Morphological and molecular characterisation of *Mycosphaerellaceae* associated with the invasive weed, *Chromolaena odorata*

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Chromolaena odorata, originating from South and Central America, is a bushy shrub invasive in many tropical and subtropical regions of the world. Several species of *Mycosphaerellaceae* have been reported on *C. odorata* in recent years as potential biological control agents. As a result, several exploratory trips were undertaken to south, north and Central America from 1988 until 1997 to survey for pathogens on *C. odorata*. Three new species of *Passalora*, and one species of *Septoria* and *Pseudocercospora*, respectively, are newly described. Furthermore, *Septoria ekmaniana* and *Passalora perfoliati* were also confirmed from *Chromolaena* during the course of this study.

Key words: biological control, morphology, plant pathogens, phylogenetics.

Introduction

Chromolaena odorata (L.) R.M. King & H. Rob. (*Asteraceae*) is an invasive bushy shrub of Neotropical origin, and is regarded as one of the worst weeds in the Old World tropics and subtropics (Holm *et al.*, 1977). It forms dense impenetrable thickets that displace other vegetation and create fire hazards due to its flammability. At present it forms two distinct centres of invasion in Africa: one in southern Africa, moving northwards, and one in West and Central Africa, moving south and east (McFadyen and Skarratt, 1996). *Chromolaena odorata* also invades most areas in the humid paleotropics and subtropics (India, South East Asia, Indonesia, Philippines, Papua New Guinea, parts of Oceania), and is predicted to spread further (McFadyen and Skarratt,

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1996). Long distance dispersal in the bodywork of long distance vehicles has been reported in Australia (Vanderwoude *et al*., 2005). In all areas it impacts seriously on biodiversity and agriculture. Due to its shrubby nature and its ability to resprout after cutting, this plant is difficult to control both chemically and mechanically.

In its native environment, *C. odorata* is attacked by a number of insects and pathogens being considered as potential candidates for biocontrol agents (Cruttwell, 1974). Insect species such as the stem gall fly *Cecidochares connexa* is used on many of the larger Indonesian islands, as well as Palau, Papau New Guinea and the Philippines, as a biological control agent (Cruttwell-McFayden *et al*., 2003). Although *C. odorata* has been the subject of biocontrol programmes for three decades (Muniappan, 1988), the potential for using plant pathogens as biocontrol agents has only recently been considered (TeBeest, 1991). Several pathogens with good potential as biological control agents on chromolaena have been identified worldwide (Elango *et al*., 1993; Barreto and Evans, 1994). Species of *Mycosphaerellaceae* are usually assumed to be host-specific (Kirschner *et al*., 2004; Schubert and Braun, 2005; Cortinas *et al*. 2006; Crous *et al*. 2004a,b, 2006a-c). Within this family, three pathogens of chromolaena have received considerable attention in the past, namely *Pseudocercospora eupatorii-formosani* U. Braun & Bagyan., *Passalora perfoliati* (Ellis & Everh.) U. Braun & Crous, and *Septoria ekmaniana* Petr. & Cif. (Barreto and Evans, 1994).

Prior to the release of any organism for use as a biological control agent, an assessment of biocontrol activity is performed *in vitro* (Avis *et al*., 2001), while the identity and relatedness of the newly found strains are also established based on morphology and molecular data. The present paper describes the morphological characteristics and phylogenetic relationship among isolates of *Mycosphaerellaceae* pathogenic to chromolaena, which were collected from several geographical origins during the course of this study.

Materials and methods

Isolates

Symptomatic leaves were placed in moisture chambers to enhance sporulation. Monoconidial cultures were subsequently established on water-agar (WA) (20 g agar / 1 L distilled H_2O). Colonies were induced to sporulate on oatmeal-agar (OA) (15 g of oatmeal, 20 g agar / 1 L distilled water), and potato-dextrose agar (PDA) (200 g potatoes, 20 g dextrose, 20 g agar / 1 L distilled water) (Gams *et al*., 1998). Dishes of all media were point inoculated and incubated at 25°C under continuous near-ultraviolet light, and inspected for sporulation at weekly intervals.

DNA isolation, amplification and phylogeny

Genomic DNA was isolated from mycelium growing on agar plates following the protocol of Lee and Taylor (1990). The primers ITS1 and ITS4 (White *et al*. 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3’ end of the 18S rDNA (SSU), the first internal transcribed spacer (ITS1), the 5.8S rDNA, the second ITS region and the 5’ end of the 28S rDNA gene (LSU). Primers CylH3F and CylH3R (Crous *et al*., 2004c) were used to amplify and sequence part of the histone H3 gene (HIS). PCR conditions and protocols follow Crous *et al*. (2004b). The nucleotide sequences generated in this study were added to other sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov) and the alignment was assembled using Sequence Alignment Editor v. 2.0a11 (Rambaut, 2002) with manual adjustments for visual improvement where necessary. Phylogenetic analyses of sequence data were done using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002) as described by Lee *et al*. (2004). Sequence data were deposited in GenBank (Table 1) and the alignment in TreeBASE (accession number SN2908).

Taxonomy

Morphological observations were based on host material were available, or on cultures sporulating on PDA or OA. Cultures were incubated at 25°C under a 12 h cool white light/dark regime, and colony colours determined according to Rayner (1970). Slide preparations were mounted in lactic acid for microscopic examination. Thirty observations (×1000) were made of each structure, the 95% intervals determined for conidial measurements, and the extremes given in parentheses. Descriptions and nomenclatural details were deposited in MycoBank (www.MycoBank.org), and the cultures and herbarium specimens in the Centraalbureau voor Schimmelcultures (CBS) in Utrecht, the Netherlands.

Results

DNA phylogeny

Approximately 520 and 390 bases were determined for the ITS and HIS loci, respectively, of the isolates listed in Table 1. Because HIS sequences were

Table 1. Species of *Mycosphaerellaceae* isolated from *Chromolaena odorata* and other hosts and included for sequence analysis.

Species	Accession number[1]	Host	Country	Collector	GenBank numbers (ITS, HIS)[2]
Passalora caribensis	CBS 113374 = 481	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676512, DQ676537
	CBS 113375 = 482	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676513, DQ676538
	CBS 113376 = 487	*Chromolaena odorata*	Cuba	S. Neser	DQ676514, DQ676539
	CBS 113380* = 498	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676515, DQ676540
	CBS 113381 = 500	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676516, DQ676541
Passalora chromolaenae	CBS 113371 = 450	*Chromolaena odorata*	Mexico	M.J. Morris	DQ676517, DQ676542
	CBS 113611* = 452	*Chromolaena odorata*	Mexico	M.J. Morris	DQ676518, DQ676543
Passalora convoluta	CBS 113377* = 488	*Chromolaena odorata*	Costa Rica	M.J. Morris	DQ676519, DQ676544
Passalora perfoliati	CBS 113378* = 494	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676520, DQ676545
	CBS 113379 = 495	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676521, DQ676546
Passalora sp.	CBS 113382 = 460	*Chromolaena odorata*	USA	M.J. Morris	DQ676522, DQ676547
	CBS 113383 = 472	*Chromolaena odorata*	USA	M.J. Morris	DQ676523, DQ676548
	CBS 113384 = 499	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676524, DQ676549
	CBS 113613 = 486	*Ageratina adenophora*	Guatemala	M.J. Morris	DQ676525, DQ676550
Pseudocercospora eupatoriella	CBS 113366 = 437	*Chromolaena odorata*	USA	M.J. Morris	DQ676526, DQ676551
	CBS 113367 = 439	*Chromolaena odorata*	USA	M.J. Morris	DQ676527, DQ676552
	CBS 113368 = 444	*Chromolaena odorata*	USA	M.J. Morris	DQ676528, DQ676553
	CBS 113369 = 447	*Chromolaena odorata*	USA	M.J. Morris	DQ676529, DQ676554
	CBS 113370 = 448	*Chromolaena odorata*	USA	M.J. Morris	DQ676530, DQ676555
	CBS 113372* = 477	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676531, DQ676556

Table 1 continued. Species of *Mycosphaerellaceae* isolated from *Chromolaena odorata* and other hosts and included for sequence analysis.

Species	Accession number[1]	Host	Country	Collector	GenBank numbers (ITS, HIS)[2]
Pseudocercospora sp.	CBS 113386 = 469	*Chromolaena odorata*	Mexico	M.J. Morris	DQ676532, DQ676557
	CBS 113387 = 502	*Lantana camara*	Jamaica	M.J. Morris	DQ676533, DQ676558
Septoria chromolaenae	CBS 113373* = 478	*Chromolaena odorata*	Jamaica	M.J. Morris	DQ676534, DQ676559
Septoria ekmaniana	CBS 113385* = 491	*Chromolaena odorata*	Mexico	M.J. Morris	DQ676535, DQ676560
	CBS 113612 = 492	*Chromolaena odorata*	Mexico	M.J. Morris	DQ676536, DQ676561

[1]CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; ex-type strains indicated with an asterisk.
[2] ITS: internal transcribed spacer region, HIS: partial histone H3 gene.

not available for the taxa downloaded from GenBank, trees were calculated for each data set individually. Neighbour-joining analysis using three substitution models (uncorrected "p", Kimura 2-parameter and HKY85) on the sequence data yielded trees with similar topology and bootstrap values.

The ITS data matrix contained 60 taxa (including the two outgroup isolates) and 500 characters including alignment gaps. Of these characters, 178 were parsimony-informative, 32 were variable and parsimony-uninformative, and 290 are constant. Parsimony analysis of the alignment yielded 670 most parsimonious trees (TL = 450 steps; CI = 0.696; RI = 0.917; RC = 0.638), one of which is shown in Fig. 1. These trees mainly differed in the order of the taxa within the two main clades, as can be seen from the thickened consensus branches and distribution of bootstrap support values in Fig. 1. The ITS phylogeny shows that isolates from *Chromolaena odorata* and the other hosts listed in Table 1 group with species of *Passalora*, *Septoria* and *Pseudocercospora*. None of the ITS sequences of the new strains is identical to any ITS sequence currently available in the NCBI GenBank sequence database.

The HIS data matrix contained 27 taxa (including the two outgroup taxa) and 371 characters including alignment gaps. Of these characters, 99 were parsimony-informative, 13 were variable and parsimony-uninformative, and 259 were constant. Parsimony analysis of the alignment yielded two most parsimonious trees (TL = 300 steps; CI = 0.647; RI = 0.849; RC = 0.549), one of which is shown in Fig. 2. The obtained HIS tree had the same general

Fig. 1. One of 670 most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and the accession numbers of type strains are shown in bold print. The tree was rooted to *Cladosporium cladosporioides* AY251070 and *Cladosporium herbarum* AY251078.

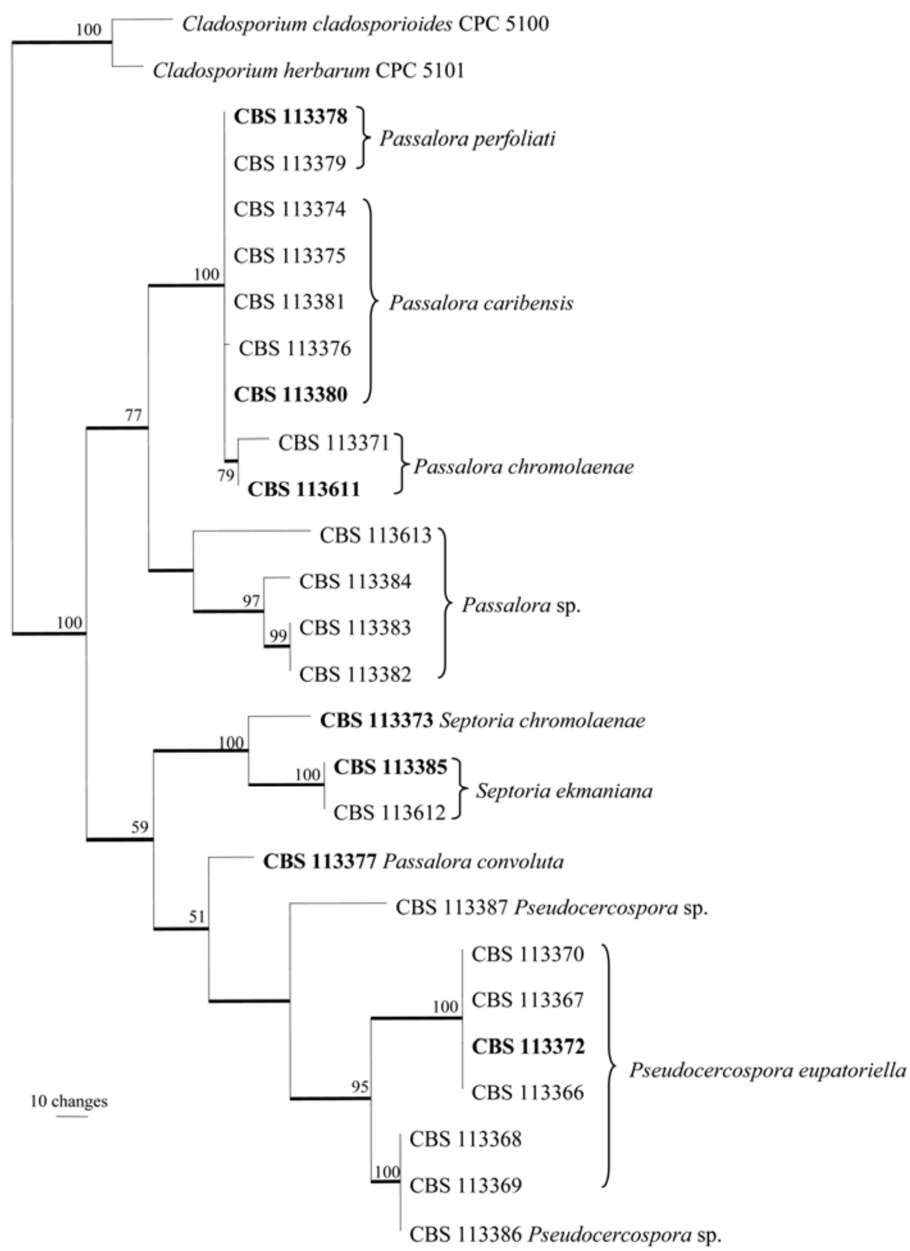

Fig. 2 One of two most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined ITS and histone H3 sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and the accession numbers of type strains are shown in bold print. The tree was rooted to *Cladosporium cladosporioides* DQ676562 and *Cladosporium herbarum* DQ676563.

topology as the ITS tree; however, it failed to clearly distinguish between *Passalora perfoliati*, *Passalora caribensis* and *Passalora chromolaenae*. Also, the HIS data shows a split between the strains of *Pseudocercospora eupatoriella*, with *Pseudocercospora* sp. CBS 113386 having the same HIS sequence as the second group of *Pseudocercospora eupatoriella* strains. The ITS sequence of *Pseudocercospora* sp. CBS 113386 differs from the ITS sequences of the *Pseudocercospora eupatoriella* strains at five nucleotide positions (98.98 % identical).

Taxonomy

Passalora convoluta Crous & Den Breeÿen, **sp. nov.** (Fig. 3)
MycoBank 500999

Etymology: Epithet referring to its prominently curled conidia.

Conidia brunnea, anguste obclavata, pluriseptata, conspicue convoluta, 150–300 × 3 µm.

Leaf spots amphigenous, grey, irregular to sub-circular, up to 1 cm diam. *Mycelium* external, consisting of medium brown, septate, branched, smooth, 3–4 µm wide hyphae. *Caespituli* not seen. *Conidiophores* arising singly from superficial mycelium, medium brown, smooth, 2–3-septate, subcylindrical, straight to curved, unbranched, 30–50 × 3–4 µm. *Conidiogenous cells* terminal, unbranched, medium brown, smooth, tapering to rounded apices with apical conidiogenous loci which are darkened, thickened, refractive, proliferating sympodially, 15–20 × 3–3.5 µm. *Conidia* solitary, medium brown, smooth, guttulate, narrowly obclavate, apex subobtuse, base long obconically subtruncate, straight to curled, multi-septate, 150–300 × 3 µm *in vitro*; hila darkened, thickened, refractive; microcyclic conidiation observed in culture.

Cultural characteristics: Colonies on OA slimy, spreading, flat, with sparse aerial mycelium, and even but irregular margins, olivaceous-black; on PDA similar, slimy with mucus droplets, spreading with sparse aerial mycelium, olivaceous-black on surface, and reverse, reaching 25 mm diam after 2 wks on OA or PDA at 25°C.

Specimen examined: Costa Rica, San Isidro between San José and Golfito, *C. odorata*, M.J. Morris, 15 Oct. 1997, CBS-H 19752 **holotype**, culture ex-type 488 = CBS 113377.

Notes: *P. convoluta* occurs with a *Mycosphaerella* sp. as well as a *Pseudocercospora* anamorph on the same lesions. As no cultures were obtained of the latter, they are not treated further. *P. convoluta* is unique among the taxa occurring on *Chromolaena* in having very long, curled conidia that are up to 350 µm long.

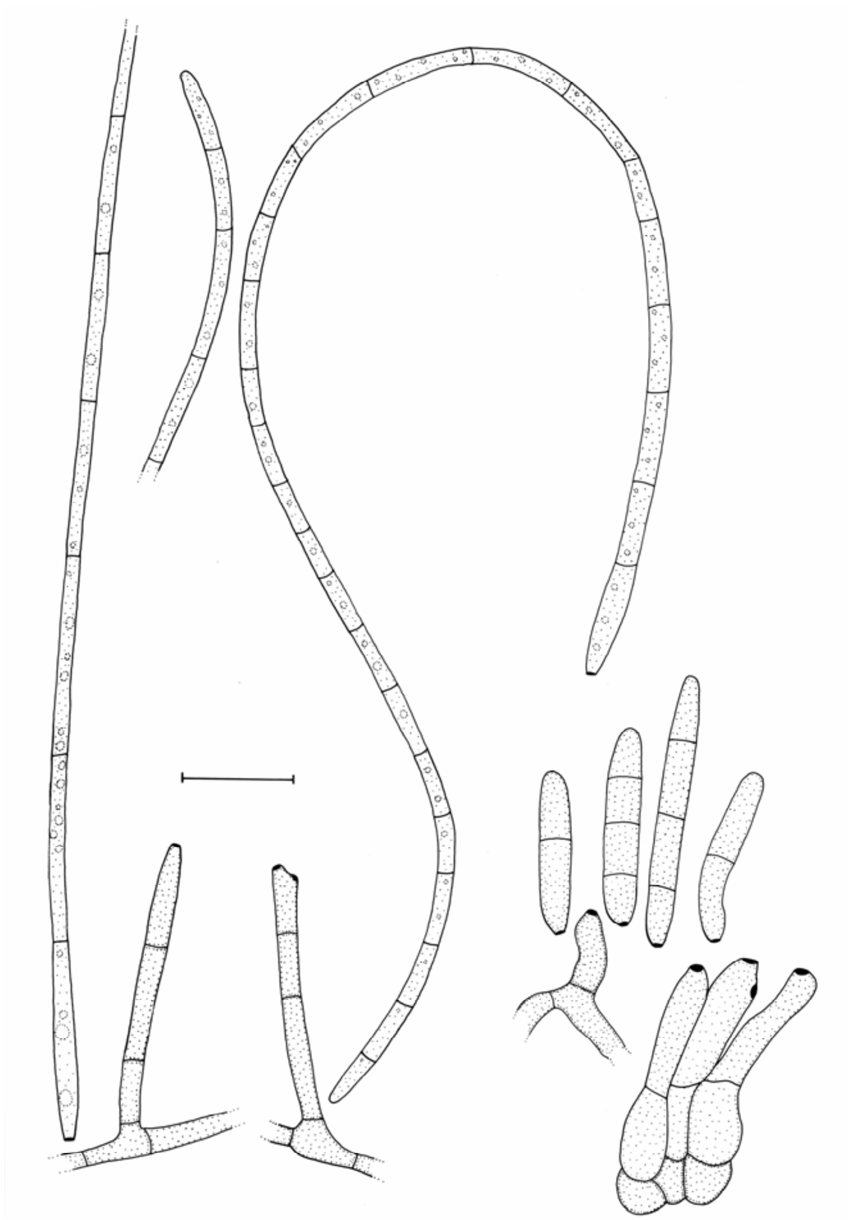

Fig. 3. Conidia and conidiogenous cells of *Passalora convoluta* (CBS 113377) (left), and *P. perfoliati* (CBS 113378) (right). Bar = 10 µm.

Passalora chromolaenae Crous & Den Breeÿen, **sp. nov.** (Fig. 4)
MycoBank 510000

Etymology: Epithet referring to its host, *Chromolaena*.

Conidia brunnea, anguste obclavata, recta vel curvata, 3–8(–pluri)-septata, (45–)60–100(–200) × 3–3.5(–4) µm.

Leaf spots amphigenous, irregular to angular, 1–4 mm diam, pale brown, surrounded by a dark brown border. *Mycelium* external, consisting of medium brown, septate, branched, smooth, 3–4 µm wide hyphae. *Caespituli* not seen. *Conidiophores* arising singly from superficial mycelium, medium brown, smooth, 2–6-septate, subcylindrical, straight to geniculate-sinuous, unbranched, 20–130 × 4 µm. *Conidiogenous cells* terminal, unbranched, medium brown, smooth, tapering to rounded apices with apical conidiogenous loci which are darkened, thickened, refractive, proliferating sympodially, 11–40 × 3.5–4 µm. *Conidia* solitary, medium brown, smooth, guttulate, narrowly obclavate, apex subobtuse, base long obconically subtruncate, straight to curved, 3–8(–multi)-septate, (45–)60–100(–200) × 3–3.5(–4) µm *in vitro*; hila darkened, thickened, refractive.

Cultural characteristics: Colonies on OA flat, spreading, aerial mycelium absent, slimy, vinaceous; margin smooth but wavy; on PDA flat, spreading, surface unevenly ridged; margins smooth but wavy, aerial mycelium absent; surface cream with iron-grey blotches, or dark vinaceous; reverse dark vinaceous or cream with iron-grey blotches; reaching 20–25 mm diam after 2 wks on OA or PDA at 25°C. The two strains available of this species differ markedly in culture.

Specimens examined: Mexico, Catemaco Lake, Veracruz Province, *C. odorata*, M.J. Morris, 12 Oct. 1997, CBS-H 19753 **holotype**, culture ex-type 452 = CBS 113611; Entrada Corretera, turnoff to Actopan from Veracruz/Zalapa road, Veracruz Province, *C. odorata*, M.J. Morris, 12 Oct. 1997, 450 = CBS 113371.

Note: *P. chromolaenae* is distinguished from other species occurring on this host by its conidial dimensions (up to 200 µm long and 4 µm wide). Conidia are also easily distinguished from that of *P. convoluta* in being shorter, wider, straight to curved, but never curled.

Passalora caribensis Crous & Den Breeÿen, **sp. nov.** (Fig. 4)
MycoBank 510001

Etymology: Epithet referring to its known distribution in the Caribbean.

Conidia brunnea, obclavata, recta vel modice curvata, 3–6-septata, (35–)40–60(–70) × 3–4(–6) µm.

Leaf spots amphigenous, resembling those of *Pseudocercospora eupatorii-formosani*. *Mycelium* internal and external, consisting of pale brown, septate, branched, smooth, 3–4 µm wide hyphae. *Caespituli* fasciculate, amphigenous, brown. *Conidiophores* arising singly from superficial mycelium,

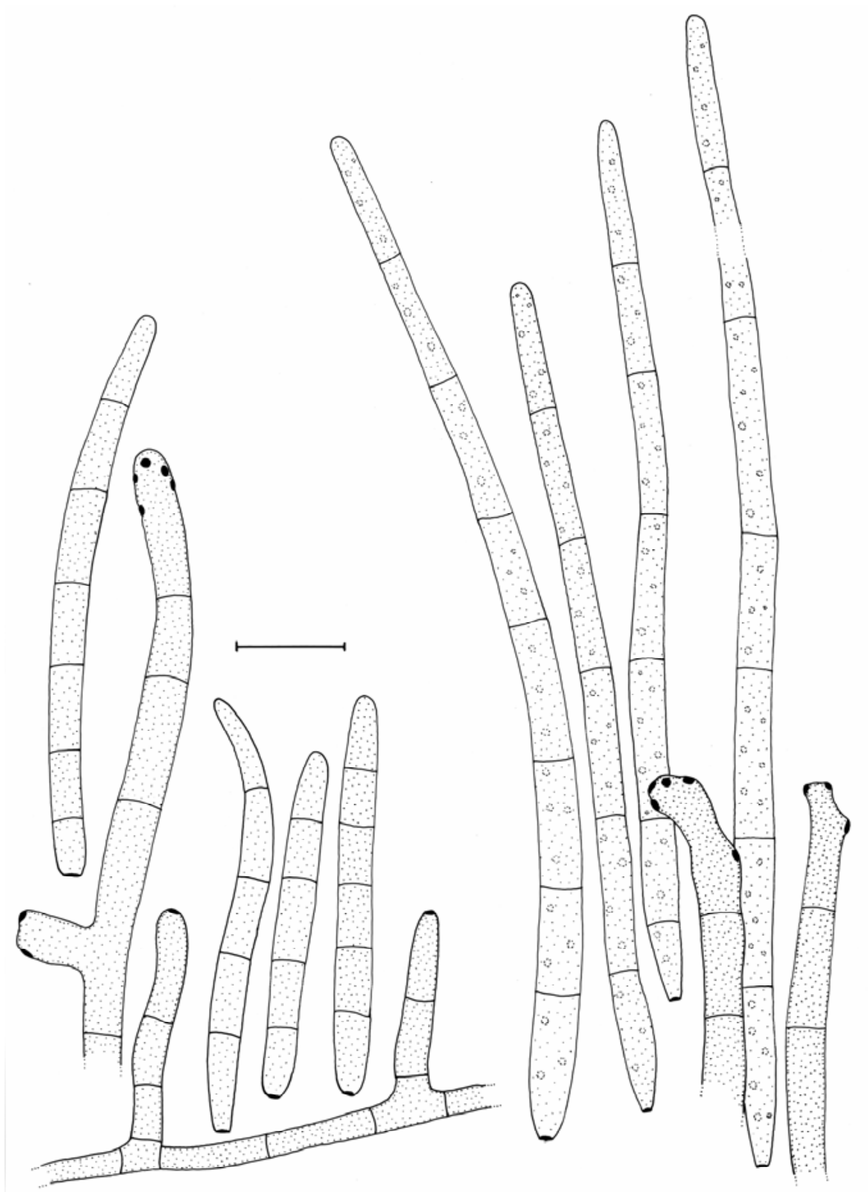

Fig. 4. Conidia and conidiogenous cells of *Passalora caribensis* (CBS 113380) (left), and *P. chromolaenae* (CBS 113611) (right). Bar = 10 µm.

or aggregated in loose fascicles arising from a brown stroma, smooth, medium brown, 1–5-septate, subcylindrical, straight to geniculate-sinuous, unbranched, 7–50 × 3–4 µm. *Conidiogenous cells* terminal, unbranched, medium brown, smooth, tapering to rounded apices with flat-tipped apical conidiogenous loci which are darkened, thickened, refractive, proliferating sympodially, 7–15 × 3–4 µm. *Conidia* solitary, medium brown, smooth, guttulate, obclavate, apex obtuse, base obconically subtruncate, straight to slightly curved, 3–6-septate, (35–)40–60(–70) × 3–4(–6) µm *in vitro*; hila darkened, thickened, refractive.

Cultural characteristics: Colonies on OA flat, spreading, slimy, with sparse aerial mycelium and smooth but wavy margins; surface livid vinaceous to cinnamon; on PDA erumpent with smooth margins and moderate aerial mycelium; surface honey to cinnamon or brick; reverse brown-vinaceous to vinaceous; reaching 20–25 mm diam after 2 wks on PDA or OA at 25°C.

Specimens examined: Jamaica, between Guinea corm and John's Hall, Central Jamaica, *C. odorata*, M.J. Morris, 31 Oct. 1997, CBS-H 19754 **holotype**, culture ex-type 498 = CBS 113380; road to Strawberry Hill off Blue Mountain road on outskirts of Kingston, *C. odorata*, M.J. Morris, 30 Oct. 1997, 481 = CBS 113374; ditto, 482 = CBS 113375; between Maypen and Chapleton, Central Jamaica, *C. odorata*, M.J. Morris, 30 Oct. 1997, 500 = CBS 113381. Cuba, near Havana, *C. odorata*, S. Neser, 28 Oct. 1997, 487 = CBS 113376.

Note: *P. caribensis* has much shorter, and slightly wider conidia (up to 70 µm long and 6 µm wide) than those of *P. chromolaenae*.

Passalora perfoliati (Ellis & Everh.) U. Braun & Crous, in Crous and Braun, *Mycosphaerella and its anamorphs*: 1: 314. 2003. (Fig. 3)

≡ *Cercospora perfoliati* Ellis & Everh. (*perfoliata*), J. Mycol. 5: 71. 1889.
≡ *Mycovellosiella perfoliati* (Ellis & Everh.) Munt.-Cvetk., Lilloa 30: 201. 1960.
= *Cercospora agerati* F. Stevens, Bull. Bernice P. Bishop Mus. 19: 154. 1925.
≡ *Ragnhildiana agerati* (F. Stevens) F. Stevens & Solheim, Mycologia 23: 402. 1931.
= *Cladosporium versicolor* Bond, Ceylon J. Sci., Sect. A, Bot., 12: 183. 1947.
= *Ramularia agerati* Sawada, Special Publ. Coll. Agric. Natl. Taiwan Univ. 8:190. 1959 (*nom. inval*.).
= *Cercosporella coorgica* Muthappa, Mycopath. Mycol. Appl. 34: 194. 1968.

Leaf spots amphigenous, pale brown with a raised border and dark brown margin, irregular to angular, 1–4 mm diam. *Mycelium* internal and external, consisting of medium brown, septate, branched, smooth, 3–4 µm wide hyphae. *Caespituli* fasciculate, amphigenous, brown, inconspicuous on leaves, up to 30 µm wide. *Conidiophores* arising singly from superficial mycelium, or aggregated in loose fascicles, arising from the upper cells of a brown stroma, up to 20 µm wide; conidiophores medium brown, smooth, 1–2-septate, subcylindrical, straight to geniculate-sinuous, unbranched, 7–20 × 2.5–3 µm. *Conidiogenous cells* terminal, unbranched, pale to medium brown, smooth, tapering to rounded apices with apical conidiogenous loci which are darkened, thickened, refractive, proliferating sympodially, 7–18 × 2.5–3 µm. *Conidia* solitary, medium brown, smooth, narrowly obclavate, apex obtuse, base obconically subtruncate, straight to curved, 1–3-septate, 17–25 × 2.5–3 µm *in vitro*; hila darkened, thickened, refractive, up to 1µm wide.

Cultural characteristics: Colonies on OA spreading with sparse, white to no aerial mycelium; margins smooth but wavy; surface smooth, white or hazel with patches of black; on PDA surface irregular with ridges, centre dirty cream to honey or brick, outer margin and reverse brown-vinaceous; reaching 15 mm diam after 2 wks on OA or PDA at 25°C.

Specimens examined: Jamaica, between Moneague and Edwarton, Central Jamaica on highway to Kingston, *C. odorata*, M.J. Morris, 1 Nov. 1997, 494 = CBS 113378; up Strawberry Hill, *C. odorata*, M.J. Morris, 30 Oct. 1997, 495 = CBS 113379.

Notes: According to the recent treatment of this fungus by Braun (1998), the present collection appears to be *Passalora perfoliati*, as also seen by Ellis (1971). *P. perfoliati* is listed by Crous and Braun (2003) as occurring on hosts such as *Ageratum conyzoides*, *Chromolaena odorata*, *Eupatorium ageratoides*, *E. perfoliatum*, *E. purpureum*, *E. repandum*, *E. rugosum*, *E. sessilifolia*, *Eupatorium* spp. in countries such as Canary Islands, China, Dominican Republ., Gabon, India, Jamaica, Kenya, Malawi, New Caledonia, Haiti, Papua New Guinea, Puerto Rico, South Africa, Sudan, Sri Lanka, Taiwan, Tanzania, Trinidad and Tobago, Uganda, and the USA (FL, HI, IL, MI, WI).

Barretto and Evans (1994) discuss morphological and pathological variation between collections, and suggest that more than one species may be involved. Judging from the description and illustrations, Barreto and Evans (1994) were clearly working with a different species than that treated here, and it seems that most published records of *P. perfoliata* will have to be interpreted with care, as this is obviously a species complex. Although conidia of the present collection are smaller than that reported by Braun (1998), we choose to treat it as *P. perfoliati* until more cultures can be obtained for a detailed comparison.

Pseudocercospora eupatoriella Crous & Den Breeÿen, **sp. nov.** (Fig. 5)
MycoBank 510002

Etymology: Epithet referring to its conidial size, being shorter than those of *P. eupatorii*.

Conidia brunnea, anguste obclavata vel subcylindrica, recta vel curvata, 3–5-septata, (40–)55–70(–80) × 2.5–3 µm.

Leaf spots amphigenous, irregular to sub-circular, 2–4 mm diam, medium brown. *Mycelium* internal, consisting of medium brown, septate, branched, smooth, 3–4 µm wide hyphae. *Caespituli* fasciculate, amphigenous, medium brown on leaves, up to 30 µm wide and 20 µm high. *Conidiophores* aggregated in dense fascicles arising from the upper cells of a brown stroma, up to 10 µm wide; conidiophores medium brown, smooth, 0–1-septate, subcylindrical to ampulliform, straight to geniculous-sinuous, unbranched, 10–20 × 2.5–4 µm.

Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to bluntly rounded loci, proliferating sympodially, 5–10 × 2.5–4 µm. *Conidia* solitary, medium brown, smooth, narrowly obclavate to subcylindrical, apex obtuse, base long obconically subtruncate to truncate, straight to curved, 3–5-septate, (40–)55–70(–80) × 2.5–3 µm *in vivo*, 45–95 × 2–3 µm *in vitro*. *Spermatogonia* also formed abundantly in culture.

Cultural characteristics: Colonies on OA flat, spreading, with moderate aerial mycelium and smooth but irregular margin; smoke-grey in centre, olivaceous-grey in outer region; on PDA similar in growth and colour, but more fluffy; reverse olivaceous-black; reaching 30 mm diam after 2 wks on OA or PDA at 25 °C.

Specimens examined: Jamaica, between St. Anna and Ocho Rios, central north coast, *C. odorata*, M.J. Morris, 1 Nov. 1997, CBS-H 19755 **holotype,** culture ex-type 477 = CBS 113372. USA, Florida, a few km along Avacado Drive, Homestead, *C. odorata*, M.J. Morris, 7 Oct. 1997, 448 = CBS 113370; ditto, along railway line off Avocado Drive on the way to airport, Homestead, Florida, M.J. Morris, 7 Oct. 1997, 439 = CBS 113367; Redlands Road, Homestead, Florida, M.J. Morris, 7 Oct. 1997, 444 = CBS 113368; ditto, 447 = CBS 113369; rolling hills Hotel, Fort Lauderdale, Florida, M.J. Morris, 6 Oct. 1997, 437 = CBS 113366.

Notes: *P. eupatoriella* has shorter conidia and smaller conidiophores than in *P. eupatorii*. Furthermore, it lacks superficial mycelium as present in *P. eupatorii-formosani*, and also has smaller conidiophores (Bagyanarayana and Braun, 1999). From what we have seen on the various specimens examined, there are numerous species of *Pseudocercospora* on this host that are presently still undescribed.

Septoria chromolaenae Crous & Den Breeÿen, **sp. nov.** (Fig. 5)
MycoBank 510003

Etymology: Epithet referring to its host *Chromolaena*.

Conidia hyalina, anguste obclavata, sursum obtusa vel subobtusa, ad basim obconica, subtruncata, recta vel modice convoluta, (1–)3-septata, (23–)26–30(–40) × 2(–2.5) µm.

Mycelium internal, consisting of hyaline to pale brown, septate, branched, smooth, 2–3 µm wide hyphae. *Conidiomata* pycnidial, unilocular, globose, medium brown, up to 150 µm diam. *Conidiophores* lining the inner layer of the conidioma, 0–1-septate, hyaline, smooth, subcylindrical, 5–15 × 3–3.5 µm. *Conidiogenous cells* terminal, unbranched, hyaline, smooth, tapering to flat-tipped apical loci, proliferating sympodially, 5–7 × 3 µm. *Conidia* solitary, hyaline, smooth, narrowly obclavate, apex obtuse to subobtuse, base long obconically subtruncate, straight to slightly curled, (1–)3-septate, (23–)26–30(–40) × 2(–2.5) µm *in vitro*; hila inconspicuous.

Cultural characteristics: On OA spreading, flat, with moderate, white aerial mycelium; margin smooth, even; surface cinnamon; on PDA flat, spreading, with white aerial mycelium in centre; outer region brick; reverse

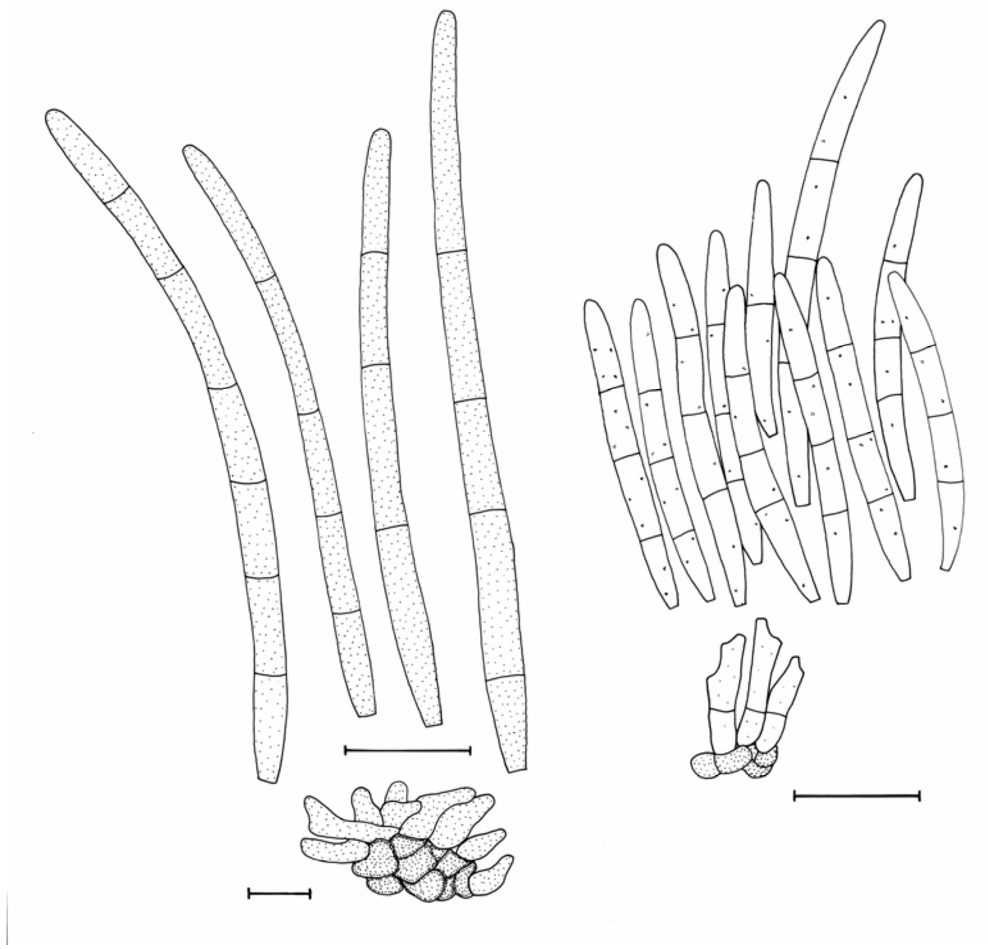

Fig. 5. Conidia and conidiogenous cells of *Pseudocercospora eupatoriella* (CBS 113372) (left), and *Septoria chromolaenae* (CBS 113373) (right). Bars = 10 µm.

brick, but dark vinaceous in central part; reaching 20–30 mm diam on OA or PDA after 2 wks at 25°C.

Specimen examined: **Cuba**, near Havana, *C. odorata*, S. Neser, 28 Oct. 1997, CBS-H 19756 **holotype**, culture ex-type 478 = CBS 113373.

Notes: *S. chromolaenae* has wider conidia that those of *S. eupatorii* (25–35 × 1.5 µm), and the latter species is restricted to true members of the genus *Eupatorium* (Barreto and Evans, 1994).

Septoria ekmaniana Petr. & Cif., Ann. Mycol. 30: 300. 1932. (Fig. 6)

= *Septoria fusarispora* Viégas, Bragantia 5: 746. 1945.

Conidiomata on OA developing after 1–2 weeks, globose, glabrous, dark brown to black, 0.25–0.8(–1.2) mm diam, without differentiated ostiolum, the whitish conidial slime released from the conidioma through dissolution or tearing of the upper wall tissues. *Conidiogenous cells* lining the inner layer of the conidioma, holoblastic, discrete, rarely integrated in 1-septate conidiophores, hyaline, smooth, cylindrical to ampulliform, 8–18 × 3.5–5 µm, monoblastic or proliferating sympodially. *Conidia* solitary, hyaline, smooth, cylindrical, widest near or just above the middle and tapering gradually towards the broadly rounded apex and truncate base, straight to strongly curved, (3–)5–11(–15)-septate, 30–105(–124) × 5–6(–7.5) µm *in vitro* (width of non-turgescent conidia 3–5 µm).

Cultural characteristics: On OA restricted or slowly spreading, cinnamon to sienna, in the centre with diffuse grey aerial hyphae, and with a luteous-glabrous margin; reverse sienna to rust; on PDA restricted, with a pale buff margin and an olivaceous-black and glabrous submarginal zone, centre covered with dark grey-olivaceous felty to woolly aerial mycelium; reverse olivaceous-black; reaching 9–14 mm diam on OA and 10 mm on PDA in 3 weeks.

Specimens examined: Mexico, Veracruz Province, *C. odorata*, M.J. Morris, Oct. 1997, 491 = CBS 113385; 492 = CBS 113612.

Notes: The description given above is based on sporulation of CBS 113612 (CBS 113385 was sterile) on OA. The conidiomata and conidia were considerably larger than reported by Barreto & Evans (1994), who observed 2–9-septate conidia, 25–67 × 2–4 µm on the host plant. It is not unusual for *Septoria* isolates to produce larger and more complex (often non-ostiolate) pycnidia after isolation in pure culture. Likewise, the conidia can be considerably longer in isolates than seen *in planta*, and (as a consequence?) also have more septa. The conidia in CBS 113612 do have the characteristic shape of *S. ekmaniana*.

Discussion

In a treatment of the mycobiota associated with *Chromolaena odorata*, Barreto and Evans (1994) provided a detailed account of the species of *Mycosphaerellaceae* reported to be pathogenic to this host. In the introduction of their paper, they refer to the confusion existing in the literature due to the

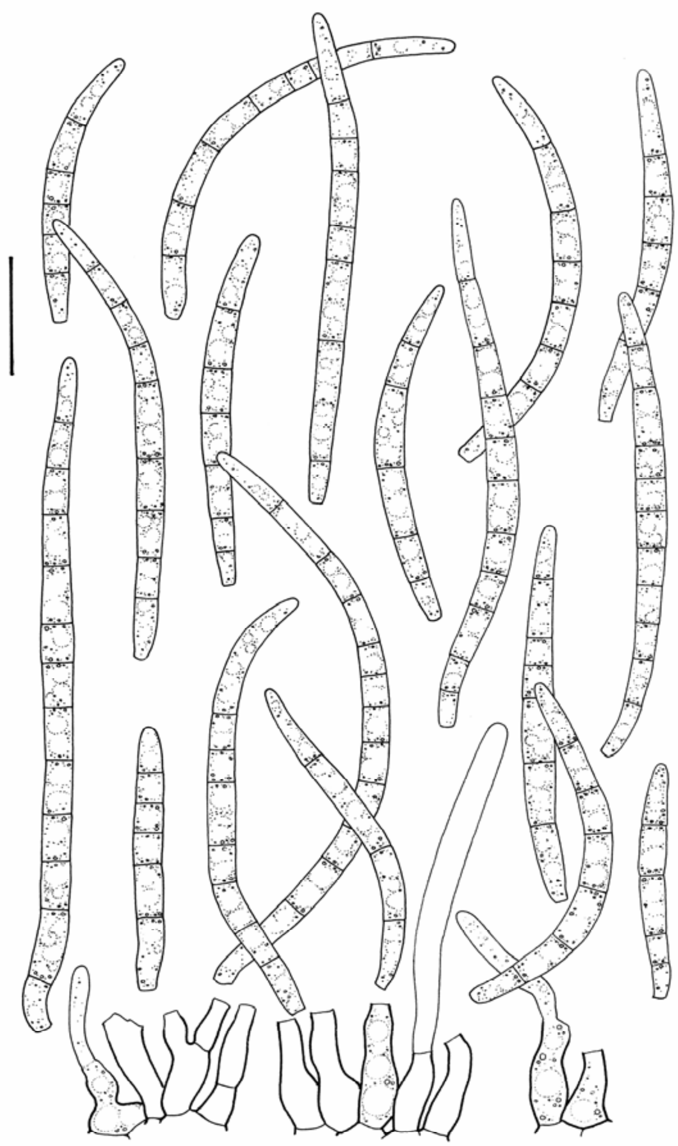

Fig. 6. Conidia and conidiogenous cells of *Septoria ekmaniana* (CBS 113612). Bar = 10 µm.

problems concerning the taxonomy of *Eupatorium* and *Chromolaena*, and specifically the synonyms of *C. odorata*. By treating *C. odorata* (= *Eupatorium odoratum*) as a species of *Eupatorium*, the literature is full of erroneous cercosporoid host records, which again cause a significant amount of confusion, especially if some of these pathogens have to be considered as potential biocontrol agents of *C. odorata*. There appears to be a high level of specificity among the cercosporoids occurring on *C. odorata* and species of *Eupatorium*, and thus it would be unlikely that a species occurring on the latter host would be a pathogen of the former. Based on inoculation studies (A. den Breeÿen, unpublished data) of the various cercosporoids isolated from different countries on *C. odorata* in this study, only some showed promise as potential biocontrol agents on the South African form of *C. odorata*. It would appear, therefore, that further specificity exists even within what is current defined as *C. odorata*. Several species of *Mycosphaerellaceae* have in the past been described from *Eupatorium* and *Chromolaena*. These are briefly discussed below.

Pseudocercospora eupatorii-formosani U. Braun & Bagyan. (as *P. eupatorii-formosani* (Sawada) J.M. Yen) was recently treated by Bagyanarayana and Braun (1999), who validated the name for the fungus originally described from *Eupatorium formosanum* in Taiwan. Furthermore, they distinguished it from *Pseudocercospora eupatorii* (Peck) U. Braun & R.F. Castañeda, based on the longer conidia and wider conidiophores of the latter. *P. eupatorii* was reported to occur on *Eupatorium*, and to be the name available for North American material. *Pseudocercospora eupatoriicola* (Govindu & Thirum.) A.Z.M. Khan & Shamsi is known from several species of *Eupatorium*, and reported by Crous and Braun (2003) to be the name available for specimens originating from Australia and New Zealand.

Pseudocercospora aciculina (Chupp) U. Braun & Crous, which occurs on *Eupatorium repandum* in Hawaii, is a somewhat uncertain name, as Crous and Braun (2003) were unsuccessful in tracing the type material. *Cercospora ageraticola* Goh & W.H. Hsieh is a true species of *Cercospora* known from China and Taiwan, different from *C. apii sensu lato* by having obclavate conidia with obconically truncate bases. *Pseudocercospora ageratoides* (Ellis & Everh.) Y.L. Guo is a species intermediate between *Pseudocercospora* and *Passalora*, known from several species of *Eupatorium* in the USA, and also reported from China, though this record could not be confirmed. *Passalora assamensis* (S. Chowdhury) U. Braun & Crous, described from *Ageratina adenophora* (*Eupatorium adenophorum*) from India, is a typical *Passalora* indistinguishable from *Cercospora eupatorii-odoratii* J.M. Yen. *Passalora castaricensis* (Syd.) U. Braun & Crous was described from *Eupatorium oerstedianium* in Costa Rica, and has also been reported on *Chromolaena odorata*, though this has not been proven. *Cercospora eupatorii-fortunei* P.K. Chi & Z.D. Jiang is a true species of *Cercospora*, known from *Eupatorium fortunei* in China. *Passalora perfoliati* (Ellis & Everh.) U. Braun & Crous, originally described from *Eupatorium perfoliatum* in the USA, is reported by

Crous and Braun (2003) to have a wide host range and distribution. The most recent treatment by Braun (1998), however, shows this fungus to be distinct from the species treated by Barreto and Evans (1994) under this name. Furthermore, comments made by the latter authors relating to morphological and pathological variation, lead us to conclude that the name *P. perfoliati* is presently used for a species complex, and that additional collections, cultures and molecular analyses would be required to fully resolve all cryptic species presently treated as "*P. perfoliati*". *Cercospora rigidipes* Munt.-Cvetk. is a relatively unstudied taxon, described from *Eupatorium hookerianum* collected in Argentina. *Cercosporella virgaureae* (Thüm.) Allesch. is a species with a wide geographic distribution and host range, including several species of *Eupatorium*.

Baretto and Evans (1994) report three species of *Septoria* from *Chromolaena*, namely *Septoria ekmaniana* Petr. & Cif. (= *S. fusarispora* Viégas) and *S. eupatorii* Roberge & Desm. Aptroot (2006) listed four species of *Mycosphaerella* on *Eupatorium*, namely *M. eupatorii* W.Y. Yen. on *Chromolaena odorata* in Malaysia, *Mycosphaerella eupatoriicola* Höhn. on *Eupatorium cannabinum* from Austria, *Mycosphaerella eupatoriicola* Petr. on *Eupatorium cannabinum* from the Czech Republic, and *Mycosphaerella tungurahuana* Petr. on *Eupatorium inulaefolium* from Ecuador.

In this study, we found that the trees obtained from the histone H3 sequence alignment have the same general topology as the ITS trees. However, the histone H3 sequence data do not distinguish between *Passalora perfoliati*, *Passalora caribensis* and *Passalora chromolaenae*. The histone H3 data indicate variation between strains of *Pseudocercospora eupatoriella*, even including the *Pseudocercospora* sp. CBS 113386 in the second group of *Pseudocercospora eupatoriella* strains. Similar variation has also been reported before for members of the *Mycosphaerellaceae*. For example, in the anamorph genus *Cercospora*, histone H3 fails to distinguish closely related species such as *C. apii* Fresen. and *C. beticola* Sacc., but it does show variation within the *C. apii* / *C. beticola* clade (Groenewald *et al*., 2006). In *Mycosphaerella marksii* Carnegie & Keane, the percentage similarity in the histone H3 gene ranges from 95.5% to 99.7% over 381 nucleotides and in *Mycosphaerella citri* Whiteside from 98.2% to 99% over 388 nucleotides (Crous and Groenewald, 2005). With the advent of multi-locus sequence typing in *Mycosphaerella* and the increasing use of histone H3 for inferring phylogenies, the conflict in phylogenies derived from this gene, coupled with the short length of obtained sequences, is leading us to believe that the histone H3 gene might not be suitable for neither phylogenetic studies nor species identification in

Mycosphaerella. Whether this will be true for all species of *Mycosphaerella* or for other families, remains to be tested and requires more data.

The present study has led to the description of several new *Mycosphaerella* anamorphs on *C. odorata*. We suspect that these species are specific to this host, and that they have in the past been confused with taxa known to occur on *Eupatorium*, which has led to the use of names of *Eupatorium* pathogens as potential candidates for biocontrol of *C. odorata*. Although several new species are introduced in this study, an examination of herbarium specimens of these have revealed several as yet undescribed cercosporoids to also be present on these leaves. Several collections made in this study, which are in fact represented in the phylogenetic trees as distinct clades, have been left undescribed due to the absence of host material, and sterile cultures. It is thus inevitable that future studies will reveal yet more species on this host. The fact that there is further specialization within *Chromolaena*, however, makes it more difficult to find cercosporoid biocontrol agents that would be able to control effectively all forms of this host, and it is possible that more than one species would be required to effectively control the various morphological forms of *C. odorata* known to exist.

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