Celoporthe dispersa gen. et sp. nov. from native Myrtales in South Africa

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Abstract: In a survey for Cryphonectria and Chrysoporthe species on Myrtales in South Africa, a fungus resembling the stem canker pathogen Chrysoporthe austroafricana was collected from native Syzygium cordatum near Tzaneen (Limpopo Province), Heteropyxis canescens near Lydenburg (Mpumalanga Province) and exotic Tibouchina granulosa in Durban (KwaZulu-Natal Province). The fungus was associated with dying branches and stems on S. cordatum, H. canescens and T. granulosa. However, morphological differences were detected between the unknown fungus from these three hosts and known species of Chrysoporthe. The aim of this study was to characterise the fungus using DNA sequence comparisons and morphological features. Pathogenicity tests were also conducted to assess its virulence on Eucalyptus (ZG 14 clones), H. natalensis and T. granulosa. Plants of H. canescens were not available for inoculation. Results showed distinct morphological differences between the unknown fungus and Chrysoporthe spp. Phylogenetic analysis showed that isolates reside in a clade separate from Chrysoporthe and other related genera. Celoporthe dispersa gen. et sp. nov. is, therefore, described to accommodate this fungus. Pathogenicity tests showed that C. dispersa is not pathogenic to H. natalensis, but that it is a potential pathogen of Eucalyptus and Tibouchina spp.

Taxonomic novelties: Celoporthe Nakab., Gryzenh., Jol. Roux & M.J. Wingf. gen. et sp. nov., Celoporthe dispersa Nakab., Gryzenh., Jol. Roux & M.J. Wingf. sp. nov.

Key words: Chrysoporthe, Heteropyxis, Holocryphia, Phylogeny, Syzygium, Tibouchina.

INTRODUCTION

The taxonomy of Cryphonectria (Sacc.) Sacc. species associated with cankers of Eucalyptus spp. and the worldwide distribution of these fungi have undergone numerous revisions and changes in recent years (Venter et al. 2002, Gryzenhout et al. 2004, 2006a, 2006b). Studies have shown that the important Eucalyptus canker pathogen, Cryphonectria cubensis (Bruner) Hodges (Sharma et al. 1985, Hodges et al. 1986, Wingfield et al. 1989, Roux et al. 2003, Wingfield 2003), is different from other Cryphonectria spp. and has been placed in a new genus, Chrysoporthe Gryzenh. & M.J. Wingf., that includes at least two distinct species, C. cubensis (Bruner) Gryzenh. & M.J. Wingf. and C. austroafricana Gryzenh. & M.J. Wingf. (Gryzenhout et al. 2004). Similarly, the opportunistic Eucalyptus canker pathogen, Cryphonectria eucalypti M. Venter & M.J. Wingf., formerly known as Endothia gyrosa (Schwein.: Fr.) Fr. (Venter et al. 2002), now resides in the new genus Holocryphia Gryzenh. & M.J. Wingf. as H. eucalypti (M. Venter & M.J. Wingf.) Gryzenh. & M.J. Wingf. (Gryzenhout et al. 2006a).

Chrysoporthe cubensis occurs in South America on native Psidium cattleianum (Hodges 1988), and on exotic Eucalyptus spp. and Syzygium aromaticum (Boerboom & Maas 1970, Hodges et al. 1976, 1986, Van der Merwe et al. 2001), all of which reside in the family Myrtaceae, as well as on native Miconia rubiginosa and M. theaezans belonging to the family Melastomataceae (Rodas et al. 2005). In South East Asia and Australia the pathogen has been reported from Eucalyptus spp. (Sharma et al. 1985, Hodges et al. 1986, Davison & Coates 1991, Myburg et al. 1999) and S. aromaticum (Hodges et al. 1986, Myburg et al. 2003). In Africa, C. cubensis has been reported from Cameroon, Republic of Congo, Democratic Republic of Congo and Unga Island, Zanzibar on Eucalyptus spp. and S. aromaticum (Nutman & Roberts 1952, Gibson 1981, Hodges et al. 1986, Micales et al. 1987, Roux et al. 2000, Myburg et al. 2003, Roux et al. 2003).

Chrysoporthe austroafricana has, until recently, been known only from South Africa. In this country, it has been reported from both native South African tree species and non-native ornamental and plantation forest trees (Wingfield et al. 1989, Myburg et al. 2002, Heath et al. 2006). The fungus was the cause of an important disease of Eucalyptus spp. in the 1990’s (Wingfield et al. 1989) and has recently also been reported from the non-native ornamental tree Tibouchina granulosa (Melastomataceae) (Myburg et al. 2002) and native Syzygium cordatum and S. guineense (Myrtaceae) (Heath et al. 2006) in South Africa.

Holocryphia eucalypti is an opportunistic pathogen of Eucalyptus spp. in South Africa, mostly resulting in only superficial bark cankers on trees (Van der Westhuizen et al. 1993, Gryzenhout et al. 2003). The fungus is also known to occur in Australia on Corymbia and Eucalyptus spp. (Walker et al. 1985, Old et al. 1986), where it has been associated with cankers and tree death (Walker et al. 1985, Davison & Coates 1991, Wardlaw 1999).

Chrysoporthe spp. can be confused with Holocryphia because species in both genera have orange stromatal tissue in their teleomorph states (Venter et al. 2002, Gryzenhout et al. 2004, Myburg et al. 2004, Gryzenhout et al. 2006a) and they share the same hosts and geographical distributions (Old et al. 1986, Wingfield et al. 1989, Davison & Coates 1991, Van der Westhuizen et al. 1993).
Table 1. Isolates included in this study.

| Species                  | Isolate number | Alternative isolate number | Host                          | Origin      | Collector            | GenBank accession numbers |
|--------------------------|----------------|---------------------------|-------------------------------|-------------|----------------------|---------------------------|
| *Amphilogia gyrosa*     | CMW 10469     | CBS 112922                | *Elaeocarpus dentatus*        | New Zealand | G.J. Samuels         | AF452111, AF525707, AF525714 |
|                          | CMW 10470     | CBS 112923                | *E. dentatus*                 | New Zealand | G.J. Samuels         | AF452112, AF525708, AF525715 |
| *Celoporthe sp.*         | CMW 10890     | CBS 115844                | *Syzygium aromaticum*         | Kalimantan, Indonesia | M.J. Wingfield     | AY084009, AY084021, AY084033 |
|                          | CMW 10779     | CBS 118785                | *S. aromaticum*               | Indonesia   | M.J. Wingfield     | AY084007, AY084019, AY084031 |
|                          | CMW 10780     | CBS 118782                | *S. arbutifolia*              | Indonesia   | M.J. Wingfield     | AY084008, AY084020, AY084032 |
| *Celoporthe dispersa*    | CMW 10978     | CBS 118781                | *Syzygium cordatum*           | Tzaneen, South Africa | M. Gryzenhout   | DQ267130, DQ267136, DQ267142 |
|                          | CMW 10976     | CBS 118782                | *S. cordatum*                 | Tzaneen, South Africa | M. Gryzenhout   | DQ267131, DQ267137, DQ267143 |
|                          | CMW 13936     | CBS 119118                | *Tibouchina granulosa*        | Durban, South Africa | M. Gryzenhout   | DQ267132, DQ267138, DQ267144 |
|                          | CMW 13937     | CBS 119118                | *T. granulosa*                | Durban, South Africa | M. Gryzenhout   | DQ267133, DQ267139, DQ267145 |
|                          | CMW 13646     | CBS 119119                | *Heteropyxis canescens*       | Lydenburg South Africa | G. Nakabonge, J. Roux & M. Gryzenhout | DQ267134, DQ267140, DQ267146 |
| *Cryphonectria parasitica* | CMW 13749    | MAFF 410158               | Castanea mollisima            | Japan       | Unknown              | AY697927, AY697943, AY697944 |
|                          | CMW 7048      | ATCC 48198                | *Quercus virginiana*          | USA         | F.F. Lombard        | AF368330, AF273076, AF273470 |
| *Cryphonectria radicalis* | CMW 10455    | CBS 238,54                | Castanea dentata              | Italy       | A. Biraghi           | AF452113, AF525705, AF525712 |
|                          | CMW 10477     | CBS 240,54                | *Quercus suber*               | Italy       | M. Orsenigo          | AF368328, AF368347, AF368346 |
|                          | CMW 10436     | CBS 165,30                | *Q. suber*                    | Portugal    | B. d’Oliveira        | AF452117, AF525703, AF525710 |
|                          | CMW 10484     | CBS 112918                | Castanea sativa               | Italy       | A. Biraghi           | AF368327, AF368349, AF368349 |
| *Chrysoporthe austroafricana* | CMW 2113    | CBS 112916                | *Eucalyptus grandis*          | South Africa | M.J. Wingfield     | AF046892, AF273067, AF273462 |
|                          | CMW 9327      | CBS 115843                | *Tibouchina granulosa*        | South Africa | M.J. Wingfield     | AF273473, AF273060, AF273455 |
| *Chrysoporthe cubensis*  | CMW 10639     | CBS 115747                | *E. grandis*                  | Colombia    | C.A. Rodas           | AY263419, AY263420, AY263421 |
|                          | CMW 10669     | CBS 115751                | *Eucalyptus sp.*              | Republic of Congo | J. Roux             | AF535122, AF535124, AF535126 |
|                          | CMW 8651      | CBS 115718                | *S. aromaticum*               | Sulawesi, Indonesia | M.J. Wingfield     | AY084002, AY084014, AY084026 |
|                          | CMW 11288     | CBS 115736                | *S. aromaticum*               | Indonesia   | M.J. Wingfield     | AY214302, AY214320, AY214266 |
| *Chrysoporthe hodgesiana* | CMW 9994      | CBS 115729                | *Tibouchina semidecandra*      | Colombia    | R. Arbelaez          | AY965968, AY965975, AY965976 |
|                          | CMW 10641     | CBS 115854                | *T. semidecandra*             | Colombia    | R. Arbelaez          | AY692322, AY692326, AY692325 |
| *Diaporthe ambigua*      | CMW 5288      | CBS 112900                | *Malus domestica*             | South Africa | W.A. Smit           | AF543817, AF543819, AF543821 |
|                          | CMW 5587      | CBS 112901                | *M. domestica*                | South Africa | W.A. Smit           | AF543818, AF543820, AF543822 |
| *Endothia gyrosa*        | CMW 2091      | ATCC 48192                | *Quercus palustris*           | U.S.A.      | R.J. Stipes         | AF046905, AF368337, AF368336 |
|                          | CMW 10442     | CBS 118850                | *Q. palustris*                | U.S.A.      | R.J. Stipes         | AF368326, AF368339, AF368338 |
| *Holocryphia eucalypti*  | CMW 7037      | CRY 45, CBS 119477        | *Eucalyptus delegatensis*      | Australia   | K.M. Old            | AF232880, AF368343, AF368342 |
|                          | CMW 14546     | CRY 287, CBS 115838       | *Eucalyptus sp.*              | South Africa | H. Smith            | AF232879, DQ368732, DQ368733 |
However, there are distinct morphological differences between the genera. For example, the conidiomata of *Chrysoporthe* are superficial, fuscous-black, pyriform to orange with attenuated necks (Gryzenhout et al. 2004, Myburg et al. 2004), whereas those of *Holocryphia* are semi-immersed, orange and globose without necks (Venter et al. 2002, Myburg et al. 2004, Gryzenhout et al. 2006a). Furthermore, the ascospores of *Chrysoporthe* are septate, whereas those of *Holocryphia* are aseptate. Phylogenetic analyses have also shown that the two genera form distinct, well-supported groups (Myburg et al. 2004, Gryzenhout et al. 2006a, 2006b), separate from each other and from the genus *Cryphonectria*, in which both had been placed previously.

Like *C. cubensis*, *C. austroafricana* is an economically important pathogen of commercially grown *Eucalyptus* spp. (Wingfield et al. 1989, Wingfield 2003). In South Africa, *C. austroafricana* has caused substantial damage to clonal plantation forestry, which has been partially mitigated through the selection and planting of disease-resistant clones (Wingfield et al. 1989, Wingfield 2003). The recent discovery of *C. austroafricana* on native *S. cordatum* and *S. guineense* in South Africa has led to a change of view regarding its possible origin. Where it was once thought to be an introduced pathogen (Wingfield et al. 1989, Van Heerden & Wingfield 2001, Wingfield 2003), there is now substantial evidence to suggest that it is a native pathogen that could have moved from native South African *Syzygium* spp. to exotic species such as *Eucalyptus* and *Tibouchina* (Hodges et al. 1986, Myburg et al. 2002, Slippers et al. 2005, Heath et al. 2006).

Although only two species of *Syzygium* are known as hosts of *C. austroafricana*, it is highly likely that this fungus occurs on other *Myrtales* in South Africa. For this reason surveys were conducted in the country to establish the occurrence of *Chrysoporthe* spp. on indigenous tree species belonging to this plant order (Roux et al. 2005). These surveys yielded a fungus similar to *C. austroafricana* that was collected from three hosts in three geographic areas of the country. The aims of this study were to characterise the unknown fungus based on morphology and DNA sequence comparisons and to assess its pathogenicity in greenhouse inoculations on plants of *Heteropyxis*, *Eucalyptus* and *Tibouchina*.

**MATERIALS AND METHODS**

**Isolates and specimens**

Isolates were obtained from symptomatic bark material that was collected from *S. cordatum* from Tzaneen, *Heteropyxis canescens* from Lydenburg and *T. granulosa* from Durban (Table 1; Fig. 1). Fungal cultures for all isolates have been deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa and duplicates in the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Bark specimens have been deposited in the National Collection of Fungi, Pretoria, South Africa (PREM).
DNA sequence comparisons

Actively growing mycelium of each isolate was scraped from the surface of one plate each containing MEA (20 g/L malt extract and 20 g/L agar, Biolab, Midland, Johannesburg) and 100 mg/L streptomycin sulfate (Sigma-Aldrich, Chemie, Gmbh, Steinheim, Germany) using a sterile scalpel, and transferred to 1.5 mL Eppendorf tubes. DNA was extracted as described by Myburg et al. (1999). Using primers ITS1 and ITS4 (White et al. 1990), the rDNA (ITS 1, 5.8S and ITS 2) regions were amplified, while primer pairs Bt1A/Bt1B and Bt2A/Bt2B (Glass & Donaldson 1995) were used to amplify the β-tubulin 1 and 2 gene regions respectively. The reactions were performed in a volume of 25 μL comprising of 2 ng DNA template, 800 μM dNTPs, 0.15 μM of each primer, 5 U/μL Taq polymerase (Roche Diagnostics, Mannheim, Germany) and sterile distilled water (17.4 μL). Polymerase chain reactions (PCR) and purification of the PCR products were carried out as described by Nakabonge et al. (2005).

The purified PCR products were sequenced in a reaction volume of 10 μL consisting of 5× dilution buffer, 4.5 μL H₂O, DNA (50 ng PCR product), 10× reaction mix BD (ABI Prism Big Dye Terminator v. 3.1 Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, CA), and ~ 2 pmol/μL of one of either the reverse or forward primers that were used in the PCR reactions. The PCR sequencing products were cleaned by using 0.06 g/mL Sephadex G-50 (Sigma-Aldrich, Amersham Biosciences Limited, Sweden) according to the manufacturer’s protocol. The products were sequenced in both directions using the Big Dye Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an ABI Prism™ 3100 DNA sequencer (Applied Biosystems).

The sequence genes were analysed and edited using Sequence Navigator v. 1.0.1™ (Perkin-Elmer Applied BioSystems, Foster City, CA). Sequences were compiled into a matrix using a modified data set (S1128, M1935) of Myburg et al. (2004) as template. Additional sequences that included those of Chrysosporthe (Gryzenhout et al. 2004), Holocryphia (Venter et al. 2002, Gryzenhout et al. 2006a), Cryphonectria (Venter et al. 2002, Myburg et al. 2004), Endothia Fr. (Venter et al. 2002, Myburg et al. 2004), Rostrareum Gryzenh. & M.J. Wingf. (Gryzenhout et al. 2005a) and Amphiloilia Gryzenh., Glen & M.J. Wingf. (Myburg et al. 2004, Gryzenhout et al. 2005b) species were added to the data matrix. Sequences representing an undescribed genus identified by Myburg et al. (2003) and originating from clove in Indonesia were also added. The alignment was executed using the web interface (http://timpani.genome.adjp/%7Emafft/server/) of the alignment program MAFFT v. 5.667 (Katoh et al. 2002), and deposited with TreeBASE as S1488 and M2673.

Phylogenetic analysis was performed using the software package PAUP (Phylogenetic Analysis Using Parsimony) v. 4.01b (Swofford 1998). A partition homogeneity test (Hulsenbeck et al. 1996) to determine the similarity and combinability of the data for the ITS and the β-tubulin 1 and 2 regions, was run. The most parsimonious trees were obtained with heuristic searches using simple stepwise addition and tree bisection and reconstruction (TBR) as the branch swapping algorithms. All equally parsimonious trees were saved and all branches equal to zero were collapsed. Gaps were treated as a fifth character. Bootstrap replicates (1000) were done on consensus parsimonious trees (Felsenstein 1985). Two isolates of Diaporthe ambiguia Nitschke (CMW 5288 and CMW 5587) were used as outgroup to root the tree (Myburg et al. 2004).

Morphology

Fruiting structures of the unknown fungus were cut from the bark under a dissection microscope, boiled for 1 min and sectioned (12 μm thick) using a Leica CM1100 cryostat (Setpoint Technologies, Johannesburg, South Africa) as described by Gryzenhout et al. (2004). Fruiting structures were also crushed on microscope slides in 85 % lactic acid or 3 % KOH in order to study the asci, ascospores, conidia, conidiophores and conidiogenous cells. Measurements were then taken for the above-mentioned structures. For the holotype
specimen PREM 58896 50 measurements were made for each character. Only 20 measurements per character were made for the remaining specimens (PREM 58897–58901). A HRc Axiocam digital camera with Axiovision 3.1 software (Carl Zeiss Ltd., Germany) was used to capture digital images and to compute measurements. Characteristics of specimens were compared with those published for Chrysoporthe and Holocryphia (Gryzenhout et al. 2004, 2006a).

Two representative isolates from H. canescens (CMW 13645 and CMW 13646), T. granulosa (CMW 13936 and CMW 13937) and S. cordatum (CMW 9976 and CMW 9978) were used for studies of cultural characteristics. Discs (4 mm diam) taken from the margins of actively growing young cultures were placed onto the centres of 90 mm diam Petri dishes containing MEA. The cultures were grown in the dark in incubators set at temperatures ranging from 15 to 35 °C in 5 ° intervals. Four plates per isolate were inoculated and two measurements perpendicular to each other were taken daily until the fastest growing culture covered the plate. For each isolate, the colony diameter was calculated as an average of eight readings. Colour notations of Rayner (1970) were used for the descriptions of cultures and fruiting bodies.

**Pathogenicity tests**

The pathogenicity of two isolates of the unknown fungus, one from H. canescens (CMW 13645) and one from T. granulosa (CMW 13936), was tested on 25 trees each of an E. grandis clone (ZG14) that is known to be highly susceptible to fungal pathogens (Van Heerden & Wingfield 2001), and T. granulosa seedlings respectively, in a greenhouse set at 25 °C. The Eucalyptus clones were approximately 2 m tall while the Tibouchina seedlings were approx. 1 m tall. In order to expose the cambium, wounds were made in the bark using a cork borer (4 mm diam). Discs of the same size from the actively growing edges of 4-d-old colonies were inserted into the wounds with the mycelium facing the xylem. To prevent desiccation and contamination, wounds were covered with parafilm (Pechiney plastic packing, Chicago, USA). Twenty-five trees each of the E. grandis clone (ZG14) and T. granulosa served as negative controls and were inoculated with sterile water agar (WA: 20 g agar Merck, South Africa / 1 L water). Lesion development was evaluated after 8 wks by taking measurements of the lengths of lesions in the xylem. The trial was repeated after four months. Re-isolations were made from lesions by plating small pieces of discoloured xylem onto MEA.

Regeneration of Heteropyxis trees such as H. canescens in nurseries is seldom achieved. Only three trees (~1 m tall) of a related species, H. natalensis, could be obtained for pathogenicity tests. Two isolates (CMW 13645 and CMW 13646) of the unknown fungus from H. canescens were inoculated into the stems of two H. natalensis trees respectively. The third tree was inoculated with a sterile agar disc to serve as a negative control. The inoculation procedure was the same as that used when inoculating Eucalyptus and Tibouchina plants, except that each of the three trees had two inoculation points, with the same isolate, on opposite sides of the stem at the same height. Lesion lengths were measured 8 wks after inoculation and re-isolations were made using the same procedures as with the Eucalyptus and Tibouchina inoculations.

Data were analysed using the general linear model of analysis of variance (ANOVA). Means were separated using the Least Significant Difference (LSD) method available in STATISTICA for Windows (StatSoft 1995).

**RESULTS**

**Isolates and specimens**

Specimens of the unknown fungus were collected from cracked stems of two S. cordatum trees near Tzaneen in the Limpopo Province. Fruiting structures occurred between structures of C. austroafricana that were also fruiting profusely on these trees. A similar fungus was collected from six native H. canescens trees exhibiting severe cankers and die-back growing in the private Buffelskloof Nature Reserve near Lydenburg in Mpumalanga Province. Some of the trees were dying or dead (Fig. 1A). Additional collections were made from the stems of two non-native T. granulosa trees from the Durban Botanic Gardens in KwaZulu-Natal Province. These trees displayed symptoms of branch die-back (Fig. 1D).

![Fig. 1. Symptoms associated with Celoporthe dispersa infection. A. Dying Heteropyxis canescens. B. Fruiting structures of C. dispersa on H. canescens. C. Cross section through trunk canker on H. canescens. D. Cracks and cankers on Tibouchina granulosa.](image-url)
DNA sequence comparisons
PCR amplicons for the two regions of the β-tubulin gene were approximately 500 bp in size. Those for the ITS 5.8S region amplified were approximately 600 bp in size. Results obtained from the partition homogeneity test showed that the data for each gene region were significantly congruent (p-value = 0.02). The aligned sequences of the combined regions generated 1532 characters of equal weight, with 812 constant characters, 32 parsimony-uninformative characters and 688 parsimony-informative characters. Five most parsimonious trees were generated with similar branch lengths and topology and one was chosen for presentation. This tree had a length of 1725, a consistency index (CI) of 0.737 and retention index (RI) of 0.922 (Fig. 4).

Isolates representing species of Amphiogella, Chrysoporthe, Cryphonectria, Endothia, Holocryphia and Rostraureum formed distinct and well-supported clades reflecting the different genera. The isolates of the unidentified fungus from H. canescens, S. cordatum and T. granulosa in South Africa grouped separately from these genera (100 % bootstrap support), specifically separate from isolates of C. austroafricanana and H. eucalypti, which also occur on Myrtales in South Africa. The isolates of the unidentified fungus formed a clade with the isolates of an undescribed fungus from S. aromaticum from Indonesia (Myburg et al. 2003). However, within this clade, isolates formed sub-clades linked to the collections from different hosts. These were based on constant single base pair differences between isolates from the different hosts. These sub-clades include the Indonesian Syzygium sub-clade (100 % bootstrap support), the South African Syzygium sub-clade (96 % bootstrap support), the Heteropyxis sub-clade (100 % bootstrap support), and the Tibouchina sub-clade (96 % bootstrap support) from South Africa. Differences were most pronounced between the South African isolates and those from Indonesia (100 % bootstrap support), strongly suggesting that they represent different species.

Morphology
The fungus on H. canescens, S. cordatum, and T. granulosa in South Africa is characterised by fruiting structures (Table 2; Figs 2–3) that are morphologically very similar to those of Chrysoporthe species and the ChrysoportheLV anamorph of Chrysoporthe (Gryzenhout et al. 2004). In the teleomorph states of both genera, the perithecial necks are covered in umber tissue as they extend beyond the bark surface (Fig. 2A–B) and limited orange to cinnamon stromatic tissue can be seen at the bases of the necks (Fig. 2A–B). Ascospores are 1-septate, hyaline, and oblong to ellipsoidal (Fig. 2C, F). In the anamorph of the unknown fungus, conidiomata are pulvinate to conical, fuscous-black and superficial (Figs 1G, 2D), similar (Table 2) to the conidiomata of the same shape and colour in ChrysoportheLV (Gryzenhout et al. 2004).

The fungus characterised in this study differs from Chrysoporthe in several morphological characters (Table 2). Perithecial necks of the fungus are about 50 μm long (Figs 2A–B, 3A–B), while Chrysoporthe spp. have long necks extending up to 240 μm (Gryzenhout et al. 2004). Conidiomata are often without a neck or have necks with slightly attenuated apices (Figs 2G, 3D), differing from those of ChrysoportheLV spp. that have long attenuated necks (Gryzenhout et al. 2004). The basal cells of the conidiophores in the unknown fungus (Figs 2J–K, 3F) are not as prominent as those of members of Chrysoporthe. Conidia are oblong to cylindrical to ovoid and occasionally allantoid (Figs 2L, 3F), differing from those of Chrysoporthe spp. that are typically oblong (Gryzenhout et al. 2004). The stromatic tissue at the base of the conidiomata is pseudoparenchymatous (Fig. 2I), differing from that of Chrysoporthe, which consists of larger cells of textura globulosa (Gryzenhout et al. 2004).

Phylogenetic analyses suggested that the collections from H. canescens, S. cordatum and T. granulosa might represent three related but cryptic species. However, no significant morphological differences were found for fruiting structures among specimens linked to the isolates used in the phylogenetic analyses. These included specimens from H. canescens (PREM 58898 and PREM 58899), S. cordatum (PREM 58896 and PREM 58897) and T. granulosa (PREM 58900 and PREM 58901). There were also no clear differences in cultural morphology.

Phylogenetic analyses showed that an unnamed fungus previously treated by Myburg et al. (2003) from clove in Indonesia is related to the unknown fungus from South Africa, which formed the focus of the present study. It was, however, not possible to compare the South African and the Indonesian fungus based on morphology, because the latter fungus is known only from culture without any connection to morphological structures on the bark (Myburg et al. 2003). Some poorly formed conidiomata obtained for the Indonesian fungus by artificially inoculating it into Eucalyptus twigs (Myburg et al. 2003), however, suggested that the fungus is similar to the South African collections and probably represents the same genus.

Taxonomy
Morphological characteristics combined with DNA sequence data show that the unknown fungus collected from H. canescens, S. cordatum and T. granulosa in South Africa can be distinguished from Chrysoporthe, Cryphonectria and other closely related genera. Based on morphology, the fungus most closely resembles Chrysoporthe but clearly represents an undescribed genus. The taxon also appears to include an unnamed fungus previously collected from clove in Indonesia (Myburg et al. 2003). Based on these differences, a new genus is thus established for the fungi from South Africa and Indonesia.

DNA sequence data showed that more than one species exists for the new genus. The sub-clade
representing the Indonesian isolates was distinctly different from the South African isolates, but could not be described because there are insufficient structures on which to base a meaningful description. The isolates from the different hosts in South Africa formed a closely related group in the genus, although three possibly cryptic species, representing the isolates from three areas (Mpumalanga, Limpopo and KwaZulu-Natal Provinces) and hosts (*H. canescens*, *S. cordatum* and *T. granulosa*), respectively, could be identified based on sequence differences. However, no morphological differences could be observed for these apparent cryptic species, and at present there is insufficient material or ecological information available regarding these groups to support the separation of three species. For the present, we have chosen to retain the South African collections in a single species. The isolates from Indonesia most likely do not belong to this species, but must remain undescribed until fresh host material bearing fungal structures can be collected.

The specimens from *S. cordatum* in Tzaneen include both the anamorph and teleomorph, while specimens from *Heteropyxis* and *Tibouchina* have only the anamorph present. For the purpose of this study, a single species is described in a new genus, and this is based on specimens from *S. cordatum* as the holotype. Descriptions of the new genus and species follow:

**Celoporthe** Nakab., Gryzenh., Jol. Roux & M.J. Wingf., *gen. nov*. MycoBank MB500886.

*Etymology*: Latin, *celo*, to hide, referring to the fact that the fungus is difficult to find deliberately, and *porthe*, destroyer, referring to its pathogenic nature.

*Ascostromata* semi-immersed in bark, recognizable by short, extending, angular, cylindrical perithecial necks, occasionally erumpent, limited, orange toumber ascostromatic tissue covering the tops of the perithecial bases; *ascostromata* extending 400–400 μm high above the bark, 320–505 μm diam (Figs 2A, 3A–B). Stromatic tissue cinnamon and pseudoparenchymatous at the edges, prosenchymatous in the centre (Fig. 2D). *Perithecia* valvated, 1–6 per stroma, bases immersed in the bark, black, globose to subglobose, 100–300 μm diam, perithecial wall 30–50 μm thick (Figs 2B–C, 3B). *Perithecial necks* black, periphysate, 80–100 μm wide (Figs 2B, 3B), emerging through the stromatal surface, covered in umber stromatic tissue of *textura porrecta* (Fig. 2A), extended necks up to 50 μm long, 100–150 μm wide. *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoidal, (19.5–)23.5–29.5– (33.5) × (4.5–)5.5–7– (7.5) μm (Figs 2E, 3C). *Ascospores* hyaline, with one median septum, oblong-ellipsoidal, with rounded ends, (4.5–)6–7– (7.5) × (2.5–)3–4– (3.5) μm (Figs 2F, 3C).

*Conidiomata* eustromatic, superficial to slightly immersed, pulvinate to conical without necks, occasionally with a neck that is slightly attenuated (Figs 2G, 3D), orange to scarlet when young, fuscous-black when mature, conidiomatal bases above the bark surface 300–500 μm high, 200–1000 μm diam. *Conidiomatal locules* with even to convoluted inner surfaces, occasionally multilocular, locules 100–550 μm diam (Figs 2H, 3E). *Stromatic tissue* pseudoparenchymatous (Fig. 2I). *Conidiophores* hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, (9.5–)12–17–(19.5) × 1.5–2.5 μm (Figs 2J, 3F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical with or without attenuated apices, (1.5–)2–3 μm wide, collarette and periclinal thickening inconspicuous (Figs 2K, 3F). *Conidia* hyaline, non-septate, oblongoid, sporulating on conidiomata to ovoid, occasionally allantoid, exuded as bright luteous tendrils or droplets.

*Celoporthe dispersa* Nakab., Gryzenh., Jol. Roux & M.J. Wingf., *sp. nov*. MycoBank MB500887. Figs 2–3.

*Etymology*: Latin, *dispersus*, scattered, referring to the conidiomata scattered on the bark surface.

*Ascostromata* perithecia nigra continentia, collis perithecibals brevius extensis textura umbrina textica, textura stromatica limitata aurantiaca vel umbrina composita. *Ascosporeae* uniseptatae, oblongo-ellipsoidae. *Conidiomata* superficiale, pulvinata vel conica collis brevis vel absentibus, fusco-nigra. *Textura stromatica* pseudoparenchymatosa. *Conidiophora* cylindrica, ramosa, cellularae conidiogenae apicibus attenuatae. *Conidia* non septata, oblonga, cylindrica vel ovoidea, interdum allantoidae.

*Ascostromata* semi-immersed in bark, recognizable by short, extending, angular, cylindrical perithecial necks, occasionally erumpent, limited, orange toumber ascostromatic tissue covering the tops of the perithecial bases; *ascostromata* extending 400–400 μm high above the bark, 320–505 μm diam (Figs 2A, 3A–B). Stromatic tissue cinnamon and pseudoparenchymatous at the edges, prosenchymatous in the centre (Fig. 2D). *Perithecia* valvated, 1–6 per stroma, bases immersed in the bark, black, globose to subglobose, 100–300 μm diam, perithecial wall 30–50 μm thick (Figs 2B–C, 3B). *Perithecial necks* black, periphysate, 80–100 μm wide (Figs 2B, 3B), emerging through the stromatal surface, covered in umber stromatic tissue of *textura porrecta* (Fig. 2A), extended necks up to 50 μm long, 100–150 μm wide. *Asci* 8-spored, biseriate, unitunicate, free when mature, non-stipitate with a non-amyloid refractive ring, fusoid to ellipsoidal, (19.5–)23.5–29.5– (33.5) × (4.5–)5.5–7– (7.5) μm (Figs 2E, 3C). *Ascospores* hyaline, with one median septum, oblong-ellipsoidal, with rounded ends, (4.5–)6–7– (7.5) × (2.5–)3–4– (3.5) μm (Figs 2F, 3C).

*Conidiomata* eustromatic, superficial to slightly immersed, pulvinate to conical without necks, occasionally with a neck that is slightly attenuated (Figs 2G, 3D), orange to scarlet when young, fuscous-black when mature, conidiomatal bases above the bark surface 300–500 μm high, 200–1000 μm diam. *Conidiomatal locules* with even to convoluted inner surfaces, occasionally multilocular, locules 100–550 μm diam (Figs 2H, 3E). *Stromatic tissue* pseudoparenchymatous (Fig. 2I). *Conidiophores* hyaline, branched irregularly at the base or above into cylindrical cells, with or without separating septa, (9.5–)12–17–(19.5) × 1.5–2.5 μm (Figs 2J, 3F). *Conidiogenous cells* phialidic, determinate, apical or lateral on branches beneath a septum, cylindrical with or without attenuated apices, (1.5–)2–3 μm wide, collarette and periclinal thickening inconspicuous (Figs 2K, 3F). *Conidia* hyaline, non-septate, oblongoid, sporulating on conidiomata to ovoid, occasionally allantoid, (2.5–)3–4– (5.5) × (1–)1.5–2.5 μm (Figs 2L, 3F), exuded as bright luteous tendrils or droplets.

*Cultural characteristics*: On MEA, *C. dispersa* appears white with grey patches, eventually becoming umber to hazel to chestnut, fluffy with an uneven margin, fast-growing, covering a 90 mm diam plate in a minimum of 5 d at the optimum temperature of 25 °C. Cultures rarely sporulate after sub-culturing and teleomorph structures are not produced in culture.
Substrates: Bark of *Heteropyxis canescens*, *Syzygium cordatum* and *Tibouchina granulosa*.

Distribution: South Africa

Specimens examined: **South Africa**, Limpopo Province, Tzaneen, *Syzygium cordatum*, 2003, M. Gryzenhout, ho*loty*pe PREM 58896, culture ex-type CMW 9976 = CBS 118782, PREM 58897, living culture CMW 9978 = CBS 118781; KwaZulu-Natal Province, Durban, Durban Botanic Gardens, *Tibouchina granulosa*, M. Gryzenhout, May 2004, PREM 58900, living culture CMW 13936 = CBS 118785, PREM 58901, living culture CMW 13937 = CBS 119118; Mpumalanga Province, Lydenburg, Buffelskloof private nature reserve, *Heteropyxis canescens*, G. Nakabonge, J. Roux & M. Gryzenhout, Oct. 2003, PREM 58899, living culture CMW 13645 = CBS 119119, PREM 58898, living culture CMW 13646.

Pathogenicity tests

Eight wks after inoculation with *C. dispersa*, lesions were observed on the stems of the *Eucalyptus* clone (ZG 14) and on those of *T. granulosa* (Fig. 5). These lesions were light to dark brown, and stretched up and down the stems from the inoculation points. Similar results were obtained in both repeats of the inoculation study. Mean lesion lengths were 106 mm for *Eucalyptus* and 29 mm for *Tibouchina* in the first experiment and 104 mm and 25 mm, respectively, in the second experiment. The differences observed between hosts were significant (*P* < 0.001) and were similar in both trials. *Celoporthe dispersa* was re-isolated from the lesions. No lesions

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**Fig. 2.** Fruiting structures of *Celoporthe dispersa*. A. Ascoma on bark. B. Longitudinal section through ascoma. C. Perithecial neck tissue. D. Stromatic tissue. E. Asci with ascospores. F. Ascospores. G. Conidioma on the bark. H. Longitudinal section through conidioma. I. Stromatic tissue of conidioma. J. Conidiophores. K. Conidigenous cells. L. Conidia. Scale bars: A–B, G–H = 100 μm; C–D, I = 20 μm; E–F, J–L = 10 μm.
developed on the negative controls, and the margins of the points of inoculation were closed by callus tissue (Figs 5D, 6).

Inoculation of *C. dispersa* on stems of *H. natalensis* showed no obvious lesion development after eight wks. Similarly, no lesions developed on the controls.

**DISCUSSION**

In this study, we have shown that the fungus isolated from *H. canescens*, *S. cordatum* and *T. granulosa* in South Africa represents a new genus and species related to, but distinctly different from, *Chrysoporthe*. Description of this new taxon, *C. dispersa*, is supported by both morphological characteristics and DNA sequence data. These have clearly shown that isolates of *C. dispersa* form a clade distinct from *Chrysoporthe*, *Holocryphia* and other taxa, which it resembles morphologically.

*Celoporthe dispersa* most closely resembles species of *Chrysoporthe* and may appear indistinguishable from *Chrysoporthe* spp. when it is observed macroscopically in the absence of light microscopy. Species of both genera have black conidiomata of similar shape. The ascostromata are in both cases semi-immersed, with limited orange to cinnamon stromatic tissue and perithecial necks covered in umber tissue as they extend beyond the bark surface. Both genera have conidia and ascospores that are expelled as bright luteous spore tendrils. The ascospores of both *Celoporthe* and *Chrysoporthe* are 1-septate, hyaline and oblong to ellipsoidal. Furthermore, *C. dispersa* occurs on the same hosts as *Chrysoporthe*. The fungus was isolated from *T. granulosa* and *S. cordatum*, two hosts on which the morphologically similar *C. austroafricana* also occurs (Myburg et al. 2002, Heath et al. 2006). However, to the best of our knowledge this is the first fungus belonging to the group that has been collected from a species of *Heteropyxis*.

Although *Celoporthe* resembles *Chrysoporthe*, distinct morphological differences separate these two fungi. The presence of short perithecial necks, pulvinate to conical conidiomata without necks, conidia that are oblong to cylindrical to ovoid, and pseudoparenchymatous stromatic tissue in the conidiomatal base, distinguish *Celoporthe* from *Chrysoporthe* spp.

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**Fig. 3.** Line drawings of *Celoporthe dispersa*. A. Shape of ascoma. B. Section through ascoma. C. Asci and ascospores. D. Shapes of conidiomata. E. Section through conidioma. F. Conidiophores and conidia. Scale bars: A–B, D–E = 100 µm; C, F = 10 µm.
Chrysoporthe spp. have long cylindrical perithecial necks, the conidiomata are pyriform to pulvinate with attenuated necks, conidia are oblong and uniform in shape, and stromatic tissue of the conidiomatal base is of textura globulosa and that of the neck of textura porrecta (Gryzenhout et al. 2004). Celoporthe dispersa produces cultures that are white with grey to chestnut-coloured patches, in contrast to Chrysoporthe spp. that have white to cinnamon-coloured cultures with hazel patches. Careful morphological and cultural comparisons thus make it relatively easy to distinguish C. dispersa from Chrysoporthe spp.

Three distinct but closely related and morphologically similar pathogenic fungi occur on exotic and native Myrtales in South Africa. These are C. austroafricana, which is a highly pathogenic fungus on Eucalyptus spp. grown in South Africa (Wingfield et al. 1989, Conradie et al. 1990) and which also occurs on T. granulosa (Myburg et al. 2002) and native S. cordatum (Heath et al. 2006). Celoporthe dispersa has been described in this study and occurs on native S. cordatum, H. canescens and exotic T. granulosa in South Africa. The third fungus, H. eucalypti, has been recorded only from Eucalyptus spp. in South Africa (Van der Westhuizen et al. 2006).

Fig. 4. A phylogenetic tree generated from combined sequence data of the ITS ribosomal DNA and β-tubulin gene sequence data and generated from heuristic searches performed on the combined data set (tree length of 1725, CI of 0.737 and RI of 0.922). Bootstrap values (1000 replicates) above 50 % are indicated on the branches. Isolates sequenced in this study are in bold. Diaporthe ambigua sequences were used as outgroup.
et al. 1993, Gryzenhout et al. 2003), but is common in and probably originates from Australia (Old et al. 1986). Holocryphia eucalyti can easily be distinguished from C. dispersa and C. austroafricana based on differences in the colour and shape of conidiomata as well as cultural morphology (Venter et al. 2002, Gryzenhout et al. 2004, Myburg et al. 2004, Gryzenhout et al. 2006a).

DNA-based comparisons in this study have shown that different phylogenetic groups are represented by the isolates now treated as the single species C. dispersa. Thus, C. dispersa is represented by isolates from Heteropyxis, Tibouchina and Syzygium spp. in South Africa, and these isolates form three closely related sub-clades. A fourth sub-clade represents isolates from clove in Indonesia and was previously studied by Myburg et al. (2003). Based on DNA sequence data, this fungus clearly represents a distinct species, which could not yet be described because of insufficient material available to characterize it. The fact that the unknown Indonesian fungus is now known to reside in Celoporthe should facilitate the collection of additional samples from clove in Indonesia.

The three closely related sub-clades consisting of isolates of C. dispersa from South Africa, were correlated with their three different host genera (Heteropyxis, Syzygium and Tibouchina) and areas of collection (Lydenburg, Tzaneen and Durban). These sub-clades are, however, represented by a limited number of isolates and a larger collection of isolates will be required to better understand the relationship among them. We were unable to detect clear morphological differences between the fungi in these three sub-clades and the comparison was also hindered by the absence of teleomorph structures on the specimens from H. canescens and T. granulosa. Description of different species for the three phylogenetic sub-clades contained in C. dispersa must await the acquisition of additional material and isolates. The ecological data and distribution of these fungi in South Africa is also largely unknown, and such information would be useful in studying the taxonomic status of these three sub-clades of C. dispersa.

Heteropyxis canescens is a rare and endangered tree species in South Africa. Currently it is found only in Mpumalanga Province (John Burrows, pers. comm., Lawes et al. 2004). Fruiting structures of C. dispersa were collected from dying trees in the Buffelskloof Nature Reserve near Lydenburg and it was thought that the fungus might be responsible for the death of the trees. However, pathogenicity tests conducted using a limited number of trees of a closely related species, H. natalensis, showed that C. dispersa is not pathogenic to that species. Although it is possible that H. canescens is more susceptible to C. dispersa than is H. natalensis, the fungus might not be the cause of tree death at Buffelskloof. However, in order to understand the pathogenicity of C. dispersa more clearly, the fungus will need to be inoculated on H. canescens and on a larger number of trees than was possible in this study. This will be difficult to achieve because H. canescens is endangered and is extremely difficult to propagate artificially. The cause of tree mortality in the Buffelskloof Nature Reserve thus remains unclear. The possibility that another organism is responsible for the death of the trees must also be investigated.

Pathogenicity trials conducted on E. grandis and T. granulosa showed that C. dispersa is pathogenic on both these hosts. In these trials, the Eucalyptus clone was more susceptible than T. granulosa. Celoporthe dispersa is thus a newly discovered pathogen of these trees and it could become important on commercially grown Eucalyptus trees in South Africa.

Celoporthe dispersa and C. austroafricana are present on both native and non-native Myrtales in South Africa. This raises many important issues pertaining to the origin and distribution of these fungi. Both fungi are currently known only from southern Africa, and they also occur on native African trees. It has already been suggested that C. austroafricana is native to South Africa (Wingfield 2003, Heath et al. 2006) and the same is probably true for C. dispersa. These fungi are virulent pathogens of exotic Eucalyptus trees and their

Fig. 5. Lesions associated with inoculation of Celoporthe dispersa on a clone of Eucalyptus grandis (ZG 14) and Tibouchina granulosa. A. Fruiting structures formed on host as a result of inoculation (arrow). B. Lesion on Eucalyptus sp. C. Lesion formed on T. granulosa. D. Control inoculation on T. granulosa showing callus formation and the absence of lesion development.

Fig. 6. Comparison of lesion lengths associated with inoculation of Celoporthe dispersa on a Eucalyptus (ZG 14) clone and Tibouchina granulosa plants under greenhouse conditions. The trees were inoculated with C. dispersa isolated from Heteropyxis canescens (CMW 13645) and T. granulosa (CMW 13936). Mean lesion lengths were determined with 98 % confidence limits (P < 0.001).
accidental introduction into Australia, where *Eucalyptus* spp. and many other *Myrtales* are native, could result in an ecological disaster. This view is based on the fact that similar canker pathogens, such as *Cryphonectria parasitica* (Murrill) M.E. Barr, have caused devastating losses to trees after being introduced into new environments (Anagnostakis 1987, Slippers et al. 2005). Both *C. austroafricana* and *C. dispersa* also potentially threaten plantation *Eucalyptus* trees wherever they are grown commercially.

Additional surveys are necessary to expand the host and geographic ranges of *Celoporthe* and *Chrysoporthe* spp. on *Myrtales* in South Africa and on other parts of the African continent. The fact that these fungi are almost indistinguishable in the field will complicate such surveys, and laboratory studies will be required for reliable identifications. New collections and associated isolates of *C. dispersa* might also lead to the subdivision of this species into additional taxa. Additional material will thus add knowledge to the relatively poorly studied fungal biodiversity on the African continent and especially on native African tree species.

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