Pestalotioid fungi from *Restionaceae* in the Cape Floral Kingdom

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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. hartigii*. 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 km^2 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 *Amphisphaeriaceae*, *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, 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 (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).

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.

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) Shoemaker 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	Cultures[1]	Host plants	GenBank accession no.	
			ITS	LSU
Pestalotiopsis matildae	CBS 118155 = CMW 18022	*Thamnochortus spicigerus*	DQ278916	
	CBS 118143 = CMW 18285	*Thamnochortus fraternus*	DQ278917	
Sarcostroma restionis	CBS 118154 = CMW 17971[2]	*Restio filiformis*	DQ278922	DQ278924
	CBS 118153 = CMW 17984	*Ischyrolepis* cf. *sieberi*	DQ278923	DQ278925
Truncatella betulae	SL1015[3, 4]	*Ischyrolepis subverticellata*	DQ278920	
T. hartigii	CBS118145 = CMW 17958	*Cannomois virgata*	DQ278912	DQ278927
	CBS118148 = CMW 18093	*Rhodocoma capensis*	DQ278913	DQ278928
T. megaspora	PREM 58870[2, 3]	*Restio egregius*	DQ278928	
T. restionacearum	CBS 118150 = CMW 17968	*Restio filiformis*	DQ278914	
	CMW 18755[2]	*Ischyrolepis* cf. *gaudichaudiana*	DQ278915	DQ278929
T. spadicea	PREM 58873[2, 3]	*Restio filiformis*	DQ278919	

[1]CBS: 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.

[2]Ex-type cultures or holotypes.

[3]Sequenced from direct PCR amplification of conidia.

[4]No 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	
					No.	Length (µm)	No.	Length (µm)
Pestalotiopsis matildae	58862	(22–)24–25(–29.5) × (6.5–)7(–8.5) (av. 24.5 × 7.2)	4	3.4 : 1	2–3	13–19	1	2–6
	58861	(19–)22–24(–27.5) × (5.5–)6.5–7(–8) (av. 22.8 × 6.7)	4	3.4 : 1	2–3	8–11	1	2–6
Sarcostroma lomatiae	58863	(15–)19–20.5(–25) × (5–)6–7 (av. 19.8 × 6.7)	4	3.0 : 1	1	30–38	1	30–36.5
S. restionis	58865[T]	(15–)17–18(–20) × (6–)7–7.5(–9) (av. 17.1 × 7.3)	4(–5)	2.3 : 1	1	27–38	1	25–40
	58864	(17–)19–20(–22.5) × (7–)8(–10) (av. 19.8 × 8.2)	4	2.4 : 1	1	37–45	1	34.5–50
Truncatella betulae	58867	(14–)16.5–17(–18) × 7–7.5(–8) (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) (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	58870[T]	(25–)30–31(–36) × (9–)11–12(–13) (av. 30.5 × 11.8)	3	2.6 : 1	2–4	9–23	-	-
T. restionacearum	58872[T]	(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)	22.5–55	-	-
T. spadicea	58873[T]	(20–)21–22(–23) × (7–)8(–8.5) (av. 21.4 × 7.8)	3	2.7 : 1	3–4	12–16(–25)	-	-

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

[T]Type specimen.

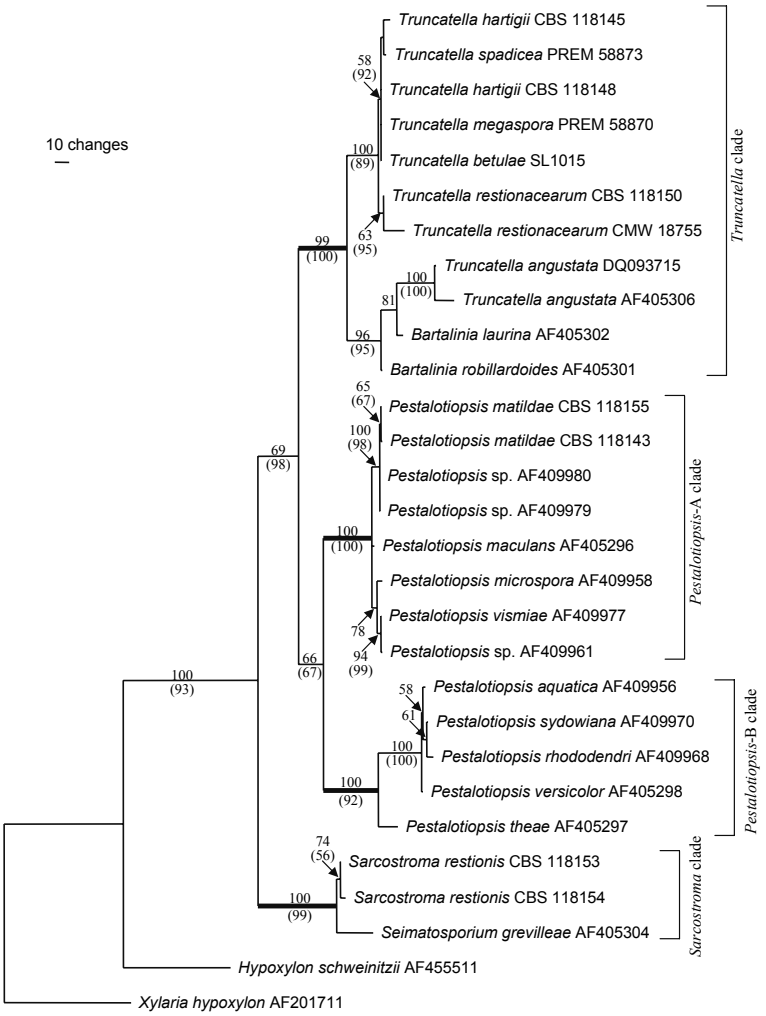


Fig. 8. One of six most parsimonious trees obtained from the ITS regions and 5.8S rDNA sequence data (TL = 788 steps, CI = 0.772, RI = 0.886, RC = 0.684). Parsimony bootstrap support values from 1000 replicates are indicated on the nodes and those from distance analysis are indicated in parentheses. 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/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 *Dyrithiopsis* L. Cai, R. Jeewon & K.D. Hyde (anamorphic *Amphisphaeriaceae*) and two *Bartalinia* 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.

Pestalotiopsis matildae (Richatt) S. Lee & Crous, **comb. nov.** MycoBank MB500857. Figs 10–14.

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

Conidiomata pycnidioid, 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-

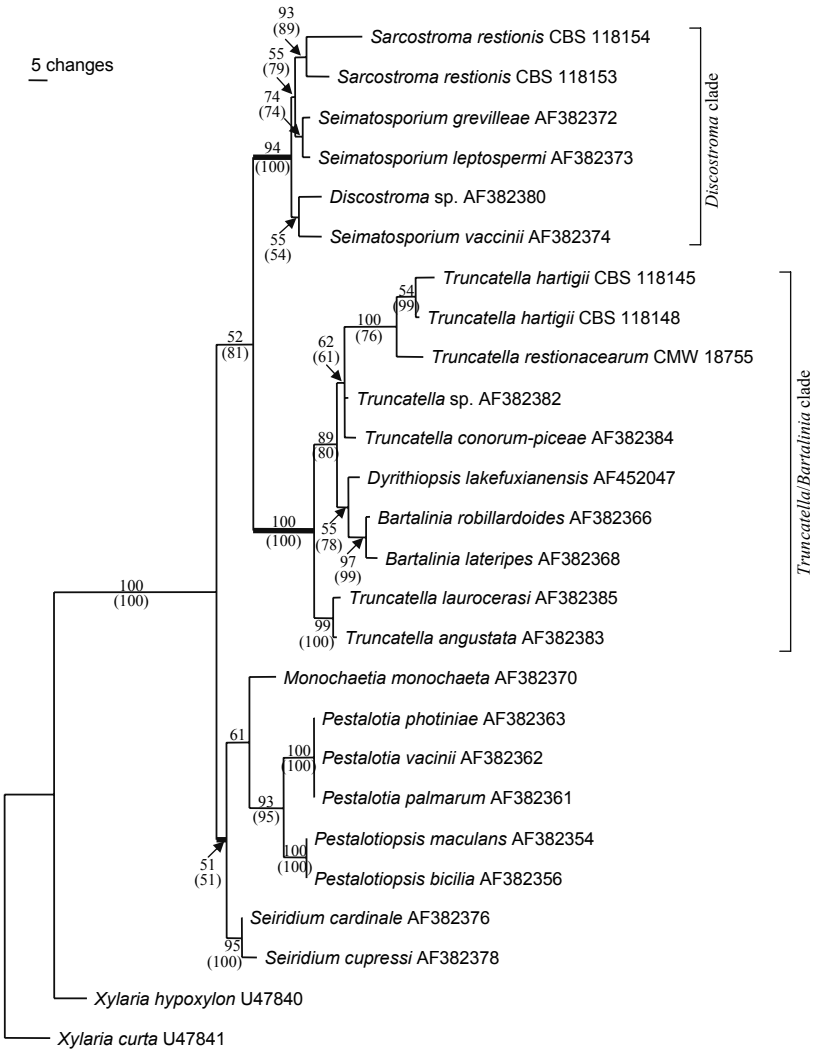


Fig. 9. One of fifty most parsimonious trees obtained from the partial 28S rDNA sequence data (TL = 272 steps, CI = 0.728, RI = 0.854, RC = 0.622). Parsimony bootstrap support values from 1000 replicates are indicated on the nodes and those from distance analysis are indicated in parentheses. Branches supporting ingroups are in bold. The tree was rooted to *Xylaria hypoxylon* and *X. curta*.

walled; median cells brown, versicoloured, with third and fourth cells from the base darker than the second cell (at times the third cell darker than the fourth cell), doliiform, 15–17 × 6–7 µm, smooth but lumpy (possibly due to desiccation), moderately thick-walled; basal cell hyaline to subhyaline, obconical, 4–5 × 4–5 µm, smooth, thin-walled. *Apical appendages* 2–3, inserted along the upper half of the apical cell, arising at different points, unbranched, flexuous, 13–19 × 1 µm, attenuated. *Basal appendage* single, centric, unbranched, 2–6 × 1 µm, attenuated.

Specimens examined: **South Africa**, Western Cape Province, De Hoop Nature Reserve, culm litter of *Thamnochortus fraternus*, 28 Feb. 2002, A. Wood, PREM 58861, living culture CBS 118143 = CMW 18285; Kirstenbosch National Botanical Garden, culm litter of *Thamnochortus spicigerus*, 3 Dec. 2001, S. Lee, PREM 58862, living culture CBS 118155 = CMW18022.

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* (Gucevič) Nag Raj, *Pestalotia* (*Pa.*) *paeoniae* Servazzi [= *Ps. paeoniae* (Servazzi) Steyaert], *Pa. batatae* Ellis & Everh., and *Pa. matildae*.

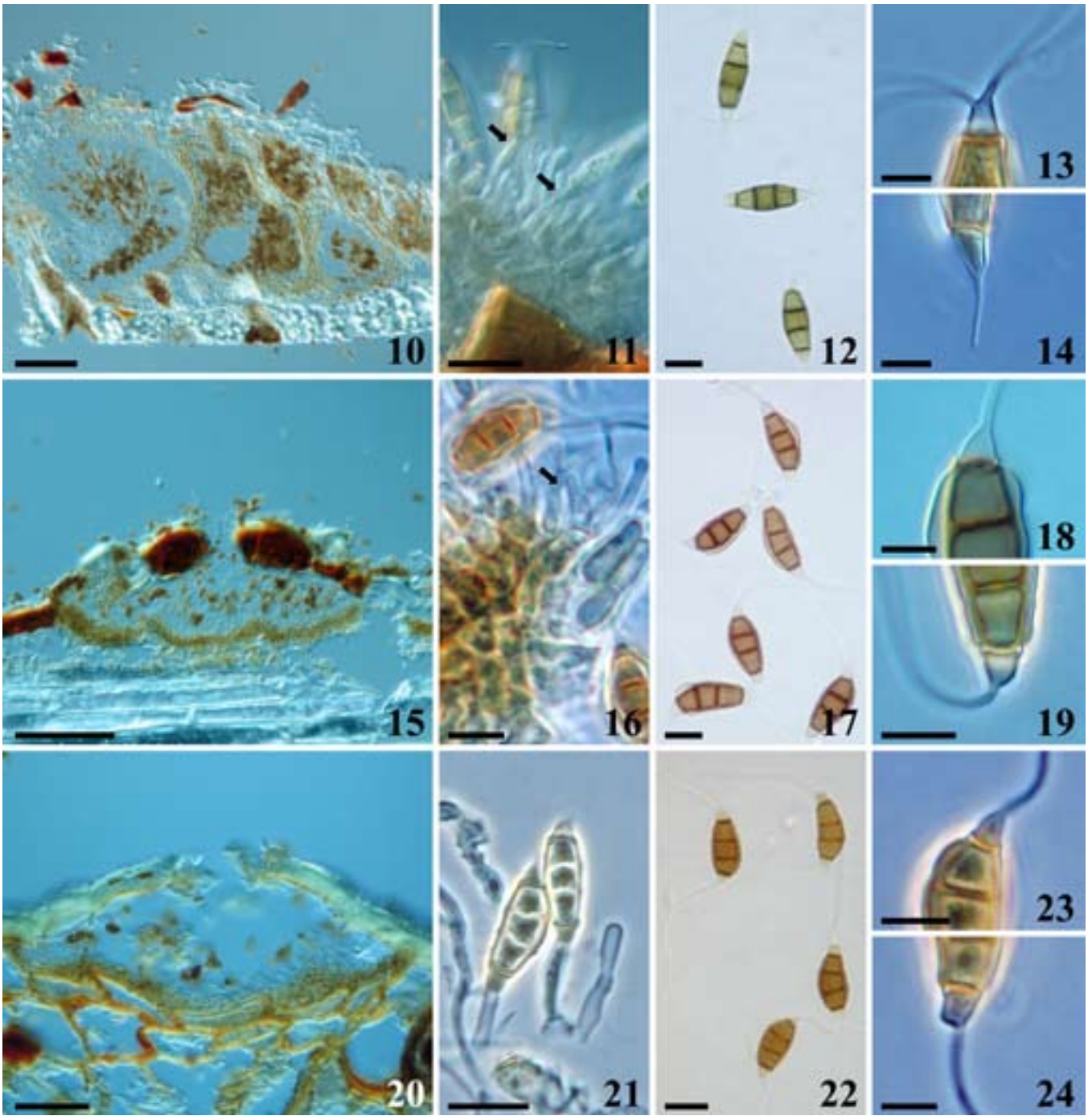
Different from our collections, *Ps. leucopogonis* has apical appendages that originate in three levels (tiers) on the apical cell, *Ps. macrospora* has larger conidia (25–10 × 9–11 µm), *Ps. palustris* has smaller conidia (25–25 × 5.5–7 µm) and distinct striations on second and fourth cells, *Ps. metasequoiae* has verruculose, pale brown second and fourth cells, and *Pa. paeoniae* has 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.

Sarcostroma lomatiae (McAlpine) Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia: 798. 1993. Figs 15–19.

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

Conidiomata acervular, scattered or gregarious, subepidermal, 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* annellidic, 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, doliiform, 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,



Figs 10–24. *Pestalotiopsis* and *Sarcostroma* species. 10–14. *Pestalotiopsis matildae* (PREM 58862). 15–19. *Sarcostroma lomatiae* (PREM 58863). 20–24. *Sarcostroma restionis* (PREM 58865). 10, 15, 20. Vertical sections of conidioma. 11, 16, 21. Conidiogenous cells (16, 21 in PhC). 12, 17, 22. Conidia (BF). 13, 18, 23. Apical appendages (13, 23 in PhC). 14, 19, 24. Basal appendages (19, 24 in PhC). Scale bars: 10 = 250 µm; 15 = 100 µm; 20 = 50 µm; 11, 12, 16, 17, 21, 22 = 10 µm; 13, 14, 18, 19, 23, 24 = 5 µm.

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 acervularia. Conidiophora cum adsunt e fundo texturaque laterali conidiomatis exorientia, debiliter evoluta vel solum cellulae conidiogenae. Cellulae conidiogenae annellidicae, hyalinae, discretae, laeves, cylindricae vel lageniformes, (5.5–)8–10(–13) × 2–3 µm. Conidia fusiformia vel ellipsoidea, recta vel subfalcata, (15–)17–18(–20) × (6–)7–7.5(–9) µm, 4(–5)-septata; cellula apicalis hyalina, conica, 2–3 × 3 µm, laevis, tenuitunicata; cellulis medianis brunneis, doliiformibus, 10–16 × 7–8 µm, echinulatis, crassitunicatis; cellula basali hyalina, obconica, truncata, 2.5–3 × 3 µm, laevi, tenuitunicata. Appendiculum apicale unicum, e centro oriens, simplex, 27–38 × 1–1.5 µm, flexuosum, attenuatum. Appendiculum basale unicum, excentricum, non ramosum, 25–40 × 1–1.5 µm, flexuosum, attenuatum.

Conidiomata acervular, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by dark exuding conidial masses, lifting up the epidermis; in section low conoid, 132–270 µm wide. *Basal stroma* pseudoparenchymatous, consisting of a few layers of brown, thick-walled, angular cells, 9–14 µm thick; lateral tissue absent or present, when present similar to the basal stroma, 8–9 µm thick. *Conidiophores* arising from the base and lateral tissue when present, often reduced to conidiogenous cells or poorly developed. *Conidiogenous cells* annellidic, hyaline, discrete, smooth, cylindrical to lageniform, (5.5–)8–10(–13) × 2–3 µm. *Conidia* fusiform to ellipsoid, straight or slightly curved, (15–)17–18(–20) × (6–)7–7.5(–9) µm (av. 17.1 × 7.3 µm, ratio 2.3 : 1), 4–(5)-septate; apical cell hyaline, conical, 2–3 × 3 µm, smooth, thin-walled; median cells brown, doliiform, 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 (Morochk.) S. Lee & Crous, **comb. nov.** MycoBank MB500859. Figs 25–29.

≡ *Pestalotia betulae* Morochk. (as "*Pestalozzia*"), J. Bot. Acad. Sci. Ukraine 2(3–4): 183. 1946 [1945].

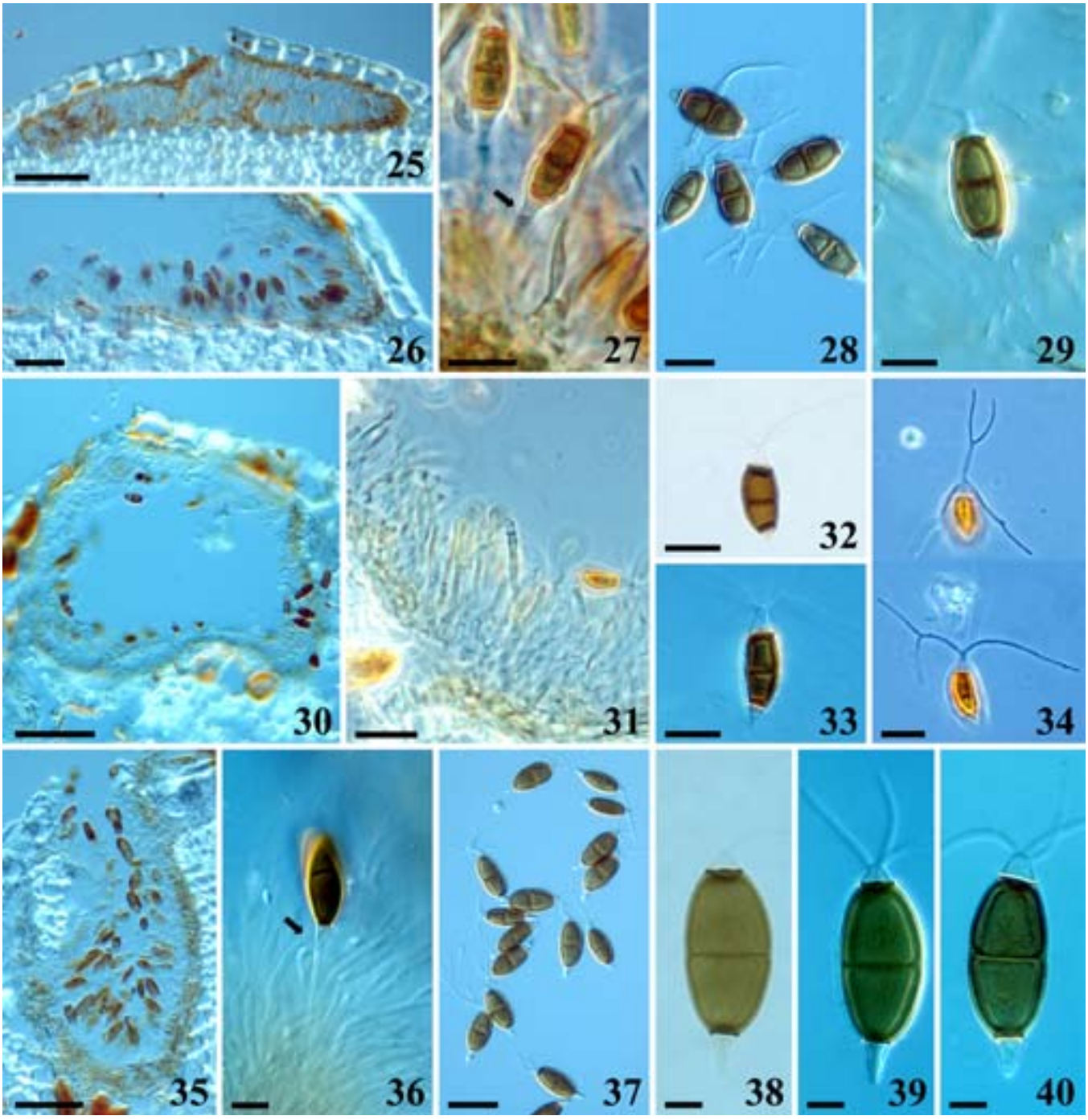
Conidiomata acervuloid, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by dark exuding conidial masses; in section low conoid, 50–67 µm high, 170–413 µm wide. *Peridium* pseudoparenchymatous, in section 4–9 µm thick throughout the conidioma, consisting of a few layers of pale brown, moderately thick-walled, compressed cells of *textura angularis*. *Conidiophores* arising from the entire periphery of the inside of the conidiomata, 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, doliiform, 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 conidioma. 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. Kenntn. Baumkrankh. 40–51. 1888.

Additional synonyms listed in Guba (1961).

Conidiomata pycnidioid, 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 conidiomata, 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 deciduous; median cells brown, doliiform, 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.

Specimens examined: **South Africa**, Western Cape Province, Jonkershoek Nature Reserve, culm litter of *Cannomois virgata*, 15 June 2001, S. Lee, PREM 58869, living culture CBS 118145 = CMW 17958; Kirstenbosch National Botanical Garden, culm litter of *Rhodocoma capensis*, 3 Dec. 2001, S. Lee, PREM 58868, living culture CBS 118148 = CMW 18093.

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

Etymology: in reference to its large conidia.

Conidiomata pycnidioidea. Conidiophora e tota peripheria interna conidiomatis exorientia, basi ramosa. 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, trapezoidea, 3–4 × 3–5 µm, laevis, tenuitunicata; cellulae medianae brunneae, doliiformes, 19–24 × 9–13 µm, echinulatae, crassitunicatae; cellula basalis hyalina, obconica, truncata, 2.5–3 × 3 µm, laevis, tenuitunicata. Appendiculi apicales (2–)3(–4), simplices, flexuosiae, 9–23 × 1–2 µm. Appendiculi basales desunt.

Conidiomata pycnidioid, 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, doliiform, 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.

Specimen 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*)

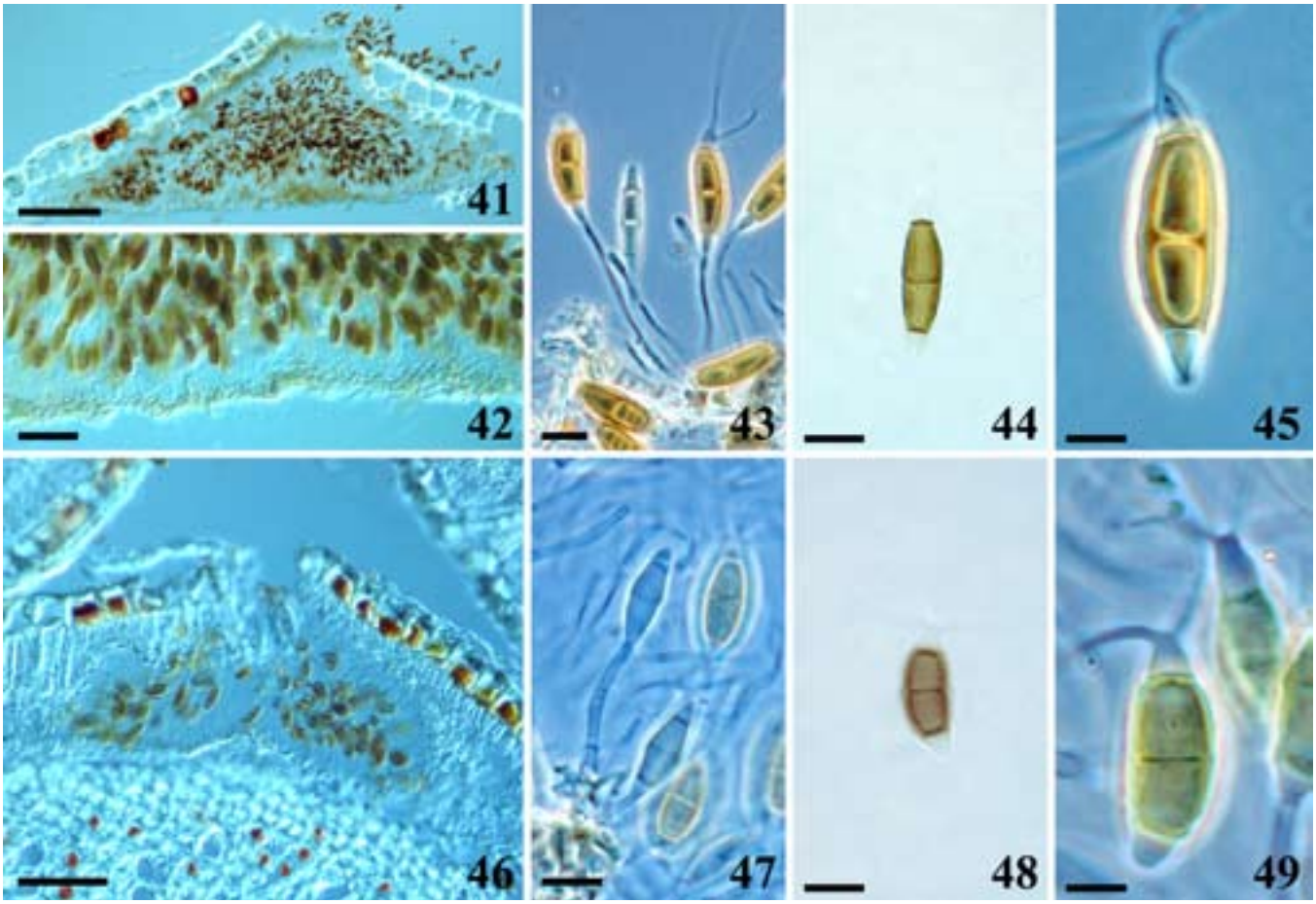
Notes: *Truncatella megaspora* is unusual in having larger conidia than any other species in this genus. The species with the most similar conidial dimensions are *Ps. torrendii* (J.V. Almeida & Sousa da Câmara) Nag Raj and *T. trevoae* (Speg.) Nag Raj (≡ *Pestalotia trevoae* Speg.). *Pestalotiopsis 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 restionacearum S. Lee & Crous, **sp. nov.** MycoBank MB500861. Figs 41–45.

Etymology: in reference to its host family, *Restionaceae*.

Conidiomata pycnidioidea. Conidiophora e tota peripheria interna conidiomatis exorientia, basi ramosa, cylindrica. Cellulae conidiogenae annellidicae, hyalinae, discretae, laeves, cylindricae, (5–)14–31 × 2–3 µm. Conidia fusiformia, (21–)24–25.5(–29) × (5–)7(–8) µm, 3-septata; cellula apicalis hyalina, oblonga vel trapezoidea, 3–4.5 × 2–4 µm, laevis, tenuitunicata; cellulae medianae brunneae, doliiformes, 14–20 × 6–8 µm, echinulatae, crassitunicatae; cellula basalis hyalina, obconica, basi 4–5 × 3–4 µm, laevis, tenuitunicata. Appendiculi apicales (2–)3(–4), e planis duobus distantibus exorientia, raro ramosi, flexuosi, 22.5–55 × 1 µm, attenuati. Appendiculi basales desunt.

Conidiomata pycnidioid, scattered or gregarious, sub-epidermal, remaining immersed, visible at the



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.

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.

Specimens examined: **South Africa**, Western Cape Province, Jonkershoek 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* (Dearn. & 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

conidiogenae annellidicae, hyalinae, discretae, laeves, cylindricae, (6–)14–31 × 2–3 µm. Conidia fusiformia, (20–)21–22(–23) × (7–)8(–8.5) µm, 3-septata; cellula apicalis hyalina, conica vel trapezoidea, 3.5–4 × 4–5 µm, laevis, tenuitunicata; cellulae medianae spadiceae, doliiformes, 14–16 × 7–8.5 µm, modice crassitunicatae; cellula basalis hyalina, obconica, 3–4 × 4–5 µm, laevis, tenuitunicata. Appendiculi apicales 3–4, apicales, simplices, 12–16(–25) × 1–1.5 µm, attenuati. Appendiculi basales desunt.

Conidiomata pycnidioid, scattered or gregarious, subepidermal, remaining immersed, visible at the surface by means of dark exuding conidial masses; in section conoid or low applanate, 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* annellidic, 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 (21–25.5 × 9–10 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 (*Bartalinia*, *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 *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

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. hartigii* than to *T. angustata*, the generic type. *Bartalinia* and *Dyrithiopsis* 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.

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