able to characterise the species, all currently known (and available) species of the genus had to be considered. However, the lack of (ex-)type material often makes it difficult to determine species names of fungi, described on morphology only, onto the modern DNA-based phylogenetic trees. To strengthen and stabilise the taxonomy of *Stemphylium*, three epitypes, one lectotype and two neotypes are proposed in the present study. However, some isolates represent names for which no ex-type isolate is present or for which it was difficult to designate an appropriate ex-epitype culture (highlighted with bold species names in Fig. 1).

Seven isolates were named *Stemphylium globuliferum* by Emory G. Simmons based on morphology. *Stemphylium globuliferum* was originally described as *Macrosporium globuliferum* from *Lotus corniculatus* (*Fabaceae*) from Gotland, Sweden (Vestergren 1896). Emory G. Simmons studied the holotype material (in UPS) and placed this species in *Stemphylium* (Simmons 1969). He described it as a common species, and isolated it from *Trifolium pratense* (*Fabaceae*). Four of the included isolates fall within the *S. botryosum* clade, and three within *Stemphylium simmonsii* sp. nov. Since none of these isolates originate from *Lotus corniculatus*, or are from Sweden (or even Europe), we choose not to use the name *Stemphylium globuliferum* for the new species, but rather provide it with a new name (*S. simmonsii* sp. nov.).

Stemphylium vesicarium, with *Helminthosporium vesicarium* as basionym, was originally described from *Allium sativum* in Germany (Wallroth 1833). Our dataset includes 25 isolates named *S. vesicarium* of which 20 were named, based on morphology, by Emory G. Simmons, who also studied the holotype specimen at STR. One isolate, not studied by him, clusters with *S. lycopersici* (CBS 436.76), two isolates (one identified by him) cluster with *S. eturmiunum,* and the other 22 all cluster within the *Stemphylium vesicarium* clade (based on ITS, Fig. 1). Since none of the isolates originate from *Allium sativum* in Germany (or from a geographically close location), no ex-epitype culture is proposed for the species.

As already mentioned in the introduction, the *Pleospora herbarum* clade *sensu* Inderbitzin *et al.* (2009) illustrated the problems with identification in the genus *Stemphylium*. Molecular studies demonstrated the phylogenetic identity of the species *S. alfalfae*, *S. herbarum*, *S. sedicola, S. tomatonis,* and *S. vesicarium* (e.g. Câmara *et al.* 2002; Inderbitzin *et al.* 2009). However, differences in RAPD fingerprints (Chaisrisook *et al.* 1995) and morphology (Simmons 1969, 1985, 1989, 2001), seemed to support them to be separate species. It should be

Table 3. Conidial characteristics of *Stemphylium* species synonymised under *S. vesicarium*.

Species	Conidial shape	Conidial size (µm)	L/B ratio[1]	Transverse septa	Longitudinal septa	Wall ornamentation	Reference
S. alfalfae	Oblong	30–40(–45) × 12–15(–18)	ND	6–7	1–2	Minutely verrucose	Simmons (1985)
	Spherical or ovoid	32–35 × 16–19	ND	3–4	variable	Minutely verrucose	Simmons (1985)
S. brassicicola	Subdoliiform, cylindrical to oblong cylindrical	32–45 × 12–19	2–3.1	1–4(–5)	3–5(–6)	Conspicuously punctulate to punctate	Pei *et al.* (2010)
S. crematthodii	Oblong to oblong-ellipsoid	18–31 × 9–19	1.5–2.6	1–3	0–3	Micromaculate	Pei *et al.* (2009)
S. herbarum	Broadly ovoid or broadly ellipsoid, sometimes inequilateral	35–45 × 20–27	ND	6–7	1–3	Conspicuously and densely verrucose	Simmons (1985)
S. mali	Oblong	18–22 × 13–16	1.3–1.6	1–3	3–5	Conspicuously punctate	Wang *et al.* (2009)
	Subspherical	14–16.5 (diam)	ND	1(–3)	2–4	Conspicuously punctate	Wang *et al.* (2009)
S. sedicola	Broadly ellipsoid or oblong	30–35 × 18–20	ND	2–3	1–3	Smooth or usually punctate	Simmons (2001)
S. tomatonis	Oblong, broadly ellipsoid (or subglobose)	46–48 × 13–16	2.4–3 (or 1–1.5)	4–7	1–2	Punctulate	Simmons (2001)
S. vesicarium	Oblong or broadly oval, sometimes inequilateral	25–42(–48) × 12–22	1.5–2.7	1–5(–6)	1–2(–3)	Conspicuously and densely verrucose	Simmons (1969)

[1] ND: not determined.

noted that the RAPD studies were only based on a small number of isolates, (including only two *S. herbarum* isolates and one *S. vesicarium* isolate) and morphologically only small differences have been used to make a distinction among these species although they also share many characters (Câmara *et al.* 2002, table 2 of Kurose *et al.* 2015). As a result, some researchers chose to retain all the species names (e.g. Inderbitzin *et al.* 2009), while others chose to synonymise them (e.g. Köhl *et al.* 2009, as *S. vesicarium*). To be able to construct a stable phylogenetic species concept in *Stemphylium* we proposed to synonymise these phylogenetically identical species under *S. vesicarium*. The conidial descriptions of the species now synonymised under *S. vesicarium* are summarised in Table 3.

The species *S. sarciniforme* (Fig. 2, clade 19) is divided in two well-supported subclades. Five isolates from *Trifolium pratense* form one branch, and three isolates from *Cicer arietinum*, Iran, all isolated by W. J. Kaiser, form a separate branch. Isolate CBS 110049, from the *Cicer arietinum* clade, was submitted to the CBS collection in 2002 as ex-holotype of "*S. kaiseri*", but this name was never published. Emory Simmons morphologically identified all isolates from this clade as *S. sarciniforme*, and also chemically the isolates from both clades are similar (B. Andersen, pers. comm.). Until more isolates become available, we choose to treat them here as *S. sarciniforme*.

After revision of the species identity and names, 28 species can be distinguished in the genus *Stemphylium* based on (parts) of the ITS, *gapdh* and *cmdA* gene regions (Fig. 2). From these 28 species, five new species are described, two new name combinations are introduced and 22 names are synonymised. Of the 22 synonymised names, seven are placed in synonymy with *S. lycopersici*, seven with *S. vesicarium*, three with *S. amaranthi*, two with *S. gracilariae* and *S. eturmiunum*, and one with *S. drummondii*. *Stemphylium subglobuliferum* might also be a synonym of *S. lycopersici* (see notes of *S. lycopersici*). The majority of the synonymised species (16 out of 22) were described from China based mostly on morphology and host-specificity. Clearly in the genus *Stemphylium*, identification on morphology and host-specificity alone is insufficient for correct species identification. Several other "new" species are described from China based solely on morphology, e.g *S. allii-cepae*, *S. basellae*, *S. descurainiae*, *S. gossypii*, *S. hydrangeae*, *S. lactucae*, *S. momordicae*, *S. pisi* and *S. turriforme* (Zhang & Zhang 2002; Zhang *et al.* 2003; Zhang & Zhang 2007; Zhou *et al.* 2012). Until molecular data of the ex-type isolates become available, the status of these species names remains unclear.

Based only on ITS sequences, 22 species can be identified to species level (Fig. 1). Only four clades (clade 1, 7, 10, and 22), containing in total 10 species names, have multiple species names associated with them. This means that for accurate species identification, an additional gene to the standard ITS barcode sequence is required in the case of these 10 species. This study will therefore be useful to other plant pathologists in the field trying to identify their *Stemphylium* species, not only by providing them with the correct name(s), but also in helping them choose appropriate loci that will ensure correct identification.

CONCLUSIONS

In the genus *Stemphylium* 28 species can be distinguished based on (parts) of the ITS, *gapdh* and *cmdA* gene regions. From these

28 species, five are described as new species and a further two new combinations are proposed. Twenty-two names are reduced to synonymy. To create a stable taxonomy for *Stemphylium*, three epitypes, one lectotype and two neotypes are designated. Morphological examination alone is not suited for species identification in *Stemphylium*. For an accurate species identification, morphological studies should be combined with molecular data.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.simyco.2017.06.001.

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