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Appressoria in *Mycoleptodiscus* Species

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Abstract

Appressoria formed by germinating conidia of *Mycoleptodiscus* species *in vitro* were examined as a possible source of taxonomic information. There were large differences in morphology between appressoria formed by the 12 taxa examined, and it is suggested that characteristics of the appressorium, such as size, shape, curvature, septation and germ pore size, provide additional differentiating criteria which are of use in the taxonomy of the genus. Three new species of *Mycoleptodiscus* are described, *M. coloratus*, *M. geniculatus*, and *M. variabilis*.

Introduction

The hyphomycete genus *Mycoleptodiscus* Ostazeski now contains 12 described species, following recent additions (Sutton and Alcorn 1990; Bills and Polishook 1992; Matsushima 1993; Cannon and Alcorn 1994). In cultures of *M. lunatus* B. Sutton & Alcorn and *M. affinis* Alcorn & P. Cannon, which were grown to compare the two taxa, mycelial appressoria similar to those produced by *Colletotrichum* spp. were sometimes observed. When germ tube appressoria were induced in both species, it was found that their morphology supported other evidence that two distinct taxa were represented (Cannon and Alcorn 1994). The study of appressorium morphology subsequently was extended to other species and un-identified isolates, and this paper reports the usefulness of this character in aiding differentiation of species within the genus *Mycoleptodiscus*.

Appressorium morphology has been found to be a useful taxonomic character in various groups of fungi (Emmett and Parbery 1975). These include the Erysiphaceae (Clare 1964), Meliolales (Parbery and Emmett 1977), *Phyllachora* spp. (Parbery 1963) and species of *Colletotrichum* (Sutton 1980). In the last-named genus, Sutton (1962) included appressorium colour and morphology as part of a suite of characters useful in separating *C. dematium* (Pers.:Fr.) Grove and *C. trichellum* (Fr.:Fr.) Duke. Similarly, qualitative and quantitative differences in mycelial appressoria helped in separating three falcate-spored *Colletotrichum* taxa from *Saccharum*, *Sorghum* and *Zea*, where use of standard spore and setal criteria had led to taxonomic confusion (Sutton 1968). In the *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. complex, Cox and Irwin (1988) found appressorium morphology, but not size, in conjunction with conidial width, assisted in discriminating between three biological groups represented among the Australian isolates studied. However, in South African isolates representing 11 species of *Colletotrichum*, Baxter *et al.* (1983) reported that appressorium morphology and dimensions could be used to characterise only one species, *C. crassipes* (Speg.) Arx. With the exception of Bezerra and Ram (1986), who illustrated appressoria of *Mycoleptodiscus indicus* (Sahni) Sutton, and Gerdemann (1954), who described their occurrence in *M. terrestris* (J.W. Gerdemann) Ostazeski, these structures in *Mycoleptodiscus* have not been reported by most authors who have worked with various species of the genus.

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Materials and Methods

Several reports indicate that *Mycoleptodiscus* spp. do not always sporulate freely in culture, or that special media are necessary to induce it. In particular, the addition of plant material to cultures has been found beneficial (Gerdemann 1953, 1954; Ostazeski 1964, 1968; Bezerra and Ram 1986), although some species do sporulate on standard agar media (Charudattan and Conway 1976; Matsushima 1987; Bills and Polishook 1992). Difficulty in inducing sporulation in some of the isolates used during this study led to some testing of various plant species as substrates, using in part as a guide, the host genera reported for *M. indicus* (Sutton 1973). Leaves or leaf pieces were dried, autoclaved, and floated on water agar or Sachs agar in Petri plates at the time of pouring. Cultures were incubated at *c.* 25°C under near ultraviolet light (nuv) on a 12 h photoperiod following inoculation. The media found suitable are indicated for each species. Abbreviations used in the results section are S for Sachs agar, and WA for water agar. Growth rates and cultural characters were assessed on potato dextrose agar (PDA) cultures grown in 9 cm plastic Petri plates and dark-incubated at *c.* 25°C.

Conidia for germination were harvested by placing two or three drops of sterile distilled water on the surface of a sporulating colony, agitating gently by aspiration and expulsion of the water with a Pasteur pipette, then flooded onto the surface of 0.5% sucrose water agar in a 9 cm plastic Petri dish. Coverslips (18 mm square, two per plate) were placed over the spore suspension and pressed firmly into place on the agar surface, then the plates were incubated in the dark at *c.* 25°C (method of R.D. Davis, pers. comm. 1992). Slides for examination were prepared by placing the coverslip over a drop of lactophenol on a slide and warming gently.

In preliminary experiments using *M. lunatus* as a test species, the temperature at which germination occurred had no effect on appressorium morphology over the range 11°–33°C in increments of *c.* 3°C. Similarly, appressoria formed on glass, cellophane, and Petri dish plastic were identical, as were those produced using conidial suspensions of different concentrations. In *M. taiwanensis* Matsushima, there was no variability in appressorium morphology with conidia from V-8 juice agar, Sachs agar + maize leaf and water agar + wheat straw.

All isolates used to produce appressoria are deposited in the Plant Pathology Herbarium, Department of Primary Industries, Indooroopilly (BRIP) as dried sporulating cultures, and are listed in Table 1. Herbarium abbreviations follow Holmgren *et al.* (1990).

Table 1. Isolates of *Mycoleptodiscus* and *Colletotrichum* used to produce appressoria

* = ex-type isolates

Species	Culture no.	Host
M. affinis	BRIP 17195*	*Panicum effusum*
M. atromaculans	HP1LM	*Chamaecyparis thyoides*
M. coloratus	BRIP 19988*	*Cattleya* sp.
M. disciformis	MFC-1P-143*	unknown
M. geniculatus	BRIP 17274*	*Stypandra glauca*
M. lateralis	BRIP 16247*	*Alloteropsis semialata*
M. lunatus	BRIP 13852*	*Carpobrotus glaucescens*
M. sphaericus	ATCC 18104*	*Lotus corniculatus*
M. taiwanensis	MFC 6T720*	*Areca catechu*
M. terrestris	BRIP 16943	*Baeckea virgata*
M. unilateralis	IMI 324533*	*Chlorophytum capense*
M. variabilis	BRIP 16983*	*Dianella congesta*
M. variabilis	BRIP 20066	*Dianella congesta*
C. gloeosporioides	BRIP 16621	*Fragaria* ×*ananassa*

Results

Appressorium morphology in the 12 species examined is described below.

Mycoleptodiscus affinis Alcorn & P. Cannon (Fig. 1*a*)

Appressoria mid-olivaceous brown, smooth, outline entire or more commonly moderately to deeply lobed, generally irregular in shape, occasionally obovoid or clavate with sinuate

margins, or cruciate, rarely 1-septate, 7.5–14 x 5–8.5(–11) µm, germ pore *c.* 1.8–2.0 µm in diameter, 2–4 µm wide at the basal septum (S + maize, WA + bean).

Mycoleptodiscus atromaculans Bills & Polishook (Fig. 1*b*)

Appressoria mid- to dark olivaceous brown, broadly ellipsoid to oblong-oval or obovoid, straight or occasionally curved, outline entire or rarely with one or two short broad lobes, 0 or 1 septate, septum transverse or occasionally oblique, germ pore *c.* 0.5–1 µm in diameter, in septate appressoria usually in the upper cell, 10–14 x 7– 10 µm, base truncate or rounded-truncate 3–4 µm in diameter (S + *Cupressus* sp.).

Mycoleptodiscus coloratus Alcorn, sp. nov. (Fig. 1*c*)

Appressoria mid-olivaceous brown, obovoid to broadly clavate, straight or commonly curved or bent, often uncinate, sometimes curved at >180°, unlobed, smooth, aseptate, 14–19 x 8–13 µm, 3–5.5 µm wide at the base, pore not visible in many appressoria, indistinct in others, *c.* 1–1.5 µm in diameter.

Mycoleptodiscus disciformis Matsushima (Fig. 1*d*)

Appressoria pale to medium brown, thin-walled, variable in shape, ellipsoid to clavate, obovoid or irregular, occasionally ± cylindrical, straight or curved, outline smooth to angular to distinctly lobed, aseptate, terminal, lateral or sometimes intercalary, pores 1(–2), 1(–1.5) µm in diameter, occasionally with short dark radial lines extending outwards from pore edge, 7–17 x 4.5–7 µm, 2.5–5 µm wide at the base (corn meal agar).

Mycoleptodiscus geniculatus Alcorn, sp. nov. (Fig. 1*e*)

Appressoria mid-olivaceous brown, irregularly obovoid to narrowly clavate or ± cylindrical, straight to commonly curved or bent, sometimes uncinate, occasionally ± sigmoid or bluntly bifurcate, unlobed, 1(–2) septate with pore *c.* 1.0 µm in diameter in upper cell, at times with pore indistinct, 9.5–15 x 6–7 µm, 3–5 µm in diameter at the base.

Mycoleptodiscus lateralis Alcorn & B. Sutton (Fig. 1*f*)

Appressoria mid-olivaceous brown, smooth, obovoid to clavate or irregular, straight to variously bent, sometimes uncinate, outline rather irregular, but margin entire, 1(–2) septate, sometimes constricted at septum, commonly with a germ pore 1.5–2.0 µm in diameter in the upper cell or occasionally in the median cell of a 2-septate appressorium, 9–16 x 7–11 µm, 2.5–4.5 (–5) µm in diameter at the base (WA + cotton).

Mycoleptodiscus lunatus B. Sutton & Alcorn (Fig. 1*g*)

Appressoria mid- to dark olivaceous brown, mostly obovoid, angular-obovoid or clavate to irregular in shape, occasionally cruciate, outline entire or margin bluntly sinuate, some lobed, but not deeply incised, aseptate, 6–15 x 4.5–8 µm, (1.5–)2–3(–3.5) µm wide at the base, pore *c.* 0.8–1.5 µm in diameter (S + maize, WA + bean).

Mycoleptodiscus sphaericus Ostazeski (Figs 2*a* and 6)

Sutton (1973) was not able to induce sporulation in the ex-type culture of *M. sphaericus* Ostazeski (ATCC 18104). A sub-culture obtained from the ATCC in November 1992 produced conidiomata freely on Sachs agar + maize leaf. Appressoria were not formed by conidia germinated under coverslips on 0.5% sucrose water agar, but some occurred when conidia were incubated in a water drop on a slide for 5 days. Most were formed terminally on hyphae remote from conidia, rather than on the germ tubes, and thus are probably more correctly regarded as mycelial appressoria. They were pale to mid-brown, thin-walled, clavate to obovoid, straight or curved, margin entire or somewhat incised, 0 or 1 septate with a pore in one or both cells, 10–25 x 6–11 µm; or complex, with large, irregular, sometimes overlapping lobes which at times are septum-delimited; pores (1.5–) 1.8–2.5 (–2.8) µm in diameter, often surrounded by radiating dark lines more or less on the same optical plane as the pore aperture.

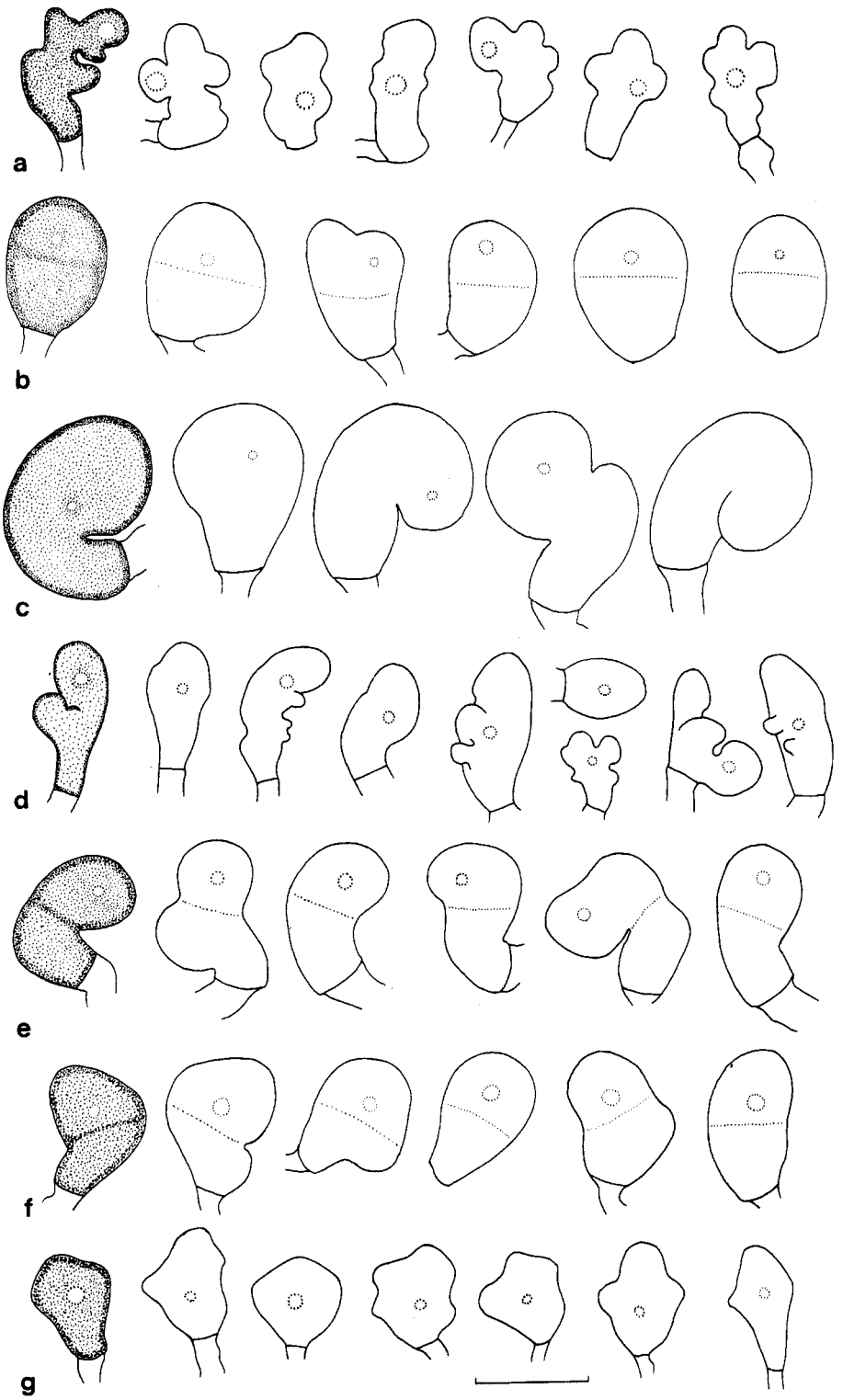

Fig. 1.

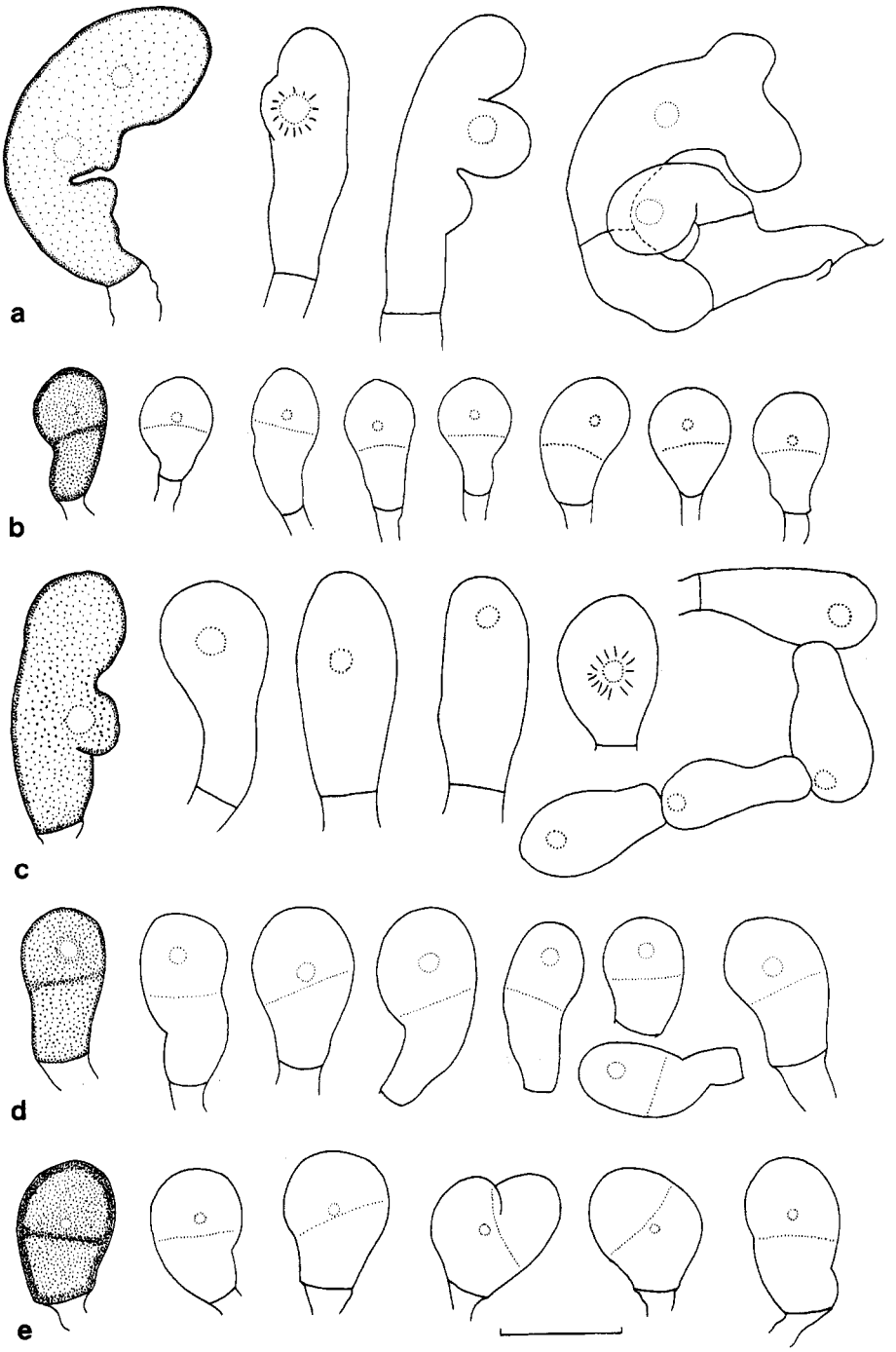

Fig. 2. Appressoria of *Mycoleptodiscus* spp. (*a*) *M. sphaericus;* (*b*) *M. taiwanensis;* (*c*) *M. terrestris;* (*d*) *M. unilateralis;* (*e*) *M. variabilis.* Scale = 10 μm.

Fig. 1. Appressoria of *Mycoleptodiscus* spp. (*a*) *M. affinis;* (*b*) *M. atromaculans;* (*c*) *M. coloratus;* (*d*) *M. disciformis;* (*e*) *M. geniculatus;* (*f*) *M. lateralis;* (*g*) *M. lunatus.* Scale = 10 μm.

Mycoleptodiscus taiwanensis Matsushima (Fig. 2*b*)

Appressoria mid-olivaceous brown, smooth, mostly clavate-obovoid with a broadly rounded apex, occasionally anvil-shaped, straight or slightly curved, outline entire or laterally somewhat sinuate, (0–)1(–2) transversely septate, septum thin, sometimes constricted slightly at septum, in 1-septate appressoria commonly with a single pore < 1.0 µm in diameter in the upper cell, 9.5–13 x 5–6.5 µm, 2.5–3.5 µm wide at the base (S + maize).

Mycoleptodiscus terrestris (J.W. Gerdemann) Ostazeski (Figs 2*c* and 6)

Appressoria pale to mid-olivaceous brown, thin-walled, obovoid to clavate or cylindrical, straight or bent, margin entire or sometimes broadly lobed, 0 (–1) septate, on some conidia produced in chains of 2–4 appressoria each of which is borne directly on the lower one or on a short germ tube originating from it, pores 1(–2), often with distinct dark radial lines around pore, 11.5–28 x 6–10.5 µm, 3–6µm wide at basal septum, pore 1.5–2 (–2.5) µm in diameter, circular or sometimes irregular in shape (WA + bean, WA + *Hippeastrum* sp., WA + wheat straw).

Mycoleptodiscus unilateralis B. Sutton & Alcorn (Fig. 2*d*)

Appressoria mid-olivaceous brown, thin-walled, mostly ± obovoid to clavate with a truncate base, straight to slightly curved or distinctly bent, margin entire, 1-septate, with a pore *c.* 1.2–1.5(–2) µm in diameter in the upper cell, 10–15.5 x 6–8(–11) µm, 2.5–4 µm wide at the base (WA + cotton).

Mycoleptodiscus variabilis Alcorn, sp. nov. (Fig. 2*e*)

Appressoria mid-brown or slightly olivaceous brown, mostly obovoid, occasionally broadly clavate, straight to slightly curved, sometimes bent into a right angle or almost uncinate, (0–) 1 septate, septum thin, transverse or oblique, sometimes slightly constricted at septum, pore *c.* 1.0 µm in diameter and usually in the upper cell, rarely one pore in each cell, 9–15 x 7–9 µm, 3.5–5 µm wide at the base.

In conjunction with the variations in appressorium form described above, differences in conidium morphology indicated that un-named isolates used in this study could be separated at species rank. As no formally published names which would accommodate these taxa are known, the new species are described below.

Mycoleptodiscus coloratus Alcorn, sp. nov. (Figs 1*c* and 3)

Conidiomata atrobrunnea, sporodochialia, interdum circularia sed generatim irregularis, 25–165 x 16–125 µm. Cellulae conidiogenae medio vel atrobrunneae, laeves, discretae vel in conidiomatibus aggregatae, ampulliformes vel late ellipsoidales, 17–25 x 13–19 (–21) µm, collo 3–10 x 5–8 µm et apertura 3–4.5 (–5) µm diam. Conidia late lunata vel ovalia-ellipsoidalia, inaequilatera, aseptata, plerumque tumida unilateralia, 16–27 x 8–13(–16) µm, cum appendicibus cellulosis simplicibus filiformibus apicalibus et basalibus usque ad 8 µm longa instructis.

Holotypus: BRIP 19988.

Colonies on PDA reddish-brown with paler margins *c.* 2 mm wide, edge somewhat scalloped, aerial mycelium flocculose, of low elevation and not very dense, pale brownish-orange; daily radial growth rate *c.* 1.5–2.0 mm; a brownish-yellow pigment diffuses into the surrounding agar; colony reverse similarly coloured; immersed hyphae 2–10 µm in diameter, aerial hyphae 2–8.5 µm in diameter.

Conidiomata varying from single conidiogenous cells up to moderately large aggregations, dark brown, sometimes rounded, but generally irregular in outline, 25–165 x 16–125 µm or larger if coalescing. Conidiogenous cells mid- to dark brown, some darker around the neck, ampulliform to broadly ellipsoidal, rounded to somewhat angular in face view, 17–25 x 13–19(–21) µm, with a cylindrical to flared collarette having ragged margins, 3–10 x 5–8 µm, enclosing a circular pore 3–4.5(–5) µm in diameter. Conidia broadly lunate to inequilaterally oval-ellipsoid, strongly convex on one side, concave to flattened to more or

less convex on other side which often has a distinct median swelling varying in degree of protrusion, aseptate, 16–27 x 8–13(–16) µm excluding appendages. Appendages cellular, polar, filiform, simple, the basal appendage often inserted eccentrically on the truncate base, 2–8 µm (apical) or 1–6 µm (basal) long, *c.* 1.0 µm in diameter.

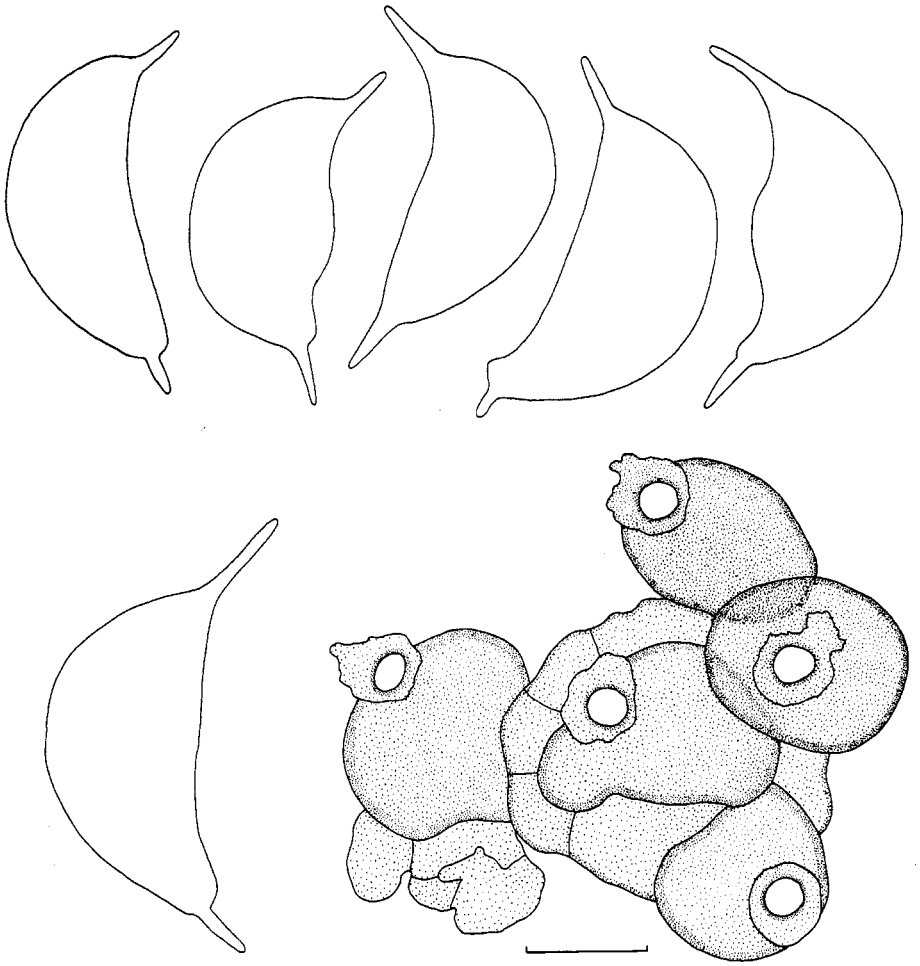

Fig. 3. Conidioma, conidiogenous cells and conidia of *M. coloratus*. Scale = 10 µm.

Notes

Bezerra and Ram (1986) reported that *M. indicus* causes leaf, fruit and stem lesions on *Vanilla fragrans* (Salisb.) Ames in Brazil. The fungus from orchids described above as *M. coloratus* differs from *M. indicus* in its larger conidiogenous cells and conidia, in appressorium morphology (Sutton 1973; Bezerra and Ram 1986), and in cultural appearance on PDA.

Plant substrates supporting moderate to abundant sporulation in culture were cotton, *Cordyline manners-suttoniae* F. Muell., *Grewia occidentalis* L. and *Hippeastrum* sp.

Etymology

(L.) *coloratus* — coloured, for the distinctly coloured cultures on PDA.

Specimens Examined

AUSTRALIA: Queensland: Bibhoora, *Cattleya* sp., 16 Jan. 1992, *J. Allen M.6076* (BRIP 19988, holotype); Windaroo, *Cattleya* sp., 28 Feb. 1993, *L. Hildebrandt* (BRIP 20717); Beenleigh, *Cattleya* hybrids (cvv. Coronation, Wallacia, Pay Dirt x La Tuckeri), 14 May 1993, *L. Forsberg* (BRIP 20908, 20909, 20910); Mareeba, *Cattleya* sp., 12 Jan. 1994, *P. Santry* (BRIP 21586).

Mycoleptodiscus geniculatus Alcorn, sp. nov. (Figs 1*e* and 4)

Conidiomata atrobrunnea, sporodochialia, forma irregularis, usque ad 125 µm diam. Cellulae conidiogenae atrobrunneae, ampulliformes, 10–15 µm diam, laeves, collo 4–6.5 µm diam et apertura 2–3.5 µm diam. Conidia hyalina, fusiformia-ellipsoidalia, aseptata, curvata vel plus minusve geniculata, 14–20 x 5.5–7.5 µm, cum appendicibus cellulosis simplicibus filiformibus apicalibus et basalibus usque ad 12.5 µm longa instructis.

Holotypus: BRIP 17274.

Colonies on PDA yellowish-grey, margins regular and entire, colourless, *c.* 3 mm wide; aerial mycelium paler, of moderate elevation, floccose and somewhat tufted; reverse olive with colour in patches, paler towards the margins; daily radial growth rate *c.* 4 mm; immersed hyphae 1.5–7 µm in diameter, aerial hyphae 1.5–6 µm in diameter.

Conidiomata sporodochial, dark brown, varying from a few conidiogenous cells to irregularly shaped aggregations up to 125 µm in diameter, or larger by confluence. Conidiogenous cells dark brown, rounded to angular in face view, ampulliform in lateral view, 10–15 µm in diameter, smooth, thick-walled, with a circular aperture 2–3.5 µm wide enclosed by a cylindrical to flared collarette 4–6.5 µm in diameter, sometimes extended into a thick-walled cylindrical neck 3–12 x 3.5–6.5 µm. Conidia hyaline, aseptate, thin-walled, fusoid-ellipsoid, curved to ± geniculate with a distinct change in curvature in the upper part, 14–20 x 5.5–7.5µm, with a simple, cellular, filiform appendage at each pole, 4–12.5 µm (apex) and 1–10 µm (base) long, *c.* 0.5–1.0 µm wide.

Notes

This taxon differs from *M. indicus* in having conidiogenous cells with the phialidic aperture sometimes at the tip of a cylindrical neck-like extension, and a proportion of conidia bent in a more or less geniculate fashion rather than smoothly curved as in *M. indicus* (Sutton 1973). I was unable to induce sporulation in a culture of *M. indicus* kindly supplied by Dr J.L. Bezerra, but appressoria of *M. geniculatus* differ markedly from those described for that species (Bezerra and Ram 1986). Two isolates of *M. indicus* were obtained from the American Type Culture Collection (ATCC 46407, ATCC 46408). Very sparse sporulation occurred in ATCC 46407, and conidia were 0 or 1 septate, ± lunate, 17.5–22.5 x 5–5.5 µm, with appendages 5–10 µm (apical) and 5–12.5 µm (basal) long. These characteristics suggest that the isolate may not be conspecific with *M. indicus* (Sutton 1973).

Some sporulation in culture was obtained on *Stypandra glauca* R.Br., *Hippeastrum* sp., *Grewia occidentalis*, *Cordyline manners-suttoniae*, bean and cotton, although on none of these species was production of conidia abundant.

Etymology

(L.) *geniculatus* — geniculate, for abrupt change in curvature of some conidia.

Specimen Examined

Australia: Queensland: Girraween National Park, *Stypandra glauca*, 13 Oct. 1990, *V.P. Cooper 55*, BRIP 17274, holotype.

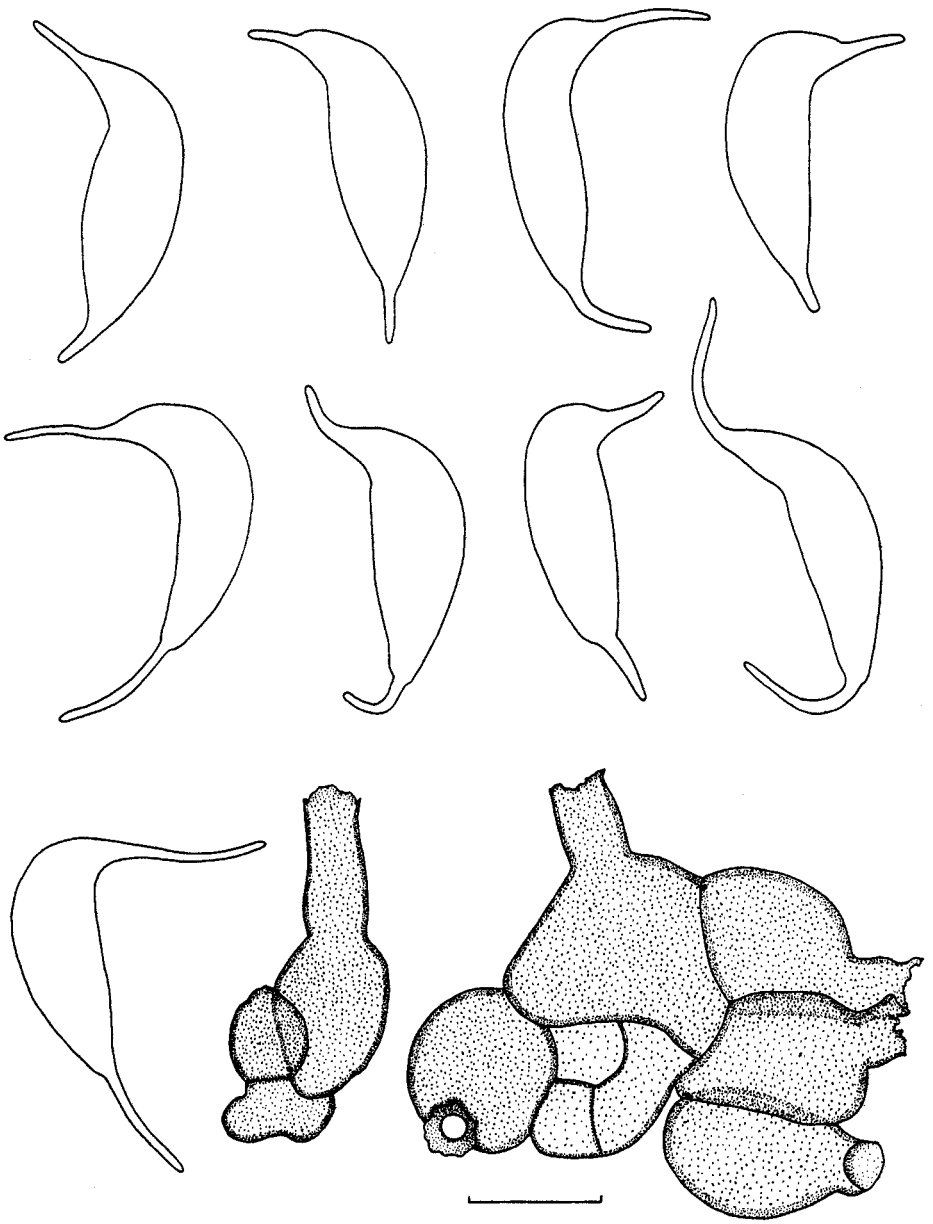

Fig. 4. Conidiomata, conidiogenous cells and conidia of *M. geniculatus*. Scale = 10 μm.

Mycoleptodiscus variabilis Alcorn, sp. nov. (Figs 2*e* and 5)

Conidiomata medio vel atrobrunnea, sporodochialia, e cellulis conidiogenis aggregatis composita, circularia vel irregularia, 125–370 x 100–225 μm. Cellulae conidiogenae medio vel atrobrunneae, laeves, ampulliformes vel elongatae, 14–24 x 9–16 μm, collo 4–6.5 μm diam. et apertura 2.5–4 μm diam. Conidia hyalina, fusiformia, curvata, aseptata, 15–25 x 5.5–8 μm, cum appendicibus cellulosis simplicibus filiformibus apicalibus, basalibus et lateralibus usque ad 22 μm longa instructis.

Holotypus: BRIP 16983.

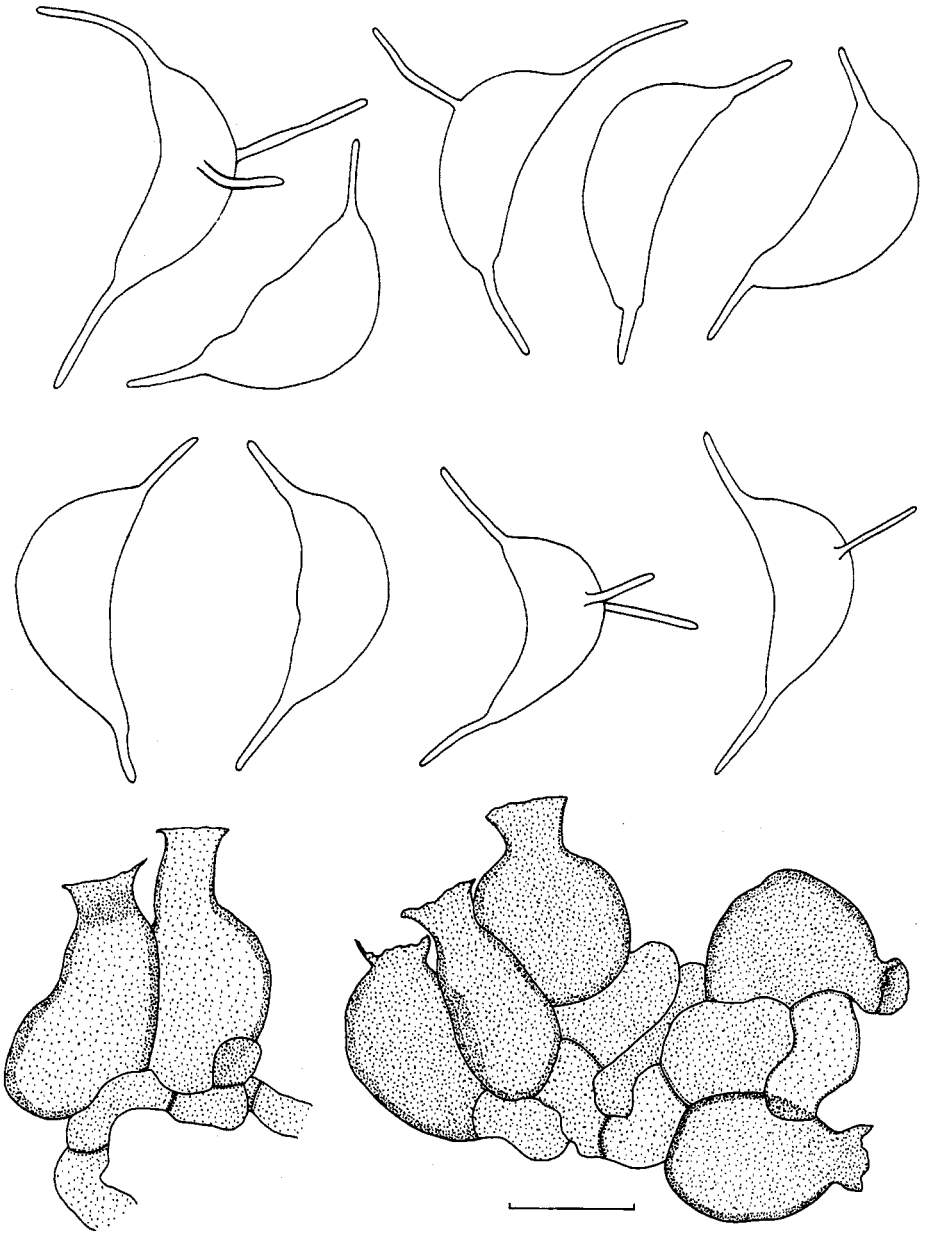

Fig. 5. Conidiomata, conidiogenous cells and conidia of *M. variabilis*. Scale = 10 μm.

Colonies on PDA greyish-yellow, edge regular and with a marginal band of appressed hyphae *c.* 5 mm wide; aerial mycelium floccose, of low elevation; reverse greyish-yellow with a more or less circular to irregular olive brown central area; daily radial growth rate *c.* 7.5 mm; immersed hyphae 1–4 μm in diameter, aerial hyphae 1.5–5.5 μm in diameter.

Conidiomata mid- to dark brown, rounded to usually irregular in outline, 125–370 x 100–225 µm in face view. Conidiogenous cells mid- to dark brown, ampulliform to elongated, rounded to angular in face view, 14–24 µm long, 9–16 µm in diameter in face view with a circular aperture 2.5–4 µm in diameter surrounded by an often flared collarette 4–6.5 µm wide. Conidia hyaline, fusiform, curved, with a single, simple filamentous cellular appendage at each pole, and 0–2 lateral appendages, 15–25 x 5.5–8 µm. The appendages measure 3–21 µm long (apical), 4–15 µm (basal), or 11–22 µm (lateral), and are up to *c.* 1.0 µm wide.

Notes

This species shows similarities to both *M. lateralis* Alcorn & B. Sutton and *M. unilateralis* B. Sutton & Alcorn. Conidia with two lateral appendages resemble those of *M. lateralis*, but are longer, and appressoria are more regular in shape with a smaller germ pore, *c.* 1.0 µm compared with 1.5–2.0 µm in *M. lateralis*. Polar conidial appendages in *M. unilateralis* are shorter than in *M. variabilis*, maximum conidial length is less, and appressoria are more elongated in shape with larger germ pores. Conidiomata of *M. variabilis* are larger than those of *M. lateralis* and *M. unilateralis*.

Sporulation in culture was obtained on leaves of maize, bean and cotton.

Etymology

(L.) *variabilis* — variable, for the number of conidial appendages.

Specimens Examined

Australia: Queensland: Peregian Beach, on *Dianella congesta* R.Br., 28 Jan. 1990, *J.L. Alcorn 9007* (BRIP 16983), holotype; 3 May 1992, *J.L. Alcorn 92/1764* (BRIP 20066).

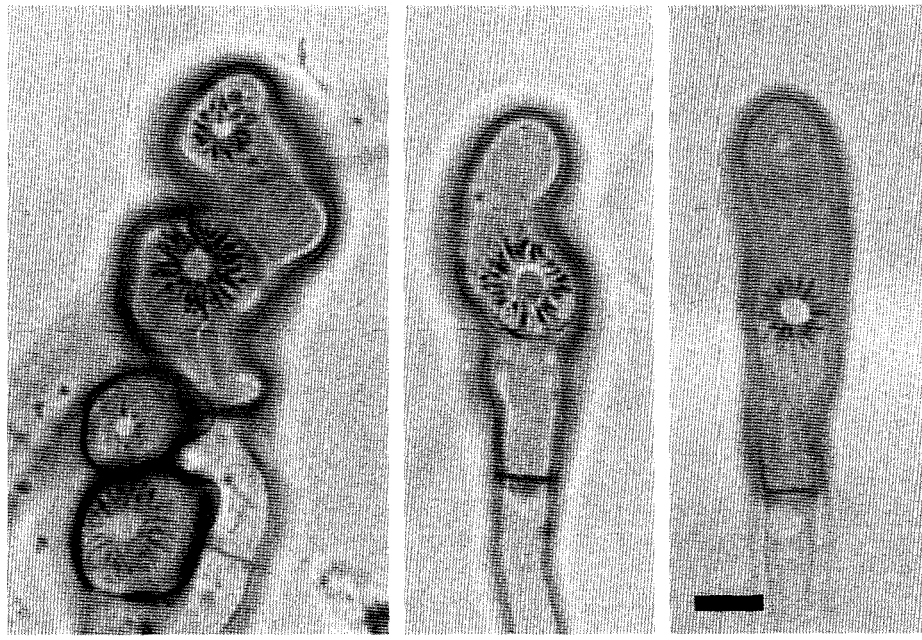

Fig. 6. Appressoria of *M. sphaericus* (left) and *M. terrestris* (right). Scale = 5 µm.

Discussion

Appressorium morphology in the genus *Mycoleptodiscus* shows considerable variation between species. Size, shape, curvature, septation, and germ pore size of appressoria provide a suite of characters which are useful in helping distinguish taxa. Thus, the use of appressorium morphology facilitated the separation of *M. affinis* and *M. lunatus,* and provided further support for the well-reasoned decision by Bills and Polishook (1992) to establish the new species *M. atromaculans* Bills & Polishook as distinct from *M. taiwanensis.*

Sutton and Alcorn (1990) have alluded to the similarities in conidiomatal and conidiogenous cell morphology between *Mycoleptodiscus* species. At that time, neither author had seen sporulating material of *M. sphaericus* or *M. terrestris* (Sutton 1973). The shield-like conidiomata of these two species, consisting of rather pale, fused conidiogenous cells, and originating from a single partially emergent cell borne at the apex of an internal hypha (Gerdemann 1953; McVey and Gerdemann 1960; Ostazeski 1968), contrast somewhat with the equivalent structures in most other species. Both *M. sphaericus* and *M. terrestris* formed appressoria sparingly under conditions favouring their development by other species, and appressorium morphology was quite distinct. If, in the future, the genus is considered unacceptably heterogeneous, such attributes may prove suitably discriminatory. The usual occurrence of *M. sphaericus* and *M. terrestris* as root pathogens may not be coincidental.

In the absence of evidence provided by transmission electron microscopy, any explanation of the distinctive radial lines surrounding the appressorial pores in *M. sphaericus, M. disciformis* and *M. terrestris* would be speculative. They may indicate structures analogous to the internal collar and cone reported for appressoria of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. (Mercer *et al.* 1971) and *C. gloeosporioides* (Brown 1977; Coates *et al.* 1993). Appressoria of the latter species produced by the method described here, and examined by light microscopy, often had a darkened area around the pore. In some appressoria, it was very dark and more or less circular, similar to that reported and illustrated for *C. truncatum* (Schw.) Andrus & Moore by O'Connell *et al.* (1993); in others it was paler (although still darker than the appressorium wall) and roughly stellate or with radial lines similar to, but not as long or pronounced as those of *M. sphaericus* or *M. terrestris* (Fig. 7).

The appressoria of *Mycoleptodiscus* spp. are generally similar to those of *Colletotrichum,* although septation is more common in the former (cf. Sutton 1962, 1980). Variability in germ pore size reported here for *Mycoleptodiscus* also has been reported (Sutton 1962, 1968) or clearly illustrated in *Colletotrichum* (Sutton 1980).

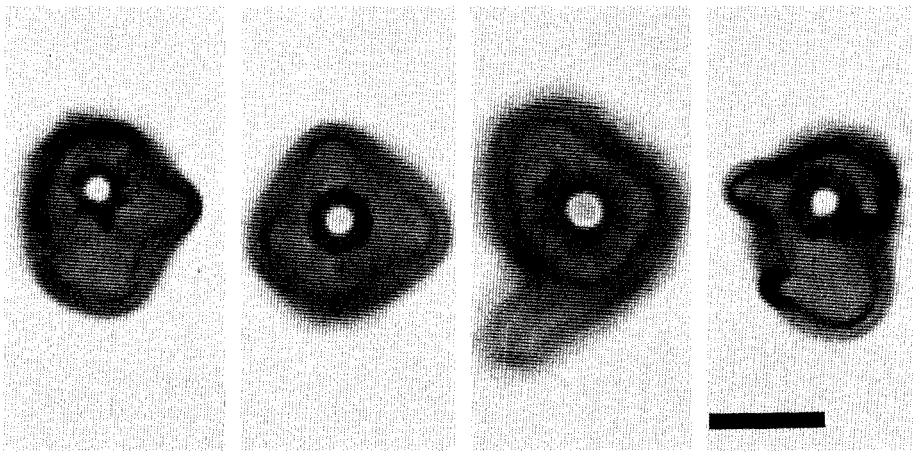

Fig. 7. Appressoria of *Colletotrichum gloeosporioides.* Scale = 5 μm.

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