A phylogenetic study of some *Septoria* species pathogenic to Asteraceae based on ITS ribosomal DNA sequences

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The phylogenetic relationships of the following 14 *Septoria* species pathogenic to Asteraceae were inferred from sequence analyses of the 5.8 S gene of nuclear rDNA and flanking internal transcribed spacers: *Septoria atropurpurea, S. astericola, S. calendulae, S. erigerontis, S. gerberae, S. helianthi, S. helianthicola, S. lactucae, S. leucanthemi, S. obesa, S. schnabliana, S. senecionis, S. socia,* and *S. taraxaci*. Fifteen additional *Septoria* species originating from other host families, eight of which have a known *Mycosphaerella* teleomorph, were included in the alignment. Also included were selected strains of *Cercospora, Pseudocercospora* (including *Paracercospora*), *Ramularia*, and several other anamorph genera linked to *Mycosphaerella* available from previous studies.

The analyses indicate that the *Septoria* species from Asteraceae are all closely related, and share an evolutionary history within the *Mycosphaerellaceae*, grouping amongst miscellaneous *Mycosphaerella* species, *Cercospora* and *Pseudocercospora* spp. *Septoria socia* is relatively distant from the other *Septoria* on Asteraceae, and the data suggest that it is more closely related to the maple pathogen *S. aceris* (tel. *M. latebrosa*) and poplar pathogen *S. populicola* (tel. *M. populicola*). Differences in the ITS sequences indicate that *S. socia* and *S. leucanthemi*, which have very similar phenotypes and have been considered to be conspecific in the past, are specifically distinct.

The fungi classified in *Septoria* Sacc. are pycnidial coelomycetes with filiform, often multiseptate hyaline conidia (SUTTON 1980, CONSTANTINESCU 1984, FARR 1991, 1992, MUTHUMARY 1999, VERKLEY & PRIEST 2000). Most taxa cause leaf spot diseases, and some inflict considerable damage to economically important crops, e.g., *Septoria apiicola* of celery (SUTTON & WATERSTON 1966, RYAN & KAVANAGH 1971), and *S. tritici* of wheat (WIESE 1987, MCDONALD et al. 1999, CUNFER & UENG 1999). Over 2000 names have been described in *Septoria*. On plants of the family Asteraceae (Compositae) alone, some 300 species have been described in the literature (Verkley & Vanev, unpublished data). Due to the limited number of useful morphological characters, and the paucity of physiological and other data *in vitro*, the taxonomy of these fungi still remains confusing and largely dependant on the host. Controlled inoculation experiments to test host specificity have only been performed for a limited number of species occurring on composite hosts (WADDELL & WEBER 1963, PUNITHALINGAM & WHEELER 1965).

Several *Septoria* spp. are responsible for leaf spot or blight of *Chrysanthemum* cultivars and other ornamental Asteraceae (JØRSTAD 1965, PUNITHALINGAM 1967a, Holliday & PUNITHALINGAM 1970, PUNITHALINGAM & HOLLIDAY 1972, HORST & NELSON 1997). *Septoria chrysanthemella, S. obesa, S. leucanthemi,* and *S. socia* cause considerable damage in *Chrysanthemum* nurseries world-wide (PUNITHALINGAM 1967 b, c, d, e, HORST & NELSON 1997). The disease is not always severe, but infected plants usually lose their commercial value. The sexual states of these pathogens are unknown, and the phylogenetic relationships still need clarification. Teleomorphs are known for only a relatively small number of *Septoria* species, and in all cases they are species of *Mycosphaerella* Johanson (Dothideales, Ascomycota), a genus with numerous plant pathogenic taxa and over 20 reported anamorph genera (VON ARX 1983, SUTTON & HENNEBERT 1994, CROUS *et al.* 2000, CROUS, KANG & BRAUN 2001). Based on sequence analyses of the ITS and D2-LSU regions of the nuclear ribosomal DNA, VERKLEY et al. (2004) found that several presumably asexual *Septoria* species from various host families group amongst *Mycosphaerella* spp., and that *Septoria* is not monophyletic within *Mycosphaerella*.

The aim of the present study was to infer a molecular phylogeny of 14 *Septoria* species pathogenic to Asteraceae by analysing 5.8S ribosomal RNA gene and flanking internal transcribed spacer (ITS1 and ITS2) sequences, and to test our hypothesis that they are closely related to other *Septoria* and belong within the main *Mycosphaerella* clade. In the analyses, we also included 15 additional *Septoria* species originating from other host families. Eight of these species have a known *Mycosphaerella* teleomorph. Also included were strains of *Cercospora* Fresen., *Pseudocercospora* Speg. (syn. *Paracer-*

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cospora Deighton), *Ramularia* Unger, and several other anamorph genera that have been linked to *Mycosphaerella*.

Materials and methods

The 17 strains of *Septoria* spp. from Asteraceae used in this study are listed in Tab. 1. These included strains from the CBS culture collection and material newly collected by the first author in Europe. The morphology of these strains *in vitro* was studied as described by VERKLEY (1998).

DNA extraction and sequencing

Strains were transferred from agar cultures to 2 mL liquid medium (2 % malt extract) and incubated on a rotary shaker (300 rpm) for 3 wk at room temperature. After transer to 2-mL tubes, liquid cultures were centrifuged and washed twice with sterile water. DNA was extracted from mycelia with the FastDNAkit (Omnilabo 6050073, BIO 101, CA) according to the manufacturer's instructions. Part of the ribosomal RNA gene cluster was amplified by PCR with primers V9G (DE HOOG & GERRITS VAN DEN ENDE 1998) and LR5 (VILGALYS & HESTER 1990). PCR was performed in 50 BL reaction volumes, each reaction containing 10-100 ng of genomic DNA, 25 pM of each primer, 40 µM dNTP, 1.0 unit Supertaq DNA polymerase and 5 µL 10× PCR buffer (SphaeroQ, Leiden, the Netherlands). PCR was performed in an Applied Biosystems (Foster City, CA) thermocycler with the following program: 1 min 95 ºC, 30× {1 min 95 ºC, 1 min 55 ºC, 2 min 72 ºC} and followed by a final extension of 5 min at 72 ºC. PCR products were cleaned using GFX columns (Amersham Pharmacia, NJ, 27-9602-01). DNA concentration was estimated on a 2 % agarose gel. ITS1 and ITS4 (WHITE et al. 1990) were used as internal sequencing primers. Sequencing was performed with the BigDye terminator chemistry (Part number 403049, Applied Biosystems) following the manufacturer's instructions. The sequencing products were cleaned with G50 Superfine Sephadex columns (Amersham Pharmacia 17-0041-01), and separated and analyzed in ABI Prism 3700 DNA Analyzer (Applied Biosystems). Forward and reverse sequences were matched using SeqMan (DNAstar Inc., WI).

Phylogenetic analyses

Pairwise and global alignment of consensus sequences were performed in Bionumerics 3.0 (Applied Maths, Kortrijk, Belgium). The alignment was manually adjusted where necessary. Parsimony analysis was performed in PAUP v. 4.0b10 (SWOFFORD 2003). The heuristic search was performed with the following parameters: characters unordered with equal weight, random taxon addition, branch swapping using tree bisection-reconnection (TBR) algorithm, with branches collapsing if the maximum branch length was zero, and maxtrees set at 10000. Alignment gaps were treated as missing characters. Parsimony bootstrap analyses were performed using the full heuristic search option, random stepwise addition, and 1000 replicates, with maxtrees set at 100.

Neighbor joining analysis was also performed in PAUP, without pairwise corrections. Stability of clusters was tested with 1000 neighbor joining bootstrap replications. BLAST searches in GenBank revealed highest similarity to species of *Mycosphaerella* and anamorph taxa known to have links with this genus. GenBank accession numbers, taxon names and other information about the sequences from GenBank used in this study are given Tab. 2. GenBank accession numbers of new ITS sequences are given in Tab. 1. A strain of *Davidiella tassiana* was defined as outgroup, as it was shown by BRAUN et al. (2003) to be a close sister group of a well-supported main *Mycosphaerella* clade.

Results

The alignment of the ITS region comprised 514 characters, of which 153 were parsimony-informative. Nineteen of the informative characters which were positioned within small insertions/deletions or regions with ambiguous position homology, were excluded from the analyses (indel comprised 54 characters). In the parsimony analysis, all autapomorphic and constant characters were excluded, while in the neighbor joining analyses only the constant characters were excluded to maintain accurate branch lengths in the phylogram.

The heuristic search involving 5000 random input orders resulted in 2724 MPT's of 474 steps (consistency index 0.508, retention index 0.849, rescaled consistency index 0.432, homoplasy index 0.492). The majority rule consensus tree is shown in Fig. 1. Branch frequencies are given above each branch (those with 100 % appear also in the strict consensus tree), bootstrap support over 50% are indicated below the branches. The neighbor joining tree with the obtained bootstrap support values over 50 % is given in Fig. 2.

The *Septoria* spp. from Asteraceae all fell within a major clade (parsimony bootstrap percentage 87/ neighbor joining 98), comprising miscellaneous *Mycosphaerella* species, *Cercospora* and *Pseudocercospora* spp., and all other included *Septoria* except the cereal pathogens *S. tritici* (tel. *M. graminicola*) and *S. passerinii*. Within this clade, high bootstrap support was only found for a clade with *Cercospora* spp. (93/ 100), and a few single-taxon clades (*S. castaneicola*, *M. populicola*, *M. latebrosa*, *M. pyri*, *M. brassicicola*, *M. musae*, *M. fijiensis*). Also well-supported were the clade of *M. graminicola* and *S. passerinii* (100/100), and a clade comprising all taxa with *Ramularia* anamorphs, including *M. punctiformis*, the type species of the genus *Mycosphaerella* (100/100).

The strains of *S. erigerontis* (CBS 109094) and *S. schnabliana* (CBS 186.93) from *Erigeron annuus* had identical ITS sequences and are probably conspecific. The sequence of *S. helianthicola* (CBS 122.81) differs by only a single base position from that of *S. scabiosicola* strains isolated from *Succissa pratensis* and *Knautia arvensis* (Dipsacaceae). Other

Tab. 1: GenBank accession numbers of ITS sequences and other data of the strains of *Septoria* spp. pathogenic to Asteraceae used in this study.

GenBank	Taxon	Host	Origin
AY489274	*Septoria astericola* Ellis & Everh.	*Aster canus*	CBS 347.58; Germany
AY489275	*S. atropurpurea* Peck	*A. canus*	CBS 348.58, Germany
AY152566	*S. calendulae* Bernaux	*Calendula arvensis*	CBS 349.58; Italy
AY489273	*S. erigerontis* Peck	*Erigeron annuus*	CBS 109094; Austria
AY152569	*S. gerberae* Syd. & P. Syd.	*Gerbera jamesonii*	CBS 410.61; Italy
AY489281	*S. helianthi* Ellis & Kellerm.	*Helianthus annuus*	CBS 123.81; Yugoslavia
AY489270	*S. helianthicola* Cooke & Harkn.	*H. annuus*	CBS 122.81; Yugoslavia
AY489282	*S. lactucae* Pass.	*Lactuca sativa*	CBS 352.58; Germany
AY489278	*S. leucanthemi* Sacc. & Speg.	*Chrysanthemum maximum*	CBS 353.58; Germany
AY489277	*S. leucanthemi* Sacc. & Speg.	*C. leucanthemum*	CBS 109090; Austria
AY489285	*S. obesa* Syd.	*C. indicum*	CBS 354.58; Germany
AY489276	*S. schnabliana* (Allescher) Died.	*Erigeron annuus*	CBS 186.93; country unknown
AY489272	*S. senecionis* Westend.	*Senecio fluviatilis*	CBS 102366; Netherlands
AY489279	*S. senecionis* Westend.	*S. fluviatilis*	CBS 102381; Netherlands
AY489271	*S. senecionis* Westend.	*S. nemorensis subsp. fuchsii*	CBS 109087; Austria
AY489284	*S. socia* Pass.	*Chrysanthemum indicum*	CBS 357.58; Germany
AY489280	*S. taraxaci* Hollós	*Taraxacum sp.*	CBS 567.75; Armenia

strains having identical sequences are those of *S. astericola* (CBS 347.58) and *S. atropurpurea* (CBS 348.58), and these differ only by a single position from the strain of *S. calendulae* (CBS 349.58). *Septoria socia* appears relatively distant from most other *Septoria* from Asteraceae, as it differs, for example, in 26 positions from *S. erigerontis*, and 16 positions from *S. obesa*. It clustered with several *Mycosphaerella* spp. with *Septoria* anamorphs, viz. *M. latebrosa* (anam. *S. aceris*) and *M. populicola* (*S. populicola*), albeit with low bootstrap support.

Discussion

The results of the ITS sequence analyses indicate that the *Septoria* species from Asteraceae are all closely related, and share an evolutionary history within the *Mycosphaerellaceae*. *Septoria socia* is relatively distant from the other *Septoria* on Asteraceae, and the ITS sequence data suggest that it is more closely related to the maple pathogen *S. aceris* (tel. *M. latebrosa*) and poplar pathogen *S. populicola* (tel. *M. populicola*) of the section *Longispora* M. E. Barr of the genus *Mycosphaerella*. KUIJPER & APTROOT (2002), who revised this section, placed *M. populicola* under 'probable synonyms' of *M. latebrosa*, based on microscopic examination of the type specimens. However, the morphology of the *Septoria* anamorphs and ITS sequences show that they are separate species. No teleomorphs are currently known for the *Septoria* species occurring on Asteraceae. If teleomorphs exist that can be connected with these species, they are predicted to have the *Mycosphaerella* phenotype.

JØRSTAD (1965) considered *Septoria obesa* and *S. leucanthemi* to be conspecific, as both have similar conidial morphologies and occur on several *Chrysanthemum* spp. Jørstad studied fresh material and herbarium specimens and observed that morphological variation in *Septoria* spp. was in part host-determined. PUNITHALINGAM (1967 c, d), however, considered *Septoria obesa* and *S. leucanthemi* to be distinct species, noting that the conidia of *S. obesa* are consistently wider than those of *S. leucanthemi*. Differences in the ITS sequences (eight positions) also indicate that they are distinct species. This is further supported by differences in host ranges: *S. obesa* is restricted to various species of *Chrysanthemum*, while *S. leucanthemi* is also capable of infecting *Tagetes*, *Achillea*, *Centaurea* and *Helianthus* (WADDELL & WEBER 1963, PUNITHALINGAM 1967 c, d). *Septoria obesa* also shows morphological similarities with the sunflower pathogen, *S. helianthi*, since the conidia are morphologically similar (*S. helianthi* 50–85 × 2–3 µm; *S. obesa* 50–90 × 2.5–3.5 µm). However, conidia in *S. obesa* form 5–11 septa, while those of *S. helianthi* seldomly form more than five. The ITS sequences differ by more than 20 base positions, which indicates that *S. obesa* and *S. helianthi* are relatively distant.

At present it is difficult to determine the significance of the ITS variation observed within *Septoria* morphospecies. For example, *M. populicola* and *M. fragariae* show infraspecific variation in ITS, whereas no infraspecific variation has been recorded so far in *M. brassicicola*, *M. latebrosa*, and *S. apiicola*. The ITS sequences of two strains of *S. leucanthemi* differed by a single base, which could be correlated with their different origins, one being from *C. maximum* in Germany,

Tab. 2: GenBank ITS sequences of fungal isolates included in analyses, in alphabetical order of the anamorph name.

| GenBank	Anamorph	Teleomorph	Origin
AY266168	*Cercospora apii* Fresen.	*Mycosphaerella* state unknown	CA1, ATCC 12246
AY152576	*C. beticola* Sacc.	*Mycosphaerella* state unknown	CBS 539.71; *Beta vulgaris*, Rumania
AY266165	*C. beticola* Sacc.	*Mycosphaerella* state unknown	CB4
AY152577	*C. kikuchii* (Matsumoto & Tomoyasu) Gardner	*Mycosphaerella* state unknown	CBS 128.27 (ex type); *Glycine max*, Japan
AY166260	*C. kikuchii* (Matsumoto & Tomoyasu) Gardner	*Mycosphaerella* state unknown	CK 39; *Glycine max*, Illinois, U.S.A.
AY266161	*C. kikuchii* (Matsumoto & Tomoyasu) Gardner	*Mycosphaerella* state unknown	CK35; *Glycine max*, Illinois, U.S.A.
AY152598	*Cercosporidium magnoliae* (J.B. Ellis & Harkn.) Sivan.	*Mycosphaerella milleri* Hodges & Haasis	CBS 541.63; *Magnolia grandiflora*, North Carolina, U.S.A.
AY152552	*Cladosporium herbarum* (Pers. : Fr.) Link	*Davidiella tassiana* (De Not.) Crous & U. Braun	CBS 289.49; *Allium schoenoprasum*, Switzerland
AY152575	*Phloeospora ulmi* (Fr. : Fr.) Wallr.	*Mycosphaerella ulmi* Kleb.	CBS 344.97; *Ulmus glabra*, Austria
AY266152	*Pseudocercospora fijiensis* (M. Morelet) Deighton	*M. fijiensis* M. Morelet	ATCC 22116, PF7 ; Philippines
AY266150	*Ps. fijiensis* (M. Morelet) Deighton**	*M. fijiensis* M. Morelet	ATCC 36054, PFD9 ; Honduras
AY266153	*Ps. cruenta* (Sacc.) Deighton	*M. creunta* Latham	PCR18, ATCC 26271, *Vigna unguiculata*, Puerto Rico
AY266149	*Ps. musae* (Zimm.) Deighton	*M. musicola* J. L. Mulder	PM 10, ATCC 22115, IMI 139520; *Musa sp*., Philippines
AY266148	*Ps. musae* (Zimm.) Deighton	*M. musicola* J. L. Mulder	PM11, ATCC 36143
AY152590	*Pseudocercospora sp*.	*Mycosphaerella laricina* R. Hartig	CBS 326.52; *Larix decidua*, Switzerland
AY152595	*Ramularia grevilleana* (Tul. & C. Tul.) Jørst.	*M. fragariae* (Tul.) Lind.	CBS 259.36; *Fragaria sp*., Netherlands
AY152597	*R. grevilleana* (Tul. & C. Tul.) Jørst.	*M. fragariae* (Tul.) Lind.	CBS 719.84; *Fragaria sp*., Netherlands
AY152596	*R. grevilleana* (Tul. & C. Tul.) Jørst.	*M. fragariae* (Tul.) Lind.	CBS 298.34; *Fragaria sp*., Netherlands
AY490762*	*Ramularia sp*.	*M. punctiformis* (Pers. : Fr.) Starb.	CBS 113871; *Quercus robur*, Netherlands
AY490763*	*Ramularia sp*.	*M. punctiformis* (Pers. : Fr.) Starb.	CBS 113265 (ex epitype); *Quercus robur*, Netherlands
AY490764*	*Ramularia sp*.	*M. punctiformis* (Pers. : Fr.) Starb.	CBS 113868; leaf endophyte *Quercus robur*, Netherlands
AY490765*	*Ramularia sp*.	*M. punctiformis* (Pers. : Fr.) Starb.	CBS 113869; leaf endophyte *Quercus robur*, Netherlands
AY152593	*Ramularia sp*.	*Mycosphaerella sp*.	CBS 943.97; *Quercus sp*., Netherlands
AY152594	*Ramularia sp*.	*Mycosphaerella sp*.	CBS 184.97; *Acer pseudoplatanus*, Netherlands
AY490768*	*Septoria aceris* (Lib.) Berk. & Br.	*Mycosphaerella latebrosa* (Cooke) Schröt.	CBS 183.97; *Acer pseudoplatanus*, Netherlands
AY490769*	*S. aceris* (Lib.) Berk. & Br.	*M. latebrosa* (Cooke) Schröt.	CBS 652.85; *Acer pseudoplatanus*, Netherlands
AY152553	*S. aceris* (Lib.) Berk. & Br.	*M. latebrosa* (Cooke) Schröt.	CBS 687.94; *Acer pseudoplatanus*, Netherlands
AY152571	*S. aciculosa* Ellis & Everh.	*Mycosphaerella* state unknown	CBS 177.77; *Fragaria sp*., New Zealand
AY152572	*S. apiicola* Speg.	*Mycosphaerella* state unknown	CBS 395.52, IMI 092627; *Apium sp*., Netherlands
AY152573	*S. apiicola* Speg.	*Mycosphaerella* state unknown	CBS 389.59; *Apium graveolens*, Italy
AY152574	*S. apiicola* Speg.	*Mycosphaerella* state unknown	CBS 400.54, IMI 092628; *Apium graveolens*, Netherlands
AY152579	*S. berberidis* Niessl	*Mycosphaerella berberidis* (Auerswald) Lind.	CBS 324.52; *Berberis vulgaris*, Switzerland
AY152566	*S. calendulae* Bernaux	*Mycosphaerella* state unknown	CBS 349.58; *Calendula arvensis*, Italy
AY152588	*S. castaneicola* Desm.	*Mycosphaerella* state unknown	CBS 102377; *Castanea sativa*, Netherlands
AY152589	*S. castaneicola* Desm.	*Mycosphaerella* state unknown	CBS 102323; *Castanea sativa*, Netherlands
AY152563	*S. lamiicola* Sacc.	*Mycosphaerella* state unknown	CBS 109113; *Lamium album*, Austria
AY152564	*S. lamiicola* Sacc.	*Mycosphaerella* state unknown	CBS 102328; *Lamium album*, Netherlands

Tab. 2: Continued

GenBank	Anamorph	Teleomorph	Origin
AY152570	S. linicola (Speg.) Garovaglio	Mycosphaerella linicola Naumov	CBS 316.37; Linum usitatissimum, Argentina
AF181697	S. passerinii	Mycosphaerella state unknown	UM011, ATCC 26516; Hordeum vulgare, Minnesota, U.S.A.
AF181699	S. passerinii	Mycosphaerella state unknown	P78; Hordeum vulgare, Minnesota, U.S.A.
AY152583	S. populicola Peck	M. populicola G. Thompson	CBS 100045; Populus trichocarpa, Washington, U.S.A.
AY152584	S. populicola Peck	M. populicola G. Thompson	CBS 100052; Populus trichocarpa, Washington, U.S.A.
AY152585	S. populicola Peck	M. populicola G. Thompson	CBS 100044; Populus trichocarpa, Washington, U.S.A.
AY152586	S. populicola Peck	M. populicola G. Thompson	CBS 100051; Populus trichocarpa, Washington, U.S.A.
AY152587	S. populicola Peck	M. populicola G. Thompson	CBS 100047; Populus trichocarpa, Washington, U.S.A.
AY152591	S. pyricola (Desm.) Desm.	M. pyri (Auerswald) Boerema	CBS 222.31; Pyrus communis
AY152592	S. pyricola (Desm.) Desm.	M. pyri (Auerswald) Boerema	CBS 640.72; Pyrus communis, Netherlands
AY152581	S. ribis (Lib.) Desm.	M. grossulariae (Fr.) Lind.	CBS 235.37; Ribes nigrum, Netherlands
AY152565	S. rubi West.***	M. rubi Roark	CBS 238.37; Rubus strigosus, Illinois, U.S.A.
AY152578	S. rubi West.***	M. rubi Roark	CBS 102327; Rubus fruticosus s.l., Netherlands
AY152580	S. rubi West.***	M. rubi Roark	CBS 109017; Rubus idaeus, Austria
AY152558	S. scabiosicola Desm.	Mycosphaerella state unknown	CBS 108981; Knautia arvensis, Austria
AY152559	S. scabiosicola Desm.	Mycosphaerella state unknown	CBS 102336; Knautia arvensis, Netherlands
AY152560	S. scabiosicola Desm.	Mycosphaerella state unknown	CBS 317.37
AY152561	S. scabiosicola Desm.	Mycosphaerella state unknown	CBS 182.93; Succissa pratensis, France
AY152562	S. scabiosicola Desm.	Mycosphaerella state unknown	CBS 102335; Knautia arvensis, Netherlands
AY152567	S. sii Rob. & Desm.	Mycosphaerella state unknown	CBS 102369; Berula erecta, Netherlands
AY152568	S. sii Rob. & Desm.	Mycosphaerella state unknown	CBS 118.96; Berula erecta, Netherlands
AY152601	S. tritici Rob.	Mycosphaerella graminicola (Fuckel) Schröt.	CBS 100330 (IPO 6566.1); Triticum aestivum
AY152602	S. tritici Rob.	M. graminicola (Fuckel) Schröt.	CBS 100335; Triticum aestivum, Netherlands
AY152603	S. tritici Rob.	M. graminicola (Fuckel) Schröt.	CBS 392.59; Triticum aestivum
AF181692	S. tritici Rob.	M. graminicola (Fuckel) Schröt.	IPO 323; Triticum aestivum, Netherlands
AF181693	S. tritici Rob.	M. graminicola (Fuckel) Schröt.	T1; Triticum aestivum, Minnesota, U.S.A.
AY152599	Stenella parkii Crous & Alfenas	Mycosphaerella parkii Crous et al.	CBS 387.92 (STE-U 353 ; ex type); Eucalyptus grandis, Brazil
AY489283*	Unknown	M. harthensis (Auersw.) Migula	CBS 325.52; Betula sp., Switzerland
AY152600	Unknown	M. marksii Carnegie & Keane	CBS 682.95 (STE-U 842); Eucalyptus grandis, South Africa
AY152554	Unknown ****	M. brassicicola (Fr.) Lind.	CBS 267.53; Brassica oleracea, Netherlands
AY152555	Unknown ****	M. brassicicola (Fr.) Lind.	CBS 228.32; Brassica oleracea, Denmark
AY152556	Unknown ****	M. brassicicola (Fr.) Lind.	CBS 174.88; Brassica oleracea, Germany
AY152557	Unknown ****	M. brassicicola (Fr.) Lind.	CBS 173.88; Brassica oleracea, Germany

*   = new sequence

**   = previously as Ps. Fijiensis var. difformis, but no longer recognized.

***   = unconfirmed

** ** = Asteromella brassicae (F. Chevallier) Boerema & van Kesteren spermatial state
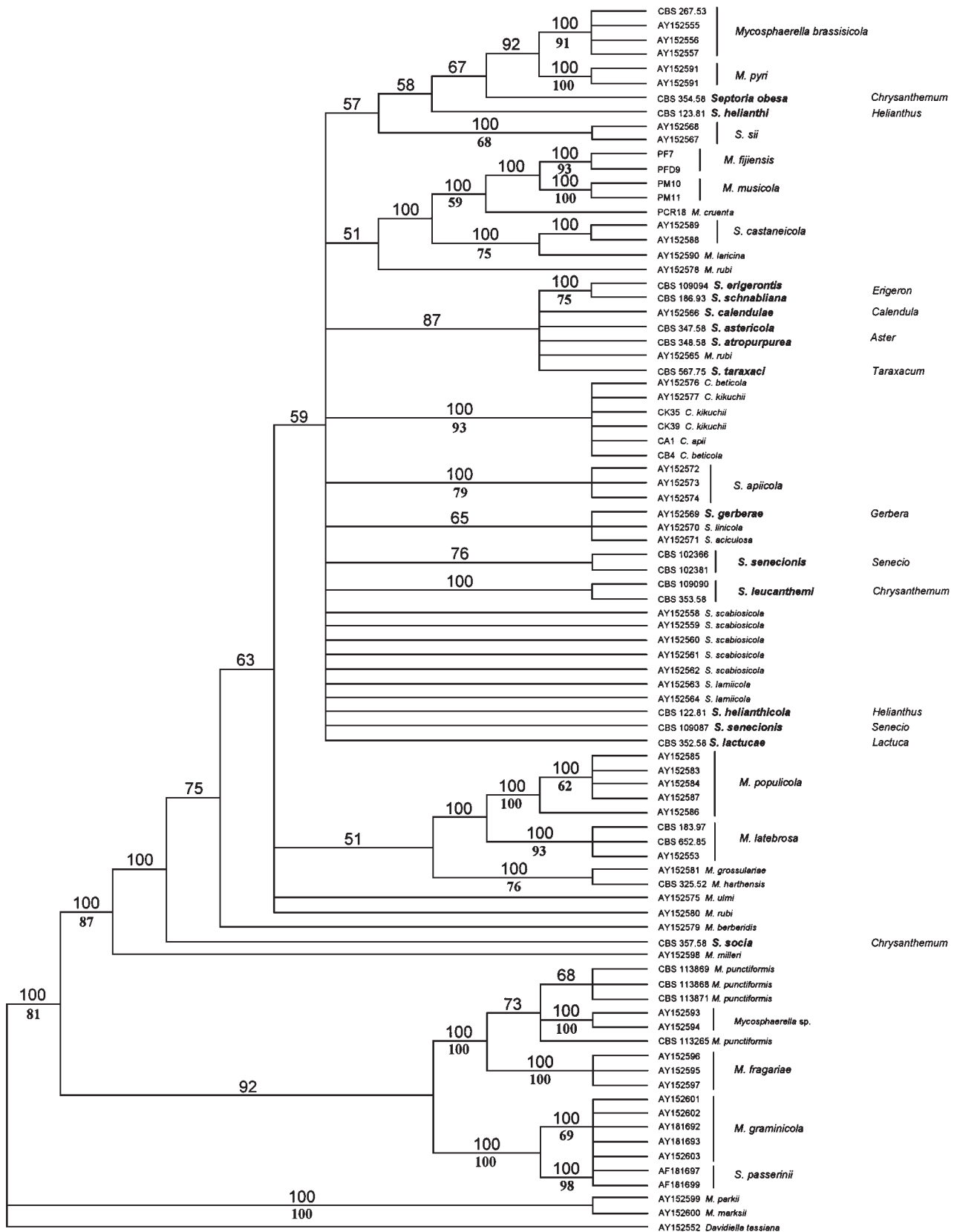
Fig. 1: Majority consensus tree of 2724 MPT's of 474 steps (CI = 0.508, RI = 0.849, RCI = 0.432, HI = 0.492), obtained in PAUP using a heuristic search of the ITS region of nuclear rDNA, using 153 parsimony-informative characters. Numbers above the branches are branch frequencies. Numbers below the branches are bootstrap values obtained from 1000 replications and rounded to the nearest integer, shown only for branches supported by more than 50 %. Names of taxa from Asteraceae are shown in bold, and the host genus is also indicated. Species are presented by teleomorph name, if known (anamorph names are given in Tab. 2). *Davidiella tassiana* was used as outgroup taxon.

— 0.01 substitutions/site
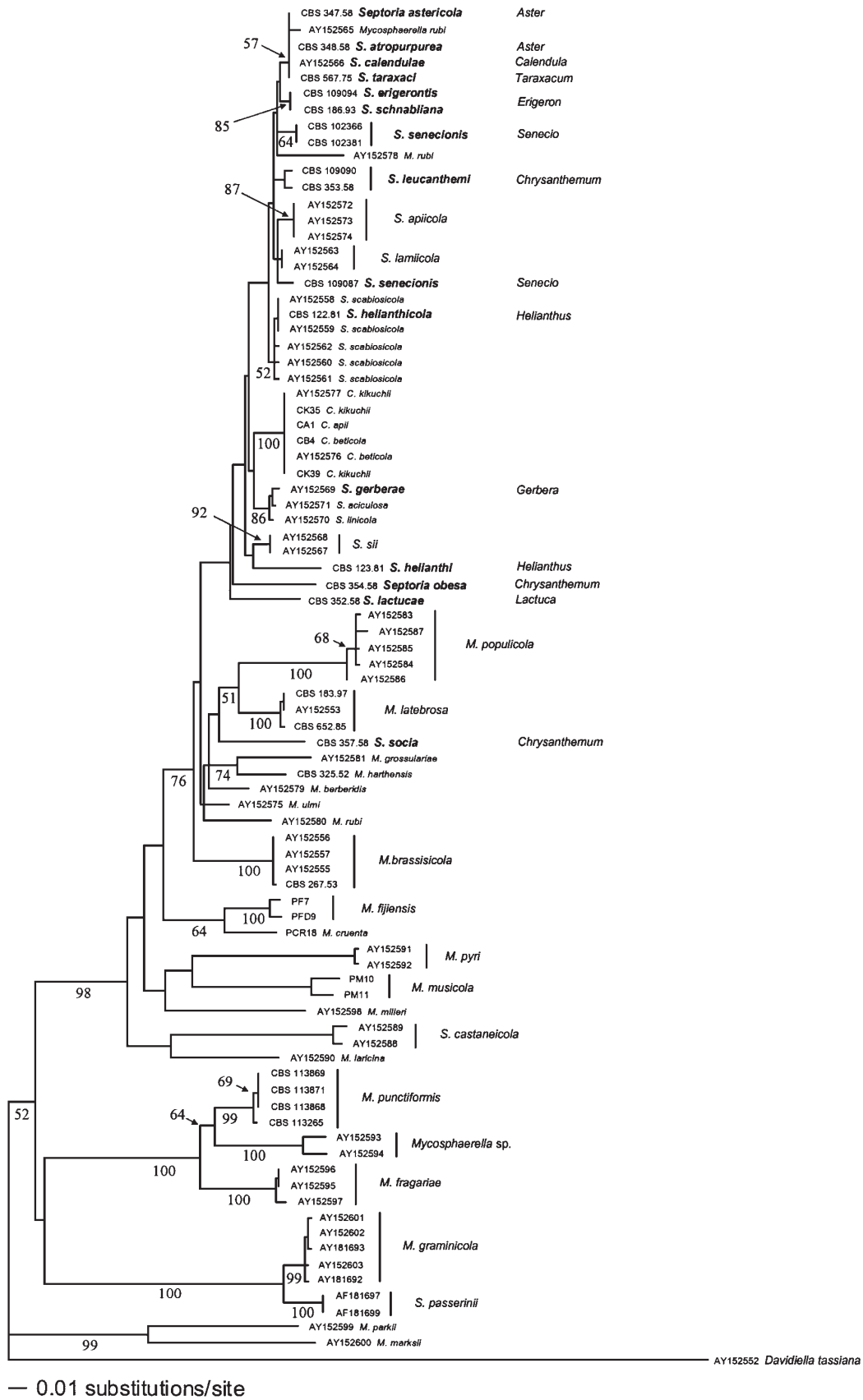
Fig. 2: Neighbor joining tree derived from 197 parsimony-informative and autapomorphic characters of the ITS region of nuclear rDNA, calculated in PAUP without pairwise corrections. Numbers below branches are bootstrap values obtained from 1000 replications and rounded to the nearest integer, shown only for branches supported by more than 50 %. Length of branches is proportional to number of changes. Species are presented by teleomorph name, if known (anamorph names are given in Tab. 2). Sequence of *Davidiella tassiana* was used as outgroup to root the tree.

and the other from wild montane *C. leucanthemum* in an alpine meadow in Austria at 1400 m elevation. Two strains of *S. senecionis*, both of which were isolated from *Senecio fluviatilis* in the same river-bank forest in the Netherlands, showed difference in two base positions. A third *S. senecionis* strain, originating from *S. nemorensis* subsp. *fuchsii* in a montane forest in Austria, differed even by five positions from the other two. In contrast, the anamorphs were all morphologically indistinguishable and the Austrian isolate only differed slightly from the Dutch isolates in colony pigmentation.

Septoria helianthicola CBS 122.81 and *S. scabiosicola* had almost identical ITS sequences (one C insertion in *S. helianthicola*), indicating that this region sometimes may not even be sufficiently variable to distinguish species. The strains of *S. astericola*, *S. calendulae*, *S. taraxaci*, *S. atropurpurea*, *S. erigerontis*, which have highly similar ITS sequences, and also *S. gerberae*, *S. senecionis* and *S. leucanthemi*, were found to be very closely related to *S. helianthicola*. We also ran analyses which included partial sequences of *S. chrysanthemella* (CBS 483.63 and 354.73). These analyses indicated that *S. chrysanthemella* also grouped among these *Septoria* species. Because ITS1 and ITS 2 sequences of the *S. chrysanthemella* strains were incomplete, they were excluded from the analyses presented here. These *Septoria* species are very difficult to discriminate morphologically *in planta*, as they all have relatively narrow and pointed conidia with largely overlapping length ranges. In culture they typically grow relatively slowly and produce brightly red or yellowish diffusable pigments. In practice it is impossible to name such *Septoria* spp. when the host remains unidentified.

Species with *Septoria* anamorphs are scattered amongst taxa with hyphomycetous anamorphs. The only exception is the clade of *S. passerinii* and *S. tritici*, which obtained maximum bootstrap support in parsimony as well as neigbor joining analysis, as was also the case in earlier work (VERKLEY et al. 2004). *Septoria* is not monophyletic within *Mycosphaerella*, and conidiomatal structure seems to have little predictive value for phylogenetic relatedness. In the neigbor joining tree, *S. lactucae* and *S. obesa* occupied a basal position in a major clade comprising most other *Septoria* spp. on Asteraceae, and other *Septoria* as well as *Cercospora* spp. from other host plant families. This major clade is insufficiently supported, and more taxa and genes need to be analyzed before any firm conlusions can be drawn. It is, nevertheless, interesting to further investigate the possible role of species on Asteraceae in the evolution of *Septoria* and *Cercospora* on various host plant families. In the present study, *Ramularia* and *Cercospora* both formed well-supported clades, but for the latter this is almost certainly due to the limited sampling in this study (GOODWIN, DUNKLE & ZISMANN 2001).

The information presented here can contribute to a better understanding of the evolution of *Septoria* pathogens of Asteraceae. The relationships between *Septoria* on wild plants and pathogens invading nurseries are still poorly understood. It is hoped that the information obtained in this study will ultimately lead to improved control of the disease of *Chrysanthemum* and other ornamentals.

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