INTRODUCTION

In his revision of coelomycetous fungi with appendage-bearing conidia, Nag Raj (1993) mentioned that in early papers dealing with these fungi, descriptions of novel taxa only rarely referred to the fact that conidia had appendages. In later years, these appendages were seen as taxonomically informative in separating species, especially in genera such as Coniella (van Niekerk et al. 2004), Phyllosticta (Wulandari et al. 2009, Gliener et al. 2011, Wikke et al. 2011), Pestalotiopsis (Mara-rachchikumbura et al. 2011), Seimatosporium (Barber et al. 2011), and Tiarosporella (Crous et al. 2006). Sutton & Sellar (1966) categorised appendages by their structure and position, whether mucilaginous or cellular, endo- or exogenous, simple or branched, as well as their position on the conidium. Nag Raj (1993) further elaborated on these concepts, and defined nine appendage types (A–I), with three subdivisions for appendage type A. He further highlighted the diversity that exists in appendage morphology, namely in shape (filiform, attenuated or podiform), position (apical, basal, lateral, or in combination), in patterns of distribution on the conidium, in branching, in integrity with the conidium, in sequence of development, and in structural changes (nucleate or not).

Appendages usually relate to an ecological function linked to spore dispersal, and the colonisation of new substrates or niches. Appendages play an important role in spore attachment to substrates to ensure that the conidium can germinate and its hyphae can infect or colonise the substrate (Nag Raj 1993). Conidial appendages are known to occur in coelomycetes in diverse habitats ranging from terrestrial to aquatic. Appendages have subsequently been used to support the combination or separation of taxa into different genera. One such genus is Dinemasporium, which forms the basis of the present study. It is characterised by superficial, stipitate to cupulate conidiomata, and small conidia with two polar, tubular, exogenous appendages. The genus Stauronema is reduced to synonymy under Dinemasporium, and an epitype designated for D. pseudostrigosum (1-septate conidia) is supported as distinct from Dinemasporium (asepitate conidia), and P. fraxini introduced as a novel species. Taxa in this generic complex differ by combination of morphological characters of conidiomata, setae, conidia and appendages. Appendage morphology alone is rejected as informative at the generic level.


The aim of the present study is to investigate the taxonomic value of appendages as defining feature at generic level in coelomycetous fungi, by using Dinemasporium as a case study. A further aim is to clarify the phylogenetic position of the genus Dinemasporium, and to revisit its circumscription in relation to its synonyms and closely allied genera, many of which are distinguished by a combination of conidium septation and appendage morphology.

Key words
Chaetosphaeriaceae
Dinemasporium systematics
LSU
Sordariomycetes
ITS

Abstract

The genus Dinemasporium is used as a case study to evaluate the importance of conidial appendages for generic level classification of coelomycetous fungi. Based on morphology and sequence data of the large subunit nuclear ribosomal RNA gene (LSU, 28S) and the internal transcribed spacers and 5.8S rRNA gene of the nrDNA operon, the genus Dinemasporium is circumscribed, and an epitype designated for D. strigosum, the type of the genus. A further five species are introduced in Dinemasporium, namely D. pseudostrigosum (isolated from Triticum aestivum, Germany and Stigmaphyllon sagraceanum, Cuba), D. americana (soil, USA), D. polygonum (Polygonum sachalinerse, Netherlands), D. pseudoindicum (soil, USA), and D. morbicum (human sputum, Netherlands and hare dung, New Zealand). Brunneodinemasporium, based on B. brasiliense, is introduced to accommodate Dinemasporium-like species with tightly aggregated brown conidiogenous cells, and pale brown conidia. Dendrophoma (= Amphitriaspora) is reinstated as distinct from Dinemasporium, and an epitype designated for D. cytisporoides, characterised by its superficial, stipitate to cupulate conidiomata, and small conidia with two polar, tubular, exogenous appendages. The genus Stauronema is reduced to synonymy under Dinemasporium. Pseudolachnea (1-septate conidia) is supported as distinct from Dinemasporium (asepitate conidia), and P. fraxini introduced as a novel species.

How important are conidial appendages?

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MATERIALS AND METHODS

Isolates
The majority of the strains used in the present study were obtained from the culture collection of the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, the Netherlands. Fresh collections were made from debris of diverse hosts by placing material in damp chambers for 1–2 d. Single conidial colonies were established from sporulating conidiomata on Petri dishes containing 2 % malt extract agar (MEA; Crous et al. 2009b) as described earlier (Crous et al. 1991). Colonies were sub-cultured onto potato-dextrose agar (PDA), oatmeal agar (OA), MEA, and pine needle agar (PNA) (Smith et al. 1996), and incubated at 25 °C under continuous near-ultraviolet light to promote sporulation. Reference strains were deposited at the CBS.

DNA isolation, amplification and analyses
Genomic DNA was extracted from fungal colonies growing on MEA using the UltraCleanTM Microbial DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA, USA) according to the manufacturer’s protocol. The primers V9G (de Hoog & Gerrits van den Ende 1998) and L5R (Vilgalys & Hester 1990) were used to amplify part (ITS) of the nuclear rDNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The primers ITS4 (White et al. 1990) and LSU1Fd (Crous et al. 2009a) were used as internal sequence primers to ensure good quality sequences over the entire length of the amplicon. The sequence alignment and subsequent phylogenetic analyses for all the above were carried out using methods described by Crous et al. (2006). Gaps longer than 10 bases were coded as single events for the phylogenetic analyses; the remaining gaps were treated as ‘fifth state’ data. Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

Morphology
Morphological descriptions are based on slide preparations mounted in clear lactic acid from colonies sporulating on PNA. 

Observations were made with a Zeiss V20 Discovery stereomicroscope, and with a Zeiss Axio Imager 2 light microscope using differential interference contrast (DIC) illumination and an AxioCam MRc5 camera and software. Colony colours and pigment production were noted after 1 mo of growth on MEA, PDA and OA (Crous et al. 2009b) incubated at 25 °C. Colony colours (surface and reverse) were rated according to the colour charts of Rayner (1970).

RESULTS

Phylogeny
Amplicons of approximately 1 700 bases were obtained of the ITS region (including the first approximately 900 bp of LSU) for the isolates listed in Table 1. The LSU sequences were used to obtain additional sequences from GenBank, which were added to the alignment (Fig. 1) and the ITS to determine species identification (Fig. 2; discussed in species notes where applicable). The manually adjusted LSU alignment contained 42 sequences (including the outgroup sequence) and 798 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 151 of these were parsi-mony-informative, 136 were variable and parsimony-uninformative, and 511 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded trees with identical topologies to one another and support the same terminal clades as obtained from the parsimony analysis. The parsimony analysis of the LSU alignment yielded 142 equally most parsimonious trees (TL = 741 steps; CI = 0.533; RI = 0.662; RC = 0.353). The manually adjusted ITS alignment contained 31 sequences (including the outgroup sequence) and 514 characters including alignment gaps (available in TreeBASE) were used in the phylogenetic analysis; 203 of these were parsimony-informative, 80 were variable and parsimony-uninformative, and 251 were constant. Neighbour-joining analyses using three substitution models on the sequence alignment yielded trees with identical topologies to one another and support the same terminal clades as obtained from the parsimony analysis. The parsimony analysis of the ITS alignment yielded 3 equally most parsimonious trees (TL = 929 steps; CI = 0.553; RI = 0.706; RC = 0.390). All the species treated below are supported as distinct in the ITS phylogeny (Fig. 2).

Table 1 Collection details and GenBank accession numbers of isolates for which novel sequences were generated in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain accession no. 1</th>
<th>Substrate</th>
<th>Country</th>
<th>Collector(s)</th>
<th>GenBank accession no. 2</th>
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<tbody>
<tr>
<td>Dendrophoma cytopsorioides</td>
<td>CBS 223.96</td>
<td>Decaying leaf branches and twigs of</td>
<td>Netherlands</td>
<td>A. Aptroot</td>
<td>JQ889273 JQ889289</td>
</tr>
<tr>
<td>Dinemasporium americana</td>
<td>CBS 127127; RMF 7507</td>
<td>A1 horizon soil of tallgrass prairie</td>
<td>USA: Iowa</td>
<td>D.E. Tuthill</td>
<td>JQ889274 JQ889290</td>
</tr>
<tr>
<td>Dinemasporium decipiens</td>
<td>CBS 592.73</td>
<td>Soil under Elaeis guineensis</td>
<td>Suriname</td>
<td>J.H. van Emde</td>
<td>JQ889275 JQ889291</td>
</tr>
<tr>
<td>Dinemasporium morbidum</td>
<td>CBS 129.66</td>
<td>Spout of man</td>
<td>Netherlands</td>
<td>N.G.M. Orie &amp; A. Kikstra</td>
<td>JQ889280 JQ889296</td>
</tr>
<tr>
<td>Dinemasporium polygonum</td>
<td>CBS 955.97; ATCC 200690</td>
<td>Hare dung</td>
<td>New Zealand</td>
<td>D.P. Mahoney</td>
<td>JQ889281 JQ889297</td>
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<td>Polygonum sachalinsense</td>
<td>Netherlands</td>
<td>A. Aptroot</td>
<td>JQ889276 JQ889292</td>
</tr>
<tr>
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<td>CBS 127402; RMF 8631</td>
<td>A1 horizon soil of tallgrass prairie</td>
<td>USA: Kansas</td>
<td>M. Christensen</td>
<td>JQ889277 JQ889293</td>
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<tr>
<td>Dinemasporium pseudostrigosum</td>
<td>CBS 717.85</td>
<td>Triticum aestivum</td>
<td>Germany</td>
<td>P. Reinecke</td>
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<td>Dinemasporium striogamus</td>
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<td>Stigmaphyllon sagaeanaum</td>
<td>Cuba</td>
<td>R.F. Castrafeda</td>
<td>JQ889279 JQ889295</td>
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<td>Netherlands</td>
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<td>Dinemasporium striogamus</td>
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<td>Dinemasporium striogamus</td>
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<td>Phragmites australis</td>
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<td>W. Quaedvlieg</td>
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<td>USA: Illinois</td>
<td>J. Batzer</td>
<td>JQ889285 –</td>
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<tr>
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<td>CPC 19796</td>
<td>Wildenovia incurvata</td>
<td>South Africa</td>
<td>A. Wood</td>
<td>JQ889286 –</td>
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<td>Pseudolachnea fraxini</td>
<td>CBS 113701; UPSC 1833</td>
<td>Fraxinus excelsior</td>
<td>Sweden</td>
<td>K. &amp; L. Holm</td>
<td>JQ889287 JQ889301</td>
</tr>
</tbody>
</table>

1 ATCC: American Type Culture Collection, Virginia, USA; CBS: CBS Fungal Biodiversity Centre, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at CBS; INIFAT: Alexander Humboldt Institute for Basic Research in Tropical Agriculture, Ciudad de La Habana, Cuba; RMF: Martha Christensen Soil Fungus Collection; UPSC: Uppsala University Culture Collection of Fungi, Botanical Museum University of Uppsala, Uppsala, Sweden.

2 ITS: Internal transcribed spacers 1 and 2 together with 5.8S rDNA; LSU: partial 28S rDNA.
**Taxonomy**

**Brunneodinemasporium** Crous & R.F. Castañeda, *gen. nov.* — MycoBank MB800158

*Type species.* *Brunneodinemasporium brasiliense* Crous.

*Etymology.* Similar to *Dinemasporium*, but conidiogenous cells and conidia brown in colour.

*Conidiomata* stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, uniocular, globose, setose; basal stroma of *textura angularis*. *Setae* abundant, brown to black, simple, septate, subulate to cylindrical, unbranched, smooth, thick-walled, multi-septate, arising randomly throughout basal stroma. *Conidiophores* lining the basal stroma in a dense layer, brown, septate, unbranched, cylindrical, thin-walled, smooth.

*Conidiogenous cells* integrated, determinate, phialidic with conspicuous periclinal thickening at an attenuated apex, brown, smooth, subcylindrical to lageniform.

*Conidia* hyaline to pale brown, aseptate, thin-walled, smooth, fusiform, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, with a single, cellular, unbranched, flexuous, with tubular appendage at each end, separated by a septum; basal appendage eccentric.

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**Fig. 1** The first of 142 equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the LSU sequence alignment. The scale bar shows 10 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. The species treated in this study are located in the coloured blocks. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Saccharomyces cerevisiae* (GenBank accession Z73326).
**Brunneodinemasporium brasiliense** Crous & R.F. Castañeda, sp. nov. — MycoBank MB800159, Fig. 3

*Conidiomata* stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular globose, up to 600 μm diam, setose with a central buff conidial mass on PNA; basal stroma of *textura angularis*. *Setae* abundant, brown to black, simple, septate, subulate to cylindrical, with obtuse apex, unbranched, smooth, thick-walled, multi-septate, 200–1300 μm long, 12–15 μm diam at base, 5–10 μm diam at obtuse apex, arising from basal stroma.

*Conidiophores* lining the basal stroma in a dense layer, brown to black, 4–6-septate, unbranched, cylindrical, thin-walled, smooth, 40–70 × 3–4 μm. *Conidiogenous cells* integrated, determinate, phialidic with clearly visible periclinal thickening at apex 1.5–2 μm diam, brown, smooth, subcylindrical to lageniform, 10–16 × 3–4 μm. *Conidia* hyaline to pale brown, aseptate, thin-walled, smooth, fusiform, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (17–)18–19(–20) × (2–)2.5–3 μm, with a single, unbranched, flexuous, tubular appendage at each end, 5–7 μm long, apparently separated by a septum; basal appendage excentric.

*Culture characteristics* — Colonies flat, spreading, with sparse to moderate aerial mycelium. On MEA smoke grey (surface), with even, lobate margins, surrounded by a zone of diffuse, red pigment; reverse olivaceous grey in centre, outer region red due to diffuse pigment; on OA iron grey with even, lobate margin; on PDA surface isabelline, in outer region smoke grey, in reverse grey olivaceous.

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**Fig. 2** The first of three equally most parsimonious trees obtained from a heuristic search with 100 random taxon additions of the ITS sequence alignment. The scale bar shows 25 changes, and bootstrap support values from 1 000 replicates are shown at the nodes. Genera are indicated in the coloured blocks and the names of genera treated in this study are written in red. Ex-type strains are printed in **bold**. Branches present in the strict consensus tree are thickened and the tree was rooted to a sequence of *Cephalotheca sulfurea* (GenBank accession AB278194).

Notes — Brunneodinemasporium has randomly distributed setae throughout the basal stroma, which differs from Dinema­sporium, which has a densely aggregated layer of brown conidiogenous cells with prominent periclinal thickening and apical taper, and conidia that appear pale brown, and have setae that separate from the conidia by a septum (though the latter is not clear under the light microscope, but differs from Dinemasporium s.str.).


Type species. Dendrophoma cytosporoides Sacc.

Conidiomata stromatic, scattered to gregarious, superficial, stipitate, globose and closed, becoming cupulate, unilocular, dark brown to black; basal excipulum brown, of dense textura intricata. Setae arising from the outer elements of excipulum, or restricted to base of conidioma, sparse, subulate to subcylindrical, apex blunt to acutely rounded, straight to curved, transversely septate, dark brown, thick-walled, smooth. Conidiophores arising from conidiomatal cavity, septate, branched, hyaline, up to 40 µm long. Conidiogenous cells discrete or integrated, terminal and lateral, lageniform to subcylindrical, hyaline, thin-walled, smooth. Conidia naviculate to botuliform, aseptate, hyaline, thin-walled, smooth, with an unbranched cellular appendage at each end.

Dendrophoma cytosporoides Sacc., Michelia 2: 4. 1880; Fig. 4

Basionym. Phoma cytosporoides Sacc. (as 'cytisporoides'), Michelia 1, 5: 522. 1879.


Conidiomata stromatic, scattered to gregarious, superficial, stipitate, at first globose and closed, then opening to become cupulate, up to 300 µm diam, unilocular, dark brown to black; basal excipulum brown, of dense textura intricata. Setae arising from the outer elements of excipulum, or restricted to base of conidioma, sparse, subulate to subcylindrical, apex blunt to acutely rounded, straight to curved, 2–8-septate, dark brown, thick-walled, smooth, 100–300 × 4–6 µm, apex 1–1.5 µm wide. Conidiophores arising from conidiomatal cavity, septate, branched, hyaline, up to 40 µm long. Conidiogenous cells discrete or integrated, terminal and lateral, lageniform to subcylindrical, frequently in terminal whorls, hyaline, thin-walled, smooth, 6–15 × 1.5 µm. Conidia naviculate to botuliform, aseptate, hyaline, thin-walled, smooth, (3.5–)4–5(–6) × (1–)1.5 µm, with an unbranched appendage at each end, 0.5–1 µm long.

Culture characteristics — Colonies flat, spreading, with sparse aerial mycelium and even, lobate margins. On OA surface grey olivaceous, reverse olivaceous grey; on PDA surface brick to cinnamon, reverse dark brick.

Notes — The characteristic features that separate Dendrophoma from Dinemasporium are conidiomata that are superficial and stipitate, becoming cupulate. The conidiogenous cells of Dendrophoma form a dense layer that gives rise to conidia with short, tubular exogenous appendages at each end, also differing from Dinemasporium s.s.

Based on an examination of the holotype specimen, Sutton (1977, 1980) regarded Amphitarospora as synonymous with Dinemasporium. The original description (conidia 4–5 × 1.5–2 μm, setae 1–2 μm; Agnihothrudu 1962), indicated that A. neotexta is synonymous with D. cytosporoides.


Conidiomata stromatic, cupulate, often discoidal, superficial (also on SNA in culture), unilocular, setose, black, with basal stroma of textura angularis. Setae arise from the basal stroma and/or from excipular margin, unbranched, subulate to cylindrical, straight or curved, brown to dark brown, smooth or verruculose, with obtuse to acute apices. Conidiophores lining the inner cavity, mostly branched, septate, hyaline (at times brown at base), smooth, invested in mucus. Conidiogenous cells discrete or integrated, lageniform, subcylindrical or cylindrical, hyaline, smooth, with visible periclinal thickening. Conidia fusiform, naviculate or allantoid, asceptate, hyaline to pale brown, smooth, with a single, unbranched, filiform, cellular appendage at each end (not separated from body via septa); lateral appendages present or absent.

Dinemasporium americana Crous & Tuthill, sp. nov. — MycoBank MB800160; Fig. 5

Etymology. Named after its country of origin, Unites States of America.

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose, up to 250 μm diam, setose with a central buff to rosy buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 μm thick. Setae brown to black, simple, septate, subulate with acute apex, unbranched, smooth, thick-walled, up to 7-septate, 50–200 × 4–10 μm, 1–1.5 μm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, prominently septate (–8), branched, cylindrical, thin-walled, smooth, base pale brown, apex hyaline, up to 70 μm long. Conidiogenous cells determinate, phialidic with periclinal thickening, hyaline, smooth, subcylindrical, 7–10 × 2–2.5 μm. Conidia hyaline, asceptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (9–)12–13(–16) × (2.5–)3 μm, with a single, unbranched, flexuosus, tubular appendage at each end, (11–)12–14(–16) μm basal appendage excentric.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium. On MEA (surface), reverse cinnamon; on OA and PDA (surface and reverse).


Notes — Isolate RMF 7507 was formerly treated as D. strigosum, but is specifically distinct. Although the conidiomata produced are similar in general morphology to those of D. strigosum, they differ by having shorter setae, larger conidia, longer appendages (setae 60–400 μm long, conidia (9–)12–13(–16) μm long, appendages 6–9 μm long in D. strigosum).

Dinemasporium morbidum Crous, sp. nov. — MycoBank MB800161; Fig. 6

Etymology. Name reflects on the unpleasant substrata from which the fungus was isolated.

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose, up to 250 μm diam, setose with a central buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 μm thick. Setae brown to black, simple, septate, subulate with acute apex, unbranched, smooth, thick-walled, up to 9-septate, 50–330 × 5–10 μm, 1–1.5 μm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, hyaline, septate, sparingly branched, cylindrical, thin-walled, smooth, 30–40 μm long. Conidiogenous cells determinate, phialidic with periclinal thickening, hyaline, smooth, subcylindri-
cal to lageniform, 8–13 × 2–2.5 µm. *Conidia* hyaline, aseptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (10–)12–14(–15) × (2.5–)3 µm, with a single, unbranched, flexuous, tubular appendage at each end, (6–)7–10(–12) µm; basal appendage excentric.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium. On MEA, OA and PDA buff (surface), reverse buff with patches of cinnamon; sporulation rosy buff.

*Fig. 5 Dinemasporium americana* (CBS 127127). a, b. Acervular conidiomata forming in culture; c–e. setae and conidiogenous cells; f. conidiogenous cells; g, h. conidia. — Scale bars = 10 µm; e applies to f.

*Fig. 6 Dinemasporium morbidum* (CBS 129.66). a, b. Acervular conidiomata forming in culture; c. setae. d, e. conidiogenous cells; f, g. bases and apices of setae; h, i. conidia. — Scale bars = 10 µm, except a = 20 µm.


Notes — *Dinemasporium morbidum* has longer conidia and appendages than *D. strigosum*, which has conidia (9–)10–12 (–13) µm long, with appendages 6–9 µm long.
Dinemasporium polygonum Crous & Verkley, sp. nov. — MycoBank MB800162; Fig. 7

Etymology. Named after the host genus from which it was isolated, Polygonum.

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose, up to 250 μm diam, setose with a central buff to rosy buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 μm thick. Setae brown to black, simple, septate, subulate with acute apex, unbranched, smooth, thick-walled, up to 9-septate, 100–300 × 5–8 μm, 1–1.5 μm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, hyaline, septate, sparingly branched, cylindrical, thin-walled, smooth, 30–60 μm long. Conidiogenous cells determinate, phialidic with periclinal thickening, hyaline, smooth, subcylindrical to lageniform, 7–16 × 2–2.5 μm. Conidia hyaline, aseptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (9–)10–12(–13) × (2–)2.5(–3) μm, with a single, unbranched, flexuous, tubular appendage at each end, (9–)10–12(–13) μm; basal appendage excentric.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium. On MEA, OA and PDA buff (surface and reverse).


Notes — Dinemasporium polygonum has conidia with longer appendages than D. strigosum though shorter than in D. americum (6–9 μm long in D. strigosum, 11–16 μm in D. americum).

Dinemasporium pseudoindicum Crous & M. Chr., sp. nov. — MycoBank MB800163; Fig. 8

Etymology. Named after its morphological similarity to Stauronema indicum.

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose to oval, irregular in outline, 300–400 μm diam, setose with a central cream conidial mass on PNA; basal stroma of textura angularis, layer 20–30 μm thick. Setae brown, simple, septate, subulate with acute to obtuse apex, unbranched, smooth, thick-walled,
up to 4-septate, 100–400 × 4–8 µm, 1–1.5 µm wide at acute apex, or 4 µm when obtuse at apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, hyaline, septate, sparingly branched, cylindrical, thin-walled, smooth, 30–40 µm long. Conidiogenous cells determinate, phialidic with periclinal thickening, hyaline, smooth, subcylindrical to lageniform, 10–15 × 2–2.5 µm. Conidia hyaline, aseptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (10–)12–13–(14) × (2.5–)3 µm, with a single, unbranched, flexuous, tubular appendage at each end; basal appendage excentric; apical and basal appendages 9–22 µm long; lateral appendages 2, inserted 4–6 µm below apex, 10–17 µm long.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium on OA, but with sparse aerial mycelium on PDA and MEA. On MEA rosy buff (surface), reverse cinnamon in middle, buff in outer region; on OA buff with profuse sporulation (peach in colour); on PDA dirty white to buff (surface and reverse).

Specimens examined. USA, Kansas, near Manhattan, Konza Prairie Research Natural Area, long-term ecological research site, A1 horizon soil, tallgrass prairie, June 1987, M. Christensen (holotype CBS H-12018, culture ex-type CBS 717.85).

Notes — Morphologically similar to Stauronema indicum, but distinct in that it has larger conidia and appendages of different length, and at different positions in relation to the conidial apex (conidia 7–13 × 2–3 µm, terminal appendages 6.5–17 µm, lateral appendages 8–20 µm long, but 3–4 µm below apex; Nag Raj 1993).

Dinemasporium pseudostrigosum Crous, sp. nov. — Myco-Bank MB800164; Fig. 9

Etymology. Named after its morphological similarity to D. strigosum.

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose, up to 250 µm diam, setose with a central buff to rosy buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 µm thick. Setae brown to black, simple, septate, subulate with acute apex, unbranched, smooth, thick-walled, up to 7-septate, 100–250 × 5–8 µm, 1–1.5 µm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, hyaline, septate, sparingly branched, cylindrical, thin-walled, smooth, 30–40 µm long. Conidiogenous cells determinate, phialidic with periclinal thickening, hyaline, smooth, subcylindrical to lageniform, 10–15 × 2–2.5 µm. Conidia hyaline, aseptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobtusely rounded, base truncate, eguttulate or guttulate, (10–)12–13–(14) × (2.5–)3 µm, with a single, unbranched, flexuous, tubular appendage at each end, (11–)13–15–(17) µm; basal appendage excentric.

Culture characteristics — Colonies spreading, erumpent, with moderate aerial mycelium. On MEA buff (surface), reverse cinnamon in middle, buff in outer region; on OA buff with profuse sporulation (peach in colour); on PDA dirty white to buff (surface and reverse).


Notes — Morphologically D. pseudostrigosum is similar to D. strigosum, but distinct in that it has larger conidia, and longer appendages.

Dinemasporium strigosum (Pers.: Fr.) Sacc., Michelia 2: 281. 1881; Fig. 10


≡ Excipula graminum (Lib.) Corda, Icon. Fungorum (Prague) 3: 29. 1839.


For additional synonyms see Sutton (1980) and Nag Raj (1993).

Conidiomata stromatic, scattered or aggregated, superficial, dark brown to black, cupulate, unilocular, globose, up to 250 µm diam, setose with a central buff to rosy buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 µm thick. Setae brown to black, simple, septate, subulate with acute apex, unbranched, smooth, thick-walled, up to 7-septate, 100–250 × 5–8 µm, 1–1.5 µm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores

Fig. 9 Dinemasporium pseudostrigosum (CBS 717.85). a, b. Acervular conidiomata forming in culture; c, d. setae; e, f. conidiogenous cells; g, h. conidia. — Scale bars = 10 µm, except a = 20 µm, applies also to b.
diam, setose with a central buff to rosy buff conidial mass on PNA; basal stroma of textura angularis, layer 20–30 µm thick. Setae brown to black, simple, sepalate, subulate with acute apex, unbranched, smooth, thick-walled, up to 9-septate, 60–400 × 7–10 µm, 1–1.5 µm wide at acute apex, arising from basal stroma or lateral from excipulum. Conidiophores lining the basal stroma, hyaline, sepalate, sparingly branched, cylindrical, thin-walled, smooth, 30–40 µm long. Conidiogenous cells determinate, phialidic with periclinial thickening, hyaline, smooth, subcyllindrical to lageniform, 12–15 × 2–2.5 µm. Conidia hyaline, aseptate, thin-walled, smooth, naviculate to fusiform or ellipsoid, gently curved or straight, apex obtuse to subobliquely rounded, base truncate, eguttulate or guttulate, (9–)10–12(–13) × (2–)2.5(–3) µm, with a single, unbranched, flexuous, tubular appendage at each end, (6–)7–8(–9) µm; basal appendage excentric.

Culture characteristics — Colonies spreading, erumpent, basal appendage excentric.

Notes — Dinemasporium strigosum is a species of the genus Dinemasporium, which was described by Crous et al. (2013). It was originally described as Pseudolachnea hispidula by Ranoj (1910) and later transferred to Dinemasporium by Mark Brandenburg (1915). The species is characterized by its acervular conidiomata, which form in vivo, and its conidia, which are fusiform or ellipsoid, gently curved or straight, with a single, unbranched, tubular appendage at each end. The basal appendage is excentric.

Although D. strigosum was linked to a sexual state described as Phomatospora dinemasporium (Webster 1955), the generic placement was questioned by Rappaz (1992), as other species of Phomatospora have been linked to hyphomycetes with Fusarium-like conidia. No sexual states were encountered or induced in the present study.

Pseudolachnea Ranoj, Ann. Mycol. 8: 593. 1910 [non Pseudolachnea Velen. 1934].

Conidiomata stromatic, scattered to gregarious, cupulate, superficial, unilocular, setose, dark brown to black; basal stroma of textura angularis. Setae divergent, subulate with blunt or acute apices, unbranched, septate, thick-walled, smooth, dark brown. Conidiophores arising from the uppermost cells of the basal stroma and the inner cells of the excipulum, branched, septate, brown at base, hyaline in upper part, smooth, invested in a thin layer of mucus. Conidiogenous cells discrete, phialidic with periclinial thickening, cylindrical, hyaline, smooth. Macroconidia fusiform with obtuse ends, 1-septate, hyaline, smooth, guttulate, bearing an unbranched, short, cellular, filiform appendage at each end; appendages not delimited by septa, basal appendage excentric. Microconidia intermixed with macroconidia in same conidioma, fusiform, curved with rounded ends, aseptate, smooth, guttulate, lacking appendages.

Type species. Pseudolachnea hispidula (Schrad.) B. Sutton (= P. bubakii Ranoj.)

Conidiomata on OA stromatic, scattered to gregarious, cupulate, superficial, unilocular, setose, dark brown to black; basal stroma of textura angularis. Setae divergent, subulate with blunt or acute apices, unbranched, septate, thick-walled, smooth, dark brown. Conidiophores arising from the uppermost cells of the basal stroma and the inner cells of the excipulum, branched, septate, brown at base, hyaline in upper part, smooth, invested in a thin layer of mucus. Conidiogenous cells discrete, phialidic with periclinial thickening, cylindrical, hyaline, smooth. Macroconidia fusiform with obtuse ends, 1-septate, hyaline, smooth, guttulate, bearing an unbranched, short, cellular, filiform appendage at each end; appendages not delimited by septa, basal appendage excentric. Microconidia intermixed with macroconidia in same conidioma, fusiform, curved with rounded ends, aseptate, smooth, guttulate, lacking appendages.

Pseudolachnea fraxini Crous, sp. nov. — MycoBank MB800165; Fig. 11

Etymology. Named after the host from which it was collected, Fraxinus.

Notes — Dinemasporium strigosum and its purported synonyms have been treated by several authors (Webster 1955, Sutton 1980, Nag Raj 1993, Yamaguchi et al. 2005, Duan et al. 2007). The synonyms proposed by Sutton (1980) and Nag Raj (1993) were accepted based on the examination of the type material cited above.
cells of the basal stroma and the inner cells of the excipulum, sparsely branched and septate at the base, up to 20 µm long, brown at base, hyaline in upper part, smooth, invested in a thin layer of mucus. Conidiogenous cells discrete, phialidic with periclinal thickening, cylindrical, hyaline, smooth, 6–12 × 2–3 µm. Macroconidia fusiform with obtuse ends, 1-septate, hyaline, smooth, guttulate, (12–)15–16(–18) × 2(–2.5) µm, bearing an unbranched, short, cellular, filiform appendage at each end, 1.5–2 µm long; appendages not delimited by septa, basal appendage excentric. Microconidia common, intermixed with macroconidia in same conidioma, fusiform, curved with rounded ends, aseptate, smooth, guttulate, (6–)7–9 × (1–)1.5 µm, lacking appendages.

Culture characteristics — Colonies flat, spreading, with sparse aerial mycelium and even, lobate margins. On MEA dirty white (surface and reverse). On OA surface honey, dark brown conidia. Furthermore, the genus Dendrophoma (= Ampliatarospora) is reinstated as distinct from Dinemasporium. Dendrophoma (which is based on D. cytosporoides) has superficial, stipitate to cupulate conidiomata, and small conidia with two polar, tubular, exogenous appendages, which separates it from Dinemasporium. The genus Stauronema is reduced to synonymy under Dinemasporium, and lateral conidial appendages rejected as a useful single character for generic separation. The genus Pseudolachnea (1-septate conidia) is supported as distinct from Dinemasporium (aseptate conidia), though no cultures were available to determine if Pseudolachnea (multi-septate conidia) needs to be reduced to synonymy with Pseudolachnea (Sutton 1980), or retained as separate genus (Nag Raj 1993).

The genus Dinemasporium is a phylogenetically well-defined genus in the Chaetosphaeriaceae. Dinemasporium is circumscribed based on D. strigosum, which is also epitified in this study. Five novel species are described from a collection of cultures formerly treated as D. strigosum. Taxa in this complex appear to differ by a combination of morphological features of the setae, conidia and conidial appendages.

Other than revising taxa in the Dinemasporium complex, a major aim of the present study was to determine the relevance of appendages as delimiting feature at generic level in coelomycetes. Conidial appendages have been used extensively to support the separation of species (e.g. Coniella, Phyllasticta, van Niekerk et al. 2004, Gliemke et al. 2011), but have also been used to separate genera of coelomycetes fungi (e.g. Discosia, Pestalotiopsis, Seimatosporium, Tiarosporella, Crous et al. 2006, Barber et al. 2011, Maharachchikumbura et al. 2011, Tanaka et al. 2011).

Results from the present study revealed that conidial appendages in coelomycetes are not informative as generic characters when looked at in isolation, as recently reported for the Seimatosporium complex (Barber et al. 2011). Although appendage morphology appeared to be highly informative at the species level (muclaiuginous or cellular, endo- or exogenous, simple or branched, as well as their position on the conidium body), they appeared to be less informative when used in isolation as generic feature. We can conclude that genera separated chiefly on the basis of appendage morphology, e.g. Dinemasporium.
and Stauronema, or Seimatosporium and Vermisporium (Barber et al. 2011), represent only two genera, namely Dinemasporium and Seimatosporium. This finding could also have implications for other more commonly encountered genera, e.g. Pestalotiopsis and Pestalotia, which are also separated solely on the basis of apical appendage morphology. Pestalotia (1839) predates Pestalotiopsis (1949), which will have serious implications for names in Pestalotiopsis, which is presently the more commonly used genus.

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