Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand

R. Cheewangkoon, P.W. Crous, K.D. Hyde, J.Z. Groenewald, C. To-anan

**Key words**

*Mycosphaerella*  
*Mycosphaerella* leaf disease  
*Penidiella*  
*Pseudocercospora*  
taxonomy

**Abstract**  
Species of *Mycosphaerella* and their related anamorphs represent potentially serious foliar pathogens of *Eucalyptus*. The fungi treated in the present study were isolated from symptomatic *Eucalyptus* leaves collected in Thailand during June–October 2007. Species were initially identified based on morphological and cultural characteristics. Identifications were confirmed using comparisons of DNA sequence data of the internal transcribed spacers (ITS1, 5.8S nrDNA, ITS2) and the 28S nrDNA (LSU) regions. To help distinguish species of *Pseudocercospora*, the dataset was expanded by generating partial sequences of the translation elongation factor 1-α and actin genes. By integrating the morphological and molecular datasets, five new taxa were distinguished, namely *Mycosphaerella* *irregulari*, *M. pseudomarksi*, *M. quasiparkii*, *Penidiella eucalypti* and *Pseudocercospora chiangmaiensis*, while *M. vietnamensis* represents a new record for Thailand.

**Article info**  
Received: 15 July 2008; Accepted: 12 September 2008; Published: 24 September 2008.

**INTRODUCTION**

Species of *Eucalyptus* (Myrtaceae) are hosts to a wide range of fungal pathogens (Sankaran et al. 1995, Crous et al. 2004a, 2006b, 2007a, Summerell et al. 2006). *Eucalyptus* spp. are commonly planted as exotics in commercial plantations for fuel wood, timber, and the paper and pulp industries in various tropical and subtropical regions (Ball 1995). Eucalypts have been cultivated extensively because of their fast growth rates and high adaptability to different soil types and climates (Turnbull 2000). In 1995, the total *Eucalyptus* plantation area in South-East Asia already exceeded 2 million ha (Old et al. 2003) and this number has continually increased over the years. In Thailand alone, a total of 443 000 ha *Eucalyptus* plantations was established by 2005 (Barney 2005). In spite of these huge areas already afforded to *Eucalyptus*, this crop is still increasingly being planted in Thailand and other Asian countries to meet rising global timber demand.

Plant pathogenic microfungi associated with *Eucalyptus* spp. can substantially decrease timber yield (Park et al. 2000, Old et al. 2003). In particular, species of *Mycosphaerella* (Mycosphaerellaceae) and *Teratosphaeria* (Teratosphaeriaceae) have proven to be serious pathogens of *Eucalyptus*, causing severe leaf spot formation, defoliation, shoot die-back and stem cankers (Crous 1998, Maxwell et al. 2003, Crous et al. 2004a, 2006a, c, 2007b, Hunter et al. 2004, 2006b, Jackson et al. 2005, Cortinas et al. 2006, Carnegie 2007). Nonetheless, information about the diversity of *Mycosphaerella* and related anamorph species on *Eucalyptus* from Thailand is generally lacking, and presently only five species have been reported, namely *Pseudocercospora basiramifera* and *Ps. flavomarginata* (Crous 1998, Hunter et al. 2006a), *Mycosphaerella heimi* (Crous et al. 2007b). Species of this pathogen complex reported from *Eucalyptus* in Asia include *Dissocioconium acciculare*, *Kirramyces destructans*, *K. eucalypti*, *M. crystallina*, *Eucalyptorum*, *M. gracilis*, *M. heimiodes*, *M. marksii*, *M. obscuris*, *M. parkii*, *M. robusta*, *M. stramenticola*, *M. sumatrensis*, *M. vrocosiafricana*, *M. vietnamensis*, *M. yunnanensis*, *Ps. deglupta*, *Ps. eucalyptorum*, *Ps. fatouae*, *Ps. paraguayensis*, *Ps. robusta*, *Septoria eucalyptorum*, *S. xenoparkii*, *Teratosphaeria fimbrata*, *T. gamsii*, *T. suberosa* and *T. suttonii* (Crous & Alfenas 1995, Crous & Wingfield 1997, Hunter et al. 2004, 2006a, Crous 1998, Crous & Braun 2003, Crous et al. 2004a, 2006c, 2007c, Burgess et al. 2007). Many *Eucalyptus* leaf pathogens originally described as related species of *Mycosphaerella* (i.e. *M. cryptica*, *M. gamsii*, *M. pseudocryptica*, *M. pseudosuberosa* and *M. suttonii*) have been re-classified into the genus *Teratosphaeria* (Teratosphaeriaceae) after substantial taxonomic revisions based on novel morphological characters integrated with their DNA phylogeny obtained by using the 28S nRNA gene (Crous et al. 2007c, 2008).

DNA sequencing of the ITS nrDNA gene has in the past proven highly effective to distinguish among species of *Mycosphaerella* (Crous et al. 2000, 2006a, b, 2007b, Cortinas et al. 2006). The main objective of the present study was to identify species of *Mycosphaerella* and related anamorphs associated with *Eucalyptus* leaves collected from plantations in Thailand, and to resolve their taxonomy and DNA phylogeny.

**MATERIAL AND METHODS**

**Isolates**

Symptomatic *Eucalyptus* leaves were collected at various locations in Thailand (Table 1). Lesions with ascomata were removed, soaked in distilled water for 2 h, and then placed in...
<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number</th>
<th>Host</th>
<th>Location</th>
<th>GenBank number</th>
<th>ITS</th>
<th>LSU</th>
<th>EF</th>
<th>ACT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycosphaerella heimii</em></td>
<td>CPC 15428</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882123</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15429</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882122</td>
<td>EU882141</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15430</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Vientein, Laos</td>
<td>EU882121</td>
<td>EU882140</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella irregulari</em></td>
<td>CPC 15408, CBS 123242</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Udonthani, Thailand</td>
<td>EU882110</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15431</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Udonthani, Thailand</td>
<td>EU882111</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15432</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Udonthani, Thailand</td>
<td>EU882112</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella pseudomarksii</em></td>
<td>CPC 15410, CBS 123241</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Chiang Mai, Thailand</td>
<td>EU882113</td>
<td>EU882137</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15435</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Chiang Mai, Thailand</td>
<td>EU882114</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15436</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Chiang Mai, Thailand</td>
<td>EU882115</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella quasiparkii</em></td>
<td>CPC 15409, CBS 123243</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882125</td>
<td>EU882143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15433</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882126</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15434</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882127</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella</em> sp.</td>
<td>CPC 15446</td>
<td><em>E. camaldulensis</em></td>
<td>Burirum, Thailand</td>
<td>EU882108</td>
<td>EU882136</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15447</td>
<td><em>E. camaldulensis</em></td>
<td>Burirum, Thailand</td>
<td>EU882109</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15448</td>
<td><em>E. camaldulensis</em></td>
<td>Ubonratchathani, Thailand</td>
<td>EU882124</td>
<td>EU882142</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella thailandica</em></td>
<td>CPC 15437</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Vientein, Laos</td>
<td>EU882120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15438</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Vientein, Laos</td>
<td>EU882119</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15439</td>
<td><em>E. camaldulensis</em></td>
<td>Mahasarakam, Thailand</td>
<td>EU882118</td>
<td>EU882139</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15440</td>
<td><em>E. camaldulensis</em></td>
<td>Ubonratchathani, Thailand</td>
<td>EU882117</td>
<td>EU882138</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15441</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882116</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mycosphaerella vietnamensis</em></td>
<td>CPC 15442</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Vientein, Laos</td>
<td>EU882104</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15443</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Burirum, Thailand</td>
<td>EU882107</td>
<td>EU882135</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15444</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Vientein, Laos</td>
<td>EU882106</td>
<td>EU882134</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15445</td>
<td><em>E. camaldulensis</em></td>
<td>Udonthani, Thailand</td>
<td>EU882105</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penidiella eucalypti</em></td>
<td>CPC 15411, CBS 123246</td>
<td><em>Eucalyptus</em> sp.</td>
<td>Mahasarakam, Thailand</td>
<td>EU882131</td>
<td>EU882145</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBS 123245</td>
<td><em>E. camaldulensis</em></td>
<td>Burirum, Thailand</td>
<td>EU882132</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15449</td>
<td><em>E. camaldulensis</em></td>
<td>Burirum, Thailand</td>
<td>EU882133</td>
<td>EU882146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudocercospora chiangmaiensis</em></td>
<td>CPC 15412, CBS 123244</td>
<td><em>E. camaldulensis</em></td>
<td>Chiang Mai, Thailand</td>
<td>EU882128</td>
<td>EU882144</td>
<td>EU882147</td>
<td>EU882150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15450</td>
<td><em>E. camaldulensis</em></td>
<td>Chiang Mai, Thailand</td>
<td>EU882129</td>
<td>–</td>
<td>EU882148</td>
<td>EU882151</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CPC 15451</td>
<td><em>E. camaldulensis</em></td>
<td>Chiang Mai, Thailand</td>
<td>EU882130</td>
<td>–</td>
<td>EU882149</td>
<td>EU882152</td>
<td></td>
</tr>
</tbody>
</table>

1 CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS.
Vilgalys & Hester (1990) were used to amplify part of the 5.8S rRNA gene, the second ITS region (ITS2) and the 5' end of the 28S rRNA gene (LSU). The primers ITS4 (White et al. 1990) and LR5 (Carbone & Kohn 1999) were used as primers for the ITS region. The Primers V9G (de Hoog & Gerrits van den Ende 1998) and LR0R (Rehner & Samuels 1994) were used as primers for the LSU region. The generated sequences were compared with other fungal DNA sequences from NCBI’s GenBank sequence database using a blast search; sequences with high similarity were added to the alignments. The additional GenBank sequences were manually aligned using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). The phylogenetic analyses of the aligned sequence data were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) and consisted of neighbour-joining analyses with the uncorrected (‘p’), the Kimura 2-parameter and the HKY85 substitution models. Alignment gaps were treated as missing data and all characters were unordered and of equal weight. Any ties were broken randomly when encountered. For parsimony analyses, alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 100 random simple taxa additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1 000 bootstrap replications (Hillis & Bull 1993). Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated and the resulting trees were printed with TreeView v. 1.6.6 (Page 1996). New sequences were lodged in GenBank and the alignments and phylogenetic trees in TreeBASE (www.treebase.org).

**Morphology**

Preparations from cultured fungal colonies were mounted on glass slides with clear lactic acid for microscopic examination. Sections of ascomata were made by hand for examination purposes. Measurements of all taxonomically relevant parameters were made at 1 000 × magnification, with 30 measurements per structure where possible. Colony colours on MEA (surface and reverse) were determined using the colour charts of Rayner (1970) after 15 d at 25 °C in the dark.

**RESULTS**

**Phylogenetic analysis**

Approximately 1 700 bases, spanning the ITS and LSU regions, were obtained for isolates listed in Table 1. These two regions were analysed separately: ITS to determine species level relationships and LSU for the generic placement. Approximately 300 and 220 bases were determined for EF-1α and ACT, respectively, and these were concatenated with the corresponding ITS sequences for a combined analysis of the *Pseudocercospora* clade. The manually adjusted ITS alignment contained 73 taxa (including the outgroup sequence) and, of the 533 characters used in the phylogenetic analysis, 228 were parsimony-informative, 59 were variable and parsimony-uninformative and 246 were constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with similar topology and bootstrap values; 1 100 equally most parsimonious trees were obtained from the heuristic search, one of which is shown in Fig. 1 (TL = 1386, CI = 0.444, RI = 0.797, RC = 0.354). The phylogenetic tree derived from the ITS region (Fig. 1) showed that some of the isolates belong to known species, whereas others appeared to be new to science. The manually adjusted LSU alignment contained 35 taxa (including the outgroup sequence) and, of the 797 characters used in the phylogenetic analysis, 121 were parsimony-informative,
**Fig. 1** One of 1 100 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment using PAUP v. 4.0b10. The scale bar shows 10 changes and bootstrap support values higher than 70 % from 1 000 replicates are shown at the nodes. Thickened lines indicate the strict consensus branches and ex-type sequences are printed in **bold** face. The tree was rooted to *Cladosporium sphaerospermum* (GenBank accession AF455481).
54 were variable and parsimony-uninformative and 622 were constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with similar topology and bootstrap values. Two equally most parsimonious trees were obtained from the heuristic search, one of which is shown in Fig. 2 (TL = 407, CI = 0.570, RI = 0.805, RC = 0.459). The phylogenetic tree of the LSU region (Fig. 2) showed two distinct groups of fungal isolates; the first was the Teratosphaeriaceae clade (97 % bootstrap support), whereas the second was the Mycosphaerellaceae clade (88 % bootstrap support).

The manually adjusted combined (ITS, EF-1α and ACT) alignment contained 16 taxa (including the outgroup sequence) and, of the 1 012 characters used in the phylogenetic analysis, 339 were parsimony-informative, 139 were variable and parsimony-uninformative and 534 were constant. Neighbour-joining analysis using the three substitution models on the sequence data yielded trees with similar topology and bootstrap values. A single most parsimonious tree was obtained from the heuristic search and is shown in Fig. 3 (TL = 876, CI = 0.822, RI = 0.852, RC = 0.700). The two trees differed only in their placement of *Ps. basiramifera*; in the one tree it is the closest sister of *Ps. chiangmaiensis* and in the other it is the sister of *Ps. flavomarginata*. 

![Phylogenetic tree](image-url)
Taxonomy

Several taxonomic novelties, namely three *Mycosphaerella*, one *Pseudocercospora* and one *Penidiella* species, were found. These species do not match any species presently described from these genera, or any linked to sequences available in GenBank, and are thus described as new below.

*Mycosphaerella irregulari* Cheewangkoon, K.D. Hyde & Crous, *sp. nov.* — MycoBank MB507001; Fig. 4, 5

*Anamorph.* Unknown.

*Mycosphaerellae tasmaniensis* similis, sed ascosporis cum tubis germinatrix irregulariter latis.

*Etymology.* Named after the irregular width of its ascospore germ tubes.

*Leaf spots* amphigenous, subcircular to oval, pale brown with grey centres, 5–12 mm diam, surrounded by a thin, medium brown margin. *Myelium* external, smooth, septate, branch, medium brown, (2–)2.5–3(–4) µm wide hyphae. *Ascomata* epiphyllous, black, subepidermal to erumpent, ovoid to sub-globose, 45–83 × 45–78 µm; apical ostiole 20–25 µm wide; wall thick (6.5–)7–10(–12) µm, consisting of 2–3 layers of medium brown *textura angularis*. *Asci* apaphysate, fasciculate, subsessile, subcylindrical to narrowly obovoid, straight to

---

**Fig. 3** Single most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the combined ITS, EF and ACT sequence alignment using PAUP v. 4.0b10. The scale bar shows 100 changes and bootstrap support values from 1 000 replicates are shown at the nodes. The tree was rooted to *Teratosphaeria cryptica* (GenBank accessions DQ239971, DQ235119 and DQ147674, respectively). *Ps.* = *Pseudocercospora*.

**Fig. 4** *Mycosphaerella irregulari*. a. Asci; b. germinating ascospores; c. ascospores. — Scale bar = 10 µm.
R. Cheewangkoon et al.: Mycosphaerella on Eucalyptus leaves

83

slightly curved, 8-spored, (25–)35–40(–45) × (6–)7–8(–10) µm. Ascospores bi- to tri-seriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid-ellipsoidal with obtuse ends, widest just above the septum, or in the middle of the apical cell, medianly 1-septate or slightly longer in the basal cell, slightly constricted at septum, tapering toward both ends, but with more prominent taper towards lower end, at times with a mucous-like coating, (8–)9–11(–13) × 2.5–3(–3.5) µm.

Ascospore germination — Germinating from both ends, remaining hyaline; germ tubes grow parallel to the long axis of the spore, with lateral branches parallel or perpendicular to the long axis of the spore; germination tubes irregular in width, constrict at the median septum of the spore, becoming (11–)13–15(–16.5) × 4–5(–5.5) µm, slightly distorting (Type I; Crous 1998).

Cultural characteristics — Colonies reach 15 mm diam on MEA after 15 d at 25 °C in the dark; circular, convex, with a slightly undulate, smooth margin, and medium aerial mycelium; pale greenish grey to pale olivaceous-grey (surface); olivaceous-black (reverse).

Specimen examined. Thailand, Udonthani, on living leaves of Eucalyptus sp, July 2007, R. Cheewangkoon, holotype CBS H-20135, cultures ex-type CBS 123242 = CPC 15408, CPC 15431, CPC 15432.

Notes — The ascospore morphology of *M. irregulari* is similar to that of *M. tasmaniensis* (Crous et al. 1998), *M. flexuosa* (Crous 1998), *M. ellipsioidea* (Crous & Wingfield 1996) and *M. heimii* (Crous & Swart 1995). However, *M. irregulari* can be distinguished from these species by its irregular germ tubes and germination pattern. Phylogenetically, it is also not closely related to any of the species cited above, but clusters near to *M. pseudomarksii* (100 % bootstrap, Fig. 1), which has a distinct morphology.

**Mycosphaerella pseudomarksii** Cheewangkoon, K.D. Hyde & Crous, sp. nov. — MycoBank MB507003: Fig. 6, 7

*Mycosphaerella marksii* similis, sed ascosporis majoribus, (12–)14–17(–18.5) × (2.5–)3(–3.5) µm.

Etymology. Named after *Mycosphaerella marksii* to which it is morphologically similar.

Leaf spots not observed. Ascomata amphigenous in apparently healthy tissue (endophyte?), occurring on greenish brown part of the leaf after incubation in moist chambers for 2 d, black, subepidermal to erumpent, globose to subglobose, 42–60 × 45–80 µm; apical ostiole 20–35 µm wide; wall consisting of 2–3 layers of medium brown textura angularis. Asci apophysate, fasciculate, bitunicate, subsessile, subcylindrical to narrowly ovoid, slightly curved, 8-spored, (40–)42–45(–48) × (6.5–)7–8(–8.5) µm. Ascospores bi- to tri-seriate overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, fusoid with obtuse ends, widest in the middle of the asymmetrical apical cell, medianly 1-septate or with slightly longer basal cell; tapering toward both ends, but with more prominent taper towards lower end, (12–)14–17(–18.5) × (2.5–)3(–3.5) µm. Spermatogonia well developed, amphigenous, dark brown, subepidermal to erumpent, globose to subglobose, up to 90 µm diam. Spermatia hyaline, smooth, rod-shaped, with obtuse ends, (3.5–)4–5(–5.5) × 1.5–2 µm.

![Fig. 5 Mycosphaerella irregulari. a. Leaf spot; b, c. sections through ascomata; d. asci; e. ascospores; f. germinating ascospores; g. colony on MEA. — Scale bars: a = 1 mm; b, c = 20 µm; d–f = 10 µm.](image-url)
Ascospore germination — Germinating from both ends, with a thin, mucous-like coat visible surrounding ascospores on agar; germ tube growing parallel to the long axis of the spore, regular in width, remaining hyaline, not distorting or becoming constricted at septum (Type B; Crous 1998).

Cultural characteristics — Colonies reach 15 mm diam on MEA after 15 d at 25 °C in the dark; subcircular, convex, with even margin, slightly folded, with sparse aerial mycelium; pale olivaceous-grey to olivaceous-grey (surface); olivaceous-black (reverse).

Specimen examined. THAILAND, Chiang Mai, Mae Tang, on living leaves of Eucalyptus sp., June 2007, R. Cheewangkoon, holotype CBS H-20134, cultures ex-type CBS 123241 = CPC 15410, CPC 15435, CPC 15436.

Notes — Mycosphaerella pseudomarksii was most similar to M. marksii (Carnegie & Keane 1994) based on its asymmetrical apical ascospore cells and ascospore germination patterns. However, germinating ascospores of M. pseudomarksii have a visible mucilaginous sheath, which was not observed in germinating ascospores of M. marksii. Furthermore, ascospores of M. pseudomarksii were slightly longer and wider (12–)14–17(–18.5) × (2.5–)3(–3.5) µm than those of M. marksii (11–)12–14(–16) × 2–2.5(–3) µm. Crous & Wingfield (1996) observed considerable variation in ascospore dimensions of several collections of M. marksii (12.5–22.5 × 2.5–5 µm) commenting that this may represent a species complex. Phylogenetically the two species are also distinct (Fig. 1).

Fig. 6 Mycosphaerella pseudomarksii. a. Vertical section through an ascoma; b. vertical section through a spermatogonium; c–f. asci; g. ascospores; h. germinating ascospores; i. colonies on MEA. — Scale bars: a, b = 10 µm; c–h = 10 µm.

Fig. 7 Mycosphaerella pseudomarksii. a. Asci; b. germinating ascospores; c. ascospores. — Scale bar = 10 µm.
Mycosphaerella quasiparkii Cheewangkoon, K.D. Hyde & Crous, sp. nov. — MycoBank MB507002; Fig. 8, 9

Anamorph. Unknown.

Mycosphaerellaceae parkii similis, sed ascosporis ellipsoideis et coloniis bubalinis in agaro MEA.

Etymology. Named after its similarity to Mycosphaerella parkii.

Leaf spots amphigenous, round to irregular, separate, becoming confluent, 5–15 mm diam, medium brown on adaxial surface, pale brown on abaxial surface, surrounded by a raised border, which is dark brown on the adaxial surface and paler brown on the abaxial surface. Ascomata epiphyllos, black, subepidermal to erumpent, subglobose, 40–60 × 40–55 µm; apical ostiole 10–15 µm wide; wall consisting of 3–4 layers of medium to dark brown textura angularis. Asci aparaphysate, fasciculate, bitunicate, subsessile, broadly ellipsoid to obclavate, straight to slightly curved, 8-spored, (30–)45–50(–57) × (7–)8.5–9(–9.5) µm. Ascospores bi- to multiseriate, overlapping, hyaline, guttulate, thin-walled, straight to slightly curved, ellipsoidal to ovoid with obtuse ends, widest in the middle of the apical cell, mediately 1-septate, not constricted at the septum, tapering toward both ends, with a thin mucilaginous sheath, (9–)10–11(–12.5) × (2.5–)3–3.5(–4.5) µm.

Ascospore germination — Germinating with more than one germ tube per cell. Initial germ tubes originating from polar ends, growing parallel to the long axis of the spore, with perpendicular germ tubes developing later; ascospores remain hyaline, but become constricted at the median septum, and distorting, (2.8–)3.5–4(–4.5) µm wide (Type D; Crous 1998).

Cultural characteristics — Colonies reach 27 mm diam on MEA after 15 d at 25 °C in the dark; circular, low convex, with entire edge and sparse aerial mycelium; buff (surface); vinaceous-buff (reverse).

Specimen examined. THAILAND, Burirum, on living leaves of Eucalyptus sp., July 2007, P. Suwannawong, holotype CBS H-20132, cultures ex-type CBS 123243 = CPC 15409, CPC 15433, CPC 15434.

Notes — Mycosphaerella quasiparkii is morphologically similar to M. parkii (Crous et al. 1993, 2006c) with which it also shares the same ascospore germination pattern. However, M. quasiparkii has more ellipsoid ascospores and paler colonies on MEA. Phylogenetically, M. quasiparkii clusters close to Phaeoramularia calotropidis (AY303969, Fig. 1).
Penidiella eucalypti Cheewangkoon, K.D. Hyde & Crous, sp. nov. — MycoBank MB507004; Fig. 10, 11

Teleomorph. Unknown.

Differt a omnibus speciebus Penidiellae conidiis mucosis in vitro (MEA) formantibus.

Etymology. Named after its host genus, Eucalyptus.

Leaf spots not observed. Mycelium consisting of branched, septate, smooth to slightly verruculose, pale to medium brown, (2.5–)3–4(–5) µm wide hyphae. Conidiophores macro- and micronematous, occasionally micronematous, arising from superficial mycelium, solitary, erect, straight to slightly curved, branched laterally or not, medium to dark brown, slightly thick-walled, wall ≤ 1 µm wide, smooth to finely verruculose, (30–)150–
200(–220) × (4–)4.5–5.5(–6.5) µm, wider at the base, (5.5–)6–7(–8.5) µm, (0–)4–6(–8) septate. Conidiogenous cells terminal, cylindrical to subcylindrical, tapering to a flattened apical region, smooth to finely verruculose, medium brown, (10–)13–17(–25) × (3–)4–5(–6) µm; scars thickened, somewhat darkened, 2–2.5 µm wide. Ramoconidia: primary ramoconidia subcylindrical or obvoid, (0–)1(–3)-septate, base truncate, with (1–)2–3(–4) apical hila, pale olivaceous to pale brown, smooth to finely verruculose, wall < 1 µm wide, (25–)35–40(–48) × (3.5–)4–5(–6.5) µm; scars thickened and slightly darkened, 2–2.5 µm wide; giving rise to chains of up to 12 conidia; secondary ramoconidia obvoid, 0–1-septate, some constricted at septum, base truncate, with 2–3(–4) apical hila, pale olivaceous, smooth, (13–15–20(–28) × (4.5–)5–6(–7.5) µm; intercalary conidia obvoid, 0–1(–2)-septate, some constricted at septa, base truncate, with 2–3 apical hila, pale olivaceous, smooth, (10–)12–15(–18) × (3.5–)4–5(–5.5) µm. Conidia in branched acropetal chains, broadly fusiform to obvoid, 0–1-septate, pale olivaceous, paler towards the apex, (9–)12–15(–19) × (4–)5–6(–7) µm; terminal conidia ovoid, 0-septate, pale olivaceous to hyaline, paler towards the apex, base truncate, conidia have a wing-like mucilaginous sheath in culture, extending up to 5 µm wide on each side, tapering towards the polar ends.

Cultural characteristics — Colonies on MEA reaching 20 mm diam after 15 d at 25 °C; margin feathery, colonies erumpent, spreading, with moderate aerial mycelium. Surface grey-olivaceous, reverse olivaceous-black.

Specimens examined. THAILAND, Phayakpoompisai, Mahasarakam, on leaves of Eucalyptus camaldulensis, July 2007, P. Suwannawong, holotype CBS H-20136, cultures ex-type CBS 123246 = CPC15411, AGI064.1, AGI064.2 (occurring on a lesion in association with several microfungi).

Notes — Penidiella eucalypti is a typical species of Penidiella by having solitary conidiophores with a branching system consisting of ramoconidia that form secondary ramoconidia and conidia, with slightly thickened, darkened scars (Crous et al. 2007c). Other than being phylogenetically distinct (Fig. 1, 2), P. eucalypti is distinct from most other species of Penidiella by having a prominent branching system which develops on a single terminal conidiogenous cell. Another character that has not previously been reported in the genus is the distinct mucilaginous sheath observed on conidia formed on MEA.

Pseudocercospora chiangmaiensis Cheewangkoon, K.D. Hyde & Crous, sp. nov. — MycoBank MB507005; Fig. 12, 13

Teleomorph. Unknown.

Pseudocercosporae basiramiferae similis, sed cellulis conidiogenis terminalibus et intercalaribus et stromatibus bene evolutis.

Etymology. Named after Chiang Mai, the province in Thailand from where it was collected.

Leaf spots amphigenous, subcircular to angular, 2–6 mm diam, pale to medium brown, surrounded by a slightly raised, dark-brown border, becoming confluent with age, leading to leaf blight from the leaf tip. Mycelium predominantly internal, consisting of smooth, septate, branched (1.5–)2–3(–4) µm wide hyphae. Caespituli amphigenous, more prominent on abaxial leaf surface, pale grey on leaves, often on dark brown to black and thickened leaf tissue, 25–50 × 50–100 µm. Stromata immersed, becoming erumpent, medium brown, 25–70 × 30–70 µm wide. Conidiophores reduced to conidiogenous

Fig. 11 Penidiella eucalypti. a. Conidia; b. conidial branching system. — Scale bars = 10 µm.
cells or one supporting cell, occasionally arising from upper cells of stroma, subcylindrical, 1–3(-6)-septate with intercalary conidiogenous cells, (13–)20–25(-60) × 3–4(-4.5) µm, situated on the superficial part of the stroma. Conidiogenous cells terminal or intercalary, subcylindrical to obclavate, medium brown, becoming paler toward apex, straight to geniculate-sinuous, tapering to a truncate or bluntly rounded apex, at times subdenticulate, smooth, medium to thick-walled, variable in length, (5–)10–11(-12) × (2–)3(-5) µm, unbranched, proliferating sympodially; conidial scars thickened at the rim, not darkened, inconspicuous. Conidia solitary, subcylindrical to narrowly obclavate, tapering toward the subobtuse apex; base obconic-subtruncate, (2–)3–5(-10)-septate, straight to slightly curved, pale to medium brown, smooth, thin-walled, guttulate, (40–)50–60(-100) × (2–)2.5–3(-3.5) µm (up to 140 µm long in moist chambers); hilum thickened and somewhat darkened at the rim (paracercospora-like), not refractive, at times inconspicuous; microcyclic conidiation observed when incubated in a moist chamber.

Cultural characteristics — Colonies reaching 18 mm diam on MEA after 15 d at 25 °C in the dark; colonies circular, convex, with entire margin and medium aerial mycelium; pale greenish grey (surface), fuscous-black (reverse).
DISCUSSION

Although numerous species of Mycosphaerella have been associated with Eucalyptus leaf diseases in tropical regions around the world (Crous 1998, Crous et al. 2000, 2006c, 2007b, c, 2008), only a few of these species have been documented from Asia. The number of novel species found in the present study from sampled Eucalyptus leaves collected in Thailand, was thus not totally unexpected. The three new Mycosphaerella spp. (i.e. M. irregulari, M. pseudomarksii and M. quasiparkii) identified here were difficult to distinguish from other species of Mycosphaerella based on their ascospore morphology and germination patterns alone, which are the characters that have been commonly used in the past for taxonomic classification of Mycosphaerella spp. (Park & Keane 1982, Crous 1998). Therefore, the DNA sequencing data generated here proved particularly helpful in distinguishing these species.

Mycosphaerella pseudomarksii has ascospores with asymmetrical apical cells similar to M. marksii, the only difference being ascospore dimensions and the presence of a mucilaginous ascospore sheath in M. pseudomarksii. Ascospores of M. irregulari are fusoid-ellipsoidal, thus being similar to those of M. ellipsoidea, M. flexuosa, M. heimii and M. tasmaniensis. Although these species have similar ascospore dimensions, M. irregulari is characterised by the distinctive irregular width of its ascospore germination tubes. Mycosphaerella quasiparkii has an ascospore morphology and germination pattern almost identical to M. parkii, but the presence of a thin mucous-like layer on ascospores of M. quasiparkii and its buff colonies, distinguishes it from M. parkii, which lacks an ascospore sheath and has olivaceous-grey colonies. Pseudocercospora chiangmaiensis, which is also newly described from Eucalyptus, shares some morphological features (conidial dimensions, hilum thickening and microcyclic conidiation) with Ps. basiramifera. It is distinct, however, by having terminal and intercalary conidigenous cells in vivo and having conidiophores arising from a well-defined stroma. Phylogenetic analyses of the ITS and ACT genes showed limited differences (only 1 nucleotide) between Ps. chiangmaiensis and Ps. assamensis, which Arzanlou et al. (2008) recently described from banana. Morphologically, however, they differ in the basal conidial cell shape and marginal thickening along the hilum. These differences were supported by analyses based on the EF gene, which indicated these two species to differ by 38 nucleotides.

Other than new species of Mycosphaerella and Pseudocercospora, the present study also led to the discovery of a novel species of Penidiella. Penidiella eucalypti is distinct in that it has a distinctive branching system developed chiefly on a single conidiogenous locus, and conidia with a persistent, characteristic mucilaginous sheath in vitro. Results from the phylogenetic analyses also indicated that this species belongs to a clade represented by an undescribed Teratosphaeria sp. (DQ632682) which could represent its teleomorph.

Two strains that were phylogenetically closely related to M. irregulari (95 % bootstrap), namely CPC 15446 and CPC 15447, represent two undescribed species of Mycosphaerella. Mycosphaerella sp. CPC 15446 differs from M. irregulari by having ascospores with more obtuse apical cells and germination tubes that are regular in width. Although insufficient material was available of Mycosphaerella sp. CPC 15447, it occurred in lesions in association with M. thailandica and M. heimii. Another undescribed Mycosphaerella species, CPC 15448, was closely related to M. quasiparkii (89 % bootstrap). More collections are required to resolve their status. In the present study, three known species were also identified from the diseased Eucalyptus leaf samples, namely M. heimii, M. thailandica and M. vietnamensis. Although M. thailandica and M. heimii were previously reported on Eucalyptus in Thailand (Crous et al. 2007b), M. vietnamensis represents a record for this country.

This study has again demonstrated that morphological characters and molecular techniques are complementary, and necessary, to uncover the diversity and geographical range of Mycosphaerella and Teratosphaeria species occurring on Eucalyptus. Although five new fungal species have been identified and one species represents a new record from diseased Eucalyptus leaves from Thailand, it is still unknown whether these species are native or exotic. We expect that more unidentified disease-causing microfungi await discovery in Thailand, because the expanding area of Eucalyptus plantations allow fungal pathogens to cross geographical barriers to infect new hosts (i.e. from exotic Eucalyptus to other native trees) more easily, and also increase the chance of infection by native fungi to the exotic plantations (Slippers et al. 2005). Some examples of introduced pathogens from exotic Eucalyptus are T. cryptica, T. nubilosa (Park & Keane 1982, Wingfield et al. 1995), and T. suttonii (Chippopa 1987, Crous et al. 1989, 1997). These species were described from Australia where Eucalyptus is native, but were found later in other countries where this host has been planted as an exotic. In addition, the study of Mycosphaerella spp. on exotic Acacia in the tropics (Crous et al. 2004b) again revealed examples of host sharing of Mycosphaerella citrin on Citrus, Acacia and Musa. More extensive research should thus be carried out to provide information concerning the fungal diversity of exotic Eucalyptus plantations in Thailand and other Asian countries to promote the understanding of the evolution of new pathogens and the movement of fungi between continents.
REFERENCES


Crous PW. 2002. Taxonomy and pathology of Cylindrocladium (Calonectria) and allied genera. APS Press, Minnesota, USA.


Park RF, Keane PJ. 1982. Leaf diseases of Eucalyptus associated with Myco-
Park RF, Keane PJ, Wingfield MJ, Crous PW. 2000. Fungal diseases of euca-
cal Research 98: 625–634.
Slippers B, Smit WA, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ. 2006. Taxonomy, phylogeny and identification of Botryosphaeriaceae asso-
tion 20: 420–421.
Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of en-