doi:10.5598/imafungus.2011.02.01.03 IMA FUNGUS · VOLUME 2 · NO 1: 13–24

ARTICLE

Diaporthaceae associated with root and crown rot of maize

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Abstract: Several isolates of coelomycetous fungi with pigmented conidia were consistently isolated from diseased roots of *Zea mays* in irrigated plots monitored in the KwaZulu-Natal Province of South Africa. Based on their morphology, these isolates could be identified as representative of *Stenocarpella macrospora*, *S. maydis*, and *Phaeocytostroma ambiguum*. Although species of *Stenocarpella* are well-known as causal agents of cob and stalk rot and leaf blight of maize in South Africa, the occurrence and importance of *P. ambiguum* is less well documented and understood. To determine the role of *P. ambiguum* as a root pathogen of maize, pathogenicity tests were conducted under glasshouse conditions at 18 °C night and 28 °C day temperatures using a pasteurised soil, river sand and perlite medium and a 0.5 % sand-bran inoculum. Based on these results, *P. ambiguum* was shown to be a primary pathogen of maize, but to be less virulent than the positive control, *S. maydis*. Furthermore, to clarify the higher-level phylogeny of these fungal genera, isolates were subjected to DNA sequencing of the nuclear ribosomal DNA (ITS & LSU). Partial gene sequences of the translation elongation factor 1-alpha gene were added to confirm the species monophyly. To resolve the generic placement of *Phaeocytostroma*, additional species such as *P. sacchari, P. plurivorum* and *P. megalosporum* were also added to the analysis. Based on these results, *Stenocarpella* and *Phaeocytostroma* were shown to be two well defined genera, belonging to *Diaporthales*, *Diaporthaceae*, being closely allied to *Phomopsis* (*Diaporthe*). All three genera were also observed to form alpha as well as beta conidia, and although this phenomenon is well documented for *Phomopsis* and *Phaeocytostroma*, it is a new observation for *Stenocarpella*. In spite of the differences in conidial pigmentation, no support could be obtained for polyphyly in *Diaporthaceae*, suggesting that as observed in *Botryosphaeriaceae* (*Botryosphaeriales*), conidial pigmentation is not informative at the family level in *Diaporthales*.

Key words:
Diplodia
diplodiosis
Phaeocytostroma
phylogeny
Stenocarpella
systematics
Zea mays

Article info: Submitted: 3 January 2011; Accepted: 15 February 2011; Published: 10 March 2011.

INTRODUCTION

Soilborne diseases significantly reduce maize yields in irrigated systems where maize follows winter wheat in the KwaZulu-Natal Province of South Africa (Lamprecht *et al.* 2008). Over the years several fungi have been consistently isolated from maize plants with symptoms of crown and root rot in fields sampled in KwaZulu-Natal. The pathogenicity of these fungi remains unresolved.

Among the isolates obtained since 2006 in the present survey were several coelomycetous fungi with dematiaceous conidia, representing the genera *Stenocarpella* and *Phaeocytostroma* (Sutton 1980). Species of *Stenocarpella* are commonly isolated from diseased maize crops worldwide, especially during humid seasons (Odriozola *et al.* 2005). The two species reported from literature to be associated with cob and stalk rot and leaf blight of maize are *S. macrospora* and *S. maydis* (Marasas *et al.* 1979, Latterell & Rossi, 1983, Crous *et al.* 2006). According to Sutton and Waterston (1966) *Diplodia maydis* (*S. maydis*) can also infect roots and cause seedling blight. Cob rot develops at the base of the maize ear, growing up to its tip. After initial infection, maize grains appear less shiny and opaque-grey or somewhat brownish, leading to seedling blight, ear or stalk rot (Kellerman *et al.* 1991). Ear rot results in yield losses, reduced grain quality, and mycotoxins may accumulate in the grain (Rheeder *et al.* 1993). Species of *Phaeocytostroma* are commonly associated with stalk rots of different hosts (Sutton 1964, 1980, Holliday 1980), with *P. ambiguum* being reported from maize in Australia, France, North America, Serbia (Stovold *et al.* 1996), Mauritius, Tanzania (Sutton 1964), South Africa (Crous *et al.* 2000) and Yugoslavia (Lević & Petrović 1998).

Although *S. macrospora* and *S. maydis* have in the past been extensively published as species of *Diplodia*, Sutton (1980) placed them in *Stenocarpella* based on their distinct conidiogenesis, a fact supported by later molecular phylogenetic studies, which revealed these taxa to belong to the *Diaporthales* rather than the *Botryosphaeriales* (Crous *et al.* 2006). Their position within the order, however, remains unresolved. Similarly *P. ambiguum* was initially described as a species of *Sphaeropsis* (suggesting *Botryosphaeriaceae*), though nothing is known about the phylogenetic position of

Table 1. Collection details and DDBJ/EMBL/GenBank accession numbers of *Phaeocytostroma* and *Stenocarpella* isolates for which novel sequences were generated in this study.

Species	Strain no.[1]	Substrate	Country	Collector	EMBL accession no. (ITS, LSU, TEF)[2]
Phaeocytostroma ambiguum	CPC 16775; Z171F	*Zea mays*	South Africa	S. Lamprecht	FR748034, —, FR748066
	CPC 16776; Z323C	*Zea mays*	South Africa	S. Lamprecht	FR748035, FR748095, FR748067
	CPC 17071; Z113V	*Zea mays*	South Africa	S. Lamprecht	FR748036, —, FR748068
	CPC 17072; Z155R	*Zea mays*	South Africa	S. Lamprecht	FR748037, FR748096, FR748069
	CPC 17074; Z182Z	*Zea mays*	South Africa	S. Lamprecht	FR748038, FR748097, FR748070
	CPC 17075; Z189Z	*Zea mays*	South Africa	S. Lamprecht	FR748039, FR748098, FR748071
	CPC 17076; Z191AB	*Zea mays*	South Africa	S. Lamprecht	FR748040, FR748099, FR748072
	CPC 17077; Z199Z	*Zea mays*	South Africa	S. Lamprecht	FR748041, FR748100, FR748073
	CPC 17078; Z213H	*Zea mays*	South Africa	S. Lamprecht	FR748042, FR748101, FR748074
	CPC 17079; Z225F	*Zea mays*	South Africa	S. Lamprecht	FR748043, FR748102, FR748075
	CPC 17083; Z432W	*Zea mays*	South Africa	S. Lamprecht	FR748044, —, FR748076
P. megalosporum	CBS 284.65; IMI 092336	Rice-field soil	India	—	FR748045, FR748103, FR748077
P. plurivorum	CBS 113835; UPSC 2042	*Helianthus annuus*	Portugal	—	FR748046, FR748104, FR748078
P. sacchari	CBS 275.34	—	Japan	—	FR748047, FR748105, FR748079
Stenocarpella macrospora	CBS 117560; MRC 8615	Rain damaged Bt maize hybrid, 2003-04 season	South Africa	J. Rheeder	FR748048, DQ377934, —
	CPC 11863	*Zea mays*	South Africa	P. Caldwell	FR748049, —, —
S. maydis	CBS 117557; MRC 8612	Bt maize hybrid from 2003-04 season	South Africa	J. Rheeder	FR748050, DQ377935, —
	CBS 117558; MRC 8613	Traditional/landrace maize from 2003/04 season	South Africa	J. Rheeder	FR748051, DQ377936, FR748080
	CBS 117559; MRC 8614	Commercial maize hybrid PAN-6043 from 2003-04 season	South Africa	J. Rheeder	FR748052, DQ377937, FR748081
	CPC 16777; Z169F	*Zea mays*	South Africa	S. Lamprecht	FR748053, FR748106, FR748082
	CPC 16778; Z178AB	*Zea mays*	South Africa	S. Lamprecht	FR748054, FR748107, FR748083
	CPC 16779; Z181R	*Zea mays*	South Africa	S. Lamprecht	FR748055, FR748108, FR748084
	CPC 16780; Z255K	*Zea mays*	South Africa	S. Lamprecht	FR748056, FR748109, FR748085
	CPC 16781; Z255AD	*Zea mays*	South Africa	S. Lamprecht	FR748057, FR748110, FR748086
	CPC 16782; Z255AE	*Zea mays*	South Africa	S. Lamprecht	FR748058, FR748111, FR748087
	CPC 16784; Z401P	*Zea mays*	South Africa	S. Lamprecht	FR748059, FR748112, FR748088
	CPC 16785; Z404K	*Zea mays*	South Africa	S. Lamprecht	FR748060, FR748113, FR748089
	CPC 16786; Z410AA	*Zea mays*	South Africa	S. Lamprecht	FR748061, FR748114, FR748090
	CPC 16787; Z422B	*Zea mays*	South Africa	S. Lamprecht	FR748062, FR748115, FR748091
	CPC 16788; Z430D	*Zea mays*	South Africa	S. Lamprecht	FR748063, FR748116, FR748092
	CPC 16789; Z434C	*Zea mays*	South Africa	S. Lamprecht	FR748064, FR748117, FR748093
	CPC 17073; Z169F	*Zea mays*	South Africa	S. Lamprecht	FR748065, FR748118, FR748094

[1]CBS: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K.; MRC: Medical Research Council Fusarium Collection, Tygerberg, South Africa; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden.

[2]ITS: Internal transcribed spacers 1 and 2 together with 5.8S nrDNA; LSU: 28S nrDNA; TEF: partial translation elongation factor 1-alpha.

the genus *Phaeocytostroma*, and it is generally regarded as *Ascomycota incertae sedis* (<MycoBank.org>). Furthermore, although pathogenicity has been confirmed for South African isolates of *S. maydis* and *S. macrospora* on maize (Marasas & Van der Westhuizen 1979, Kellerman *et al.* 1991, Rheeder *et al.* 1993), this has not been done for *P. ambiguum*. The aim of the present study was thus to resolve the higher order phylogeny of *Stenocarpella* and *Phaeocytostroma*, and also determine the importance of *P. ambiguum* as pathogen on maize, when compared to *S. maydis*, which is regarded as an important pathogen of this host.

MATERIALS AND METHODS

Isolates

Maize roots and crown pieces were surface sterilised in 1 % sodium hypochlorite for 1 min, rinsed twice in sterile distilled water and allowed to dry in a laminar flow bench. Pieces of tissue (5–10 mm) were placed on potato-dextrose agar (PDA) and water agar (WA) containing 0.02 % novostreptomycin. Petri dishes were incubated at 25 °C in the dark for 7 d. Fungi that developed were transferred to divided Petri dishes containing carnation leaf agar (WA with sterile carnation leaves; Fisher *et al.* 1981) in one half and PDA in the other. Plates containing colonies of *Stenocarpella* and *Phaeocytostroma* isolates were incubated at 20 °C under near-ultraviolet light radiation (12 h/d) for 21–28 d, when sporulating colonies could be positively identified. Colonies were sub-cultured onto 2 % PDA, 2 % malt extract agar, oatmeal agar (OA) and pine needle agar (PNA) (Crous *et al.* 2009), and incubated under continuous near-ultraviolet light at 25 °C to promote sporulation. To help resolve the generic position of *Phaeocytostroma*, isolates of additional species such as *P. sacchari* (CBS 275.34), *P. plurivorum* (CBS 113835) and *P. megalosporum* (CBS 284.65) were added to the analysis. Representative cultures obtained in this study are maintained in the culture collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands (Table 1).

DNA phylogeny

Genomic DNA was extracted from mycelia taken from fungal colonies on MEA using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Solana Beach, CA, USA). A part of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene (SSU), the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region (ITS2) and the first 900 bp at the 5' end of the 28S rRNA gene (LSU) was amplified and sequenced as described by Cheewangkoon *et al.* (2008). Partial gene sequences for the translation elongation factor 1-alpha gene (TEF) were generated as described by Bensch *et al.* (2010). The generated ITS and LSU sequences were compared with other fungal DNA sequences from NCBI's GenBank sequence database using a megablast search of the nr database; sequences with high similarity were added to the alignments. The *Diaporthales* LSU phylogeny of Tanaka *et al.* (2010) was used as starting point for Fig. 1 in this study. Novel sequences were lodged in the DDBJ/EMBL/GenBank nucleotide database (Table 1) and the alignments and phylogenetic trees in TreeBASE (<treebase.org>).

Taxonomy

Wherever possible, 30 measurements (× 1000 magnification) were made of structures mounted in lactic acid, with the extremes of spore measurements given in parentheses. Colony colours (surface and reverse) were assessed after 1 mo on MEA, OA and PDA at 25 °C in the dark, using the colour charts of Rayner (1970).

Pathogenicity trial

Sand-bran inoculum was prepared according to Lamprecht (1986). Autoclaving times were adapted to 60 min on the first day followed by 30 min on two consecutive days. Ten plugs (2 mm diam) of each isolate were used to inoculate two 2 L flasks. Control flasks were inoculated with plugs of WA only. The inoculum was incubated for 11 d at 22 °C without being directly exposed to light. The mixture was shaken every fourth day to ensure even growth of the mycelium throughout the medium.

The pathogenicity trial was conducted in a glasshouse (18 °C night and 28 °C day temperatures) using plastic pots, 22.5 cm diam, with a holding capacity of 1 500 g planting medium. The planting medium was made up of equal amounts of soil, perlite and sand, which was pasteurised (30 min at 83 °C) and left for 3 d before being mixed with inoculum. An inoculum concentration of 0.5 % (wt/wt) was used. The inoculum was mixed with the planting medium and pots were watered and left to stand overnight in the glasshouse before being planted to 10 maize seeds (cv PHI 32D96B) the next day. Maize seeds were treated with hot water at 60 °C for 5 min (Daniels 1983) to ensure that clean seed was used. Pots were watered every alternate day to field capacity. Pathogenicity and relative virulence of each isolate were determined by calculating the percentage survival and plant growth (shoot length) as well as the percentage plants with crown and root rot severity using a 0–4 scale with 0 = no root rot, 1 = > 0–25 % root rot, 2 = > 25–50 % root rot, 3 = > 50–75 % root rot and 4 = > 75–100 % root rot, 3 wk after planting. To confirm the presence of the different fungi, re-isolations were made by plating 5 mm pieces of tissue excised from crowns and roots of plants with crown and root rot representatively selected from each treatment on PDA. The experimental design was a randomised block design with three replicates for each treatment.

Statistical analysis

Data were subjected to analysis of variance using SAS (v. 9.3, SAS Institute, Inc) and the Shapiro-Wilk test (Shapiro & Wilk 1965) was performed to test for normality. The Student's t test for least significant differences were calculated to compare means at the 5 % significance level.

Fig. 1. The first of 23 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions (PAUP v. 4.0b10). Bootstrap support values are shown at the nodes and strict consensus branches are thickened. Families are indicated in different coloured boxes. The tree was rooted to *Gaeumannomyces graminis* var. *avenae* (GenBank AF362556) and *Magnaporthe grisea* (GenBank AB026819).

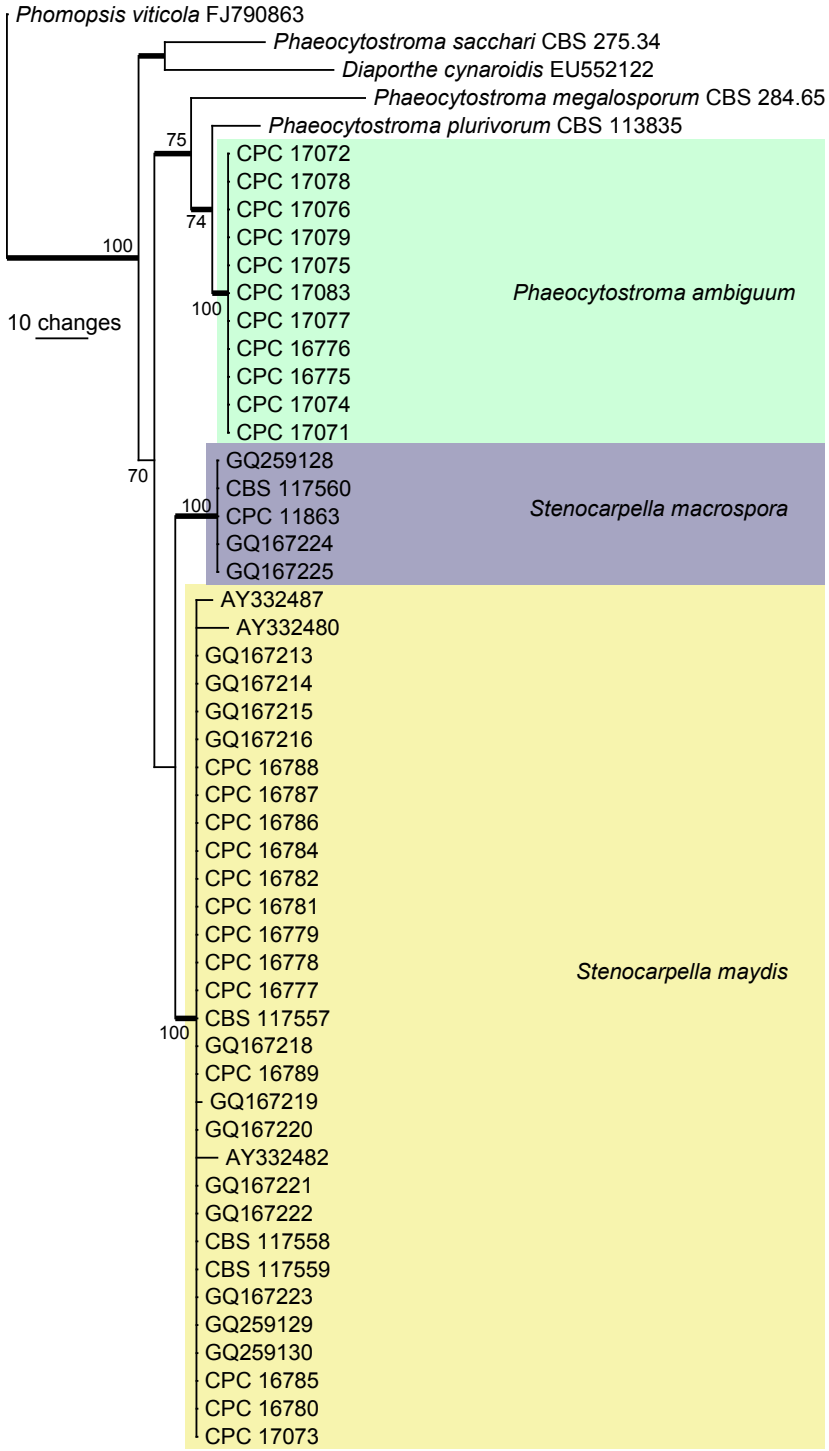

Fig. 2. The first of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions (PAUP v. 4.0b10). Bootstrap support values > 69 % are shown at the nodes and strict consensus branches are thickened. The three species from maize are indicated in different coloured boxes. The tree was rooted to *Phomopsis viticola* (GenBank FJ790863).

RESULTS

Phylogenetic analyses

Approximately 1 700 bases, spanning the ITS and LSU regions, and approximately 650 bp for TEF, were obtained. The LSU region was used in the phylogenetic analysis for the generic placement (Fig. 1) and ITS and TEF to determine species-level relationships (Figs 2, 3).

The manually adjusted LSU alignment contained 72 taxa (including the two outgroup sequences) and, of the 836 characters used in the phylogenetic analysis, 179 were parsimony-informative, 54 were variable and parsimony-uninformative and 603 were constant. Twenty-three equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 1 (TL = 578, CI = 0.516, RI = 0.834, RC = 0.430). The phylogenetic tree

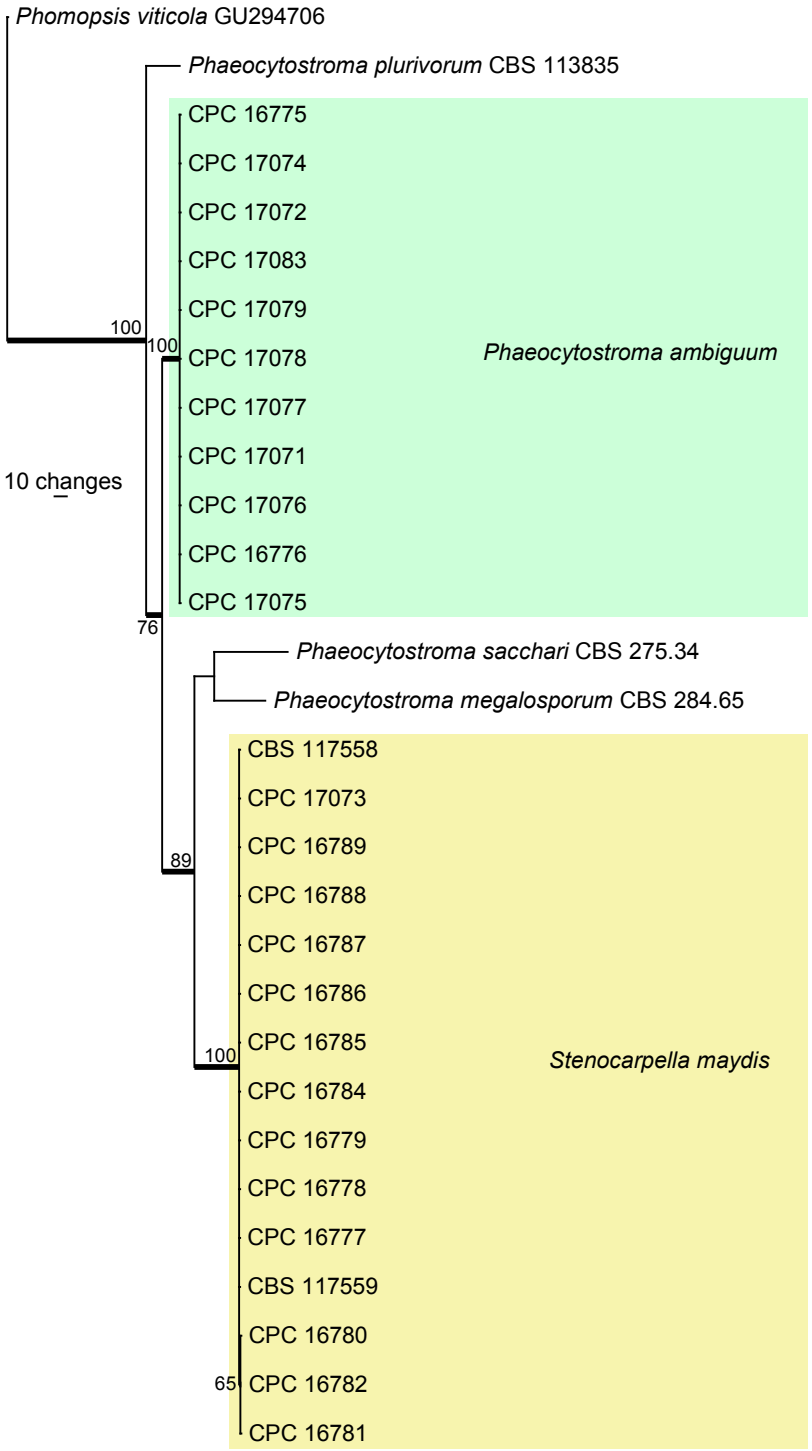

Fig. 3. The first of two equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions (PAUP v. 4.0b10). Bootstrap support values > 69 % are shown at the nodes and strict consensus branches are thickened. The two species from maize for which TEF sequences were available are indicated in different coloured boxes. The tree was rooted to *Phomopsis viticola* (GenBank GU294706).

of the LSU region (Fig. 1) shows that *Stenocarpella* and *Phaeocytostroma* are embedded with the *Diaporthaceae* and could not be distinguished phylogenetically from *Diaporthe*.

The manually adjusted ITS alignment contained 52 taxa (including the outgroup sequence) and, of the 491 characters used in the phylogenetic analysis, 34 were parsimony-informative, 84 were variable and parsimony-uninformative and 373 were constant. Two equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 2 (TL = 170, CI = 0.876, RI = 0.935, RC = 0.819). The phylogenetic tree of the ITS region (Fig. 2) shows that the sequences of species of *Phaeocytostroma* form a monophyletic lineage with a bootstrap support value of 75 % whereas the monophyletic lineage for *Stenocarpella* was poorly supported (51 %, not shown on tree).

The manually adjusted TEF alignment contained 30 taxa (including the outgroup sequence) and, of the 317 characters (due to the inclusion of a much shorter outgroup sequence

Fig. 4. *Phaeocytostroma ambiguum* (CPC 17079). **A.** Conidiomata on potato-dextrose agar. **B, C.** Conidiomata on pine needle agar. **D–F.** Conidiophores and paraphyses. **G.** Hyaline conidiophores giving rise to brown conidial mass. **H, I.** Alpha conidia. **J.** Conidiogenous cells giving rise to beta conidia. **K.** Beta conidia. Scale bars = 10 µm.

compared to the length of the ingroup sequences) used in the phylogenetic analysis, 102 were parsimony-informative, 102 were variable and parsimony-uninformative and 113 were constant. Two equally most parsimonious trees were retained from the heuristic search, the first of which is shown in Fig. 3 (TL = 345, CI = 0.884, RI = 0.956, RC = 0.845). The phylogenetic tree of the TEF region (Fig. 3) shows very little intraspecific variation for *S. maydis* and *P. ambiguum*.

Taxonomy

Phaeocytostroma ambiguum (Mont.) Petr., *Feddes Repert*. **42**: 457 (1927)
Basionym: *Sphaeropsis ambigua* Mont., *Ann. Sci. Nat., Bot*. **12**: 308 (1849)
Synonyms: *Phaeocytostroma istrica* Petr., *Ann. Mycol*. **19**: 45 (1921)
Phaeocytosporella zeae Stout, *Mycologia* **22**: 280 (1930)
(Fig. 4)

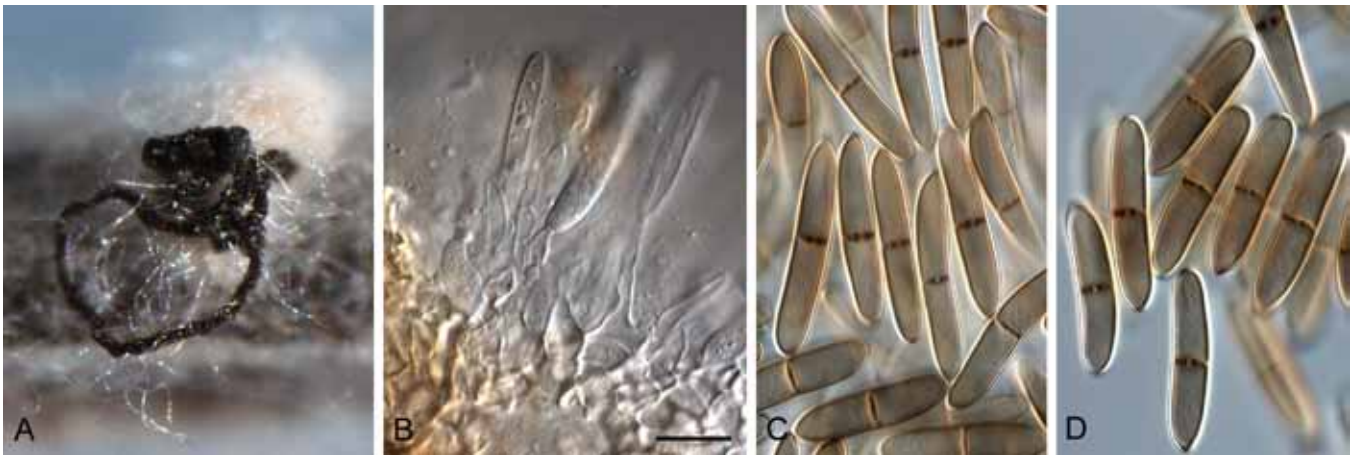

Fig. 5. *Stenocarpella maydis* (CBS 117559). **A.** Conidioma with exuding black conidial cirrhus on pine needle agar. **B.** Conidiogenous cells giving rise to conidia. **C, D.** Conidia. Scale bar = 10 µm.

Conidiomata on PNA and OA immersed, initially solitary, but forming a stroma up to 2 mm diam, becoming multilocular with one to several clearly defined black necks extending above the stroma, up to 300 µm tall (after 2 wk, but becoming more elongated with age), up to 170 µm wide with terminal ostiole, up to 100 µm wide. *Conidiomatal wall* black, consisting of several layers of *textura intricata* to *textura angularis*, up to 40 µm wide; forming an inner pale brown to hyaline layer, up to 30 µm wide. *Alpha conidiophores* tightly aggregated, subcylindrical, branched in mid region, consisting of 2–3 supporting cells, giving rise to septate, cylindrical conidiogenous cells or paraphyses, 1–5-septate, 40–100 × 2–4 µm. *Alpha conidiogenous cells* hyaline, subcylindrical, terminal and lateral, 15–50 × 2–4 µm; apex with minute periclinal thickening and collarette. *Paraphyses* intermingled between conidiophores or arising from same conidiophores that give rise to conidiogenous cells, subcylindrical, hyaline, branched or not, 1–3 transversely septate, 40–120 × 2–4 µm; apex bluntly rounded. *Alpha conidia* medium brown, smooth, ellipsoid to pyriform (somewhat clavate on PNA), widest in middle of conidium, apex bluntly rounded, base truncate, (14–)15–16(–18) × (4.5–)5–6(–6.5) µm. *Beta conidiophores* interspersed among alpha conidiophores, hyaline, subcylindrical, branched, 1–3-septate, 10–30 × 2–4 µm; *Beta conidiogenous cells* phialidic, integrated, terminal and lateral, 10–20 × 2–3 µm. *Beta conidia* subcylindrical, straight to slightly curved, hyaline, smooth, widest in middle, tapering to acutely rounded apex; base truncate, 15–20 × 1.5–2 µm.

Culture characteristics: *Colonies* on OA flat, spreading with smooth margins and sparse aerial mycelium; surface flat, with a dull black layer and patches of flat white mycelium, forming a layer on the surface, covering the plate within 2 wk. On PDA similar, except that the black layer extends from the centre outwards, with the white layer in the outer region, less dense than on OA; reverse dull black in middle, pale white in outer region. On MEA appearing olivaceous-black due to woolly, grey aerial mycelium; reverse similar as on PDA.

Specimens examined: France ?: from stems of *Zea mays*, (PC 206294 – holotype). South Africa: KwaZulu-Natal, Winterton, Gourton farm, on roots of *Zea mays*, 2008, *S. Lamprecht*, (CBS H-20547 – ***epitypus hic designatus***); culture ex-epitype CPC 17071 = CBS 128784.

Notes: The beta conidia described above for *P. ambiguum* were recently reported by Lević & Petrović (1998), and seem to be commonly produced by isolates of this species. Other taxa in the *Diaporthaceae* (Figs 1, 2), such as *Phomopsis* (*Diaporthe*) also produce beta conidia, suggesting that the putative link between *Phaeocytostroma iliau* and *Clypeoporthe iliau* (Barr 1978), could be correct.

Stenocarpella maydis (Berk.) B. Sutton, *Coelomycetes*: 432 (1980)
Basionym: *Sphaeria maydis* Berk., *Hooker's J. Bot., London* **6**: 15 (1847)
Synonyms: Additional synonyms are listed in Sutton (1980).
(Fig. 5)

Specimens examined: South Africa: KwaZulu-Natal, Simdlangentsha, Bt *Zea mays* hybrid from 2003-04 season, *J. Rheeder* (ex-epitype CBS 117558 = MRC 8613, designated in Crous *et al.* 2006); ibid. CBS 117557 = MRC 8612; Hlabisa, commercial hybrid PAN-6043, MRC 8614 = CBS 117559.

Note: Conidia subcylindrical to narrowly ellipsoid, straight, curved, occasionally irregular, 0–2-septate, smooth-walled, pale brown, apex obtuse, base truncate, 15–34 × 5–8 µm (Sutton 1964).

Stenocarpella macrospora (Earle) B. Sutton, *Mycol. Pap*. **141**: 202 (1977)
Basionym: *Diplodia macrospora* Earle, *Bull. Torrey Bot. Cl*. **24**: 29 (1897)
Synonyms: Additional synonyms are listed in Sutton (1980).
(Fig. 6)

Fig. 6. *Stenocarpella macrospora* (CPC 11863). **A.** Conidioma with exuding conidial mass on pine needle agar. **B, C.** Conidiogenous cells giving rise to conidia. **D.** Hyaline layer of conidiogenous cells giving rise to brown conidial mass. **E, F.** Alpha conidia. **G.** Conidiogenous cells giving rise to beta conidia. **H.** Beta conidia. Scale bars = 10 µm.

Specimens examined: South Africa: KwaZulu-Natal, Hlabisa, rain damaged Bt *Zea mays* hybrid, 2003-04 season, *J. Rheeder* (ex-epitype, CBS 117560 = MRC 8615, designated in Crous *et al.* 2006); KwaZulu-Natal, *Zea mays* kernels, 2005, *P. Caldwell*, CPC 11863 = CBS 128560.

Notes: Conidia subcylindrical to narrowly ellipsoid, straight, curved, occasionally irregular, 0–3-septate, smooth-walled, pale brown, apex obtuse, base truncate, 44–82 × 7.5–11.5 µm (Sutton 1964). Several cultures also formed hyaline, scolecosporous, curved beta conidia, which is a new observation for *S. macrospora*, but not uncommon in the *Diaporthaceae* (Fig. 6).

Pathogenicity trial

Stenocarpella maydis significantly reduced the survival of seedlings compared to the control and *P. ambiguum* (Table 2). *Stenocarpella maydis* isolates Z169F, Z178AB, Z181R, Z430D and Z434C significantly reduced seedling survival compared to the control, with the lowest survival rates recorded for Z178B, Z181R and Z430D (Table 3).

Both *P. ambiguum* and *S. maydis* caused significantly more crown and root rot, and growth reduction, than the control. However, *S. maydis* was the most virulent, causing significantly more crown and root rot and growth reduction than *P. ambiguum* (Table 2). Of the isolates included in this study, *P. ambiguum* isolates Z113V, Z182Z, Z191AB, Z323C and Z432W and all *S. maydis* isolates except Z422B significantly reduced plant growth (shoot length). The highest growth reductions were recorded for *S. maydis* isolates Z181R and Z430D, but growth reduction caused by these isolates did not differ significantly from that caused by isolates Z401P and Z434C. All isolates of both fungi caused significant crown rot compared to the control, except for *P. ambiguum* isolates Z182R, Z199Z, Z213H and Z222AS, and all isolates tested except Z213H (*P. ambiguum*) caused significant root rot. The highest root rot severities were recorded for isolates Z181R, Z401P, Z430D and Z434C (Table 3).

Table 2. Survival, shoot length and crown and root rot recorded for maize seedlings inoculated with *Phaeocytostroma ambiguum* and *Stenocarpella maydis* under glasshouse conditions.

Fungus	Survival (%)[x]	Shoot length (mm)[x]	Crown rot (%)[x y]	Root rot severity[x z]
Control	100.0a	489.7a	0.00c	0.00c
P. ambiguum	97.6a	446.0b	45.1b	1.40b
S. maydis	74.8b	367.7c	67.2a	2.59a

[x]Means within a column followed by the same letter do not differ significantly (P = 0.05)

[y]Percentage plants with crown rot

[z]Root rot severity rated on a scale of 0–4 with 0 = no root rot, 1 = > 0–25 % root rot, 2 = >25–50 % root rot, 3 = > 50–75 % and 4 >75–100 % root rot.

Table 3. Effect of different isolates of *Phaeocytostroma ambiguum* and *Stenocarpella maydis* on survival, plant growth (shoot length) and crown and root rot of maize seedlings under glasshouse conditions.

Fungus	Isolate	Survival (%)[x]	Shoot length (mm)[x]	Crown rot (%)[x y]	Root rot severity[x z]
Control		100.0a	489.7ab	0.0j	0.00h
P. ambiguum	Z113V	100.0a	416.9defg	46.7def	1.27g
	Z171F	100.0a	442.9bcd	60.0cd	1.10g
	Z182Z	96.7a	434.1cd	13.7hij	1.14g
	Z189Z	96.7a	452.1abcd	23.7ghi	1.28g
	Z191AB	100.0a	422.6cdef	100.0a	2.00f
	Z199Z	100.0a	497.8a	13.3hij	1.13g
	Z213H	96.7a	487.9ab	17.0hij	1.07h
	Z222AS	93.3ab	496.4a	14.2hij	0.82g
	Z225F	96.7a	455.1abcd	7.0ij	1.21g
	Z323C	100.0a	375.8fgh	100.0a	2.30def
	Z432W	93.3ab	428.3cde	100.0a	2.04ef
S. maydis	Z169F	76.7bc	381.4efgh	100.0a	2.21ef
	Z178AB	40.0e	370.2gh	50.6de	2.83dc
	Z181R	56.7de	290.2j	100.0a	3.41b
	Z255K	83.3abc	376.6fgh	77.8bc	2.36def
	Z255AD	100.0a	377.9fgh	73.3bc	2.23ef
	Z401P	83.3abc	313.7ij	59.7cd	3.32bc
	Z404K	86.7abc	361.2hi	100.0a	2.58de
	Z410AD	83.3abc	420.6cdef	27.8fgh	2.20ef
	Z422B	96.7a	465.8abc	38.5efg	1.00g
	Z430D	46.7e	289.0j	20.8ghi	3.79ab
	Z434C	70.0cd	315.5ij	90.5ab	4.00a

[x]Means within a column followed by the same letter do not differ significantly (P = 0.05)

[y]Percentage plants with crown rot

[z]Root rot severity rated on a scale of 0–4 with 0 = no root rot, 1 = > 0–25 % root rot, 2 = > 25–50 % root rot, 3 = > 50–75 % and 4 = >75–100 % root rot.

DISCUSSION

Stenocarpella maydis is well documented as a major cause of cob rot of maize (Ullstrup 1977), and the chief organism associated with diplodiosis (Kellerman *et al.* 1985). In contrast, *S. macrospora* has been seen as of less importance when compared to *S. maydis* (Virtanen *et al.* 1956). In Latin America and Africa, however, both pathogens have been regarded as important ear-rotting pathogens, because of their ability to produce toxins in infected grain, which may be used to feed livestock and poultry (Marasas *et al.* 1979). A later study by Latterell & Rossi (1983), however, produced results contradictory to those of Hoppe (1936), actually suggesting that *S. macrospora* was more virulent on young stalks than isolates of *S. maydis*. The contrasting results were partially explained by the fact that there may be strains

with differing vigour within each species. In spite of their virulence, *S. maydis* is more commonly observed in the USA (Latterell & Rossi 1983), as well as South Africa (Marasas *et al.* 1979). Although commonly associated with root and stalk rot of maize, not much is known about the pathogenicity of *P. ambiguum*, other than the study by Stovold *et al.* (1996) in Australia. Its potential role as primary pathogen was, however, confirmed in the present study, though strains of *P. ambiguum* generally appeared to be less virulent than the strains of *S. maydis* tested (Tables 2, 3). Nevertheless, *P. ambiguum* should be considered as an important pathogen of maize, and certainly as part of a soilborne disease complex could result in significant damage to maize plants. Surveys conducted for a number of seasons in the KwaZulu-Natal province showed that the incidences of both fungi increase significantly towards the end of the growing season when maize plants are often subjected to moisture stress (Results not shown). Stovold *et al.* (1996) reported that while *P. ambiguum* can cause extensive infection of maize roots the fungus did not significantly affect the growth of plants under optimal conditions of soil moisture and nutrition. Although these fungi may overwinter in infected maize residue, from where they infect the roots, mesocotyl, crown and eventually the stalks of new plants, not much is known about their host specificity, and whether they could also be isolated from grasses that grow in the vicinity of maize fields.

Based on their pigmented conidia and *Diplodia*-like morphology, both *Stenocarpella* and *Phaeocytostroma* have in the past been suspected to be members of the *Boytyosphaeriaceae*, being initially described in genera such as *Diplodia* and *Sphaeropsis*. However, Crous *et al.* (2006) revealed *Stenocarpella* to belong to the *Diaporthales*, though the phylogenetic relationships of *Phaeocytostroma* remained obscure until the present study. From the taxa treated here (Figs 1, 2), it is clear that both anamorph genera are best allocated to the *Diaporthales*, *Diaporthaceae*. This is somewhat surprising, as their pigmented conidia suggests that they might represent a separate family within the *Diaporthales*. In spite of these differences, however, no support could be obtained for polyphyly in *Diaporthaceae*. These findings suggest that as observed earlier in the *Botryosphaeriaceae* (*Botryosphaeriales*) (Crous *et al*. 2006, Phillips *et al.* 2008), conidial pigmentation appears to be uninformative at the family level, while conidiogenesis, and the ability to produce both alpha and beta conidia, appear more informative at family level in *Diaporthaceae* (*Diaporthales*).

ACKNOWLEDGEMENTS

We thank the technical staff, Alta Schoeman, Almarie Van den Heever, Thabo Phasoana, Sheryldene Williams, Gregory Anthony and John Deysel (isolations, purifications and conducting the pathogenicity test), Arien van Iperen (cultures), Marjan Vermaas (photographic plates), and Mieke Starink-Willemse (DNA isolation, amplification and sequencing) for their invaluable assistance.

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