# Phylogeny of some cercosporoid fungi from *Citrus*

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This study examines several cercosporoid species that are known to cause foliar diseases of *Citrus*. A cercosporoid fungus causing a new fruit and leaf spot disease on *Citrus* in South Africa was identified. From morphological and rDNA sequence data (ITS1, 5.8S and ITS2), it was concluded that the new disease was caused by *Cercospora penzigii*, belonging to the *Cercospora apii* species complex. It was subsequently compared with a similar organism, *Pseudophaeoramularia angolensis*, which is of quarantine significance to the citrus industry. The genus *Pseudophaeoramularia* is regarded as synonym of *Pseudocercospora*, and subsequently a new combination is proposed in *Pseudocercospora* as *P. angolensis*. *Cercospora gigantea* was shown to not represent a species of *Cercospora*, while *Mycosphaerella citri* was found to be morphologically variable, suggesting that it could represent more than one taxon. A key is also provided to the cercosporoid species occurring on *Citrus*.

Keywords: *Cercospora*, *Citrus*, Leaf spot, *Mycosphaerella*, *Pseudocercospora*, systematics.

A wide range of *Mycosphaerella* Johanson species with cercosporoid anamorphs are commonly associated with fruit and leaf spot diseases of species of *Citrus* L. Of these, two are regarded as being particularly serious. Greasy spot, caused by *Mycosphaerella citri* Whiteside (anamorph *Stenella citri-grisea* (F.E. Fisher) Sivan.) (Sivanesan, 1984), occurs in Florida and Texas (USA), the Caribbean, and Central and South America (Timmer & Gottwald, 2000). Phaeoramularia fruit and leaf spot, caused by *Pseudophaeoramularia angolensis* (T. Carvalho & O. Mendes) U. Braun, is common in sub-Saharan Africa, the Comoro Islands, and has also been reported from Yemen on the Arabian Peninsula (Seif, 2000). The most devastating effect of Phaeoramularia fruit and leaf spot is the development of fruit spots, which render the crop unmarketable. A yield loss of 50–100% is common in highly effected areas (Seif, 1995). As

Phaeoramularia fruit and leaf spot also occurs in Zimbabwe, which borders South Africa, it is of particular concern to the local citrus industry. Although the disease is presently restricted to two areas north of Harare in Zimbabwe, it has not yet spread to South Africa (Crous & al., 2000b), presumably due to unfavourable climatic conditions. This organism, however, is still regarded as of extreme phytosanitary importance.

During the course of 2000, previously unknown leaf and fruit spot disease symptoms were found associated with species of *Citrus* cultivated in Swaziland, and the Northern and Mpumalanga Provinces of South Africa. Although symptoms were not as severe as for Phaeoramularia fruit and leaf spot, the new cercosporoid disease was still regarded as a potential threat for *Citrus* cultivation. The aim of the present study, therefore, was to compare the *Cercospora* Fresen. isolates from Swaziland and South Africa to determine whether they belong to the same species, and to determine their identity. These isolates were also compared with other cercosporoid fungi occurring on *Citrus* spp., and specifically to *C. apii* Fresen., to which they were morphologically similar.

## Materials and methods

### Morphology

Herbarium and type specimens were obtained from USDA U.S. National Fungus Collections, Beltsville (BPI), CABI Bioscience, Egham, England (IMI), and the Department of Plant Pathology at the University of Florida (F). Morphological observations were made on structures mounted in clear lactophenol, and descriptions were based on collections from host material. All measurements were derived from at least 30 observations of each respective structure. Cultures were obtained from freshly collected field material (*Cercospora* sp. and *P. angolensis*) by establishing colonies from single conidia on 2% malt extract agar (MEA) (Biolab, Midrand, Johannesburg). Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U) and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

### PCR amplification and sequencing

The isolation protocol of Crous & al. (2000a) was used to isolate genomic DNA from fungal mycelia grown on MEA plates. The primers ITS1 and ITS4 were used to amplify part of the nuclear rRNA

operon using the PCR conditions recommended by the authors (White & al., 1990). The amplified region included the 3' end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5' end of the 28S (large subunit) of the rRNA gene. PCR products were separated by electrophoresis at 75 V for 1 h in a 0.8% (w/v) agarose gel in 0.5 × TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

PCR products were purified by using a NucleoSpin Extract 2 in 1 Purification Kit (Macherey-Nagel GmbH, Germany). The cycle sequencing reaction of purified PCR products was carried out with an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA) following the instructions of the manufacturer. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). Isolates subjected to molecular analysis are listed in Tab. 1. The unidentified *Cercospora* isolates from *Citrus* were compared to other species of *Cercospora*, and to *C. apii*, from which they were morphologically indistinguishable.

## Phylogenetic analysis

The nucleotide sequences generated in this study were added to a previously published data matrix (TreeBase M691, Stewart & al., 1999). *Mycocentrospora acerina* (R. Hartig) Deighton AY266155 served as outgroup. Sequences were assembled using the editor in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8a (Swofford, 2000), and aligned using the CLUSTAL W software (Thompson & al., 1994). Adjustments for improvement were done manually where necessary. Phylogenetic analyses were undertaken using PAUP. Gaps were treated as a new state and all characters were unordered and weighted equally. Heuristic searches were conducted using stepwise simple addition and tree bisection and reconstruction (TBR). The robustness of the branches was evaluated by 1,000 bootstrap replications (Hillis & Bull, 1993). A second parsimony analysis was also performed for which all missing and ambiguous characters were excluded. Tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively) were also calculated. Resulting trees were printed with TreeView Version 1.6.5 (Page, 1996) and decay indices were calculated with AutoDecay Version 4.0.2 (Eriksson, 1998). Sequences were deposited at GenBank (Tab. 1), and the alignment in TreeBase (submission number SN1397).

### Nucleotide differences between and within *Cercospora* species

The number and type of nucleotide differences between the 25 *Cercospora* sequences used in this study were tabulated using *C. apii* Fresen. AY266168 (TreeBase matrix M691; Stewart & al., 1999) as reference sequence. The differences within isolates of *C. penzigii* Sacc. were calculated separately. Separate counts for transversions, transitions, insertions and deletions in the ITS1, 5.8S and ITS2 regions, respectively, were made for all of the *Cercospora* sequences included in this paper.

## Results

### Morphology

Isolates causing the new disease on *Citrus* in Swaziland and South Africa were morphologically similar and were indistinguishable from *C. penzigii*, which is the common species of *Cercospora* occurring on this host (Chupp, 1954). They had long, fasciculate, septate, smooth, pigmented conidiophores with thickened, darkened and refractive loci. Fully developed long conidia were acicular with truncate bases, whereas young, shorter conidia were obclavate to subcylindrical with obconically subtruncate bases and darkened, thickened, refractive hila. *Cercospora apii* is a species with a wide host range and geographical distribution (Pons & Sutton, 1988), with which *C. penzigii* appears to be synonymous.

### Sequence alignment

All the *Cercospora* sequences used in the phylogenetic analysis, except for '*C. oryzae*' STE-U 4303 (one nucleotide shorter) and *C. asparagi* Sacc. AF297229 (one nucleotide longer), were exactly the same length (462 bp, including 5 bp of the 3' end of the 18S rDNA gene and 11 bp of the 5' end of the 28S rDNA gene) when alignment gaps were excluded. The alignment contained the complete sequences of the 5.8S rRNA gene, the second ITS (ITS2) region and the 5' end of the 28S (large subunit) of the rRNA gene. The complete ITS1 region was not included in the phylogenetic analysis of this study as the sequences of *P. angolensis* STE-U 4115, 4116 and 4118 included in the alignment did not contain the first eighteen nucleotides of the ITS1 region. For counting the nucleotide changes between the *Cercospora* species, however, the complete ITS1 was included. The manually adjusted alignments of the nucleotide sequences contained 520 sites for the data set (data not shown). Of

Tab. 1. – Isolates of cercosporoid species sequenced.

| Anamorph | Teleomorph | Host | Origin | Collector | Date isolated | Accession no. | GenBank no. |
|---|---|---|---|---|---|---|---|
| *Cercospora canescens* | Unknown | *Vigna* | Free State, South Africa | P. S. Van Wyk | 1995 | STE-U 1137 | AY260065 |
| *C. canescens* | Unknown | *Vigna* | Free State, South Africa | P. S. Van Wyk | 1995 | STE-U 1138 | AY260066 |
| '*C. oryzae*' | '*Sphaerulina oryzina*' | *Oryza* | Arkansas, U.S.A. | E. C. Tullis | – | STE-U 4303, IMI 303642, CBS 145.37 | AY260064 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | K. Serfontein | 2000 | STE-U 4408 | AY260067 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | K. Serfontein | 2000 | STE-U 4409 | AY260068 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | M. C. Pretorius | 2000 | STE-U 4410 | AY260070 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | M. C. Pretorius | 2000 | STE-U 4411 | AY260071 |
| *C. penzigii* | Unknown | *Citrus* | Swaziland | M. C. Pretorius | 2000 | STE-U 3946 | AY260072 |
| *C. penzigii* | Unknown | *Citrus* | Swaziland | M. C. Pretorius | 2000 | STE-U 3947 | AY260073 |
| *C. penzigii* | Unknown | *Citrus* | Swaziland | M. C. Pretorius | 2000 | STE-U 3945 | AY260074 |
| *C. penzigii* | Unknown | *Citrus* | Mpumalanga, South Africa | M. C. Pretorius | 2000 | STE-U 3948 | AY260075 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | M. C. Pretorius | 2000 | STE-U 3949 | AY260076 |
| *C. penzigii* | Unknown | *Citrus* | Northern Province, South Africa | M. C. Pretorius | 2000 | STE-U 3950 | AY260077 |

Tab. 1. – Cont.

| Anamorph | Teleomorph | Host | Origin | Collector | Date isolated | Accession no. | GenBank no. |
|---|---|---|---|---|---|---|---|
| *C. populicola* | Unknown | *Populus* | KwaZulu-Natal, South Africa | M. J. Wingfield | 1995 | STE-U 1051 | AY260069 |
| *C. zebrina* | Unknown | *Trifolium pratense* | Ottawa, Canada | K. A. Seifert | 2000 | STE-U 3955 | AY260078 |
| *C. zebrina* | Unknown | *Trifolium repens* | Ottawa, Canada | K. A. Seifert | 2000 | STE-U 3957 | AY260079 |
| *C. zebrina* | Unknown | *Trifolium repens* | Ottawa, Canada | K. A. Seifert | 2000 | STE-U 3958 | AY260080 |
| *Pseudocercospora angolensis* | Unknown | *Citrus* | Zimbabwe | M. C. Pretorius | 2000 | STE-U 4116 | AY260061 |
| *P. angolensis* | Unknown | *Citrus* | Zimbabwe | M. C. Pretorius | 2000 | STE-U 4115 | AY260062 |
| *P. angolensis* | Unknown | *Citrus* | Zimbabwe | M. C. Pretorius | 2000 | STE-U 4118 | AY260063 |
| *Pseudocercospora* sp. | *Mycosphaerella* sp. | *Acacia* | Venezuela | M. J. Wingfield | 2000 | STE-U 3837 | AY260060 |

the aligned nucleotide sites for the data set, 166 characters were parsimony-informative, 127 variable characters were parsimony-uninformative and 227 were constant.

## Phylogenetic relationships

The aligned sequences of 37 isolates and an outgroup were subjected to maximum parsimony analysis, and only a single most parsimonious tree was obtained and evaluated with 1,000 bootstrap replications. All 25 *Cercospora* sequences grouped in a strongly supported clade (99% support) (Fig. 1) as did *Pseudocercospora* Speg. (99%) and *Stenella* Syd. (100%). In the main *Cercospora* clade, '*C. oryzae*' [= *Passalora janseana* (Racib.) U. Braun] STE-U 4303 and '*C. canescens*' AY266164 (TreeBase matrix M691; Stewart & al., 1999) (identifications could not be confirmed) were found outside a clade containing the rest of the *Cercospora* species (74%). *Cercospora zebrina* Pass., a species with acicular to cylindrical-filiform conidia, formed a clade with a bootstrap support value of 63% within the larger *Cercospora* clade. Excluding all missing and ambiguous characters from the analysis did not change the topology of the tree.

## Nucleotide differences between and within *Cercospora* species

The decrease in length for '*C. oryzae*' STE-U 4303 can be ascribed to a deletion of a G at character 357, and the increase in length for *C. asparagi* AF297229 can be accounted for by an extra C at character 101 of the alignment (Tab. 2). Eight isolates had sequences identical to *C. apii* CA1 (TreeBase matrix M691; Stewart & al., 1999): *C. canescens* Ellis & G. Martin STE-U 1137 & 1138, *C. nicotianae* Ellis & Everh. AF297230, *C. sorghi* Ellis & Everh. *f. maydis* AF297232, *C. beticola* Sacc. AF222827, *C. penzigii* STE-U 4408 & 4409 and *C. hayi* Calp. CH6 (TreeBase matrix M691, Stewart & al. 1999). Of the remaining sixteen isolates, '*C. oryzae*' STE-U 4303 and '*C. canescens*' AY266164 (TreeBase matrix M691, Stewart & al. 1999) differed most from *C. apii* AY266168 (TreeBase matrix M691, Stewart & al. 1999), with changes at nine and three positions respectively. Eighteen changes were observed for the eleven *Cercospora* species studied (Tab. 2), resulting in a difference of 1.64 (18 changes over 11 species) nucleotides between species. Goodwin & al. (2001) calculated an overall mean of 1.27 differences between taxa in their *Cercospora* cluster, which is slightly lower than what we found. This might be ascribed to the sampling of 18 isolates representing 11 species by Goodwin & al. (2001) whereas 25 isolates representing 11

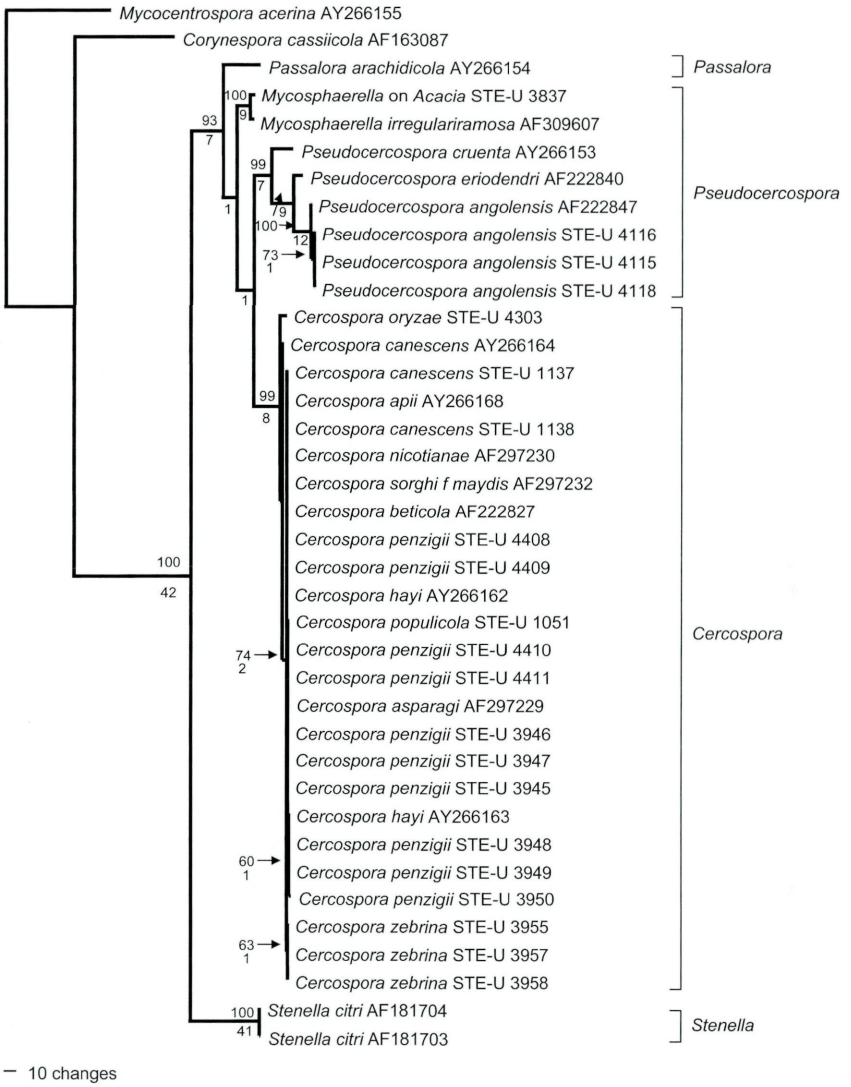


Fig. 1. – Single most parsimonious tree obtained from a heuristic search using simple taxon additions (TL = 550 steps, CI = 0.836, RI = 0.852, RC = 0.712). Bootstrap and decay values are shown at the nodes, above and below the branches, respectively. *Mycocentrospora acerina* was included as outgroup.

species were sampled in the present study. Within *Cercospora*, twelve transitions, four transversions and a single duplication and deletion were observed. Goodwin & al. (2001) also observed more transitions than transversions for *Cercospora* and *Mycosphaerella* based on the ITS region.

Tab. 2. – Nucleotide differences observed for *Cercospora* species included in this study. Base positions include spaces caused by alignment gaps.

| | **ITS1** | | | | | **5.8S rRNA gene** | | | | | **ITS2** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | base 69 | base 101 | base 147 | base 148 | base 149 | base 280 | base 293 | base 334 | base 357 | base 360 | base 464 | base 500 | base 502 |
| *C. apii* CA1[5,6] | T | – | A | G | T | C | G | C | G | T | G | C | C |
| *C. asparagi* AF297229 | | C[3] | | | | | | | | | | | T[1] |
| *C. canescens* CCA196 | | | | | | T[1] | A[1] | A[2] | | | | | |
| *C. oryzae* STE-U 4303 | C[1] | | C[2] | A[1] | C[1] | T[1] | A[1] | A[2] | -[4] | C[1] | | | |
| *C. penzigii* STE-U 4410 | | | | | | | | | | | | | T[1] |
| *C. penzigii* STE-U 4411 | | | | | | | | | | | | | T[1] |
| *C. penzigii* STE-U 3946 | | | | | | | | | | | | | T[1] |
| *C. penzigii* STE-U 3947 | | | | | | | | | | | | | T[1] |
| *C. penzigii* STE-U 3945 | | | | | | | | | | | | | T[1] |
| *C. populicola* STE-U 1051 | | | | | | | | | | | | | T[1] |
| *C. penzigii* STE-U 3948 | | | | | | | | | | | | T[1] | T[1] |
| *C. penzigii* STE-U 3949 | | | | | | | | | | | | T[1] | T[1] |
| *C. penzigii* STE-U 3950 | | | | | | | | | | | | T[1] | T[1] |
| *C. hayi* CH5[6] | | | | | | | | | | | | T[1] | T[1] |
| *C. zebrina* STE-U 3955 | | | | | | | | | | | C[2] | | |
| *C. zebrina* STE-U 3957 | | | | | | | | | | | C[2] | | |
| *C. zebrina* STE-U 3958 | | | | | | | | | | | C[2] | | |

[1] Transition.
[2] Transversion.
[3] Insertion / duplication of leading nucleotide.
[4] Deletion.
[5] Sequences identical to *C. apii*: *C. canescens* STE-U 1137, *C. canescens* STE-U 1138, *C. nicotianae* AF297230, *C. sorghi* f. *maydis* AF297232, *C. beticola* AF222827, *C. penzigii* STE-U 4408, 4409, *C. hayi* CH6*.
[6] Sequences obtained from TreeBase matrix M691.

Based on the ITS sequence, *C. penzigii* is distributed over three groups (Tab. 2): the first group contains two isolates (STE-U 4408 & 4409) identical to *C. apii* AY266168 (TreeBase matrix M691); the second group contains five isolates (STE-U 4410, 4411, 3946, 3947 & 3945) as well as *C. populicola* Tharp STE-U 1051, that differed from *C. apii* AY266168 (TreeBase matrix M691) at character 502; and the final group consisted of three isolates (STE-U 3948, 3949 & 3950) that contained the same two changes as in *C. hayi* AY266163 (TreeBase matrix M691). The third group has the same change at character 502 as the second group, but also an additional change at character 500. The *C. penzigii* of the first group was isolated on citrus fruit, whereas the *C. penzigii* isolates in groups two and three were isolated from leaf spots.

There was no difference between the number of changes in the ITS1 and ITS2 regions of the *Cercospora* sequences (5 changes each between the eleven species). However, eight changes in the sequence of the 5.8S gene were observed among the eleven species. All eight changes occurred in '*C. oryzae*' STE-U 4303 and '*C. canescens*' AY266164 (TreeBase matrix M691), whereas no changes were observed for this region in the rest of the *Cercospora* isolates. Goodwin & al. (2001) also reported a very small difference in the number of changes between the ITS1 and ITS2 region, but found no changes in the 5.8S gene. As '*C. oryzae*' STE-U 4303 and '*C. canescens*' AY266164 (TreeBase matrix M691) clustered outside the main *Cercospora* clade, it appears that they are not part of the *C. apii* complex, and that the *Cercospora* isolates in the main clade (74 % bootstrap support) represent *C. apii sensu lato*.

## Treatment of species

*Cercospora gigantea* F. E. Fisher, Phytopathology 51: 300. 1961. – Fig. 2.

Hosts and distribution. – *Citrus sinensis* Pers., *C. paradisi* Macfad. (Rutaceae), USA (FL).

Specimen examined. – USA, Florida, Orange County, Winter Park, on grapefruit leaves, F. Fisher, 28 May 1957, F-46419 (holotype).

*Cercospora gigantea* was described as having straight, fasciculate conidiophores with broad, 3–12-septate, brown conidia with rounded apices and beveled bases, 80–180 × 6–8 µm (Fisher, 1961). Although the specimen is in a poor condition, a few conidia fitting this description were found. However, the conidia are distoseptate with darkened, thickened hila, and resemble those of *Corynespora citricola* M. B. Ellis (Ellis, 1971). The poor quality of the type specimen, however, made it impossible to resolve this issue.

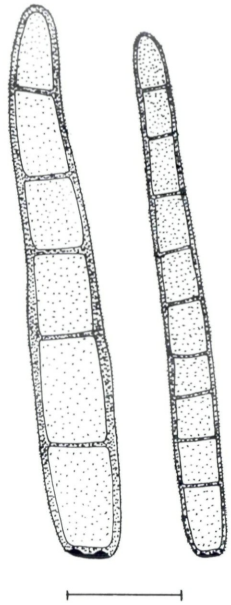

Fig. 2. – Conidia of *Cercospora gigantea* (F 46419). – Bar = 10 µm.

*Cercospora penzigii* Sacc., Syll. Fung. 15: 84. 1901. – Fig. 3.

≡ *Cercospora fumosa* Penz., Michelia 2: 476. 1882, non *C. fumosa* Speg., 1880.

= *Cercospora aurantia* Heald & F.A. Wolf, Mycologia 3: 15. 1911.

= *Cercospora daidai* Hara, List of Japanese Fungi ed. 4: 400. 1954.

Leaf spots amphigenous, circular to irregular, 2–30 mm diam., pale to dark brown, margin raised on lower surface, medium brown, surrounded by a chlorotic zone. – Caespituli chiefly hypophyllous, fascicles dense to loose and divergent; more compact with shorter conidiophores on epiphyllous surface. – Stromata medium to dark brown, erumpent, up to 70 µm diam.; fascicles grey (compared to brown tufts of *P. angolensis*). – Mycelium internal, pale brown, consisting of septate, branched, smooth hyphae, 3–4 µm. – Conidiophores in loose to dense fascicles, arising from stromata, straight to geniculate-sinuous, subcylindrical, unbranched, 20–300 × 4–6.5 µm, multi-septate, pale to medium brown, smooth. – Conidiogenous cells terminal, pale brown, smooth, tapering to a subobtuse or swollen apex, 20–60 × 3–5 µm; scars thickened, darkened and refractive. – Conidia solitary, long, fully developed conidia acicular, short conidia obclavate or subcylindrical, 50–300 × 2.5–5 µm, multi-septate, hyaline, apex obtuse to subacute to subobtuse, base truncate in acicular conidia or long obconically

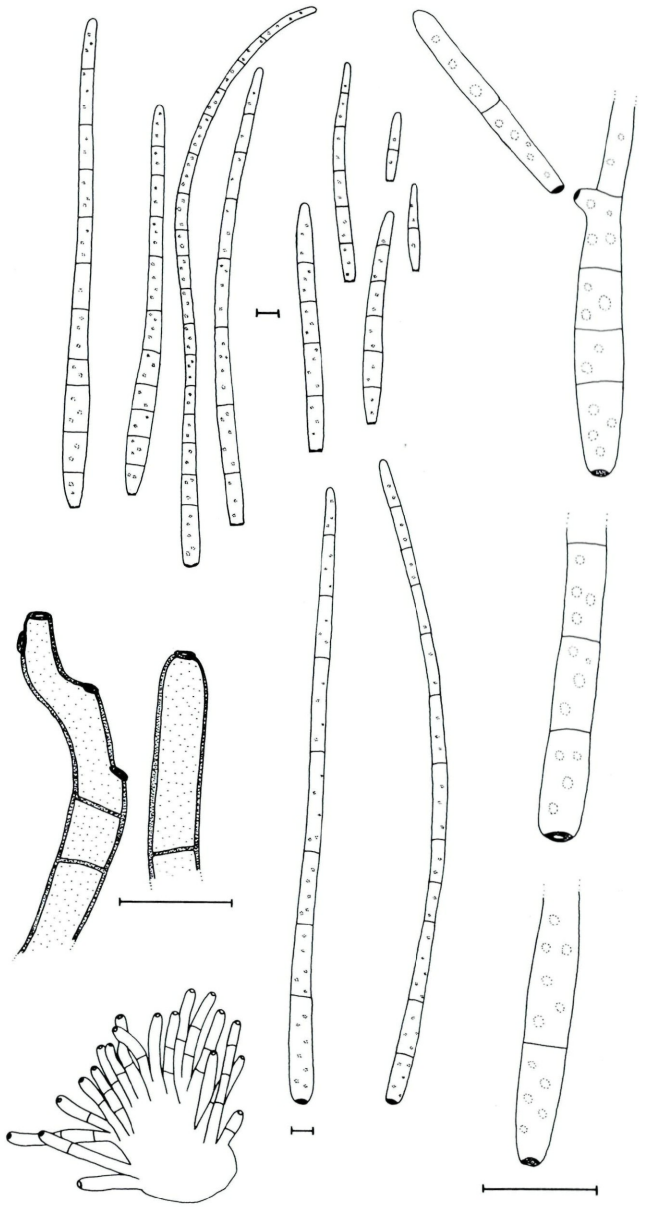

Fig. 3. – Fascicles, conidiophores and conidia of *Cercospora penzigii* (herb CBS 6591). – Bars = 10 µm.

subtruncate in obclavate-cylindrical conidia, hilum thickened, darkened and refractive; secondary conidia arising via microcyclic conidiation hyaline, subcylindrical to acicular or obclavate, 1–3-

septate, 15–35 × 2–3.5 µm, with thickened, darkened and refractive hila.

Hosts and distribution. – *Araujia albens* G. Don, *Citrus aurantium* L., *C. junos* Sieb. ex Tanaka, *C. limon* (L.) Burm. f, *C. natsudaidai* Hayata, *C. nobilis* Lour., *C. paradisi* hybrid, *C. sinensis*, *Dictamnus dasycarpus* Turcz., *Poncirus trifoliatus* Rafin. (Rutaceae).

Algeria, Argentina, Azerbaijan, Bhutan, Caucasus, China, Cuba, Dominican Republic, India, Italy, Japan, Mexico, Papua New Guinea, Senegal, South Africa, Swaziland, USA (FL, MS, TX), Venezuela.

Specimens examined. – ITALY: Padova, 'R. Horto Agrario', on leaves of *Citrus medica*, O. Penzig, 15 Jun. 1881, ex herb. Penzig [Staz. Patol. Veg. Roma], IMI 47696 (slide), type material of *C. penzigii*. BHUTAN, on leaves of *Citrus medica* v. *limon*, W.T.H. Peregrine SIB 385, 28 May 1985, IMI 295922. DOMINICAN REPUBLIC: intercepted at JFK airport, USA, on leaves of *Citrus* sp., K. Uchida, 6 Jun. 1975, BPI 439366; *Citrus* sp., R. Green, 29 Jun. 1983, BPI 439362. MEXICO, intercepted at Nogales, on living leaves of *Citrus* sp., W. Jackson, 15 Oct. 1965, BPI 439359. PAPUA NEW GUINEA: Laloki Q Stn., Port Moresby, on leaves of *Citrus* sp., A. Williams 6234b, 10 Oct. 1968, IMI 136538b. SOUTH AFRICA: Komatipoort, on leaves of *Citrus sinensis*, M. C. Pretorius, 6 Sep. 2000, herb CBS 6591, culture STE-U 3948-3950; Northern Province, Tsipise, on leaves and fruit of citrus pomelo, K. Serfontein, Sep. 2001, STE-U 4408, 4409; Northern Province, Messina, on leaves and fruit of *Citrus* sp., M. C. Pretorius, Sep. 2001, STE-U 4410, 4411. SWAZILAND: on leaves of *Citrus sinensis*, M. C. Pretorius, 6 Sep. 2000, herb CBS 6592, culture STE-U 4001; on leaves of *Citrus sinensis*, M. C. Pretorius, Oct. 2000, herb CBS 6593, culture STE-U 4002. USA, Texas, Falfurrias, on leaves of *Citrus aurantium*, F. D. Heald & F. A. Wolf, no. 2446, 14 Sept. 1909, BPI 433199 (holotype of *C. aurantia*), BPI 433198, photomicrographs of type.

*Cercospora penzigii* is morphologically similar to other cercosporoid species that are commonly referred to as part of the *Cercospora apii*-complex. This suggests that *C. penzigii* could have a wide host range (other than Rutaceae) and distribution. Morphologically this is a highly variable taxon with regards to conidiophore length, arrangement of scars on the conidiogenous cells (*in vitro* vs. *in vivo*), conidium length, shape, basal cell taper and fascicle morphology.

As shown in the present study, numerous *Cercospora* species are indistinguishable from the *C. apii* complex based on morphology and ITS sequence data (Fig. 1). It is tempting to reduce them all to synonymy with *C. apii*, as inoculation studies have also shown many of these taxa to exhibit cross-pathogenicity between hosts (Johnston & Valleau, 1949; Berger & Hanson, 1963; Kaiser & Lukezic, 1965), but as we presently only have one molecular data set at our disposal, we will refrain from doing this step formally until a multi-locus DNA data set has been established for the *Cercospora* complex surrounding *C. apii*. ITS sequence data is a valuable tool for species

identification, but insufficient as sole data set on which to base species synonymies. Additional data sets are therefore presently being generated to address host specificity in *Cercospora*.

*Mycosphaerella citri* Whiteside, Phytopathology 62: 263. 1972. – Fig. 4.

Anamorph: *Stenella citri-grisea* (F.E. Fisher) Sivan., In Sivanesan, The bitunicate ascomycetes and their anamorphs: 226. 1984.

≡ *Cercospora citri-grisea* F.E. Fisher, Phytopathology 51: 300. 1961.

This species was treated in detail by Sivanesan (1984, pp. 226–228).

Hosts and distribution. – Species of *Aeglopsis* Swingle, *Citrus*, *Fortunella* Swingle, *Murraya* L., *Poncirus* Rafin. (Rutaceae).

Brazil, Costa Rica, Cuba, Dominican Republic, El-Salvador, Gabon, Haiti, Hong Kong, Japan, Puerto Rico, Surinam, Taiwan, Thailand, USA (FL, HI, TX), Venezuela, Virgin Islands.

Specimens examined. – USA, Florida, Lake Alfred & Haines City, on leaves of *Citrus* sp., F. E. Fisher, May 1970, IMI 148810. VENEZUELA: on leaves of *Citrus sinensis*, R. Urtiaga 1560, 5 Jun. 1972, IMI 166609. DOMINICAN REPUBLIC: intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 19 Oct. 1967, BPI 439357; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 23 Nov. 1969, BPI 439358; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 21 Dec. 1968, BPI 439360; intercepted at JFK airport, USA, on leaves of *Citrus* sp., D. Walters, 19 Jun. 1970, BPI 439361; intercepted at JFK airport, USA, on leaves of *Citrus* sp., Heliczer, 23 Oct. 1969, BPI 439364; intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Locklear, 14 Nov. 1969, BPI 439365; intercepted at JFK airport, USA, on leaves of *Citrus* sp., R. Iwamoto, 3 Oct. 1967, BPI 439355; intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Smock, 26 Feb. 1968, BPI 439354; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Wong, 4 Apr. 1968, BPI 439356. HAITI, on leaves of *Citrus* sp., N. R. Manalo, 14 Apr. 1985, BPI 439367. WEST INDIES: intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Locklear, 17 Aug. 1968, BPI 439363. PUERTO RICO, intercepted at San Juan airport, on leaves of *Citrus* sp., C. M. Looke, 24 Oct. 1950, BPI 439353. HONG KONG: on leaves of *Citrus sinensis*, H. R. Mills, 13 Mar. 1970, BPI 431901. EL SALVADOR, on leaf of *Citrus* sp., J. Okamura, 1 Feb. 1983, BPI 420196; on leaf of *Citrus sinensis*, D. Bickell, 11 Aug. 1984, BPI 607682. HAWAII, Poamoho, on leaves of *Citrus sinensis*, J. Fine & L. M. Chilson, 20 Dec. 1954, BPI 602133.

A similar disease, also known as greasy spot, has been observed on *Citrus* in Australia (Timmer & Gottwald, 2000). Examination of a voucher specimen (Australia, Nambour, on leaves of *Citrus latifolia*, R. Thomas, BRIP 14527, 13 Jun. 1984, IMI 290702) found ascospores to be similar in size (10–12 × 2.5–3 µm) to those of *M. citri*, guttulate and fusiform, widest in the middle of the apical cell, and not con-

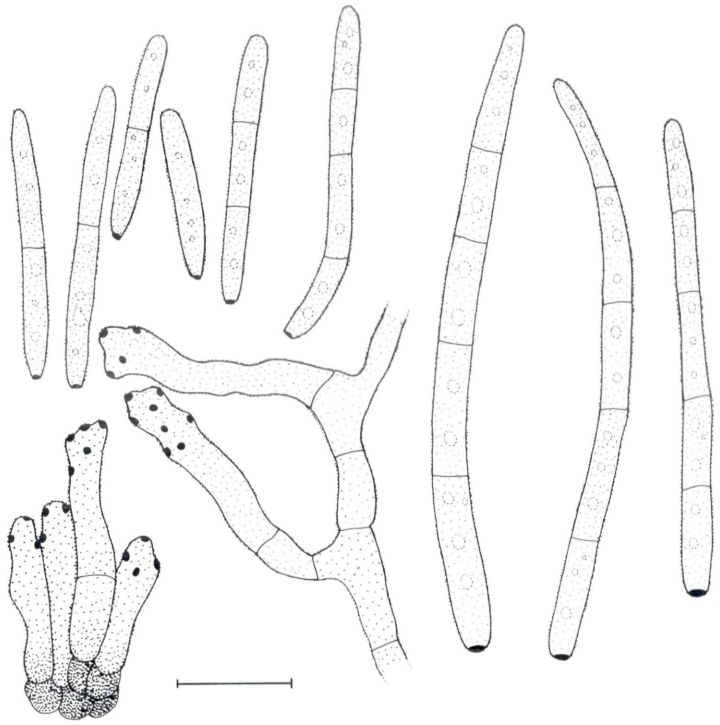

Fig. 4. – Conidiophores and conidia of *Stenella citri-grisea*, the anamorph of *Mycosphaerella citri* (IMI 148810). – Bar = 10 μm.

stricted at the median septum. Symptoms vary, however, in being small, hypophyllous black specks surrounded by chlorotic halos (Timmer & Gottwald, 2000). It appears, therefore, that the Australian species represents yet another distinct species on *Citrus*. A further species of *Mycosphaerella* known to occur on *Citrus* in Japan is *M. horii* Hara (Timmer & Gottwald, 2000). After numerous attempts, however, we were unable to obtain any type material. Ascospores were reported to be 9–12.5 × 2.5–3 μm (Corlett, 1991).

Conidiophore fascicles of *S. citri-grisea* tend to be predominantly associated with spermatogonia or pseudothecia. Specimen BPI 439371 showed a lot of variation regarding ascospore size, with ascospores being up to 15 μm long and 4 μm wide. The anamorph is also highly variable, suggesting that this may, in fact, be a species complex. In some collections there is abundant superficial mycelium (BPI 420196), and short, narrow conidia, while in others conidia are borne on fascicles, and are long, wide and flexuous. Cultures and molecular studies would be required, however, to resolve the variation observed within *S. citri-grisea*.

***Pseudocercospora angolensis*** (T. Carvalho & O. Mendes) Crous & U. Braun, **comb. nov.** – Fig. 5.

≡ *Cercospora angolensis* T. Carvalho & O. Mendes, Bol. Soc. Brot. 27: 201. 1953.

≡ *Phaeoramularia angolensis* (T. Carvalho & O. Mendes) P. M. Kirk, Mycopathologia 94: 177. 1986.

≡ *Pseudophaeoramularia angolensis* (T. Carvalho & O. Mendes) U. Braun, Crypt. Mycol. 20: 171. 1999.

This species was treated in detail by Kirk (1986).

Host range and distribution. – *Citrus sinensis*, *Citrus* spp. (Rutaceae).

Angola, Burundi, Cameroon, Central African Republic, Comoros, Congo, Ethiopia, Gabon, Gambia, Guinea, Ivory Coast, Kenya, Mozambique, Nigeria, Tanzania, Togo, Uganda, West Africa, Yemen, Zaire, Zambia, Zimbabwe.

Specimens examined. – ANGOLA: Mozambique Province, on leaves of *Citrus sinensis*, T. Carvalho & O. Mendes, Dec. 1951, BPI 432660, BPI 442839 (isosyntype), BPI 442837 (type). CAMAROON: Yaoundé, on leaves of *Citrus sinensis*, E. Milla, 17 Mar. 1978, IMI 252792. ETHIOPIA: on leaves of *Citrus* sp., ??, IMI 361170. KENYA: on leaves of *Citrus sinensis*, A. Seif W3753, 15 Nov. 1991, IMI 351626. UGANDA: on leaves of *Citrus sinensis*, W.T.H. Peregrine, 14 Jun. 1991, IMI 384297. WEST AFRICA: intercepted at San Pedro, California, USA, on leaves of *Citrus* sp., L. A. Hart, 2 Oct. 1953, BPI 432661, BPI 432659. ZAMBIA: on leaves of *Citrus* sp., R. H. Raemakers 7837, 18 Jun. 1973, IMI 176562; Chilanga, on leaves of *Citrus aurantium*, D. M. Naik, 28 Sep. 1983, IMI 280618; Chilanga, on leaves of *Citrus* sp., B. K. Patel, 18 Jul. 1975, IMI 196889; Lusaka, on leaves of *Citrus* sp., I. Javaid, 17 Jun. 1977, IMI 214501. ZIMBABWE, Bindura, on leaves of *Citrus* sp., A. Rothwell, 13 Aug. 1979, IMI 240682; on leaves of *Citrus* sp., M. C. Pretorius, Sep. 2000, STE-U 4111–4118.

*Cercospora angolensis* was originally described as having hyaline, subclavate conidia (De Carvalho & Mendes, 1953). For this reason it was seen as distinct from a *Phaeoisariopsis* Ferraris species causing a severe disease on *Citrus* in Nigeria (Emechebe, 1980). Kirk (1986) found this to be the same organism, and placed the fungus in *Phaeoramularia* Munt.-Cvetk. as *P. angolensis* based on its conspicuous, slightly pigmented scars, and pale brown catenulate conidia. Braun & Mel'nik (1997) established the genus *Pseudophaeoramularia* U. Braun for species with unthickened or only very slightly thickened, but somewhat darkened-refractive scars (intermediate between *Pseudocercospora* and *Phaeoramularia*) and hence proposed the combination *Pseudophaeoramularia angolensis*. In a later molecular study, however, Crous & al. (2001) reported that genera with such scars as in *Paracercospora* and *Pseudophaeoramularia* belong in *Pseudocercospora* (Fig. 1).

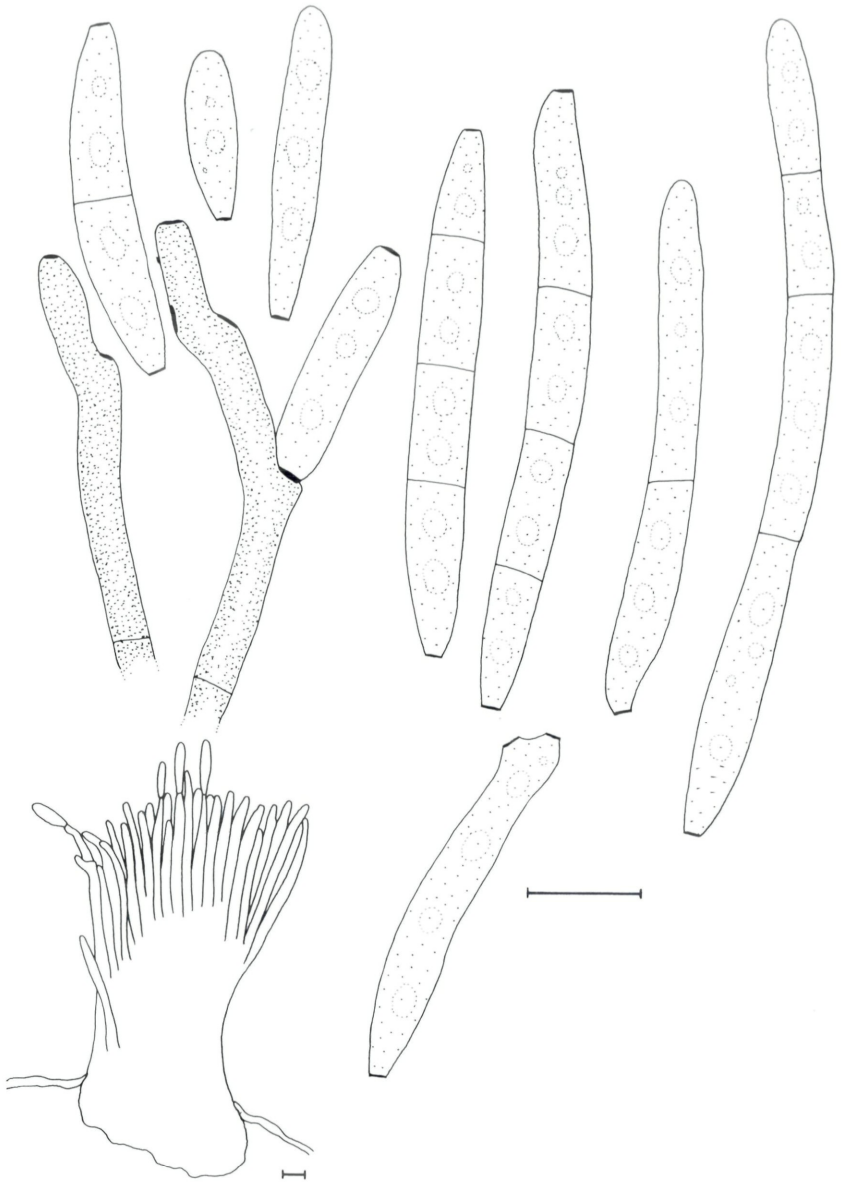

Fig. 5. – Conidiophores, conidiogenous cells and conidia of *Pseudocercospora angolensis* (IMI 176562). – Bars = 10 µm.

Because this has again been confirmed in the present study, a new combination for *Cercospora angolensis* is herewith proposed in *Pseudocercospora*.

Key to cercosporoid species on *Citrus*[1]

1. Conidia hyaline, acicular or obclavate to subcylindrical, 50–300 × 2.5–5 µm, multiseptate, with thickened, darkened, refractive hila ..... *Cercospora penzigii* (= *C. apii s.lat.*)
1*. Conidia pigmented ..... 2
2. Conidia and superficial mycelium verruculose; conidia pale olivaceous, subcylindrical to narrowly obclavate, catenulate, hila thickened, darkened, refractive, 6–50 × 2–4.5 µm, (0–)3–6(–9)-septate ..... *Stenella citri-grisea* (*M. citri*)[2]
2*. Conidia and superficial mycelium smooth ..... 3
3. Conidial hila and scars inconspicuous, or minutely thickened . 4
3*. Conidial hila and scars prominently thickened, darkened and refractive ..... 5
4. Conidia solitary, narrowly obclavate, base narrowly subtruncate, 3–4 µm wide, scars inconspicuous; occurring on leaves only .... ..... *Pseudocercospora citri* Crous & U. Braun[3]
4*. Conidia solitary or catenulate, cylindrical to obclavate, base truncate, 4–5(–6.5 µm) wide, scars inconspicuous or minutely thickened; occurring on leaves and fruit ..... ..... *Pseudocercospora angolensis*
5. Conidia 1–6-septate, 28–60 × (1.5–)2(–2.5) µm ..... ..... *Passalora citrigena* Crous & U. Braun (*Mycosphaerella citrigena* Crous & U. Braun)[1]
5*. Conidia 0–1(–3)-septate, 18–35 × 4–5 µm .... *Passalora citricola*[1]

[1] For additional species on *Citrus* and Rutaceae see Crous & Braun (2003).
[2] Regarded as a species complex.
[3] Described in Braun & al. (2003).

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