Pestalotioid fungi from Restionaceae in the Cape Floral Kingdom

Seonju Lee1*, Pedro W. Crous2 and Michael J. Wingfield1

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

*Correspondence: Seonju Lee, seonju.lee@fabi.up.ac.za

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 saprobic 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 Amphishphaeraceae, 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 regions (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 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 x 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, 1.00, 5.5792 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, collapsed and all multiple, equally parsimonious trees 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. restionis S. Lee & Crous and Seimatosporium (Se.) grevilleae (Loos) Shoeemaker which has a characteristic of Sarcostroma, centric apical and excentric 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

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. restionis S. Lee & Crous and Seimatosporium (Se.) grevilleae (Loos) Shoeemaker which has a characteristic of Sarcostroma, centric apical and excentric 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).
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.

Figs 1–7. Restios in natural habitats and their economic use (Western Cape Province, South Africa). 1. Hypodiscus aristatus in mountain fynbos growing among other major fynbos plants: Leucadendron and Protea species (Proteaceae), and species of Asteraceae and Ericaceae. 2–4. Restio species. 5. Inflorescence of Elegia capensis consisting of many spikelets. 6. Restio festuciformis. 7. Thatched roof made of culms of a Thamnochortus insignis.
Table 1. List of species for which DNA sequence data were generated in this study.

| Fungal species            | Cultures1 | Host plants                  | GenBank accession no. |
|---------------------------|-----------|------------------------------|-----------------------|
| **Pestalotiopsis matildae** | CBS 118155 = CMW 18022 | *Thamnochortus spicigerus* | DQ278916             |
|                           | CBS 118143 = CMW 18285 | *Thamnochortus fraternus*  | DQ278917             |
| **Sarcostoma restionis**  | CBS 118154 = CMW 179712 | *Restio filiformis*       | DQ278922 DQ278924    |
|                           | CBS 118153 = CMW 17984 | *Ischyrolepis cf. sieberti* | DQ278923 DQ278925    |
| **Truncatella betulae**   | SL10153, 4 | *Ischyrolepis subverticellata* | DQ278920            |
| **T. hartigii**           | CBS 118145 = CMW 17938 | *Cannomois virgata*       | DQ278912 DQ278927    |
|                           | CBS 118148 = CMW 18093 | *Rhodocoma capensis*      | DQ278913 DQ278928    |
| **T. megaspora**          | PREM 588703, 5 | *Restio egregius*          | DQ278928             |
| **T. restionacearum**     | CBS 118150 = CMW 17968 | *Restio filiformis*       | DQ278914             |
|                           | CMW 18755   | *Ischyrolepis cf. gaudichaudiana* | DQ278915 DQ278929 |
| **T. spadicea**           | PREM 588733, 5 | *Restio filiformis*       | DQ278919             |

1CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Forest and Agriculture Biotechnology Institute, University of Pretoria, Pretoria, South Africa; PREM: National Collection of Fungi, Pretoria, South Africa; SL: Collection of S. Lee.
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.

| Species                   | PREM no.1 | Conidial dimensions in μm (Length × Width) | No. of septa | Ratio (L : W) | Apical appendages | Basal appendages |
|---------------------------|-----------|--------------------------------------------|--------------|---------------|-------------------|------------------|
| **Pestalotiopsis matildae** | 58862     | (22–)24–25(–29.5) × (6.5–)7(–8.5) (av. 24.5 × 7.2) | 4            | 3.4 : 1       | 2–3              | 12–19            |
|                           | 58861     | (19–)22–(27.5) × (5.5–)6.5–7(–8) (av. 22.8 × 6.7) | 4            | 3.4 : 1       | 2–3              | 8–11             |
| **Sarcostoma lomatiae**   | 58863     | (15–)19–20.5(–25) × (5–)6–7(–8) (av. 19.8 × 6.7) | 4            | 3.0 : 1       | 1                | 30–38            |
| **S. restionis**          | 588651    | (15–)17–(18–20) × (6–)7–7.5(–9) (av. 17.1 × 7.3) | 4(–5)        | 2.3 : 1       | 1                | 27–38            |
|                           | 58864     | (17–)19–(22–22.5) × (7–)8(–10) (av. 19.8 × 8.2) | 4            | 2.4 : 1       | 1                | 37–45            |
| **Truncatella betulae**   | 58867     | (14–)16.5–17(–18) × (7–)8(–9) (av. 16.8 × 7.3) | 3            | 2.3 : 1       | 2–4              | 8–16             |
|                           | 58866     | (15–)16–17(–19.5) × (5–)6–7(–8) (av. 16.5 × 6.5) | 3            | 2.5 : 1       | 3–5              | 8–15             |
| (SL1015)                  |           | (14–)16–17(–18) × (5–)6–7(–8) (av. 16.3 × 6.2) | 3            | 2.6 : 1       | 2–5              | 8–13.5           |
| **T. hartigii**           | 58869     | (16–)17–18(–20) × (6–)7(–8) (av. 17.8 × 7.1) | 3            | 2.5 : 1       | 2–4(–5)          | 26–31            |
|                           | 58868     | (15.5–)18–19(–20.5) × (6–)7(–8) (av. 18.3 × 7.1) | 3            | 2.6 : 1       | 2–4              | 24–33.5          |
| **T. megaspora**          | 588701    | (25–)30–31(–36) × (9–)11–12(–13) (av. 30.5 × 11.8) | 3            | 2.6 : 1       | 2–4              | 9–23             |
| **T. restionacearum**     | 588721    | (20–)22–23(–26.5) × (6–)7(–8) (av. 22.8 × 7.1) | 3            | 3.3 : 1       | 2–4              | 30–44            |
|                           | 58871     | (21–)24–25.5(–29) × (5–)7(–8) (av. 24.9 × 6.8) | 3            | 3.7 : 1       | (2–)3(–4)        | 22.5–65          |
| **T. spadicea**           | PREM 58873 | (20–)21–22(–23) × (7–)8(–8.5) (av. 21.4 × 7.8) | 3            | 2.7 : 1       | 3–4              | 12–16(–25)       |

1PREM: National Collection of Fungi, Pretoria, South Africa.
2Type specimen.
The Discostroma clade accommodated Sa. restionis, three Seimatosporium Corda species and a Discostroma species (teleomorphic state of either Seimatosporium or Sarcostroma). The Truncatella/Bartalinia 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/Bartalinia clade contained a species of Dynthiopsis L. Cai, R. Jeewon & K.D. Hyde (anamorphic Amphisphaeriaceae) and two Bartalinia species (teleomorphic 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 %).

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**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.

**Pestalotiopsis matildae** (Richatt) S. Lee & Crous, comb. nov. MycoBank MB500857. Figs 10–14. = 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 ellipsoid, 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 conidiogenous cells or poorly developed, branched at the base, ampulliform. Conidiogenous cells annellidic, hyaline, discrete or integrated, smooth, lageniform to cylindrical, 7–12 × 1–2 µm. Conidia fusiform, (22–)24–25(–29.5) × (6.5–)7(–8.5) µm (av. 24.5 × 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):

similar to the following seven species as treated by Nag Raj (1993), it is clear that recircumscription of Pestalotia batatae Nag Raj, 1993, is not justified. From the species description by Guba (1961), and the characteristics of the morphological comparisons, our collections best fit Pestalotia fraternus (Servazzi) Steyaert, 1994. Based on host studies, the third cell being darker than the fourth cell. The second cell differs from our collections, has third and fourth cells that are always darker than the second cell, whereas our collections often had pale brown second and fourth cells, and has larger conidia

**Hosts:** Boldoa boldus (Nyctaginaceae), Thamnochortus fraternus, T. spicigerus (Restionaceae).

**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 (Gucevic) Nag Raj, Pestalotia (Pa.) paeoniae Servazzi [= Ps. paeoniae (Servazzi) 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 (25–25×9–11 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 longer 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.

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**Fig. 9.** One of fifty most parsimonious trees obtained from the partial 28S rDNA sequence data (TL = 272 steps, CI = 0.728, RI = 0.854, RC = 0.622). 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 Xylaria hypoxylon and X. curta.
**Sarcostroma lomatiae** (McAlpine) Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 798. 1993. Figs 15–19.

≡ *Monochaeta lomatiae* McAlpine, Proc. Linn. Soc. N. S. W. 79: 140. 1954.

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 µm wide. **Basal stroma** pseudoparenchymatous, consisting of a few layers of brown, thick-walled, globose to angular cells, 9.5–21 µm thick; lateral tissue absent. **Conidiophores** arising from the basal stroma, cylindrical, 4–10 × 2–3 µm.

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

Specimen examined: South Africa, Western Cape Province, Jonkershoek Nature Reserve, culm litter of *Ischyrolepis cf. gaudichaudiana*, 31 July 2001, S. Lee, PREM 58863.

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 acervuloid. Conidiophora cum adunt e fundo texturaeque laterali condionum exsensit, debiliter evoluta vel solum cellulae conidiales. *Cellulae conidiales annellidicae, hyalinae, discreteae, laeves, cylindraceae vel lageniformes, (5.5–)8–10(–13) × 2–3 µm. Conidia fusiformia vel ellipsoidea, recta vel subfalcata, (15–)17–18(–19.5) × (5–)6–7.5(–8) µm, 4(–5)-septata; cellula apicalis hyalina, conica, 2–3 × 3 µm, laevis, tenunticulata; cellulae medianis brunneis, doliformibus, 10–16 × 7–8 µm, echinulatis, crassitunicatis; cellulae basalis hyalinae, obconica, truncate, 2.5–3 × 3 µm, laevis, tenunticulata. Appendiculum apicale unicum, e centro oriens, hyalina, obconica, truncate, 2.5–3 × 3 µm, laevis, tenunticulata. Appendiculum basale unicum, eccentricum, non ramosum, 25–40 × 1–1.5 µm, flexuosum, attenuatum*.

Conidiomata acervuloid, scattered or gregarious, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section low conoid, 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(–19.5) × (5–)6–7(–8) µm (av. 16.5 × 6.5 µm, ratio 2.5 : 1), 3-septate; apical cell hyaline, conical, 2–3 × 3 µm, smooth, thin-walled; median cells brown, doliform, 10–16 × 7–8 µm, echinulate, thick-walled; basal cell hyaline, obconical with truncate end, 2.5–3 × 3 µm, smooth, thin-walled. Apical appendage single, centric, unbranched, 27–38 × 1–1.5 µm, flexuous, attenuated. Basal appendage single, excentric, unbranched, 25–40 × 1–1.5 µm, flexuous, attenuated.

Specimens examined: South Africa, Western Cape Province, Jonkershoek Nature Reserve, culm litter of *Restio filiformis*, 15 June 2001, S. Lee, PREM 58865, holotype, living ex-type culture CBS 118154 = CMW 17971; culm litter of *Ischyrolepis cf. sieberi*, 15 June 2001, S. Lee, PREM 58864, living culture CBS 118153 = CMW 17984.

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

Notes: Three known species are morphologically close 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.

*Truncatella betulae* (Morch.) S. Lee & Crous, comb. nov. MycoBank MB500859. Figs 25–29.

≡ *Postaloria betulae* Morch. (as “*Postalozia*”), J. Bot. Acad. Sci. Ukraine 2(3–4): 183. 1946 [1945].

*Conidiomata* acervuloid, scattered or gregarious, sub-epidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section low conoid, 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(–19.5) × (5–)6–7(–8) µm (av. 16.5 × 6.5 µm, ratio 2.5 : 1), 3-septate; apical cell hyaline, conical to trapezoid, 2–3 × 3–3.5 µm, smooth, thin-walled, at times deciduous; median cells brown, doliform, 12–15 × 7–8 µm, echinulate, thick-walled; basal cell hyaline, 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.
Specimens examined: South Africa, Western Cape Province, Kirstenbosch National Botanical Garden, culm litter of Ischyrolepis subverticellata, S. Lee, 3 Dec. 2001, SL1015; Kogelberg Nature Reserve, culm litter of Elegia filacea, 3 Nov. 2000, S. Lee, PREM 58867; culm litter of Elegia juncea, 11 May 2000, S. Lee, PREM 58866.

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.

Figs 25–40. Truncatella species. 25–29. Truncatella betulae (PREM 58866). 30–34. Truncatella hartigii (PREM 58869). 35–40. Truncatella megaspora (PREM 58870). 25, 30, 35. Vertical sections of conidiomata. 26. Peridial structure. 27, 31, 36. Conidiogenous cells (27, 31 in PhC). 28, 29, 32, 33, 37, 38. Conidia (32, 38 in BF). 34, 39, 40. Apical appendages (PhC). Scale bars: 25, 30, 35 = 50 µm; 26 = 25 µm; 31, 37 = 20 µm; 27, 28, 32–34, 36 = 10 µm; 29, 38–40 = 5 µm.
Truncatella hartigii (Tubeuf) Steyaert, Bull. Jard. Bot. État Bruxelles 19: 298. 1949. Figs 30–34.
≡ Pestalotia hartigii Tubeuf, Beitr. Kennntr. Baumkrankh. 40–51. 1888.

Additional synonyms listed in Guba (1961).

Conidiomata pycnidioïde, 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 conidiomata, branched at the base, 8–10 × 2 μm. Conidiogenous cells annellidic, hyaline, integrated, smooth, cylindrical, 0–3-septate, 7–26 × 2–3 μm. Conidia fusiform, (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, 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.

Specimens examined: South Africa, Western Cape Province, Kogelberg Nature Reserve, culm litter of Restio egregius, 3 Nov. 2000, S. Lee, PREM 58870, holotype.

Host: Restio egregius (Restionaceae).

Notes: The two collections obtained are very similar to T. laurocerasi, T. angustata and T. hartigii. 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. hartigii have more than one apical appendage, often irregularly or dichotomously branched. However, T. hartigii 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. hartigii.

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

Eymology: in reference to its large conidia.}

Conidiomata pcyndidioidae. Conidiophora e tota peripheria interna conidiomatis exorizontia, basi ramosa, cylindrica. Cellulae conidiogenae annellidicae, hyalinae, discretae, laeves, cylindricae, 0–3-septatae, 7–26 × 2–3 μm. Conidia fusiformia, (25–30–31(–36) × (9–)11–12(–13) μm, 3-septata; cellula apicalis hyalina, trapezoida, 3–4 × 3–5 μm, laevis, tenutinicatae; cellulae medianae brunneae, doliformes, 19–24 × 9–13 μm, echinulatae, crassitunicatae; cellulae basalis hyalina, obconica, trunca, 2.5–3 × 3 μm, laevis, tenutinicatae. Appendiculi apicales (2–3(–4), simplices, flexuosae, 9–23 × 1–2 μm. Appendiculi basales desunt.

Conidiomata pycndidioidae, scattered or gregarious, subepidermal, remaining immersed, visible at the
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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, doliform, 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.

Specimens examined: South Africa, Western Cape Province, Jonkershoeck Nature Reserve, culm litter of *Ischyrolepis* cf. *gaudichaudiana*, 31 July 2001, S. Lee, PREM 58871, holotype, living ex-type culture CMW 18755; culm litter or *Restio filiformis*, 15 June 2001, S. Lee, PREM 58872, living culture CBS 118150 = CMW 17968.

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* (Dearm. & 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. Cellulae

**Figs 41–49.** *Truncatella* species. 41–45. *Truncatella restionacearum* (PREM 58871). 46–49. *Truncatella spadicea* (PREM 58873). 41, 46. Vertical sections of conidioma. 42. Peridial structure. 43, 47. Conidiogenous cells (PhC). 44, 48. Conidia (BF). 45, 49. Apical appendages (PhC). Scale bars: 41 = 100 µm; 46 = 50 µm; 42 = 25 µm; 43, 44, 47, 48 = 10 µm; 45, 49 = 5 µm.
Conidiomata pycnidiod, scattered or gregarious, subependral, remaining immersed, visible at the surface by means of dark exuding conidial masses; in section conoid or low planate, some laterally joined, (96–)200–238 × 105–136 µm. *Peridium* pseudoparenchymatous, (4–)6–9 µm thick throughout the conidioma, consisting of a few layers of hyaline or slightly pigmented, moderately thick-walled, compressed cells. *Conidiophores* arising from the entire periphery of the inside of the conidiomata, branched at the base, cylindrical, 0–2–septate, 11–20 × 2–3 µm. *Conidiogenous cells* anellidic, hyaline, integrated, smooth, cylindrical, (6–)14–31 × 2–3 µm. *Conidia* fusiform, (20–)21–22–(23) × (7–)8–(8.5) µm (av. 21.4 × 7.8 µm, ratio 2.7 : 1), 3-septate; apical cell hyaline, conical to trapezoid, 3.5–4 × 4–5 µm, smooth, thin-walled; median cells pale brown, doliiform, 14–16 × 7–8.5 µm, echinulate, moderately thick-walled; basal cell hyaline, obconical, 3–4 × 4–5 µm, smooth, thin-walled. *Apical appendages* 3–4, inserted in the top part of the apical cell, arising at different points, unbranched, 12–16(–25) × 1–1.5 µm, attenuated. *Basal appendages* absent.

**Specimen examined:** *South Africa*, Western Cape Province, Jonkershoek Nature Reserve, culm litter of *Ischyrolepis capensis*, 5 Apr. 2001, S. Lee, PREM 58873, **holotype**.

**Host:** *Ischyrolepis capensis* (Restionaceae).

**Notes:** *Truncatella spadicea* is unique in having pale brown median cells, and apical appendages originating at distant loci on the apical cell. Four species, *Ps. citrina* (McAlpine) Nag Raj, *Ps. gastrolobi* (Tassi) Nag Raj, *Ps. jacksoniae* (Henn.) Nag Raj and *Ps. stevensonii*, are morphologically similar to *T. spadicea* (Nag Raj 1993). However, *Ps. gastrolobi* has elongated, obconical basal cells and narrower conidia (17–24 × 5–7.5 µm), *Ps. jacksoniae* has larger conidia (19–23 × 5.5–7.5 µm) with constricted septa, *Ps. stevensonii* has brown median cells and narrower conidia (19–23 × 5.5–7.5 µm), and *Ps. citrina* has larger conidia (19–26 × 7–9 µm) and a distinctly different origin of the apical appendages distinguishing them from *T. spadicea*.

**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).

**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 *Se. grevilleae* and *Se. 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 *Diplorceras* (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 *Quadrilocularia*. When Sutton (1980) reinstated the genus, he considered that the species placed in *Pestalotia* (sect *Quadriloculariae*) and *Monochaetia* (sect. *Quadriloculariae*) 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...
Pestalotiopsis (e.g. Ps. besseyi (Guba) Nag Raj, Ps. casuarinae (Cooke & Massee) 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 conidia 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 only 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. hartii than to T. angustata, the generic type. Bartalinaia and Dyrithiopsis clustered within the Truncatella/Bartalinaia 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 pestalotoid 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.

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