Septoria epambrosiae sp. nov. on *Ambrosia artemisiifolia* (common ragweed)

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Recently an unidentified *Septoria* was reported to be pathogenic on *Ambrosia artemisiifolia* L. (common ragweed) in Hungary and may have potential as a biocontrol agent for this noxious weed. After reviewing the two previously known species of *Septoria* on *Ambrosia* (Asteraceae), the *Septoria* from Hungary is described as a new species, *Septoria epambrosiae*. A synopsis of the three species of *Septoria* on *Ambrosia* is provided. Based on a molecular analyses of the nSSU and complete ITS region of the rDNA, the taxonomic relationships of *S. epambrosiae* are determined. *Septoria epambrosiae* clusters with members of the Dothideales with the teleomorph most likely a species of *Mycosphaerella*.

Keywords: Ascomycetes, molecular taxonomy, biocontrol, taxonomy.

Ragweed (*Ambrosia artemisiifolia* L., Asteraceae) is a noxious weed common throughout temperate regions that produces quantities of pollen to which many humans have an allergic reaction, often referred to as hay fever. A species of *Septoria* Sacc. in Hungary was discovered and tested as a potential biocontrol agent of ragweed (Bohár & Schwarczinger, 1999). Ragweed plants grown in the greenhouse were inoculated with a conidial suspension of this fungus. After 3-4 weeks the infected leaves became completely necrotic and occasionally the entire plants died (Bohár & Schwarczinger, 1999). The possibility of developing this fungus as a biocontrol agent necessitated an accurate identification.

The coelomycetous genus *Septoria* includes about 1,000 described species for which no monographic account exists (Rossman & al., 1987), thus identification is difficult. Host-specificity in *Septoria* has been assumed and the few recent studies suggest that this assumption is justified, at least, at the level of host genus (Farr, 1991, 1992; Goodwin & Zismann, 1999). Two species of *Septoria* have been described from *Ambrosia*, differing primarily in conidial characteristics; none of these previously known species, however, agree with the potential biocontrol fungus of ragweed. The fungus studied by Bohár & Schwarczinger (1999) and three

specimens misidentified as *Septoria bacilligera* Winter on *Ambrosia* in BPI are described herein as a new species of *Septoria*. The taxonomic relationships and the potential teleomorph of this new asexual species were determined by an analysis of sequences of the nSSU and complete ITS region of the rDNA.

Materials and methods

For microscopic examination material was rehydrated and mounted in 3% KOH. Conidiomata were sectioned at about 10 µm thick using a freezing microtome. Sections were mounted in lactic acid with cotton blue. Observations of microscopic features were made using a Zeiss Axioplan 2 microscope with both bright-field and fluorescence illumination. Calcofluor was used as the fluorescent dye. Measurements were taken using a digital camera and ImagePro software (Media Cybernetics, Silver Spring, MD). Scatterplots of conidial dimensions were constructed using Systat 7.0 for Windows. The Gaussian bivariate ellipses are graphed for the samples in each plot. The resulting ellipse is centered on the sample means of the x and y variables. The unbiased sample standard deviations of x and y determine its major axes and its orientation is determined by the sample covariance between x and y. A probability of 0.6827 was used to determine the size of the ellipses.

Cultures were isolated from nature on Difco Potato Dextrose Agar (PDA). To produce and observe pycnidia in culture the fungus was grown on autoclaved 20–40 × 1–2 mm stems of *Medicago* (alfalfa) placed on distilled water agar in petri dishes. All isolates were maintained on Difco Corn Meal Agar slants with an alfalfa stick at 4 C and in water cultures (Burdsall & Dorworth, 1994). For the growth studies, cultures were grown on Difco Czapek Solution Agar (CZ), Difco Corn Meal Agar (CM) and PDA at 25 C in the dark. Color names and numbers were determined using Kornerup & Wanscher (1978).

DNA was extracted from lyophilized mycelium using the Qiagen Plant DNeasy kit (Qiagen Inc., Chatsworth, CA). The internal transcribed spacer (ITS) regions 1 and 2, including the 5.8S rDNA, were amplified in 50 µL reactions on a GeneAmp 9700 thermal cycler (Perkin Elmer/ABI, Foster City, CA) under the standard reaction conditions with 15 ng of genomic DNA, 2.5 units Amplitaq Gold™ (PE Biosystems, Foster City, CA), 25 pmoles each of primers ITS5 and ITS4 (White & al., 1990) and the supplied PCR buffer. The thermal cycler program was as follows: 10 min at 95 C followed by 35 cycles of 30 s at 94 C, 30 s at 55 C, 1 min at 72 C, with a final extension period of 10 min at 72 C. The nuclear small subunit ribosomal (nSSU) RNA genes were amplified under the same

conditions, using primers NS1 and NS8 (White & al., 1990). Following amplification, the PCR products were purified with QIAquick (Qiagen Inc., Chatsworth, CA) columns according to the manufacturer's instructions. Purified PCR products were sequenced on an ABI 310 automated DNA sequencer using the BigDye Terminator kit (PE Biosystems) according to the manufacturer's instructions. Sequencing primers were as follows: ITS5 and ITS4 for the ITS region and NS1, NS3, NS4, NS5, NS6, and NS8 for the nSSU genes. The ITS sequence has been deposited at GenBank with the number AF 279582 and the SSU sequence with the number AF 279583.

The resulting sequences were edited, joined, and aligned using the Sequencher software program (Gene Codes Corp, Ann Arbor, MI). Additional sequences of *Septoria, Mycosphaerella*, *Capnodium* Mont., *Communitispora* Ramaley and *Dothidea* Fr. were obtained from GenBank. The sources of these sequences along with the original isolate number followed by the GenBank accession number are: Crous & al. (1999), *M. africana* Crous & M. J. Wingf. (STE-U 794, AF173314), *M. ellipsoidea* Crous & M. J. Wingf. (STE-U 1225, AF173303), *M. ellipsoidea* (STE-U 1224, AF173302), *M. fragariae* (Tul.) Lindau (STE-U 656, AF173312), *M. juvenis* Crous & M. J. Wingf. (STE-U 1005, AF173299), *M. keniensis* Crous & T. Coutinho (STE-U 1084, AF173300), *M. marksii* Carnegie & Keane (STE-U 935, AF173316), *M. molleriana* (Thüm.) Lindau (STE-U 784, AF173313), *M. molleriana* (STE-U 1214, AF173301), *M. parkii* Crous & M. J. Wingf. (STE-U 353, AF173311), *M. suttoniae* Crous & al. *(STE-U 1346, AF173306), M. tasmaniensis* Crous & M. J. Wingf. (STE-U 1457, AF173307); Goodwin & Zismann (Unpublished), *M. citri* Whiteside (Fellsmere, AF181703), *M. citri* (GS8, AF181704), *M. fijiensis* M. Morelet (ATCC 22116, AF181705), *M. graminicola* (Fuckel) Schroet. (IPO323, AF181692), *M. graminicola* (T1, AF181693), *M. graminicola* (T48, AF181694), *M. musicola* J. L. Mulder (ATCC 22115, AF181706), *S. passerinii* Sacc. (P70, AF181700), *S. passerinii* (P78, AF181699), *S. passerinii* (P83, AF181698), *S. passerinii* (ATCC 26516, AF181697), *S. passerinii* (ATCC 26515, AF181696), *S. passerinii* (ATCC 22585, AF181695), *S. passerinii* (P71, AF181701); Hoog & al. (Unpublished), *Capnodium coffeae* Pat. (CBS 147.52, AJ244239) *C. salicinum* Mont. (CBS 131.34, AJ244240), *Comminuntispora agavaciensis* Ramaley (AJ244250, CBS 619.95); Jacobs & Rehner (1998), *D. hippophaeos* (Pass.) Fuckel (CBS 186.58, AF027763), *D. insculpta* Wallr. (CBS 189.58, AF027764); Schenck & Frey (not published), *M. dearnessii* Barr (MDUS1, AF211196), *M. dearnessii* (MD038, AF211195), *M. dearnessii* (MD002, AF211194), *M. pini* Rostr. (MP002, AF211197), *M. tassiana* (De Not.) Johanson (CBS 111.82, AF 238469); Ueng & Subramanian (Unpublished), *M. graminicola* (Unknown, U77363).

Alignments were manually adjusted using MacClade 3.0 (Maddison & Maddison, 1992) and GeneDoc 2.5 (Nicholas & Nicholas, 1997). Maximum parsimony trees were inferred using the heuristic search (simple addition sequence) and branch swapping (tree bisection-reconnection) options of PAUP 4.0b4a (Swofford, 1999). All molecular characters were unordered and given equal weight during analysis. Relative support for the branches was estimated with 100 bootstrap replications (Felsenstein, 1985).

Description

Septoria epambrosiae D. F. Farr, **sp. nov.** – Figs. 1-7.

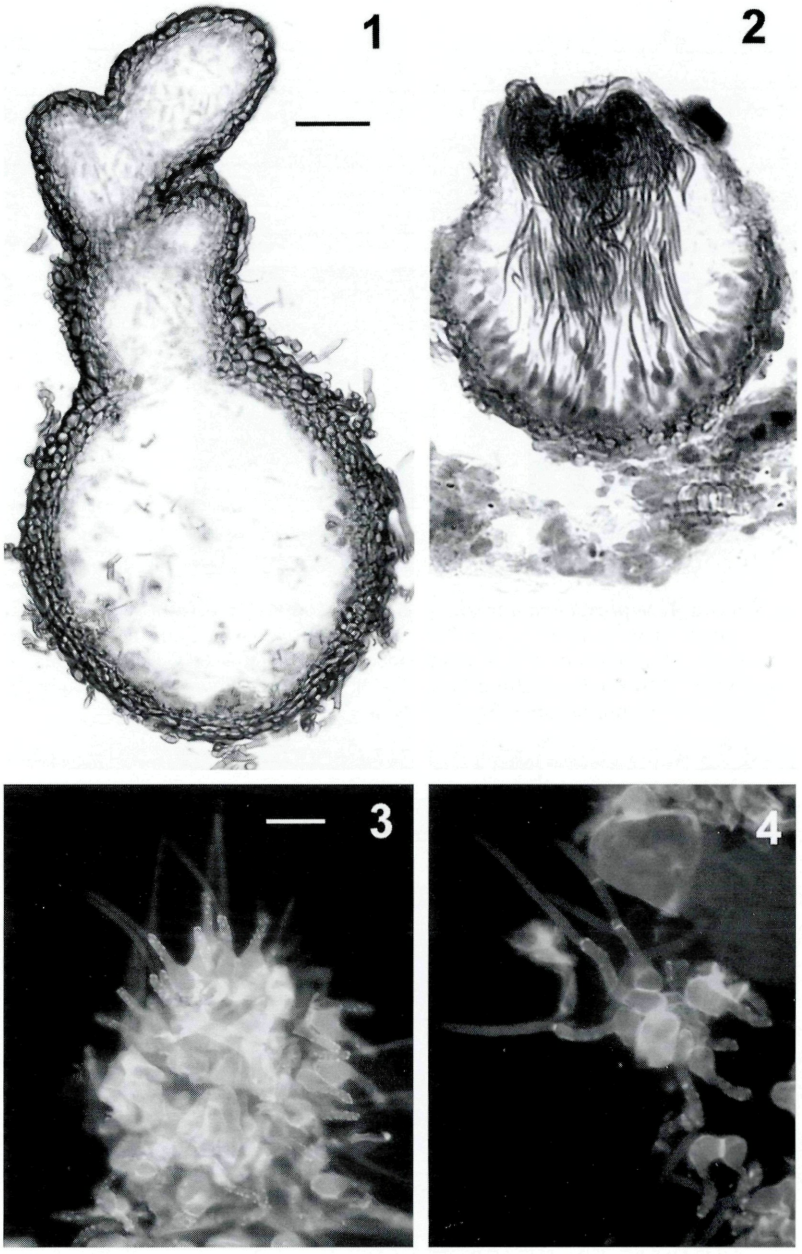
Pycnidia globosa, fusca, 48–104 µm diametro, ostiolata; cellulis conidiogenis pyriformibus, hyalinis, 3–5 × 7–17 µm, supera elongatio cellulae parietibus irregulariter incrassatis; conidiogenesis holoblastica, sympodialis; conidia hyalina, recta vel parum arcuata, interdum flexuosa, 17–41 × 1.0–2.3 µm, 0–4 septata, basi truncata, gradatim dimidiata versus rotundatum apicem.

Holotype. – HUNGARY. Pest County, parasitic on leaves of *Ambrosia artemisiifolia*, 1997, Bohár G. & I. Schwarczinger, BPI 747030, ex-type culture ATCC MYA-970.

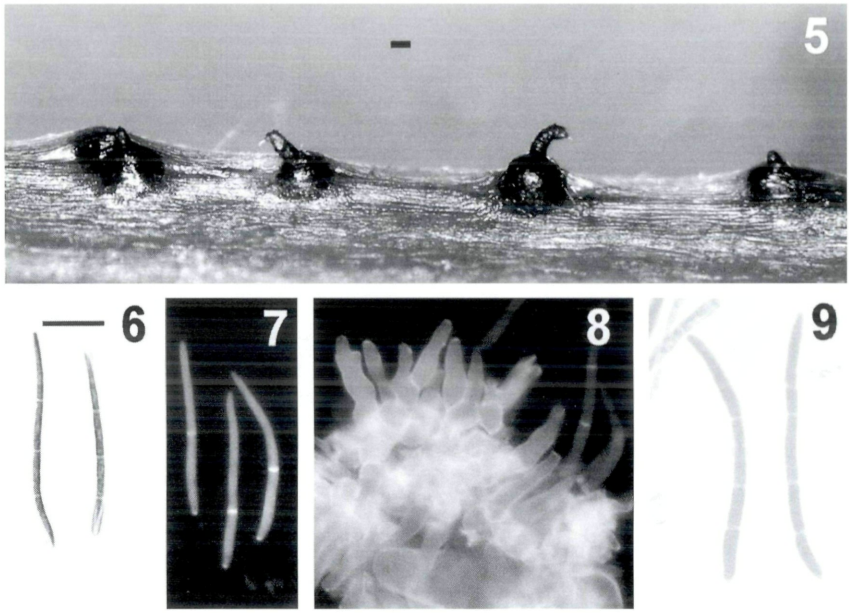
Lesions 0.5–5 mm in longest dimension, angular to irregular, white with a brown margin or more uniformly brown without a distinct margin, with 1 to several, scattered, immersed pycnidia. – Pycnidia 48–104 µm diameter, globose, brown-black, ostiolate, wall of one to two layers of pale brown cells, outer layer darker than inner layer, cell walls of outer layer slightly thickened (Fig. 2). – Conidiogenesis holoblastic, sympodial. – Conidiogenous cells lining inner wall, 3–5 × 7–17 µm, pyriform, hyaline, upper portion covered with irregular wall thickenings, number of scars 2–6 per cell (Figs. 3, 4). – Conidia 17–41 µm ($\bar{x}$ = 27.4, SD = 2.6, number = 130) × 1.0–2.3 ($\bar{x}$ = 1.7, SD = 0.15), 0–4 ($\bar{x}$ = 2.1, SD = 0.83) septate, straight to slighty falcate, occasionally flexuous, base truncate, gradually tapering to a rounded apex, hyaline (Figs. 6, 7).

Pycnidia of ex-type culture forming on alfalfa have one or more beaks up to 110 µm long × 10 µm wide, occasionally branching (Fig. 5). – Pycnidial walls thicker than on leaves, with 3–6 layers of dark brown cells; cell walls somewhat thickened; innermost 1 or 2 layers hyaline (Fig. 1). – Conidia longer, more flexuous than on leaves, 21–46 µm ($\bar{x}$ = 32.0, SD = 8.7, number = 49) x 1.1–1.8 µm ($\bar{x}$ 1.4, SD = 0.16), 0–5 septate ($\bar{x}$ = 2.4, SD = 0.81).

Cultural characteristics of ex-type culture, growth after 6 weeks: PDA: – Colony 44 mm diam, brown (6E5) to black with white cottony mycelium around plug, margin uneven to feathery,



Figs. 1–4. *Septoria epambrosiae*. – 1. Longitudinal section of conidiomata on alfalfa stem in cultures. – 2. Longitudinal section of conidiomata on *Ambrosia* leaves. – 3–4. Conidiogenous cells viewed with fluorescence illumination. – Scale bars: 1–2 = 20 µm; 2–3 = 10 µm.



Figs. 5–9. – 5–7. *Septoria epambrosiae*. – 5. Conidiomata on alfalfa twigs in culture. – 6. Conidia viewed with bright-field illumination. – 7. Conidia viewed with fluorescence illumination. 8–9. – *Septoria ambrosiicola*. – 8. Conidiogenous cells viewed with fluorescence illumination. – 9. Conidia viewed with bright-field illumination. – Scale bars: 5 = 20 µm; 6–9 = 10 µm.

pycnidia common. CM: – Colony 30 mm diam, pale brown (5D5) with no surface mycelium, margin even, sparse pycnidia around plug. CZ: Colony 33 mm diam, dark brown (6F6) to black, dark brown felty mycelium around plug, margin uneven, scattered pycnidia.

Habitat. – Parasitic on leaves of *Ambrosia* (Asteraceae).

Known hosts. – *Ambrosia trifida* L. and *A. artemisiifolia* L.

Known distribution. – Europe (Hungary) and North America (Canada, United States-Wisconsin).

Additional specimens examined. – CANADA: Ontario, London, 1910 Sep 19, on *A. trifida* leaves, J. Dearness, as *Septoria bacilligera*, BPI 377153; Ontario, London, 1923 Sep, on *A. trifida*, J. Dearness, as *Septoria bacilligera*, BPI 377156. – UNITED STATES: Wisconsin, Jefferson Co., Milford, 1948 May 15, on *A. trifida* leaves, H. C. Greene H. C., as *Septoria bacilligera*, BPI 377155.

Discussion

The pycnidial genus *Septoria,* based on the type species *S. cytisi* Desm., has conidiogenous cells that are holoblastic, determinate or indeterminate, with a limited number of sympodial proliferations and each locus with a broad, flat, unthickened scar (Sutton, 1980). The conidia are characterized as hyaline, multiseptate and filiform. *Septoria epambrosiae* agrees with this generic description.

Two species of *Septoria* were previously described on *Ambrosia*; the fungus on *Ambrosia* from Hungary, however, is determined to be different from these species. The principal characteristics of the three species of *Septoria* on *Ambrosia* are summarized in Tab. 1. Only the original description of *Septoria ambrosiae* Hemmi & Naito in Naito (1940) was available while two specimens of *S. ambrosiicola* Speg. (1910) were examined (*S. ambrosiicola* – UNITED STATES: Kansas, Rooks Co., 1895 Sep 27, on *A. trifida,* Elam Bartholomew, misidentified as *Septoria bacilligera*, BPI 377161; Texas, Austin, 1916 Jun 17, B. C. Tharp, on *A. aptera,* BPI 376564). Conidia of *Septoria ambrosiae* have a greater number of septa than those of *S. epambrosiae* and *S. ambrosiicola* Speg. While Spegazzini (1910), in the original description, and later Tharp (1917) described the conidia of *S. ambrosiicola* as being non-septate, conidia from the Tharp specimen at BPI were 0–5 septate and agreed in all other aspects with the description of *S. ambrosiicola* (Figs. 8, 9). Conidia of *S. ambrosiae* are wider than those of *S. ambrosiicola* and *S. epambrosiae*. Conidia of *S. ambrosiicola* are longer and somewhat wider than those of *S. epambrosiae* as shown by scatterplots of conidial length and width measurements (Fig. 10). In addition, conidia of *S. epambrosiae* are straight to slightly falcate while those of *S. ambrosiae* and *S. ambrosiicola* are flexuous or undulate.

A striking feature of *S. epambrosiae* and *S. ambrosiicola* is the numerous knobby protuberances on the conidiogenous cells; these represent the scars left when the conidia are discharged (Figs. 3, 4, 8). Sympodial conidiogenesis has been reported from other *Septoria* sp. (Sutton & Pascoe, 1987; Verkley & Priest, 2000) but is uncommon in the genus (Sutton, 1980; Farr, 1991; Farr, 1992). The number of scars per conidiogenous cell varied from 2–6 or more in specimens of both S. *epambrosiae* and *S. ambrosiicola* suggesting that this is not a character useful for defining species in *Septoria*.

A GenBank BLAST search (Altschul & al., 1990) of the small SSU rDNA of *S. epambrosiae* revealed similarities to *Coccodinium bartschii*, *Dothidea insculpta* and *D. hippophaeos*. A cladogram based on the nSSU rDNA shows *S. epambrosiae* grouping with the Dothideales in a clade with the taxa mentioned above (DePriest,

Tab. 1. – Comparison of species of *Septoria* on *Ambrosia*.

Name	Host	Lesion	Pycnidia	Conidia
Septoria epambrosiae Farr	*Ambrosia trifida* and *A. artemisiifolia*	Angular to irregular, white with brown margin or entirely brown	Brown-black, globose, 48-104 µm diam	17-41 ($\bar{x}$ = 27.4) × 1.0-2.3 ($\bar{x}$ = 1.7) µm, 0-4- ($\bar{x}$ = 2.1) septate, straight to slightly falcate
Septoria ambrosiae Hemmi & Naito	*Ambrosia artemisiifolia*	Oblong to irregular, sometimes confluent, at length occupying a large part of the leaf, black brown	Brown, globose, 95.65–121.70 x 86.95-121.70 µm diam, membranous, epiphyllus	40-64 × 2-2.6 µm, 3-7-septate, attenuate-acutate, filiform, straight to flexuous
Septoria ambrosiicola Speg.	*Ambrosia aptera, A. trifida*, originally described on *A. scabrae*	White above, tan below, tan-brown margin, 0.5-2 mm, angular to suborbicular	Brown-black, globose, 57-123 µm diam	21-62 ($\bar{x}$ = 44) x 1.7-2.6 ($\bar{x}$ = 2) µm, 0-5-($\bar{x}$ = 2.3) septate, subarcuate to flexuous

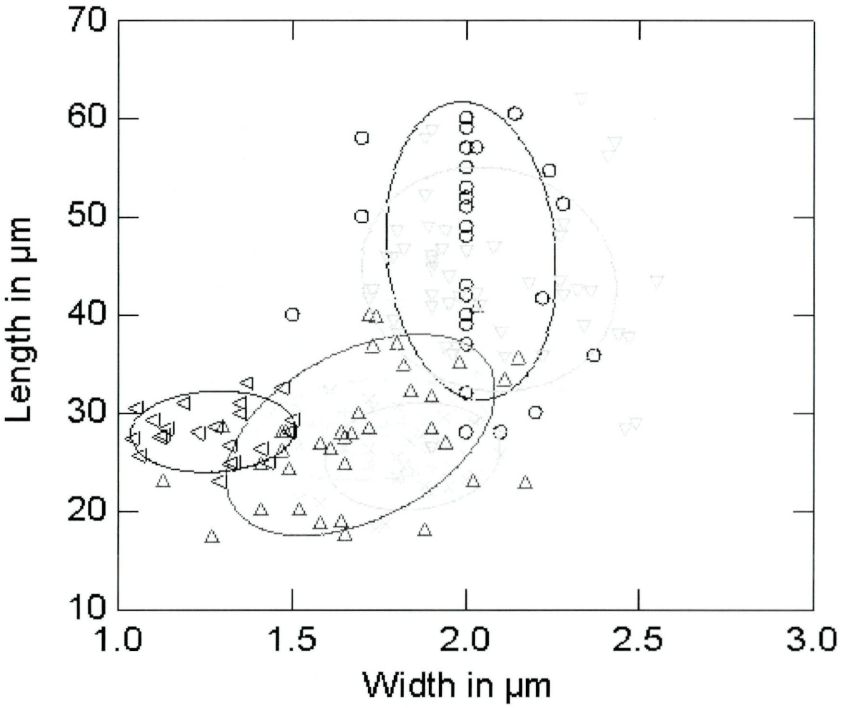


Fig. 10. – Scatterplot of the conidial length and width of *Septoria epambrosiae* (× = BPI 377153, △ = BPI 377156, + = BPI 377155, ◁ = BPI 747030 and *Septoria ambrosiicola* (▽ = BPI 377161, ○ = BPI 376564).

pers. comm.). A GenBank BLAST search of the ITS sequence placed *S. epambrosiae*, among species of *Mycosphaerella* (Dothideales). A cladogram of ITS sequences of species of *Mycosphaerella* and related species in the Dothideales was constructed to investigate the relationships of *S. epambrosiae* (in Fig. 11). If known, the anamorph for the species of *Mycosphaerella* is listed in the cladogram. Based primarily on sequences obtained and analyzed by Crous & al. (1999, 2000), *S. epambrosiae* clusters with species of *Mycosphaerella* having *Pseudocercospora* Speg. and *Mycovellosiella* Rangel anamorphs. Thus, the teleomorph of *S. epambrosiae*, if found, would most likely belong to *Mycosphaerella*. A number of *Septoria* species have been reported to have *Mycosphaerella* teleomorphs (Rossman & al., 1987; Farr & al., 1989; Brzustowski, 2000). *Septoria tritici* Rob., the anamorph of *M. graminicola*, and *S. passerini*, cluster within the main *Mycosphaerella* clade but are not particularly close to *S. epambrosiae*. Within *Mycosphaerella*, the known anamorphs are classified in both hyphomycetous genera such as *Cercospora* Fresen. and

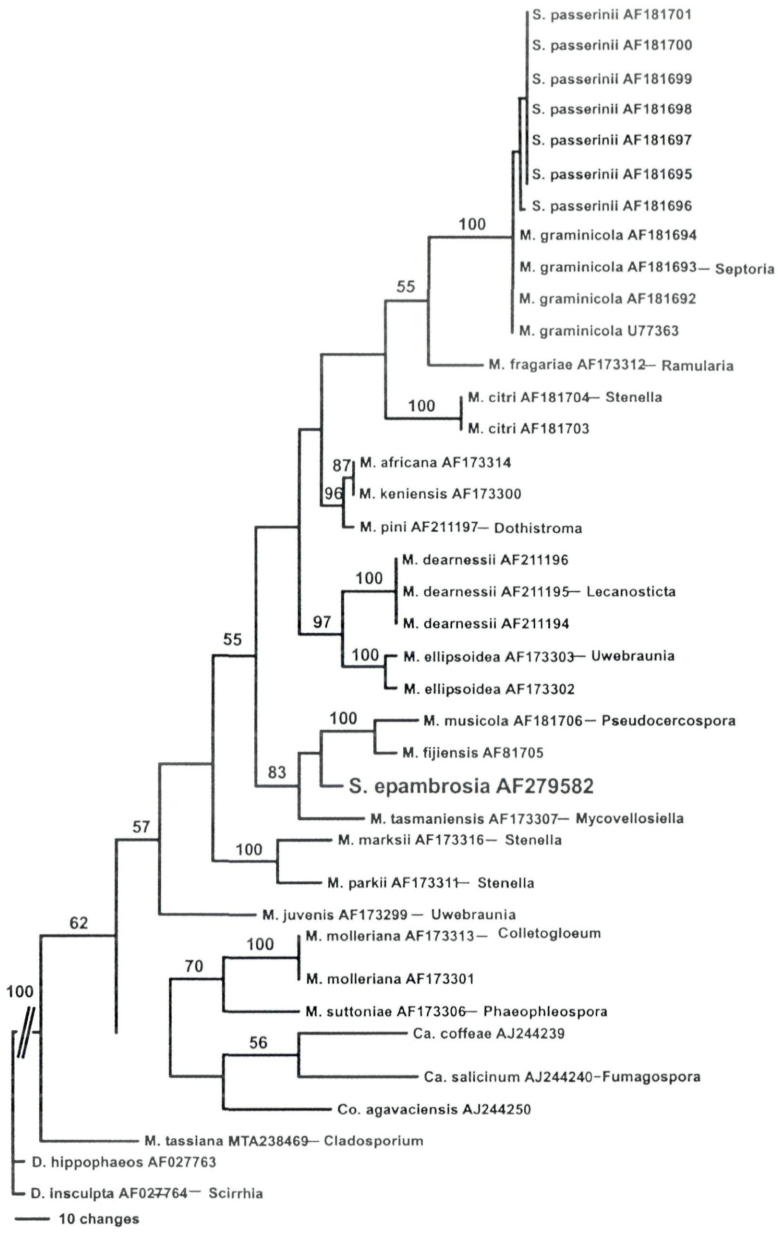


Fig. 11. – One of six most parsimonious trees from analysis of the complete ITS region of rDNA. Bootstrap values greater than 50% are listed above the branches. – Length = 860 steps, CI = 0.492, RI = 0.714, RCI = 0.351, alignment = 568 total characters, 93 ambiguously aligned positions excluded. 199 parsimony informative characters analyzed.

Passalora Fr. and coelomycetous genera, such as *Colletogloeum* Petr. and *Phaeophleospora* Rangel.

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