Pestalotioid fungi from Restionaceae in the Cape Floral Kingdom

Seonju Lee, Pedro W. Crous and Michael J. Wingfield

1Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Lumnor Road, Hillcrest, Pretoria 0002, South Africa; 2Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Abstract: Eight pestalotioid fungi were isolated from the Restionaceae growing in the Cape Floral Kingdom of South Africa. Sarcostroma restionis, Truncatella megaspora, T. restionacearum and T. spadicea are newly described. New records include Pestalotiopsis matildae, Sarcostroma lomatiae, Truncatella betulae and T. hartigi. To resolve generic affiliations, phylogenetic analyses were performed on ITS (ITS1, 5.8S, ITS2) and part of 28S rDNA. DNA data support the original generic concept of Truncatella, which encompasses Pestalotiopsis species having 3-septate conidia. The genus Sarcostroma is retained as separate from Seimatosporium.

Taxonomic novelties: Pestalotiopsis matildae (Richatt) S. Lee & Crous comb. nov., Truncatella betulae (Morochk.) S. Lee & Crous comb. nov., Sarcostroma restionis S. Lee & Crous sp. nov., Truncatella megaspora S. Lee & Crous sp. nov., Truncatella restionacearum S. Lee & Crous sp. nov., Truncatella spadicea S. Lee & Crous sp. nov.

Key words: Fungi imperfecti, fynbos, microfungi, South Africa, systematics.

INTRODUCTION

The Restionaceae (restios) is a monocotyledonous family distributed in the Southern Hemisphere, which includes more than 30 genera and about 400 species (Figs 1–6). In Africa approximately 330 species are found, mostly in the south-western tip of South Africa (Haaksma & Linder 2000). This area, comprising 90 000 km² and known as the Cape Floral Kingdom, is home to more than 8 500 plant species, of which 5 800 are endemic (Cowling & Richardson 1995). Fynbos is the dominant vegetation type of the Kingdom contributing 80 % of its species. Approximately 94 % of the restios growing in fynbos are indigenous. Locally, the stems of the plants are used for thatching, matting or brooms (Fig. 7). Research on the diversity of saprobiic microfungi in fynbos was initiated in 2000 with an emphasis on two major plant groups: the dicotyledonous Proteaceae and the Restionaceae. About 500 fungal specimens have been collected from restios, of which 40 % represent coelomycetous anamorphs including the so-called pestalotioid fungi. Pestalotioid fungi are defined as those having multi-septate, more or less fusiform conidia with appendages at both or either ends, resembling those taxa accommodated in Pestalotia De Not. or Pestalotiopsis Steyaert, of which teleomorphic connections are found with the members of the Ampisphaeriaceae, Broomella Sacc., Discostroma Clem., and Pestalosphaeria M.E. Barr.

The aim of this study was to characterise pestalotioid fungi from restios growing in fynbos. Four new and four known species are treated. To clarify the phylogenetic relationships between these and other related pestalotioid fungi, DNA sequence data were generated for the partial 28S gene and ITS region (ITS1, 5.8S, ITS2) and phylogenetic analyses were applied.

MATERIALS AND METHODS

Isolates

Field collections were made in Western Cape Province nature reserves and in undisturbed areas of the fynbos during 2000–2002. Culm litter was collected in paper bags. Host identification was done either with the assistance of curators of the Kirstenbosch Botanical Garden or by using Intkey (Linder 2001). Specimens were either studied immediately or air-dried for later use. Dried specimens were re-hydrated in damp chambers with wet filter paper. Single-conidium isolations were made from spore suspensions on 2 % malt extract agar (Merck, Gauteng, South Africa) supplemented with 0.04 g/L streptomycin sulfate, and incubated at room temperature. Reference cultures are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. Herbarium specimens have been deposited in the National Collection of Fungi, Pretoria (PREM), South Africa.

DNA amplification and phylogeny

Fungal isolates were grown in 1 mL 2 % malt extract broth in three 2 mL Eppendorf tubes for up to 7 d. Mycelium was collected and DNA was isolated following a modification of the method of Möller et al. (1992). The primers ITS1 and ITS4 (White et al. 1990) were used to amplify part of the nuclear rDNA spanning the 3’ end of the 18S rDNA, the internal transcribed spacers, the 5.8S rDNA and a part of the 5’ end of the 28S rDNA. The primers LR0R and LR7 were used to amplify part of the large subunit nuclear rDNA (Vilgalys & Hester 1990). Amplification reactions were started with 3 min denaturation in 94 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 1 min annealing at 55 °C and 1.5
min extension at 72 °C, and 10 min extension at 72 °C. For the amplification of partial 28S rDNA, the annealing temperature was adjusted to 50 °C. For specimens that could not be cultivated, direct PCR was performed from conidia with increased cycles (40 cycles). PCR products were separated by electrophoresis at 80–90 V for 15 min in 1 % (w/v) agarose gel in 1× TAE running buffer (0.1 mM Tris, 0.01 mM EDTA, 2 % SDS, pH 8.0) and visualised under UV light.

The amplification products were purified using a modified PEG method (Steenkamp et al. 2005). The purified products were sequenced in both directions using the same primers used in the amplification reactions except for the reverse primer of the partial 28S rDNA where LR5 was used (Vilgalys & Hester 1990). Sequencing reactions were performed using a PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Warrington, U.K.). Nucleotide sequence data were generated with an ABI Prism 3100™ automated DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). The raw sequence data were processed using the Sequence Navigator v. 1.0.1 software package (Perkin-Elmer Applied BioSystems, Foster City, California).

Sequences were assembled and aligned using ClustalW algorithm in MEGA v. 3.1 (Kumar et al. 2004) and finally optimised by eye. Phylogenetic analyses of sequence data were done in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). For parsimony analysis, alignment gaps were treated as fifth character and all characters were unordered and of equal weight. Maximum parsimony was performed for all data sets using the heuristic search option with 100 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. Neighbour-Joining (NJ) with the Tamura-Nei parameter model (Tamura & Nei 1993) was performed with adjusted settings: proportion of invariable sites (I) = 0.6169, gamma distribution (G) = 0.5970, base frequency equal, rate matrix 1.00, 2.3919, 1.00, 2.5792 for partial 28S rDNA; I = 0, G = 0.3769, base frequency equal, substitution model (Ti/tv ratio) 1.6846 for ITS regions. These models were chosen as suggested by MODELTEST v. 3.5 (Posada & Crandall 1998). Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). Other measures calculated included tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC). GenBank accession numbers of sequences generated in this study are listed in Table 1. The DNA sequence alignment is deposited in TreeBASE (Study accession number S1442).

**Phylogenetic analyses**

*ITS:* Approximately 550 bases were determined for the isolates as indicated in Table 1. The manually adjusted alignment consisted of 29 taxa (including the two outgroups) and 612 characters including alignment gaps, of which 247 were parsimony-informative, 111 were variable and parsimony-uninformative, and 254 were constant. Parsimony analysis of the alignment yielded six most parsimonious trees, one of which is presented (Fig. 8). Ingroups consisted of four clades referred to as a *Truncatella* Steyaert clade, a *Pestalotiopsis*-A clade, a *Pestalotiopsis*-B clade and a *Sarcostroma* Cooke clade with 99 %, 100 %, 100 % and 100 % bootstrap support, respectively.

The *Truncatella* clade consisted of two sub-clades. The one sub-clade included five *Truncatella* species from our collections (100 % bootstrap support). And the other included *T. angustata* (Pers.) S. Hughes and species of *Bartalinia* Tassi with 96 % bootstrap support. The *Pestalotiopsis*-A clade included six *Pestalotiopsis* (Ps.) species having conidia with concolorous median cells, and *Ps. matildae* (Richatt) S. Lee & Crous having conidia with versicolorous median cells. The *Pestalotiopsis*-B clade included four *Pestalotiopsis* species having conidia with versicolorous median cells, and formed a sister clade to *Ps. theae* (Sawada) Steyaert, which had conidia with concolorous median cells and knobbed apical appendages (R. Jeewon, pers. comm.). The *Sarcostroma* (Sa.) clade included *Sa. restiosis* S. Lee & Crous and *Seimatosporium* (Se.) *grevillea*e (Loos) Shoemaker which has a characteristic of *Sarcostroma*, centric apical and eccentric basal appendages. The distance tree gave the same topology. Similar bootstrap values were obtained for both parsimony and distance analyses except for the branches supporting two *T. restionacearum* isolates and four *Truncatella* species within the *Truncatella* clade. These branches have higher support in distance analysis (95 % and 92 %, respectively) than in parsimony analysis (63 % and 58 %, respectively).

**RESULTS**

**Taxonomy**

A Zeiss Axioskop 2 Plus microscope was used with differential interference contrast to examine specimens. For some observations, phase contrast (PhC) or bright field (BF) was employed and indicated. Images were captured using a Canon digital camera equipped with a Canon Utilities Remote Capture v. 2.7.3.23. Measurements were done using Axiovision software (AxioVs 40 v. 4.3.0.101). Where possible, thirty measurements were made of all structures. Apical and/or basal appendages were excluded in measurements of conidial length, and were measured separately. For conidial dimensions the 95 % confidence levels were calculated, and extremes provided in parentheses.

To study the internal and peridial structures, vertical sections of conidiomata were made. Small pieces of plant tissue containing conidiomata were taken from dried herbarium material, placed on water agar with a drop of water, and incubated overnight. Tissues were mounted on a disc with Jung tissue freezing medium™. Sections were made (10–12 μm thick) using a Cryomicrotome (Leica CM1100). Sections were lifted onto a coverslip, mounted in lactic acid (85 %), and slides were placed on a heated plate to remove trapped air bubbles.
28S: Approximately 850 bases were determined for the isolates as indicated in Table 1. The manually adjusted alignment contained 26 taxa (including the two outgroups), and 856 characters including alignment gaps, of which 106 were parsimony-informative, 55 were variable and parsimony-uninformative, and 695 were constant. Parsimony analysis yielded fifty most parsimonious trees, one of which is presented (Fig. 9). Ingroups consisted of three clades: a Discostroma clade, a Truncatella/Bartalinia clade, and a basal clade with 94 %, 100 % and 51 % bootstrap support, respectively.

### Table 1. List of species for which DNA sequence data were generated in this study.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Cultures1</th>
<th>Host plants</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ITS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSU</td>
</tr>
<tr>
<td>Pestalotiopsis matildae</td>
<td>CBS 118155 = CMW 18022</td>
<td>Thamnochortus spicigerus</td>
<td>DQ278916</td>
</tr>
<tr>
<td></td>
<td>CBS 118143 = CMW 18285</td>
<td>Thamnochortus fraternus</td>
<td>DQ278917</td>
</tr>
<tr>
<td>Sarcostrama restiosis</td>
<td>CBS 118154 = CMW 17968</td>
<td>Restio filiformis</td>
<td>DQ278922 DQ278924</td>
</tr>
<tr>
<td></td>
<td>CBS 118153 = CMW 17994</td>
<td>Ischyrolepis cf. sieberi</td>
<td>DQ278923 DQ278925</td>
</tr>
<tr>
<td>Truncatella betulæ</td>
<td>SL10153, 4</td>
<td>Ischyrolepis subverticellata</td>
<td>DQ278920</td>
</tr>
<tr>
<td>T. hartigii</td>
<td>CBS 118145 = CMW 17958</td>
<td>Cannomois virgata</td>
<td>DQ278912 DQ278927</td>
</tr>
<tr>
<td></td>
<td>CBS 118148 = CMW 18093</td>
<td>Rhodocoma capensis</td>
<td>DQ278913 DQ278928</td>
</tr>
<tr>
<td>T. megaspora</td>
<td>PREM 588703</td>
<td>Restio egregius</td>
<td>DQ278928</td>
</tr>
<tr>
<td>T. restionacearum</td>
<td>CBS 118150 = CMW 17968</td>
<td>Restio filiformis</td>
<td>DQ278914</td>
</tr>
<tr>
<td></td>
<td>CMW 18755</td>
<td>Ischyrolepis cf. gaudichaudiana</td>
<td>DQ278915 DQ278929</td>
</tr>
</tbody>
</table>


2Ex-type cultures or holotypes.

3Sequenced from direct PCR amplification of conidia.

4No herbarium specimen left after examination.

### Table 2. Conidial characteristics of the species described in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>PREM no.1</th>
<th>Conidial dimensions in μm (Length × Width)</th>
<th>No. of septa</th>
<th>Ratio (L : W)</th>
<th>Apical appendages</th>
<th>Basal appendages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
<td>Length (μm)</td>
</tr>
<tr>
<td>Pestalotiopsis matildae</td>
<td>58862</td>
<td>(22–)24–25(-29.5) × (6.5–)7(–8.5) (av. 24.5 × 7.2)</td>
<td>4</td>
<td>3.4 : 1</td>
<td>2–3</td>
<td>13–19</td>
</tr>
<tr>
<td></td>
<td>58861</td>
<td>(19–)22–24(-27.5) × (5.5–)6.5–7(–8) (av. 22.8 × 6.7)</td>
<td>4</td>
<td>3.4 : 1</td>
<td>2–3</td>
<td>8–11</td>
</tr>
<tr>
<td>Sarcostrama lomatiae</td>
<td>58863</td>
<td>(15–)19–20.5 (-25) × (5–)6–7 (av. 19.8 × 6.7)</td>
<td>4</td>
<td>3.0 : 1</td>
<td>1</td>
<td>30–38</td>
</tr>
<tr>
<td>S. restiosis</td>
<td>58865T</td>
<td>(15–)17–18–(20) × (6–)7–7.5(–9) (av. 17.1 × 7.3)</td>
<td>4–5</td>
<td>2.3 : 1</td>
<td>1</td>
<td>27–38</td>
</tr>
<tr>
<td></td>
<td>58864</td>
<td>(17–)19–20–(22.5) × (7–)8(–10) (av. 19.8 × 8.2)</td>
<td>4</td>
<td>2.4 : 1</td>
<td>1</td>
<td>37–45</td>
</tr>
<tr>
<td>Truncatella betulæ</td>
<td>58867</td>
<td>(14–)16.5–17(–18) × 7–7.5(–8) (av. 16.8 × 7.3)</td>
<td>3</td>
<td>2.3 : 1</td>
<td>2–4</td>
<td>8–16</td>
</tr>
<tr>
<td></td>
<td>58866</td>
<td>(15–)16–17–(19.5) × (5–)6–7(–8) (av. 16.5 × 6.5)</td>
<td>3</td>
<td>2.5 : 1</td>
<td>3–5</td>
<td>8–15</td>
</tr>
<tr>
<td>(SL1015)</td>
<td></td>
<td>(14–)16–17(–18) × (5–)6(–7) (av. 16.3 × 6.2)</td>
<td>2</td>
<td>2.6 : 1</td>
<td>2–5</td>
<td>8–13.5</td>
</tr>
<tr>
<td>T. hartigii</td>
<td>58869</td>
<td>(16–)17–18–(20) × (6–)7(–8) (av. 17.8 × 7.1)</td>
<td>3</td>
<td>2.5 : 1</td>
<td>2–4</td>
<td>26–31</td>
</tr>
<tr>
<td></td>
<td>58868</td>
<td>(15.5–)18–19(–20.5) × (6–)7(–8) (av. 18.3 × 7.1)</td>
<td>3</td>
<td>2.6 : 1</td>
<td>2–4</td>
<td>24–33.5</td>
</tr>
<tr>
<td>T. megaspora</td>
<td>58870T</td>
<td>(25–)30–31(–36) × (9–)11–12(–13) (av. 30.5 × 11.8)</td>
<td>3</td>
<td>2.6 : 1</td>
<td>2–4</td>
<td>9–23</td>
</tr>
<tr>
<td>T. restionacearum</td>
<td>58872T</td>
<td>(20–)22–23(–26.5) × (6–)7(–8) (av. 22.8 × 7.1)</td>
<td>3</td>
<td>3.3 : 1</td>
<td>2–4</td>
<td>30–44</td>
</tr>
<tr>
<td></td>
<td>58871</td>
<td>(21–)24–25.5(–29) × (5–)7(–8) (av. 24.9 × 6.8)</td>
<td>3</td>
<td>3.7 : 1</td>
<td>(2–)3(–4)</td>
<td>22.5–55</td>
</tr>
<tr>
<td>T. spadicea</td>
<td>58873T</td>
<td>(20–)21–22(–23) × (7–)8(–8.5) (av. 21.4 × 7.8)</td>
<td>3</td>
<td>2.7 : 1</td>
<td>3–4</td>
<td>12–16(–25)</td>
</tr>
</tbody>
</table>

1PREM: National Collection of Fungi, Pretoria, South Africa.

2Type specimen.
Fig. 8. One of six most parsimonious trees obtained from the ITS regions and 5.8S rDNA sequence data (TL = 788 steps, CI = 0.772, RI = 0.886, RC = 0.684). Parsimony bootstrap support values from 1000 replicates are indicated on the nodes and those from distance analysis are indicated in parentheses. Branches supporting ingroups are in bold. The tree was rooted to Hypoxylon schweinitzii and Xylaria hypoxylon.

The Discostroma clade accommodated Sa. restionis, three Seimatosporium Corda species and a Discostroma species (teleomorphic state of either Seimatosporium or Sarcostroma). The Truncatella/Bartalnia clade had two sub-clades with T. angustata and T. laurocerasi (Westend.) Steyaert as basal taxa. The one sub-clade included Truncatella sp., T. conorum-piceae (Tubeuf) Steyaert, and a group of T. hartigii (Tubeuf) Steyaert and T. restionacearum S. Lee & Crous with 100 % bootstrap support. The other sub-clade of the Truncatella/Bartalnia clade contained a species of Dyriothrips L. Cai, R. Jeewon & K.D. Hyde (anamorphic Amphisphaeriaceae) and two Bartalnia species (teleomorph connection unknown). The topology of the NJ tree was essentially similar to the parsimony trees in grouping three clades, except for the rearrangement of taxa within each clade. Bootstrap values were similar for both analyses, except for the branch supporting two T. hartigii isolates which received higher support in distance analysis (99 %) than in parsimony analysis (54 %).

Taxonomy
A total of 14 specimens with pestalotioid conidia and acervuloid–pycnidioid conidiomata were collected in this study. They were identified as belonging to three known genera representing eight species. Of these, four are treated as new taxa, and they are described below. Conidial characteristics of the respective species are summarised in Table 2.

≡ Pestalotia matildae Richatt, Agricultura Técnica (Chile) 13: 91. 1953.

Conidiomata pycnidial, scattered or gregarious and laterally joined, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section subglobose to ellipsoidal, 193–366 × 178–215 μm. Peridium pseudoparenchymatous, in section 13–16(–28) μm thick, consisting of 3–several layers of pale brown, moderately thick-walled cells of textura angularis. Conidiophores arising from the entire periphery of the inside of the conidiomata, reduced to conidigenous cells or poorly developed, branched at the base, ampulliform. Conidiogenous cells annelidic, hyaline, discrete or integrated, smooth, lageniform to cylindrical, 7–12 × 1–2 μm. Conidia fusiform, (22–)24–25(–29.5) × (6.5–7) × 7.2 μm, ratio 3.4 : 1, 4-septate; apical cell hyaline, conical to trapezoid, 3–5 × 3–4 μm, smooth, thin-
Nag Raj (1993) and Guba (1961) treated seven species similar to the following:

**Hosts**


**Specimens examined**

From the species description by Guba (1961), and the characteristics of the third cell being darker than the fourth cell. Based on the species description by Nag Raj (1993), it is clear that recircumscription of *Pestalotia* is supported by the DNA sequence data presented in this study.

**Notes**

The two collections are morphologically most similar to the following seven species as treated by Nag Raj (1993) and Guba (1961): *Pestalotiopsis leucopogonis* Nag Raj, *Ps. macrospora* (Ces.) Steyaert, *Ps. palustris* Nag Raj, *Ps. metasequoiae* (Gucevič) Nag Raj, *Pestalotia* (Pa.) *paeoniae* Servazzii [= *Ps. paeoniae* (Servazzii) Steyaert], *Pa. batatae* Ellis & Everh., and *Pa. matildae*.

Different from our collections, *Ps. leucopogonis* has apical appendages that originate in three levels (tiers) on the apical cell, *Ps. macrospora* has larger conidia (25–10 × 9–11 μm), *Ps. palustris* has smaller conidia (25–25 × 5.5–7 μm) and distinct striations on second and fourth cells, *Ps. metasequoiae* has verruculose, pale brown second and fourth cells, and *Pa. paeoniae* has larger apical appendages (16–26 μm). *Pestalotia batatae* has third and fourth cells that are always darker than the second cell, whereas our collections often had the third cell being darker than the fourth cell. Based on the morphological comparisons, our collections best fit the characteristics of *Pa. matildae*.

From the species description by Guba (1961), and the recircumscription of *Pestalotia* and *Pestalotiopsis* by Nag Raj (1993), it is clear that *Pa. matildae* resides in *Pestalotiopsis*, a decision that is also supported by the DNA sequence data presented in this study.

Conidiomata acervular, scattered or gregarious, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses, lifting up the epidermis; in section low conoid, 187–366 \( \mu \)m wide. *Basal stroma* pseudoparenchymatous, consisting of a few layers of brown, thick-walled, globose to angular cells, 9.5–21 \( \mu \)m thick; lateral tissue absent. *Conidiophores* arising from the basal stroma, cylindrical, 4–10 × 2–3 \( \mu \)m.

**Conidiogenous cells** annelidic, hyaline, discrete, smooth, cylindrical to lageniform, 14–20 × 2–4 \( \mu \)m. **Conidia** fusiform, straight or slightly curved, (15–)19–20.5(–25) × (5–)6–7 \( \mu \)m (av. 19.8 × 6.7 \( \mu \)m, ratio 3 : 1), 4-septate; apical cell hyaline, conical, 2–3 \( \mu \)m long, 2.5–3.5 \( \mu \)m wide at the base, smooth, thin-walled; median cells brown, concoloured, doliiform, 12.5–16 × 7–8 \( \mu \)m (second cell from the base (4–)5–6(–7) \( \mu \)m long, av. 5.4 \( \mu \)m; fourth cell (3–)5(–7) \( \mu \)m long, av. 5.0 \( \mu \)m), echinulate, thick-walled, at times wall extended like bubbles; basal cell hyaline, obconical with truncate end, 2–4 \( \mu \)m long, 3–3.5 \( \mu \)m wide at the top, smooth, thin-walled. **Apical appendage** single, centric,

---

unbranched, 30–38 × 1–1.5 μm, flexuous, attenuated. Basal appendage single, excentric, unbranched, 30–36 × 1–1.5 μm, flexuous, attenuated.


Hosts: *Lomatia ilicifolia* (Proteaceae), *Ischyrolepis cf. gaudichaudiana* (Restionaceae)

Notes: Our collections from the Restionaceae resulted in three *Sarcostroma* specimens representing two species. All of these had long, single appendages at both ends. Based on its conidial and appendage dimensions, one *Sarcostroma* species (PREM 58863) matched the descriptions of *Sa. lomatiae* and *Sa. berberidis* (Lind) Nag Raj (Nag Raj 1993). The main character separating these two species in Nag Raj (1993) is the length of second and fourth conidial cells from the base. *Sarcostroma lomatiae* has equal length of cells (4–6 μm, av. 5 μm), whereas *Sa. berberidis* has unequal length (second cell (3.5–)4–6 μm, av. 5 μm; fourth cell 4–4.5(–5) μm, av. 4.3 μm). However, this difference is not obvious from Nag Raj’s line drawings of these species (Nag Raj 1993), as some of these cells in the depicted conidia of *Sa. lomatiae* are also unequal in length. Our collection has unequal length of second and fourth conidial cells. But the difference is not as noteworthy as in *Sa. berberidis* and furthermore the range of length fits best that of *Sa. lomatiae*.

*Sarcostroma restionis* S. Lee & Crous, sp. nov. MycoBank MB500858. Figs 20–24.

Etymology: in reference to its host genus, Restio.

Conidiomata acervulata. Conidiophora cum adunt e fundo texturata laterali conidiomatis exorientia, debiliter evoluta vel solum conidiomata acervulares, scariosa, non ramosa, 25–40 × 2–3 μm, echinulatis, crassitunicatis; basal cellula hyalina, obconica, 10–16 × 1–2 μm, echinulata, thick-walled, short-walled; apical cellula hyalina, obconica, 6–10 × 1–1.5 μm, flexuous, attenuated. Apical appendages absent.

Conidiomata acervulata, scattered or gregarious, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section low conidial cells, 50–67 μm high, 170–413 μm wide. Peridium pseudoparenchymatous, in section 4–9 μm thick throughout the conidioma, consisting of a few layers of pale brown, moderately thick-walled, compressed cells of *textura angularis*. Conidiophores arising from the entire periphery of the inside of the conidioma, branched at the base, cylindrical, 10–12(–20) × 1–2 μm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, 4–7 × 2–2.5 μm. *Conidia* fusiform, (15–)16–17–18(–19.5) × (5–)6–7(–8) μm (av. 16.6 × 6.5 μm, ratio 2.5 : 1), 3-septate; apical cellula hyalina, obconica, 10–12 × 7–8 μm, echinulata, thick-walled; basal cellula hyalina, obconica, 2–3 × 2–4 μm, smooth, thin-walled, at times deciduous; median cells brown, doliiform, 10–16 × 7–8 μm, echinulate, thick-walled; basal cellula hyalina, obconical with truncate end, 2.5–3 × 3 μm, smooth, thin-walled. Apical appendage single, excentric, unbranched, 27–38 × 1–1.5 μm, flexuous, attenuated. Basal appendage single, excentric, unbranched, 25–40 × 1–1.5 μm, flexuous, attenuated.


Hosts: *Ischyrolepis cf. sieberi*, Restio filiformis (Restionaceae).

Notes: Three known species are morphologically closest to the two collections of *Sa. restionis*. They are *Sa. cadicola* (B. Sutton) M. Morelet (1985), [= *Sa. cadicola* (B. Sutton) Nag Raj 1993], *Sa. grevilleae* (Loos) M. Morelet (1985) [= *Sa. grevilleae* (Loos) Nag Raj 1993] and *Sa. lomatiae*.

Based on Nag Raj’s (1993) descriptions, *Sa. cadicola* has shorter appendages (basal 12–29 μm, apical 18–33 μm) and smaller conidia (13–16.5 × 6–7.5 μm), and *Sa. lomatiae* has appendages of similar length (basal 14–40 μm, apical 13–40 μm), but larger conidia (18–24 × 6–7 μm) than those of *Sa. restionis*. *Sarcostroma grevilleae* is the closest in terms of conidia and appendages, but the variable shapes of conidia with visible septal pores clearly differentiate it from our collections (Nag Raj 1993). Thus, *Sa. restionis* is introduced as a new species to accommodate these two specimens.


Conidiomata acervuloid, scattered or gregarious, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section low conidial cells, 50–67 μm high, 170–413 μm wide. Peridium pseudoparenchymatous, in section 4–9 μm thick throughout the conidioma, consisting of a few layers of pale brown, moderately thick-walled, compressed cells of *textura angularis*. Conidiophores arising from the entire periphery of the inside of the conidioma, branched at the base, cylindrical, 10–12(–20) × 1–2 μm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, 4–7 × 2–2.5 μm. *Conidia* fusiform, (15–)16–17–18(–19.5) × (5–)6–7(–8) μm (av. 16.6 × 6.5 μm, ratio 2.5 : 1), 3-septate; apical cellula hyalina, obconica, 2–3 × 3–3.5 μm, smooth, thin-walled, at times deciduous; median cells brown, doliiform, 12–15 × 7–8 μm, echinulata, thick-walled; basal cellula hyalina, obconical, 2–3 × 3–4 μm, smooth, thin-walled, at times deciduous. Apical appendages 3–4, inserted in the topmost part of the apical cell, arising at the same point, occasionally branched, flexuous, 8–16 × 1 μm. Basal appendages absent.

Hosts: Betula alba (Betulaceae), Elegia filacea, Elegia juncea, Ischyrolepis subverticellata (Restionaceae).

Notes: The three collections are morphologically similar to two known species: Pestalotiopsis puyae (Henn.) Nag Raj and Pa. betulae (Guba 1961, Nag Raj 1993). Pestalotiopsis puyae has similar conidial dimensions (15–18 × 7–7.5 µm) as the fungi in these three collections, but it has much shorter and unbranched apical appendages (3–8 µm). The description of the type specimen of Pa. betulae provided by Guba (1961) (conidia 15–22 × 5.5–8 µm, apical appendages 8–21 µm) closely matches the dimensions of our collections.

The circumscription of Truncatella (Nag Raj 1993) suggests that Pa. betulae should be allocated to this genus. The specimens collected in the present study also clustered in the Truncatella clade (Fig. 1) with a high bootstrap support.

Additional synonyms listed in Guba (1961).

Conidiomata pycnidiod, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section spherical or occasionally conical, at times laterally joined, 106–156 × (73–)124–177 μm. Peridium pseudoparenchymatous, in section 9–12 μm thick throughout the conidioma, consisting of 3–5 layers of pale brown, moderately thick-walled, compressed cells of textura angularis. Conidiophores arising from the entire periphery of the inside of the conidioma, branched at the base, cylindrical, 0–4-septate, 11–25 × 2–3 μm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, 6–19 × 2 μm. Conidia fusiform, (16–)17–18(–20) × (6–)7–8 (μm (av. 17.8 × 7.1 μm, ratio 2.5 : 1), 3-septate; apical cell hyaline, conical to trapezoid, 2.5–3 × 2.5–4 μm, smooth, thin-walled, at times decidual; median cells brown, doliform, 13–14 × 7 μm, echinulate, thick-walled; basal cell hyaline, obconical, 2–3 × 2–3 μm wide, at times deciduous. Apical appendages 2–4(–5), inserted in the topmost part of the apical cell, arising at the same point, flexuous, 26–31 × 1 μm, attenuated, 1–2 appendages often dichotomously branched. Basal appendages absent.


Hosts: Abies alba (Pinaceae), Cannomois virgata, Rhodocoma capensis (Restionaceae).

Notes: The two collections obtained are very similar to T. laurocerasi, T. angustata and T. hartigi. The only obvious difference between these taxa is in the branching patterns of their apical appendages (Guba 1961, Nag Raj 1993). Truncatella laurocerasi has 1–3 simple or staghorn-like branches. Truncatella angustata and T. hartigi have more than one apical appendage, often irregularly or dichotomously branched. However, T. hartigi often has two equal branches that branch dichotomously again. Based on their conidial dimensions and the branching pattern of their apical appendages, our collections are best accommodated in T. hartigi.

Truncatella megaspora S. Lee & Crous, sp. nov. MycoBank MB500860. Figs 35–40.

Etymology: in reference to its large conidia.


Conidiomata pycnidiod, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section subglobose to ellipsoid, 141–245 × 85–136 μm. Peridium pseudoparenchymatous, in section 8.5–18 μm thick throughout the conidioma, occasionally becoming thinner towards the apex, consisting of 3–5 layers of pale brown to brown, moderately thick-walled, highly and moderately compressed cells of textura angularis. Conidiophores arising from the entire periphery of the inside of the conidioma, branched at the base, 8–10 × 2 μm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, 0–3-septate, 7–26 × 2–3 μm. Conidia fusiforma, (25–)30–31(–36) × (9–)11–12(–13) μm (av. 30.5 × 11.8 μm, ratio 2.6 : 1), 3-septate; apical cell hyaline, trapezoid, 3–4 × 3–5 μm, smooth, thin-walled; median cells brown, doliform, 19–24 × 9–13 μm, echinulate, thick-walled; basal cell hyaline, obconical, 5–7 × 3–4.5 μm, smooth, thin-walled. Apical appendages (2–)3(–4), inserted in the top part of the apical cell, arising at different points, unbranched, flexuous, 9–23 × 1–2 μm. Basal appendages absent.


Host: Restio egregius (Restionaceae)

Notes: Truncatella megaspora is unusual in having larger conidia than any other species in this genus. The species with the most similar conidial dimensions are Ps. torrendii (J.V. Almeida & Sousa da Câmara) Nag Raj and T. trevoae (Speg.) Nag Raj (≡ Pestalotia trevoae Speg.). Pestalotiopsis torrendii is, however, different from T. megaspora in having smaller conidia (23–32 × 7.5–10 μm) and more roughly ornamented median conidial cells (verruculose to rugulose) (Guba 1961, Nag Raj 1993). Truncatella trevoae has similar conidial dimensions (25–33.5 × 8–11.5 μm), but can be distinguished from T. megaspora by having 4-septate conidia as opposed to the 3-septate (Nag Raj 1993).

Truncatella restionacea S. Lee & Crous, sp. nov. MycoBank MB500861. Figs 41–45.

Etymology: in reference to its host family, Restionaceae.


Conidiomata pycnidiod, scattered or gregarious, subepidermal, remaining immersed, visible at the
PESTALOTIOID FUNGI FROM RESTIONACEAE

surface by dark exuding conidial masses; in section conoid, convoluted, 200–270 × 520–573 µm. Peridium pseudoparenchymatous, 9–12.5 µm thick throughout the conidioma, consisting of 3–5 layers of pale brown, moderately thick-walled cells of textura angularis. Conidiophores arising from the entire periphery of the inside of the conidiomata, branched at the base, cylindrical, 5–12.5 × 2–3 µm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, (5–)14–31 × 2–3 µm. Conidia fusiform, (21–)24–25.5(–29) × (5–)7(–8) µm (av. 24.9 × 6.8 µm, ratio 3.6 : 1), 3-septate; apical cell hyaline, oblong to trapezoid, 3–4.5 × 2–4 µm, smooth, thin-walled; median cells brown, doliiform, 14–20 × 6–8 µm, echinulate, thick-walled; basal cell hyaline, obconical, 4–5 × 3–4 µm wide at the base, smooth, thin-walled. Apical appendages (2–)3(–4), inserted in the top part or along the upper half of the apical cell, arising at different points, rarely branched, flexuous, 22.5–55 × 1 µm, attenuated. Basal appendages absent.


Hosts: Ischyrolepis cf. gaudichaudiana, Restio filiformis (Restionaceae).

Notes: Truncatella restionacearum is distinct in having 3-septate conidia with relatively long apical appendages. Five species are considered close to the species. These are Ps. eupyrena (Tassi) Nag Raj, Ps. moorei (Harkn.) Nag Raj, Ps. pestalozzioides (Deam. & Fairm.) Nag Raj, Ps. stevensonii (Peck) Nag Raj and Ps. torrendii (Nag Raj 1993). The conidia of Ps. moorei (25–36 × 8–10 µm), Ps. pestalozzioides (25–32 × 8–10 µm) and Ps. torrendii (23–32 × 7.5–10 µm) are larger than those of T. restionacearum. In contrast Ps. stevensonii has smaller conidia (19–23 × 5.5–7.5 µm), and could thus be excluded from the comparisons. Truncatella restionacearum closely matches the description of Ps. eupyrena, although there are some differences between these two species. Pestalotiopsis eupyrena is reported to have up to five apical appendages, and to also have a basal appendage. In contrast, T. restionacearum only developed up to four apical appendages, and basal appendages were never observed. ITS rDNA sequence comparisons also showed T. restionacearum to be congeneric with other species of Truncatella.

Truncatella spadicea S. Lee & Crous, sp. nov. MycoBank MB500862. Figs 46–49.

Etymology: in reference to its pale brown conidia.

Conidiomata pycnidioidea. Conidiophora e tota peripheria interna conidiomatis exorientia, basi ramosa, cylindrica. Cellulare
Conidiomata pycnidial, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by means of dark exuding conidial masses; in section conoid or flat, laterally joined, occasionally overlapping morphological characters used to delineate the genera (Steyaert 1949, Guba 1961, Sutton 1980, Nag Raj 1993, Jeewon et al. 2002). Recent studies employing rDNA sequence data have, however, clarified the confusion, and provided a more complete understanding of the generic circumscriptions for pestalotioid fungi (Jeewon et al. 2002, 2003, 2004).

Sarcostroma

The genus Sarcostroma was introduced by Cooke in 1872. Sutton (1980) reduced Sarcostroma to synonymy with Seimatosporium that accommodated species having 2–5-septate conidia with only a basal appendage, or without any appendages. He acknowledged the heterogeneity of the genus, and anticipated that Seimatosporium would later be subdivided. Sarcostroma was reintroduced by Nag Raj (1993) to accommodate some of the species classified under Seimatosporium. He retained Seimatosporium for species having a mixture of conidia with and without appendages in a single isolate, and Sarcostroma for species having multi-septate, fusiform conidia with attenuated centric apical and excentric basal appendages. Three collections treated in this study had 4-septate conidia with single centric apical and excentric basal appendages. We have adopted the generic concepts of Nag Raj (1993) and placed our species in Sarcostroma as Sa. lomatiae and Sa. restionis.

Phylogenetic data suggest that our new taxon, Sa. restionis is sister to Sa. grevilleae and Sa. leptospermi. The Discostroma clade resolved in this study consists of morphologically heterogeneous taxa, but is well supported in parsimony and distance analyses. Seimatosporium grevilleae has centric apical and excentric basal appendages, and was recognised as a member of Sarcostroma by Nag Raj (1993). Seimatosporium leptospermi R.G. Bagn. & Sheridan has conidial morphology completely different to that of either Sarcostroma or Seimatosporium. This fungus has cylindrical to acerose, mostly hyaline conidia with a tubular basal appendage. The species was placed in Diploceras (Sacc.) Died. as D. leptospermi (R.G. Bagn. & Sheridan) Nag Raj (Nag Raj 1993). Seimatosporium vaccinii (Fuckel) B. Erikss. has conidia devoid of appendages. Sarcostroma restionis has conidia with single appendages at each end. Judging from their diverse conidial morphology, it is surprising that these morphologically different taxa group closely together. As additional species are added, it is possible that more distinct groups will emerge to subdivide this clade.

Truncatella versus Pestalotiopsis

Truncatella was introduced by Steyaert (1949) to accommodate five former Pestalotia species having 3-septate conidia with 1–4-branched or unbranched apical appendages. Later Guba (1961) reduced it to synonymy with Pestalotia section Quadriloculatae. When Sutton (1980) reinstated the genus, he considered that the species placed in Pestalotia (sect. Quadriloculatae) and Monochaetia (sect. Quadriloculatae) as defined by Guba (1961) should be relocated to Truncatella. Nag Raj (1993) agreed with Sutton’s view but still accommodated some species with 3-septate conidia in

**DISCUSSION**

The intergeneric relationships and generic status of pestalotioid fungi (Bartalina, Monochaetia (Sacc.) Allesch., Pestalotia, Pestalotiopsis, Sarcostroma, Seimatosporium, Truncatella) have been the subject of considerable debate in the past. This has been largely due to different generic concepts, and inadequate or overlapping morphological characters used to delineate the genera (Steyaert 1949, Guba 1961, Sutton 1980, Nag Raj 1993, Jeewon et al. 2002). Recent studies employing rDNA sequence data have, however, clarified the confusion, and provided a more complete understanding of the generic circumscriptions for pestalotioid fungi (Jeewon et al. 2002, 2003, 2004).
Pestalotiopsis (e.g. Ps. besseyi (Guba) Nag Raj, Ps. casuarinae (Cooke & Massey) Nag Raj, Ps. citrina and Ps. eupyrena). Recently, the generic distinctiveness of this fungus was confirmed using comparisons of partial 28S rDNA (Jeewon et al. 2002). In the present study, a comparison of ITS rDNA sequence data revealed that isolates with 3-septate conidial cluster in the Truncatella clade, distant from those of the Pestalotiopsis clade with 4-septate conidia. Jeewon et al. (2002) also argued that all species with 3-septate conidia should be accommodated within Truncatella. Our results support this opinion, and agree with Steyaert's original concept of the genus, that Truncatella should be restricted to fungi with 3-septate conidia. More than 80 % of the currently known Pestalotiopsis species have 4-septate conidia (thus Pestalotiopsis), whereas around 34 species (15 %) have 3-septate conidia, and thus belong in Truncatella.

Phylogenies also reveal that Truncatella restionacearum, T. megaspora and T. spadicea are more closely related to T. betulae and T. hartigii than to T. angustata, the generic type. Bartalinia and Dyriothiopsis clustered within the Truncatella/Bartalinia clade, a result similar to that of Jeewon et al. (2002).

Pestalotiopsis is a species-rich genus occurring as pathogens, endophytes and saprobes (Jeewon et al. 2004, Kumar & Hyde 2004, Wei & Xu 2004). It includes approximately 220 published names (www.indexfungorum.org). Many of these were established based on slight morphological differences and host affiliation. Jeewon et al. (2004) studied a number of selected Pestalotiopsis spp. from different origins and host plants using comparisons of sequences for the nuclear rDNA. They concluded that species of Pestalotiopsis were typically not host-specific and recommended that morphological characters should be given priority over host association, in identifications.

The pestalotioid fungi treated in this study were collected from restios in the Cape Floral Kingdom (fynbos) and are recorded for the first time from this niche. The fynbos vegetation represents a floral “island”, geographically and climatically separated from the rest of South Africa. In addition to the isolation, abiotic factors such as summer drought, nutrient-poor soils, recurring fires, strong winds and a Mediterranean climate have influenced the development of a remarkably high level of endemism in plant and small invertebrate animal species. Although there are no other data available for microfungi, the results of this study suggest that the island effect has also positively influenced endemism of microfungi in the fynbos.

REFERENCES


ACKNOWLEDGEMENTS

We are grateful to the Western Cape Nature Conservation Board for permission to work in nature reserves and the NRF/ DST Centre of Excellence in Tree Health Biotechnology for financial support. Dr. Rajesh Jeewon of the Department of Ecology & Biodiversity, University of Hong Kong provided valuable insights into the taxonomy of pestalotioid fungi and reviewed a preliminary draft of the manuscript, for which we are deeply indebted.