Stemphylium revisited

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Abstract: In 2007 a new Stemphylium leaf spot disease associated with a 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. betica 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.

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 (Hanze 2013). The causal agent was recently formally named as Stemphylium betica (Crous et al. 2016), but the sexual itself was not treated in that study.

Stemphylium is a dematiaceous hyphomycete, which can be distinguished from other hyphomycetes forming phaeoidicyospores based on the percurrent rejuvenation of its conidiophores, and apically swollen conidigenous 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 (Cámara 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 assexual genera (e.g. Inderbitzin et al. 2006; De Gruyter et al. 2013; Aryawansa 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 (act4), beta-tubulin (tub2), calmodulin (cmd4), translation elongation factor 1-alpha
(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%) 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 and 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, 1989)ITS4 (White et al. 1990), gpd1/gpd2 (Berbee et al. 1999), EF1-728/EF1-986R (Carbone & Kohn 1999), and RPB2-5F2 (Sung et al. 2007)/RPB2-7cR (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 MgCl2, 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-Martens-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 PCR 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 Bio Numerics v. 4.61 (Applied Maths) and by eye (Table 2).

**Phylogenetic analyses**

In Bio Numerics 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/alignme nt/server/index.html) using the FFT-NS-i method. With Findmodel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmo del.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 name¹ | Strain number² | Other collection number² | Host/Substrate            | Country   | GenBank accession numbers |
|---------------------------------------|-----------|----------------|--------------------------|---------------------------|-----------|---------------------------|
|                                        |           |                |                          |                           |           | ITS gapdh cmdA             |
| *Alternaria alternata*                |           | GV14-634a1     |                          | *Chenopodium album*       | Netherlands | KU850502 KU850649 KU850790 |
| *Stemphylium amaranthi*               | S. phaseolina⁷ | CBS 124650    | HSAUP VI1538             | *Phaseolus vulgaris*      | China     | KU850503 KU850650 KU850791 |
|                                       |           | CBS 124651     | HSAUP VII1682            | *Phaseolus vulgaris*      | China     | KU850504 KU850651 KU850792 |
|                                       |           | CBS 124746⁷    | HSAUPyf1835             | *Amaranthus retroflexus*  | China     | KU850505 KU850652 KU850793 |
|                                       |           | CBS 124750     | HSAUPyf1902             | *Malus sieversii*         | Chile     | KU850506 KU850653 KU850794 |
| *S. microsporum*⁷                     |           | CBS 124984     | HSAUPyf2018             | *Raphanus sativus*        | China     | KU850508 KU850655 KU850796 |
| *Stemphylium astragali*               |           | CBS 136589     | E.G.S. 48.098           | *Lotus pendunculatus*     | New Zealand | KU850510 KU850657 KU850798 |
| *Stemphylium armeriae* comb. nov.    | P. armeriae | CBS 338.73     |                          | *Ameria martima*          | UK        | KU850511 KU850658 KU850799 |
| *Stemphylium beticola*                |           | CBS 378.54     | E.G.S. 08.174           | *Astragalus sp.*          | Japan     | KU850512 KU850659 KU850800 |
|                                        |           | CBS 116699     | UAMH 10489              | *Lychnis sp.*             | Canada    | KU850513 KU850660 KU850801 |
|                                        |           | CBS 133512     | E.G.S. 30.152           | *Pisum sativum*           | Canada    | KU850514 KU850661 KU850802 |
|                                        |           | 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 141024⁷    | 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 | Strain number | Other collection number | 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<sup>T</sup> | Medicago sativa | E.G.S. .04.118c; IMI 135456; MUCL 11717; QM 1379 | Canada | KU850538 | KU850685 | KU850827 |
| Stemphylium callistephi | CBS 116596 | Medicago sativa | E.G.S. .08.069; QM 7066 | USA | KU850539 | KU850686 | KU850828 |
| Stemphylium canadense sp. nov. | CBS 116602<sup>T</sup> | Salicornia sp. | UAMH 10491 | Canada | KU850641 | KU850782 | KU850932 |
| Stemphylium chrysanthemicola sp. nov. | CBS 118081 | Salicornia sp. | UAMH 10491 | Canada | KU850642 | KU850783 | KU850933 |
| Stemphylium drummondii | CBS 346.83<sup>T</sup> | Phlox drummondii | E.G.S. 31.008 | New Zealand | KU850640 | KU850781 | KU850931 |
| Stemphylium etumianum S. vesicarium | CBS 668.80 | Solanum lycopersicum | E.G.S. 29.099; IMI 386969 | Greece | KU850540 | KU850688 | KU850830 |
| Stemphylium etumianum S. variabilis<sup>T</sup> | CBS 109845<sup>T</sup> | Solanum lycopersicum | E.G.S. 29.099; IMI 386969 | New Zealand | KU850541 | KU850689 | KU850831 |
| Stemphylium etumianum S. vesicarium | CBS 122124 | Solanum lycopersicum | HSAUPIV1508 | Allium sativum | France | KU850542 | KU850690 | KU850832 |
| Stemphylium etumianum S. capsici<sup>T</sup> | CBS 122641 | Solanum lycopersicum | HSAUP1559 | Asphodelus aestivus | Greece | KU850543 | KU850691 | KU850833 |
| Stemphylium gracilariae P. lycopersici | CBS 133528 | Solanum lycopersicum | E.G.S. 30.002 | India | KU850545 | KU850693 | KU850835 |
| Stemphylium gracilariae P. herbarum f. lactucum<sup>T</sup> | CBS 308.36 | Solanum lycopersicum | E.G.S. 53.123 | Capsicum annuum | China | KU850546 | KU850694 | KU850836 |
| Stemphylium halophilum comb. nov. | CBS 273.35 | Lactuca sp. | ATCC 10737 | Unknown | USA | KU850547 | KU850695 | KU850837 |
| Stemphylium halophilum comb. nov. | CBS 482.90<sup>T</sup> | Gracilaria sp. | E.G.S. 37.073; ATCC 669721 | Israel | KU850549 | KU850549 | AF443883 | KU850839 |
| Stemphylium halophilum comb. nov. | CBS 115179 | Leucospermum sp. | STE-U 5216; CPC 5216 | Spain | KU850550 | KU850697 | KU850840 |
| Stemphylium halophilum comb. nov. | CBS 115180 | Leucospermum sp. | STE-U 5217; CPC 5217 | Spain | KU850551 | KU850698 | KU850841 |
| Stemphylium halophilum comb. nov. | CBS 125060 | Cucumis melo | HSAUPpyf2377 | China | KU850552 | KU850699 | KU850842 |
| Stemphylium halophilum comb. nov. | CBS 337.73<sup>T</sup> | Limonium vulgare | E.G.S. 37.073; ATCC 669721 | UK | KU850553 | KU850700 | KU850843 |
| Stemphylium halophilum comb. nov. | CBS 410.73 | Armeria maritima | E.G.S. 37.073; ATCC 669721 | UK | KU850554 | KU850701 | KU850844 |
| Species                                      | Strain Code | Host                          | Country       | Accession Numbers |
|----------------------------------------------|-------------|-------------------------------|---------------|-------------------|
| Stemphylium ixeridis                         | CBS 124748<sup>T</sup> | Ixeris denticulata            | China         | KU850590, KU850737, KU850881 |
| Stemphylium lancipes                         | CBS 101217  | Aquilegia sp.                  | New Zealand   | KU850594, KU850741, KU850885 |
|                                             | CBS 116584  | Aquilegia sp.                  | New Zealand   | KU850595, AF443886, KU850896 |
|                                             | CBS 133341<sup>TT</sup> | Aquilegia canadensis          | USA           | KU850596, KU850742, KU85087 |
| Stemphylium loti                             | CBS 407.54<sup>T</sup> | Lotus corniculatus            | USA           | KU850597, KU850743, KU850888 |
| Stemphylium lycii                            | CBS 115192  | Protocarpa cynaroides         | Portugal      | KU850598, KU850744, KU850899 |
|                                             | CBS 116582  | Pistacia vera                  | USA           | KU850599, KU850745, KU850890 |
|                                             | CBS 124982  | Apium graveolens              | China         | KU850600, KU850746, KU850891 |
|                                             | CBS 125240  | Cucurbita moschata             | China         | KU850601, KU850747, KU850892 |
| Stemphylium lycopersici                      | S. lancipes | PD 72/1118                    | Netherlands   | KU850603, KU850749, KU850894 |
| S. vesicarium                                | CBS 436.76  | Unknown                       | Indonesia     | KU850604, KU850750, KU850895 |
| S. lycopersici                               | CBS 463.78  | Solarum tuberosum             | Senegal       | KU850606, KU850752, KU850897 |
| S. xanthosomatis<sup>T</sup>                 | CBS 116585  | Xanthosoma sagittifolium      | New Caledonia | KU850607, AY317010, KU850898 |
|                                             | CBS 116587  | Solarum lycopersicum          | Dominant Republic | KU850608, KU850753, KU850899 |
| S. sophorae<sup>T</sup>                      | CBS 120325  | Sophora microphylla           | China         | KU850609, KU850754, KU850900 |
| S. oblongum<sup>T</sup>                      | CBS 120326  | Gossypium hirsutum            | China         | KU850610, KU850755, KU850901 |
|                                             | CBS 122639<sup>XT</sup> | Solarum lycopersicum         | China         | KU850611, KU850756, KU850902 |
| S. pyrina<sup>T</sup>                        | CBS 122803  | Pyrus sinkiangensis           | China         | KU850612, KU850757, KU850903 |
|                                             | CBS 123008  | Brassica pekinensis           | China         | KU850613, KU850758, KU850904 |
| S. pruni<sup>T</sup>                         | CBS 124980  | Prunus persica                | China         | KU850614, KU850759, KU850905 |
| S. plantaginis<sup>T</sup>                   | CBS 124981  | Plantago major                | China         | KU850615, KU850760, KU850906 |
|                                             | CBS 124983  | Clinopodium polycephalum     | China         | KU850616, KU850761, KU850907 |
|                                             | CBS 135778  | Salvia officinalis            | New Zealand  | KU850617, AY317026, KU850908 |
| Stemphylium majusculum                       | CBS 717.68<sup>T</sup> | Lathyrus maritimus            | USA           | KU850618, AF443891, KU850909 |
|                                             | CBS 133424  | Lathyrus maritimus            | USA           | KU850619, AF443891, KU850910 |

(continued on next page)
| Name                           | Old name¹ | Strain number² | Other collection number² | Host/Substrate | Country       | GenBank accession numbers |
|-------------------------------|-----------|----------------|--------------------------|----------------|---------------|---------------------------|
| *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 | ATCC 10828     | Trifolium pratense       | USA             |               | KU850621 KU850763 KU850912 |
|                               | CBS 364.49 | ATCC 10828     | Trifolium pratense       | USA             |               | KU850622 KU850764 KU850913 |
|                               | CBS 110049 | E.G.S. 31.011  | Cicer arietinum          | Iran            |               | KU850591 KU850738 KU850882 |
|                               | CBS 116579 | E.G.S. 38.121  | Trifolium pratense       | USA             |               | KU850623 KU850739 KU850883 |
|                               | CBS 116581 | E.G.S. 29.188  | Cicer arietinum          | Iran            |               | KU850592 KU850765 KU850915 |
|                               | 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. | CBS 716.68 | ATCC 18518; IMI 135458; MUCL 11718; QM 8729 | Commelina sp. | USA             |               | KU850632 KU850773 KU850923 |
| *S. globuliferum*              | CBS 116598 | UAMH 10487     | Phragmites sp.           | Canada          |               | KU850633 KU850774 KU850924 |
|                               | CBS 116603 | UAMH 10493     | Lactuca muralis          | Canada          |               | KU850634 KU850775 KU850925 |
|                               | CBS 116604 | UAMH 10949     | Guem macrophyllum        | Canada          |               | KU850635 KU850776 KU850926 |
|                               | CBS 133515 | E.G.S. 30.153  | Solarum lycopersicum     | Canada          |               | KU850636 KU850777 KU850927 |
| *S. globuliferum*              | CBS 133518^T | E.G.S. 30.154  | Fragaria sp.            | Canada          |               | KU850637 KU850778 KU850928 |
|                               | 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     | Solarum lycopersicum     | USA             |               | KU850626 KU850767 KU850917 |
|                               | CBS 116586^T | E.G.S. 41.135  | Solarum 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 | E.G.S. 52.041  | Symphytum uplandicum     | New Zealand     |               | KU850643 KU850784 KU850934 |
|                               | CBS 118796 | E.G.S. 52.041  | Symphytum uplandicum     | New Zealand     |               | KU850644 KU850785 KU850935 |
|                               | CBS 138069 | E.G.S. 52.042  | 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 1227746; IMI 135460; MUCL 11716; MUCL 18569; NRRL 5270; QM 8752 | Triglochin maritima | UK             |               | KU850648 KU850789 KU850939 |
| Species               | Accession Numbers | Location          | Country       | GenBank Accession Numbers |
|----------------------|-------------------|-------------------|---------------|--------------------------|
| Stemphylium vesicarium | CBS 155.24        | Allium sp.        | Unknown       | KU850555, KU850702, KU850845 |
|                      | CBS 157.24        | Abies sp.         | Unknown       | KU850556, KU850703, KU850846 |
| *P. pomorum*         | 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 327.36        | Citrus sp.        | Tunisia       | KU850560, KU850707, KU850850 |
|                      | CBS 156.45        | Dianthus canaryphyl| Netherlands | KU850561, KU850708, KU850851 |
|                      | CBS 322.49        | Lathyrus odoratus| Netherlands | KU850562, KU850709, KU850852 |
|                      | CBS 370.51        | Trigonella foenum-graecum| Netherlands | KU850563, KU850710, KU850853 |
|                      | CBS 386.59        | Linum usitatissimum| Denmark     | KU850564, KU850711, KU850854 |
| S. vesicarium         | CBS 715.68        | Unknown           | Unknown       | KU850558, KU850705, KU850848 |
|                      | CBS 406.76        | Pisum sativum     | Canada        | KU850565, KU850712, KU850855 |
|                      | CBS 205.82        | Allium cepa       | Netherlands   | KU850567, KU850714, KU850857 |
| S. herbarum           | CBS 191.86        | Medicago sativa   | India         | KC584239, AF443884, KU850858 |
|                      | CBS 192.96        | Medicago sativa   | Australia     | KU850568, KU850715, KU850859 |
| S. vesicarium         | CBS 311.92        | Allium cepa       | Netherlands   | KU850569, KU850716, KU850860 |
|                      | CBS 486.92        | Allium cepa       | Netherlands   | KU850570, KU850717, KU850861 |
| P. sedicola           | CBS 109843        | Sedum spectabile  | New Zealand   | KU850571, KU850718, KU850862 |
|                      | CBS 109844        | Solanum lycopersicum | USA         | KU850572, KU850719, KU850863 |
| P. tomatonis          | CBS 115182        | Leucadendron sp.  | South Africa  | KU850573, KU850720, KU850864 |
|                      | CBS 115204        | Leucadendron sp.  | Portugal      | KU850574, KU850721, KU850865 |
| S. maili             | CBS 122840        | Malus sieversii   | China         | KU850575, KU850722, KU850866 |
|                      | CBS 123005        | Fabaceae          | China         | KU850576, KU850723, KU850867 |
| S. alfalfae          | CBS 123803        | Allium sativum    | China         | KU850577, KU850724, KU850868 |
| S. etrumnum          | CBS 124279        | Malus domestica   | Denmark       | KU850578, KU850725, KU850869 |
| S. cremanthodii       | CBS 124747        | Cremanthodium discoideum | China    | KU850579, KU850726, KU850870 |
| S. brassicicola       | CBS 124749        | Brassica pekinensis | China       | KU850580, KU850727, KU850871 |
|                      | CBS 124751        | Pyrus sikhingensis | China         | KU850581, KU850728, KU850872 |
|                      | CBS 124752        | Populus tomentosa | China         | KU850582, KU850729, KU850873 |
| S. tomatonis          | CBS 133474        | Dahlia pinnata    | China         | KU850583, KU850730, KU850874 |
| S. alfalfae          | CBS 133737        | Solanum lycopersicum | USA        | KU850584, KU850731, KU850875 |
|                      | CBS 406.76        | Medicago sativa   | Australia     | KU850585, KU850732, KU850876 |
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* isolate 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 7502 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...
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 phylogenically recognised, this difference splits the CBS isolates in two subgroups, with seven isolates, CBS 378.54, CBS 116599, CBS 124651; Sinkiang Province, Tashkurgan, from Phloeospora vulgaris leaves, Oct. 2006, Y. Wang, CBS 124651; Sinkiang Province, Korla, from Amanthuretus 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).

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 | actA | rpb2 | tef1 | cmdA | gapdh | tub2
|----------------|--------------|------|----------|-----|------|------|------|------|-------|------|
| CBS 116599     | Pleospora sp.| Herbaceous dicot | Canada | 1   | 1    | 1    | 1    | 1    | 1     | np    |
| GV11-196a1-3   | Stemphylium sp. | Beta vulgaris | Netherlands | 1 | 1 | 1 | 1 | 1 | 1 | np |
| CBS 378.54     | P. armeriae | Lycnhs 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. | Phragmites sp. | Canada | 2 | 2 | 2 | 2 | 3 | 4 | np |
| CBS 134496     | S. globuliferum | Malus syvlestris | Australia | 2 | 2 | np | 3 | 4 | 5 | np |

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 (KUB506565) 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 (KUB506565) 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.

**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.

**Stemphylium armeriae** (Corda) Woudemb. & Crous, comb. nov. MycoBank MB820657.
CBS 11691\textsuperscript{*} Stemphylium lucomagnoense  
CBS 133935  
CBS 17.68\textsuperscript{*} Stemphylium majusculum  
CBS 133424  
CBS 136799  
CBS 136943  
CBS 137081  
CBS 137085  
CBS 482.90\textsuperscript{*} Pleospora gracillariæ  
CBS 308.39  
CBS 273.55\textsuperscript{*} Pleospora herbarum f. lactucum  
CBS 138503  
CBS 138502  
CBS 137479  
CBS 137045  
CBS 136908  
CBS 136796  
CBS 136726  
CBS 136570  
CBS 133406  
CBS 116583\textsuperscript{**} Stemphylium astragali  
CBS 115186  
CBS 133472  
CBS 133403  
CBS 133463  
CBS 137460  
CBS 115179  
CBS 133479  

CBS 125060\textsuperscript{*} Stemphylium cucumis  
CBS 338.73 Pleospora armeriae  

CBS 124747\textsuperscript{*} Stemphylium cromanthoidi  
CBS 124751, CBS 124803  
CBS 136743 Stemphylium vesicarium  
CBS 136732 Stemphylium vesicarium  
CBS 133821, CBS 139800  
CBS 156.45, CBS 406.76  
CBS 191.98\textsuperscript{*} Stemphylium herbarum  
CBS 136900, 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 133826 Stemphylium vesicarium  

CBS 136815, CBS 136138  
CBS 133541, CBS 133478  
CBS 136951 Stemphylium vesicarium  
CBS 136887, CBS 136955  
CBS 133834, CBS 125242  
CBS 134977, CBS 133677  
CBS 133914, CBS 133737  
CBS 71.06 Stemphylium vesicarium  
CBS 136802 Stemphylium vesicarium  
CBS 109844\textsuperscript{*} Stemphylium tomatonis  
CBS 133467, CBS 133672  
CBS 133477, CBS 370.51  
CBS 368.59, CBS 136953  

CBS 135741 Stemphylium vesicarium  
GV11-355-a-5, CBS 136935  

CBS 109843\textsuperscript{*} Stemphylium sedicola  
CBS 115182, CBS 135787  
CBS 136588 Stemphylium vesicarium  
CBS 136587 Stemphylium vesicarium  
CBS 133905, CBS 136308  
CBS 133659, CBS 136813  
CBS 138988, CBS 137082  
CBS 133668, CBS 124752  
CBS 138421, CBS 136804  
CBS 138824, CBS 137139  

CBS 137922, CBS 133519  
CBS 322.49, CBS 139010  
CBS 133663, CBS 133893  

CBS 138817 Stemphylium vesicarium  
CBS 122640\textsuperscript{*} Stemphylium malii  

CBS 138069, CBS 133481  

CBS 192.88\textsuperscript{*} Stemphylium alfalfae  
CBS 133474, CBS 137490  
CBS 133172 Stemphylium vesicarium  
CBS 136851 Stemphylium vesicarium  
CBS 133633, CBS 136897  

CBS 124748\textsuperscript{*} Stemphylium brassicicola  
CBS 138938, CBS 136934  
CBS 136813 Stemphylium vesicarium  
CBS 133640 Stemphylium vesicarium  
CBS 133473, CBS 133475  
CBS 137155, CBS 133459  
CBS 136950, CBS 123005  
CBS 134279, CBS 134156  
CBS 136071 Stemphylium vesicarium  
CBS 135759 Stemphylium vesicarium  
CBS 136760 Stemphylium vesicarium  
CBS 136745, CBS 136814  
CBS 137145, CBS 138620  

CBS 154.20\textsuperscript{*} Pleospora pomoorum  
CBS 138625, CBS 136353  
CBS 136724 Stemphylium vesicarium  
CBS 133652, CBS 136586  
CBS 274.31, CBS 307.36  

CBS 136744 Stemphylium vesicarium  
CBS 136736 Stemphylium vesicarium  
CBS 136807, CBS 136733  
CBS 115204, CBS 133676  
CBS 133673, CBS 136812
Fig. 1. (Continued).

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STEMPHYLIUM etumum

STEMPHYLIUM botryosum

STEMPHYLIUM lyopersici

STEMPHYLIUM lancipes

STEMPHYLIUM solani

STEMPHYLIUM symphyli

STEMPHYLIUM callistephi

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**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, 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
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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, MBT375577), 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 an 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 olive-brown, 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, pseudeoclips 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, fimbricate, colourless with abundant black ascomatal initials in the agar, aerial mycelium is scarce, 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, GV12-286-2 = CBS 141025; Drenthe, Eerste Eelde, 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. Myco-Bank 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 solitario, 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 Columbia, Hornby Island, beach of Cape Gumey, 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 sp. 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 chrysanthemicos Woudenb. & Crous, sp. nov. Myco-Bank 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-hyline, (71–)106–186(–282) × (3–)4–5 μm, bearing multiple darkened percurrent rejuvenation sites. Conidiogenous cells swollen at the apex, sub-hyline, (5–) 5.5–7(–7.5) μm wide. Conidia solitario, conidium body brown, verrucose, ellipsoid to cylindrical, (24.5–)26–29(–30.5) × (11–)
A. alternata synonymised species names. Ex-type strains are in 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

**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.

**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

13.5–15.5 (–16.5) μm, LW = 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.

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**Fig. 2.** (Continued).
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...
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 ellipsoidal 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.
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 lucamagnoense** Woudenb. & Crous, sp. nov. MycoBank MB820661. Fig. 7.

Etymology: Named after the place of isolation, Lucamagno, 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
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 outgrowths, 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 28–40 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 outgrowths 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 (Niesl 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 “xanthosoma”, Bull. Soc. Mycol. France 81: 697. 1966. Stemphylium planatinis 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 sphaerai Yong Wang bis & X.G. Zhang, Nova Hedwigia 88: 200. 2009. Stemphylium platycodonitis 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, MBT37506, 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); Sichuan Province, Koria, from Pyrus sinyangensis leaves, 9 Aug. 2006, Y. Wang (culture ex-type of S. pyrina CBS 122203). 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
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 *Plantycodon* 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 *Plantycodon*, 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....
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. novaezelandiae 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 outgrowths, 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, wooly, 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.
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 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 novae-zelandiae 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 116802 = E.G.S. 42.055; South Carolina, Charleston, from Solanum lycopersicum, 1952, C.F. Andrus, CBS 133894 = E.G.S. 42.055.

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: Macrosorium vesicarium (Wallr.) Sacc., Syll. Funqorum 4: 537. 1886.
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, Harvard Pap. Bot. 6: 202. 2001. 
Pleospora 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 discoidium 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 122648); 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. 48.005 = 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 (Camara 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.
**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 *Macrosorium 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. 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 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 *Macrosorium 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) | Transverse septa | Longitudinal septa | Wall ornamentation | UB ratio | Reference                          |
|--------------------------|--------------------------------------------------------------------------------|-------------------|-----------------|-------------------|------------------|----------|------------------------------------|
| *S. alfalfae*            | Oblong, subglobose                                                            | 30–40 × 12–16      | 1–2             | 2–3               | Minutely verrucose | ND       | Simmons (1989)                     |
| *S. brassicicola*        | Oblong, broadly ellipsoid                                                    | 32–45 × 12–19     | 1–4–5           | 1–4               | Conspicuously punctate to punctate | 1–3      | Simmons (1989)                     |
| *S. crematodii*          | Oblong, sometimes inequilateral                                               | 18–31 × 9–19      | 1–3             | 3–5               | Micromaculate     | ND       | Simmons (2001)                     |
| *S. herbarum*            | Oblong, elongated                                                             | 25–42 × 12–22     | 1–2             | 3–6               | Conspicuously and densely verrucose | 1–3      | Simmons (1969)                     |
| *S. momordicae*          | Oblong, broadly ellipsoid                                                    | 35–55 × 18–20     | 2–3             | 4–7               | Smooth or usually punctate | ND       | Simmons (2001)                     |
| *S. sedicola*            | Oblong, sometimes inequilateral                                               | 14–30 × 13–16     | 2–3             | 4–7               | Punctate          | ND       | Wang et al. (2008)                 |
| *S. tomatonis*           | Oblong, broadly ellipsoid or oblong                                           | 18–22 × 13–16     | 2–3             | 4–7               | Conspicuously and densely verrucose | ND       | Simmons (2009)                     |

Note: ND: not determined.

CONCLUSIONS

In the genus *Stemphylium* 28 species can be distinguished based on (parts) of the ITS, *gapdh* and *cmdA* gene regions. From these noted that the RAPD studies were only based on a small number of isolates, (including only two *S. herbarum* isolates and one *S. vesicarum* isolate) and morphologically only small differences have been used to make a distinction among these species although they also share many characters (Camara 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. 2008, 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. kaiserii*”, 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. etumunium*, 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. turiniforme* (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.
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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