New coelomycetes occurring on Restionaceae

Seonju Lee & Pedro W. Crous¹

Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa

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During a study of the saprobic fungi occurring in the fynbos of the Western Cape province of South Africa, several previously undescribed species of coelomycetes were collected from members of Restionaceae. These are described as Libartania ischyrolepis from Ischyrolepis capensis, Parahyalotiopsis elegiae from Elegia capensis, Psammina elegiae from Elegia juncea, and Septoriella restionis from a Restio sp.

Keywords: Biodiversity, South African fynbos, systematics.

Fynbos is the dominant vegetation type in the Cape Floristic Region at the south-western tip of South Africa. The word comes from the Dutch 'fijn bosch', which describes the narrow-leaved bushes characterizing much of this vegetation. Fynbos, however, also supports broad-leaved bushes, for example members of the family Proteaceae. The ca. 7,700 fynbos species can be divided into several groups according to their growth form. One of these is the restioid group including members of the family Restionaceae (Cowling & Richardson, 1995).

The Restionaceae is a typical family of the southern hemisphere and comprises grass-like members that are perennial, evergreen, wind-pollinated and dioecious. They are found on all the southern continents, with ca. 330 species in Africa, ca. 150 species in Australia, four species in New Zealand, one species in South America and one species occurring throughout South-East Asia. In the Cape Floristic Region of South Africa, however, the Restionaceae dominate the vegetation over large areas of the fynbos (Haaksma & Linder, 2000). They are considered excellent ecological indicators, and the species of a specific region correlate with climate and soil moisture conditions (Haaksma & Linder, 2000). Despite its unique distribution and ecology, the microfungi occurring on this family have hardly been studied. The aim of this study was to collect and

¹ Present address: Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

describe the saprobic fungi occurring on this family. Part of these data are presented in the present paper.

Materials and methods

Dead culms attached to plants or lying on the ground were collected from nature reserves in the Western Cape province of South Africa over the period 2000-2001. The samples were inspected immediately for fungal structures, and air-dried for further study. Air-dried samples were incubated in moisture chambers for 2-3 d before examination. Single spore isolations were made from all collections: cultures were established on 5% malt extract agar (MEA; Biolab, Midrand, Johannesburg) supplemented with 0.04 gl⁻¹ streptomycin sulphate. Cultural characteristics were determined for each isolate in triplicate from MEA plates after 10 d of incubation at 25 °C in the dark, and colours determined according to Rayner (1970). General observations, measurements and photography of characteristic structures were made from structures mounted in lactophenol when not stated otherwise. Wherever possible, 30 observations were made of each type of structure, from which the 95% confidence intervals were calculated with the extremes given in parentheses. Sections of conidiomata were made on a cryotome (Leica CM1100) and mounted with Jung tissue freezing mediumTM. Photographic images were taken with a digital camera (Nikon DXM 1200) on a Nikon Eclipse E600 light microscope or a Nikon SMZ800 dissecting microscope. Herbarium specimens are deposited in PREM and reference cultures are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

Taxonomy

Libartania ischyrolepis S. Lee & Crous, sp. nov.– Figs. 1–12.

Conidiomata pycnidica, subepidermalia, alte immersa, ostiolata, in sectione verticali lenticularia vel ellipsoidalia, usque ad 150 μm alta et ad 60 μm lata. Peridium circa ostiolum pseudoparenchymatosum, crassitunicatum, fuscum, ad 17.5 μm crassum, undique et basi tenuitunicatum, hyalinum, indistinctum cellulis hospitalibus, usque ad 5 μm crassum. Conidiophora circa cavitatem conidiomatis enascentia, ad cellulas conidiogenas reducta. Cellulae conidiogenae discretae, ampulliformes, piriformes vel irregulares, hyalinae, tenuitunicatae, laeves, $7-8\times2-3$ μm . Conidia holoblastica, monoblastica, fusiformia, anguste basi truncata, (2-)4(-5)-septata, hyalina, tenuitunicata, laevia $(22-)24-26(-30)\times(4-)4.5(-5)$ μm , solum appendices apicales ferentia. Appendices cellulares, dichotome vel irregulariter ramosae (2-)3(-4) ramis divergentibus et attenuatis, raro septis paucis in parte basali ramorum $(14-)18.5-22.5(-24)\times0.5-1$ μm . Ratio conidii long./lat. = 5.5:1.

Etymology. – in reference to its host, *Ischyrolepis*.

Holotype. – South Africa: Western Cape province, Jonkershoek Nature Reserve, on dead culms of *Ischyrolepis capensis* (L.) Linder (Restionaceae), 15 Jun. 2001, S. Lee, PREM 57456, cultures ex-type STE-U 5258, CBS 111027.

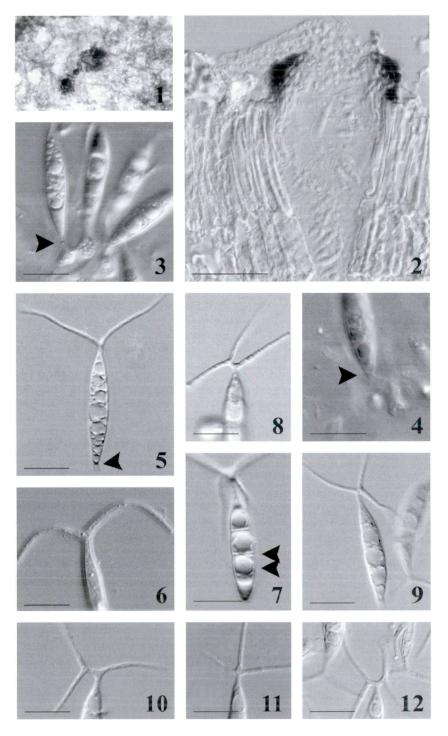
Conidiomata caulicolous, pycnidial, amphigenous, scattered to gregarious, subepidermal, deeply immersed, unilocular, glabrous, ostiole wide open, in vertical section lenticular to ellipsoidal, up to 150 μm high, up to 60 μm wide (Figs. 1, 2). - Peridium around ostiole pseudoparenchymatous, thick-walled, dark brown, up to 17.5 µm thick, on the sides and at the base thin-walled, hyaline, indistinct from the host cells, up to 5 µm thick. - Conidiophores lining the cavity of the conidioma, reduced to conidiogenous cells (Fig. 2). - Conidiogenous cells discrete, ampulliform, pyriform or irregular, hyaline, thin-walled, smooth, $7-8 \times 2-3 \mu m$ (Figs. 3, 4). - Conidia holoblastic, monoblastic, fusiform with a narrowly truncate base, at times with part of conidiogenous cell attached at the base (Fig. 5), (2-)4(-5)-septate, cells often unequal, hyaline, thin-walled, smooth, covered with a thin, inconspicuous gelatinous sheath (Fig. 7), with slight constrictions at the septa, (22-)24- $26(-30) \times (4-)4.5(-5)$ µm ($\bar{x} = 24.7 \times 4.5$ µm), bearing apical appendages only. - Appendages cellular, arising as tubular extensions of the conidium body (Fig. 6), branched dichotomously or irregularly with (2-)3(-4) divergent, attenuated branches, rarely with a few septa in the basal part of the branches (14-)18.5- $22.5(-24) \times 0.5-1$ µm (Figs. 8-12). - Mean conidium length/ width ratio = 5.5:1.

Cultural characteristics. – Colonies fertile, reaching 3.2 mm diam. on MEA after 10 d at 25 °C in the dark, flat with papillate center, circular with entire margin, pale luteous (19d), same in reverse. Mycelium dense, superficial with edge immersed.

Known host. – Ischyrolepis capensis (L.) Linder (Restionaceae).

Known distribution. - Western Cape province, South Africa.

While trying to classify the present species from Restionaceae, several genera, namely *Hyalotiella*, *Truncatella*, *Labridella*, *Libartania* and *Hyalotiopsis* were considered. Species of *Hyalotiella* resemble our fungus in having branched, apical, cellular appendages, but differ by having hyaline to pale brown conidia with long median cells, and a hyaline, conical apical cell, and long, cylindrical conidiogenous cells. Species of *Truncatella* can be distinguished by their enteroblastic-percurrent proliferation and conidia with dark brown median cells. Similarly, species of *Hyalotiopsis* are distinguished by their apical appendages originating from separate loci, and the annellations pre-



sent on their conidiogenous cells. Although species of *Labridella* share some characteristics with the fungus from Restionaceae such as holoblastic conidiogenous cells, and branched, apical, cellular appendages, they are distinguished by having a coloured conidium body and long, cylindrical conidiogenous cells. The fungus from Restionaceae is thus best accommodated in the genus *Libartania*.

The genus Libartania was introduced by Nag Raj (1979) for two species, namely Libartania laserpiti (Bres.) Nag Raj on stems of Laserpitium siler L. (Umbelliferae) from Italy, and Libartania themedae (Hansf.) Nag Raj on culms of Themeda australis Stapf (Gramineae) from Australia. In L. laserpiti, the type of the genus, the conidiogenous cells are predominantly asynchronously polyblastic, and the conidia bear simple or bifurcate apical appendages, and occasionally a simple excentric basal appendage. In L. themedae, however, the conidiogenous cells are monoblastic, and the conidia bear dichotomously or irregularly branched apical appendages only. Nag Raj (1979) defined the genus to include species with pycnidioid. stromatic conidiomata, discrete or indeterminate conidiogenous cells, and monoblastic or asynchronously polyblastic, fusiform, septate, hyaline conidia, bearing simple and branched apical appendages, rarely with a basal appendage. The introduction of a third species with a persistent basal appendage, namely L. phragmiticola Nag Raj on culms of *Phragmites* spp. (Gramineae) from Canada and Latvia, further broadened the generic concept (Nag Raj, 1993). Presently, species of Libartania are characterized by having hyaline conidia, and apical cellular appendages with cytoplasmic continuity.

The peridial composition of L. ischyrolepis is quite different from that of the other species in the genus, because it lacks a well-defined, thick-walled, dark brown outer layer. In contrast, the conidiomatal wall consists of 2–3 layers of hyaline to pale brown cells. A basal conidial appendage is absent in L. ischyrolepis.

Key to Species of Libartania

Figs. 1–12. Libartania ischyrolepis (holotype). – 1. Conidioma on the host surface. – 2. Section through a conidioma. – 3, 4. Conidia and conidiogenous cells (arrowheads, in water). – 5. Conidium with the part of conidiogenous cell attached (arrowhead). – 6. Young conidium showing cytoplasmic continuity of apical appendage (in water). – 7. Thin gelatinous sheath (arrowheads, in water). – 8–12. Conidia with variously branched apical appendages. – Bars 2 = 50 μ m; 3–12 = 10 μ m.

- 3. Conidia $20-29 \times 2.5-3.5 \mu m$, rarely with basal appendage; mean conidium length/width ratio = $8.3:1 \dots L. laserpiti$
- 3*. Conidia $22-30 \times 4-5$ µm, without basal appendage; mean conidium length/width ratio = 5.5:1....L. ischyrolepis

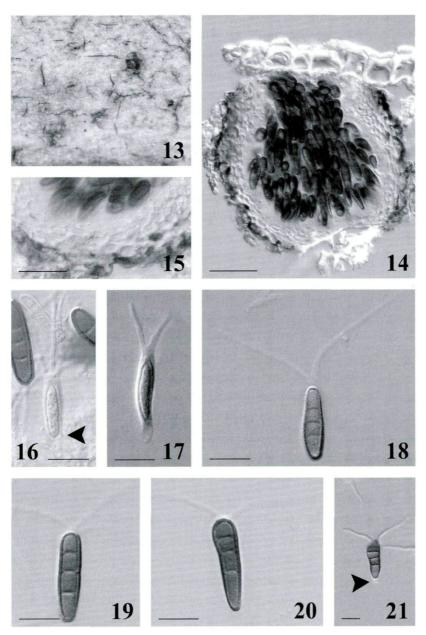
Paraphyalotiopsis elegiae S. Lee & Crous, sp. nov.– Figs. 13–21.

Conidiomata pycnidica, immersa, subepidermalia, ostiolata, in sectione verticali subglobosa vel globosa, usque ad 100 μ m alta et ad 130 μ m lata. Peridium pseudoparenchymatosum, complectens duo strata cellularum, 18–19 μ m crassum. Conidiophora circa cavitatem conidiomatis enascentia, ad cellulas conidiogenas reducta. Cellulae conidiogenae discretae, ampulliformes vel subcylindricae, hyalinae, tenuitunicatae, laeves, $5.5-6.5\times1.5-2.5$ μ m. Conidia holoblastica, cylindrica vel oblongata, interdum medio vel apicaliter turgida, apice obtuso et base truncata, (2-)3(-4)-septata, pallide brunnea, crassitunicata, minute verruculosa, $(16-)19-21(-23)\times(5-)6(-7.5)$ μ m, appendices apicales ferentia. Appendices cellulares (2-)3(-4), fere non ramosae, gradatim contractae versus apicem, flexuosae, (18-)22-27(-28) μ m longae, 1-1.5 μ m latae base. Ratio conidii long./lat.= 3.5:1.

Etymology. – in reference to its host, Elegia.

Holotype. – South Africa: Western Cape province, Jonkershoek Nature Reserve, on dead culms of *Elegia capensis* (Burm. f.) Schelpe (Restionaceae), 5 Apr. 2001, S. Lee, PREM 54547, cultures ex-type STE-U 5255.

Conidiomata caulicolous, pycnidial, amphigenous, scattered to gregarious, immersed, subepidermal, unilocular, glabrous, ostiolate, in vertical section subglobose to globose, up to 100 µm high and 130 μm wide (Figs. 13, 14). – Peridium pseudoparenchymatous, comprising 2 layers of cells, 18-19 µm thick, cells of outer layer thick-walled, brown, darker in the ostiolar region, up to 13 µm thick, cells of inner layer thin-walled, hyaline, up to 5 μm thick (Fig. 15). – Conidiophores lining the cavity of the conidioma, reduced to conidiogenous cells. - Conidiogenous cells discrete, subcylindrical to ampulliform, hyaline, thin-walled, smooth, 5.5- $6.5 \times 1.5 - 2.5 \, \mu m$ (Fig. 16). – Conidia holoblastic, cylindrical to oblong, sometimes medianly or apically inflated, with a blunt apex and a truncate base, at times with part of conidiogenous cell attached at the base, (2-)3(-4)-septate, median cells shorter than end cells, light brown, thick-walled, minutely verruculose, without constrictions at the septa, $(16-)19-21(-23)\times(5-)6(-7.5)$ µm, $(\bar{x}=19.8\times$ 5.7 μm), bearing apical appendages (Figs. 17–21). – Appendages cellular, arising as tubular extensions of the conidium body before conidium maturation, (2-)3(-4), mostly unbranched, gradually



Figs. 13–21. Parahyalotiopsis elegiae (holotype). – 13. Conidioma on the host surface. – 14. Section through a conidioma. – 15. Peridium. – 16. Conidiogenous cell (arrowhead). – 17. Young conidium. – 18–20. Conidia with apical appendages. – 21. Conidium with the part of conidiogenous cell attached (arrowhead). – Bars 14, $15=25~\mu m;~16–21=10~\mu m.$

tapering to the tip, flexuous, $(18-)22-27(-28) \mu m \log 1-1.5 \mu m$ wide at the base. – Mean conidium length/width ratio = 3.5:1.

Cultural characteristics. — Colonies sterile, reaching 12 mm diam. on MEA after 10 d at 25 °C in the dark, convex, circular with entire margin, mouse grey (13""i) to lavender grey (45""f) with greyish sepia (21""i) in the centre, reverse moderate yellow (19"b) with greyish olive (21"k) in the centre. Aerial mycelium dense and fluffy.

Known host.— Elegia capensis (Burm. f.) Schelpe (Restionaceae).

Known distribution. - Western Cape province, South Africa.

Paraphyalotiopsis elegiae is the second species known for this genus. Paraphyalotiopsis Nag Raj was originally established to accommodate Hyalotiopsis borassi Thaung, which occurred on dead leaves of Borassus flabellifer L. (Palmae) from Burma (Nag Raj, 1979). Hyalotiopsis Punith. can be distinguished from Paraphyalotiopsis by its non-ostiolate conidiomata, separately originating apical appendages, and versicoloured conidia with unequal cells. The mean conidium length/width ratio of P. elegiae (3.5:1) is very similar to P. borassi (Thaung) Nag Raj (3.4:1), but the range of conidial size is distinctly greater than that of P. borassi (11–18 × 4–6 μ m). Conidia of P. elegiae are thick-walled and sometimes medianly or apically inflated, which was not reported for P. borassi. Furthermore, P. borassi sometimes has branched appendages (Nag Raj, 1993), which has not been observed in P. elegiae.

Psammina elegiae S. Lee & Crous, sp. nov.– Figs. 22–30.

Conidiomata acervulata vel indeterminata, in origine intra-epidermalia, epiphylla vel amphigena, prosenchymatica, irregularia in lineamento, usque ad 75 μm lata et ad 50 μm alta. Conidiophora septata et parce ramosa vel ad cellulas conidiogenas reducta. Cellulae conidiogenae discretae vel integratae, determinatae, cylindricae vel doliiformes, hyalinae, (6–)7–12 × 2.5–3.5(–4) μm . Conidia palmata, multiseptata, pallide brunnea, laevia, constantia ex circiter 33–56 brachiis radiantibus de corpore medio cellularum brevium ramosarum, (52–)58–66(–70) μm diam. Brachia cylindrica, contracta et pallidiora versus apicem, pallide brunnea, plerumque duo brachia unita in V-figura, tunica tenui gelatinosa induta, 0.5 μm crassa, omnia (2–)3(–4) septis transversis, (19–)26–30(–35) × 2 μm , recta vel exigue arcuata, plerumque paulo inaequalia in diametro.

Etymology. – in reference to its host, *Elegia*.

Holotype. – South Africa: Western Cape province, Kogelberg Nature Reserve, on dead culms of *Elegia juncea* L. (Restionaceae), 11 May 2001, S. Lee, PREM 57458, cultures ex-type STE-U 5256, CBS 112112.

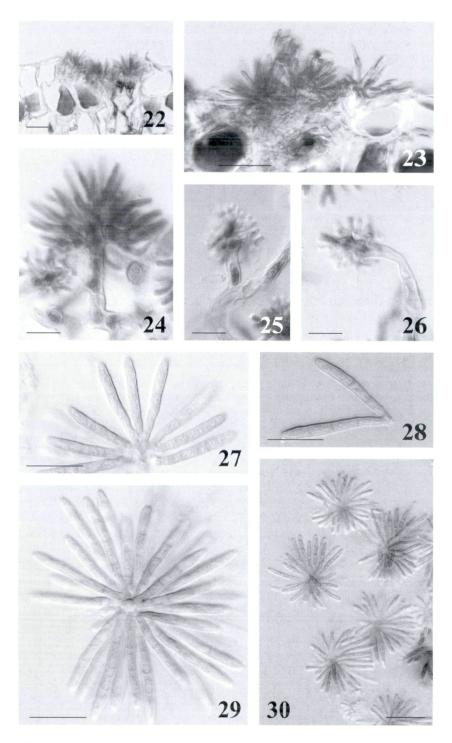
caulicolous, acervuloid to indeterminate, Conidiomata intraepidermal in origin, epiphyllous to amphigenous, prosenchymatous, scattered to gregarious, irregular in outline, up to 75 µm wide and 50 μm high (Figs. 22, 23). - Conidiophores septate and sparsely branched or reduced to conidiogenous cells. - Conidiogenous cells discrete to integrated, determinate, cylindrical to doliiform, hyaline, $(6-)7-12\times2.5-3.5(-4)$ µm (Figs. 24-26). - Conidia palmate, multiseptate, pale brown, smooth, consisting of about 33-56 arms radiating from a central complex of short, branched cells, (52-)58-66(-70) µm diam. ($\bar{x} = 61.7$ µm) when lightly squashed (Figs. 29, 30). - Arms cylindrical, tapering and paler towards the tip, pale brown, mostly two arms joined in V-shape (Figs. 27, 28), at times slightly constricted at septa, covered with thin gelatinous sheath, 0.5 µm thick, each arm with (2-)3(-4) transverse septa, $(19-)26-30(-35)\times 2$ µm, straight or slightly curved, generally somewhat uneven in diameter.

Cultural characteristics. – Colonies sterile, reaching 1.9 mm diam. on MEA after 10 d at 25 °C in the dark, convex, circular with entire margin, iron grey (23""k) with reverse black. Mycelium very dense, velvety, superficial.

Known host.- Elegia juncea L. (Restionaceae).

Known distribution. - Western Cape province, South Africa.

The genus Psammina Sacc. & M. Rousseau ex E. Bommer & M. Rousseau, which includes species characterized by their palmate conidia, was introduced to accommodate the saprobic fungus P. bommeriae Sacc. & M. Rousseau, which occurs on Ammophila arenaria (L.) Link (Gramineae) and Juncus effusus L. (Juncaceae) (Saccardo, 1892). Only two further species, P. mariae-theresiae Dias & Teixeira on Smilax nigra Willd. (Smilacaceae), and P. stipitata D. Hawksw. on Schismatomma decolorans (Turner & Borrer ex Sm.) Clauz. & Vezda (lichen), were known until recently, when two algicolous and lichenicolous species, P. inflata Earl.-Benn. & D. Hawksw. and P. simplex Earl.-Benn. & D. Hawksw., were discovered in the British Isles (Earland-Bennett & Hawksworth, 1999). Conidiomata were regarded as acervuli by Sutton (1980), while Hawksworth (1979) regarded these structures as rudimentary stromata. Earland-Bennett & Hawksworth (1999) stated that all three lichenicolous species lacked acervuli. Vertical sections through conidiomata of P. elegiae also showed the absence of a distinct hymenium layer. Psammina elegiae differs from other species in the genus in the range of arm length, which is regarded as being more significant than the overall dimensions of conidia in species separation as men-



tioned by Earland-Bennett & Hawksworth (1999). They observed the three-dimensional arrangement of the conidial arms varied depending on the level of pressure applied to the cover glass, and they emphasized the size, shape, and septation of the individual conidial arms as being more consistent characters.

Key to species of Psammina

1. Occurring on lichens or algae	
2. Conidial arms 3–7-septate	
3. Conidial arms 0–1(–2)-septate, (7–)15–25(–29) × (1.5–)2–4 μm	x
4. Average length of conidial arms up to 25 μm 4*. Average length of conidial arms longer than 25 μm	
5. Conidial arms $27.5-45\times 2.5-3~\mu m$	

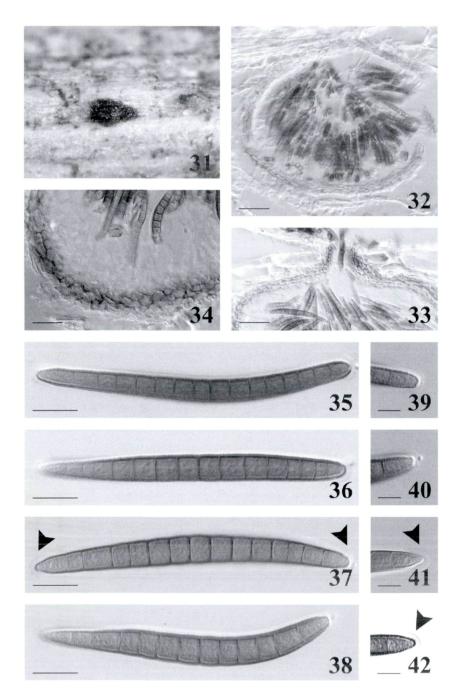
Septoriella restionis S. Lee & Crous, sp. nov.—Figs. 31–42.

Conidiomata pycnidica, alte immersa, ostiolata, ellipsoidalia in sectione verticali, usque ad 190 μm alta, ad 240 μm lata. Peridium pseudoparenchymatosum, complectens duo strata cellularum, ad 25 μm crassum. Conidiophora circa cavitatem conidiomatis enascentia, ad cellulas conidiogenas reducta. Cellulae conidiogenae discretae, ampulliformes vel subconicae, hyalinae, tenuitunicatae, laeves, 5.5–6.5 \times 1.5–2.5 μm . Conidia holoblastica, cylindrica, apice lato et attenuato vel apiculato et basi truncata vel rotundata, crassiusculiora in dimidio superno, recta, parce curvata vel sigmoidea, plerumque (9–)13–14(–15)-septata, pallide brunnea, glabro-tunicata, (52.5–)66–70(–77.5) \times (5–)6(–7) μm , utrinque appendices pileatas gelatinosas ferentia. Appendices 1–1.5 μm crassae.

Etymology. – In reference to its host, *Restio*.

Holotype. – South Africa: Western Cape province, Kogelberg Nature Reserve, on dead culms of *Restio* sp. (Restionaceae), 11 May 2001, S. Lee, PREM 57459, cultures ex-type STE-U 5254, CBS 110663

Figs. 22–30. Psammina elegiae (holotype). – 22, 23. Sections through a conidioma. – 24–26. Conidiophores with conidiogenous cells (in lactophenol cotton-blue). – 27. Part of conidium. – 28. Two arms joined in V-shape. – 29, 30. Conidia. – Bars 22, 23, 30 = 25 μ m; 24–29 = 10 μ m.



Conidiomata caulicolous, pycnidial, scattered to gregarious, deeply immersed, unilocular, glabrous, ostiolate with dark tendrils of conidia extruding through the ostiole, in vertical section ellipsoidal, up to 190 μm high and 240 μm wide (Figs. 31-33). - Peridium pseudoparenchymatous, comprising 2 layers of cells, up to 25 µm thick, cells of outer layer thick-walled, brown, darker in the ostiolar region, cells of inner layer thin-walled, hyaline (Fig. 34). - Conidiophores lining the cavity of the conidioma, reduced to conidiogenous cells, invested in mucus. - Conidiogenous cells discrete, ampulliform to subconical, hyaline, thin-walled, smooth, $6.5 \times 1.5 - 2.5 \mu m$. - Conidia holoblastic, cylindrical with a broad, tapered or apiculate apex and a truncate or rounded base, somewhat broader in the upper half, straight, slightly curved or sigmoid, mostly (9-)13-14(-15)-septate, light brown, smooth-walled, often slightly constricted at the septa, $(52.5-)66-70(-77.5)\times(5-)6(-7)$ µm (= $67.9 \times 6.0 \mu m$), bearing cap-like, gelatinous appendages at both ends. - Appendages developing by gelatinisation of appendage primordia on the developing conidia, 1–1.5 µm thick (Figs. 35–42).

Cultural characteristics. – Colonies sterile, reaching 15 mm diam. on MEA after 10 d at 25 °C in the dark, convex, circular with entire margin, grey olivaceous (21""b) with reverse brick (9"b). Mycelium dense, velvety, aerial, with fluffy margin.

Known host. – Restio sp. (Restionaceae).

Known distribution. - Western Cape province, South Africa.

The genus *Septoriella* Oudem. can easily be recognized by its coloured phragmospores with gelatinous sheaths at both ends. This fungus is very similar to the species of *Callistospora* in its morphological characters except for its euseptate conidia. Nag Raj (1993) discussed the necessity of further taxonomic research in this genus, mentioning that most of the species had been erroneously allocated to it. In addition to the nine species recognized by Saccardo (1909; 1931), a further seven species, six from Nag Raj (1993) and one from Sutton & Mel'nik (1999), have been described from various host plants worldwide. *Septoriella restionis* can easily be distinguished by its (9–)13–14(–15)-septate conidia, and cap-like, gelatinous polar appendages, versus the fringe-like gelatinous appendages that occur in other species. Besides the large, dark-brown conidia, hyaline,

Figs. 31–42. Septoriella restionis (holotype). – 31. Conidioma on the host surface. – 32. Section through a conidioma. – 33. Ostiole. – 34. Peridium. – 35–38. Conidia with gelatinous appendages at both ends (arrowheads). – 39–42. Close view of caplike appendages (arrowheads). – Bars 32, 33 = 50 μm ; 34 = 25 μm ; 35–38 = 10 μm ; 39–42 = 5 μm .

unicellular microconidia were reported in some other species near the wall of the ostiolar channel. In the current species, however, no microconidia were detected, as was the case for the most recently published species, *S. halensis* B. Sutton & Mel'nik (Sutton & Mel'nik, 1999).

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